



# 22<sup>nd</sup> INTERNATIONAL DIATOM SYMPOSIUM

Ghent, Belgium 26-31 August 2012

## ABSTRACTS

22nd International Diatom Symposium

Ghent, Belgium

26-31 August 2012

VLIZ SPECIAL PUBLICATION 58

Organized by:

Lab. Protistology & Aquatic Ecology  
Biology Department  
Ghent University  
Krijgslaan 281, S8  
B-9000 Ghent, Belgium  
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### **This publication should be cited as follows:**

Sabbe Koen, Van de Vijver Bart & Vyverman Wim (Eds). 2012 Twentysecond International Diatom Symposium, Aula Academica, Ghent, 26-31 August 2012. Abstracts. VLIZ Special Publication 58, 260p.

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ISSN 1377-0950

## **Contents**

Preface	5
Keynote presentations	7
Oral presentations	19
Poster presentations	119
Author index	253



## **Preface**

The Laboratory of Protistology & Aquatic Ecology (Ghent University) and the National Botanic Garden of Belgium are proud to welcome over 210 diatom scientists and students to the 22<sup>nd</sup> International Diatom Symposium in Ghent, August 26-31<sup>st</sup> 2012.

The IDS 2012 program will host sessions on the following themes in diatom research: DNA Barcoding, Biodiversity & Biogeography, Ecology and Physiology, Ecotoxicology, Evolution and Phylogeny, Fossils, Molecular Biology, Morphology, Paleoecology, Taxonomy and Water Quality.

During the 22<sup>nd</sup> International Diatom Symposium, keynote lectures by 9 eminent diatom researchers will highlight trends and opportunities in contemporary diatom science. In addition to 97 abstracts which will be given as oral presentations, over 131 abstracts will be presented as posters in three sponsored poster sessions.

We wish all participants a pleasant and fruitful symposium,

The organizers,

Koen Sabbe  
Bart Van de Vijver  
Wim Vyverman



**KEYNOTE  
PRESENTATIONS**





## DATA RICH, INFORMATION POOR? 10 YEARS OF THE EU WATER FRAMEWORK DIRECTIVE IN EUROPE.

Martyn Kelly

Bowburn Consultancy

The Water Framework Directive (WFD) provides the legal basis for water management in the European Union (EU). Twelve years after it was passed, all but five out of 27 EU Member States now have phytobenthos assessment methods for rivers, whilst only nine have methods for lakes. Most of these methods are based on diatoms, although a few are supplemented by evaluations of non-diatom algae and some states include macroalgae as part of their macrophytes assessment methods. Norway is the exception, having a phytobenthos assessment system based on non-diatom algae alone. Over half of all states have methods based wholly or partly on weighted average metrics developed before the onset of the WFD, with nine states choosing the Indice de Polluosensibilité Spécifique. These metrics generally have high correlations with the predominant nutrient/organic pollution gradient found across Europe and, as such, represent pragmatic solutions to ecological status assessment. However, their widespread use also raises questions about what, exactly, “ecological status” means. Diatom-based metrics are often strongly correlated with chemical pressure gradients yet this may be a mixed blessing, particularly when we move from describing spatial patterns of ecological status to prescribing solutions for water bodies that fail to achieve good ecological status. Such pressure gradients are often composed of several intercorrelated variables, making it difficult to disentangle “correlation” and “causation” in the absence of ecophysiological studies of individual diatom species. Moreover, the focus on strong relationships with chemical gradients means that most phytobenthos metrics describe the scale of hazard at a site rather than the risk that the hazard poses to other trophic levels, and to ecosystem services. This first generation of phytobenthos assessment tools are, at best, “fit for purpose” but may be inadequate when catchment managers need detailed guidance on remediation steps needed for particular water bodies. A second generation of assessment tools, focused on the fitness of the phytobenthos as part of aquatic ecosystems, rather than just as indicators of chemical conditions, is needed if the goal of good ecological status around Europe is to be achieved.

## ASPECTS OF DIATOM METABOLISM

Peter G. Kroth

Department of Biology, University of Konstanz

Algal research to date experiences a strong boost from actual genome projects and the development of molecular tools to study algae and algal processes, like large scale transcription analyses, transformation techniques and gene silencing approaches. For diatoms there are two genomes published (for the pennates *Phaeodactylum tricorutum* and the centric *Thalassiosira pseudonana*) including two more genomes close to publication (*Pseudonitzschia* sp. and *Fragilariopsis cylindrus*). Diatoms are very interesting organisms for various reasons, including their ecological relevance, their silicified cell walls and their peculiar evolution via secondary endocytobiosis (the incorporation of a eukaryotic alga into a eukaryotic host cell, followed by transformation of the endosymbiont into a plastid). Especially the latter process resulted in various differences with respect to the physiology and cell biology of these algae when compared to green algae and land plants. Comparative analyses revealed relocated metabolic pathways, modified regulation processes and unexpected proteins:

- (i) In diatoms very few enzymes of the Calvin cycle are redox regulated by small proteins termed thioredoxins that function as a “light switch” for CO<sub>2</sub> fixation by reducing target enzymes.
- (ii) Essential pathways like the oxidative pentose phosphate together with nucleotide synthesis pathways have been reallocated in diatoms.
- (iii) Diatoms possess a urea cycle.
- (iv) Diatoms possess the bacterial Entner-Doudoroff pathway.
- (v) As a consequence, additional plastidic translocators for nucleotides and carbohydrates were established to allow the essential transport of triosephosphates as well as nucleotides into and out of the plastids.
- (vi) Enzymes of the second half of glycolysis can be found in the cytosol, in the plastids as well as in the mitochondria.
- (vii) There are first indications that enzymes might be located in the periplastidic space of diatoms, including an NADP-dependent thioredoxin reductase and a phosphogluconate dehydrogenase.
- (viii) Diatoms also show a very unusual distribution of carboxylating and decarboxylating enzymes possibly involved in photosynthesis.

## DIATOM-BASED BIOMONITORING IN CANADA: REFLECTIONS ON 10 YEARS OF DEVELOPMENT, IMPROVEMENT AND PERSUASION

Isabelle Lavoie, Stéphane Campeau & Claude Fortin

Institut national de la recherche scientifique, Centre Eau Terre Environnement

Canadian provinces and territories have the primary jurisdiction over most areas of freshwater management and protection, and establish their own guidelines, objectives and regulations following the recommendations of the Canadian Council of Ministers of the Environment. These different jurisdictional roles have led to a number of laws and policies specific to each province and territory (eg., *Sustainable Development Act* in Québec, *Clean Water Act* in Ontario, *Water Management Framework* in Saskatchewan, etc.).

All these regulations require integrated water management systems with a good knowledge and understanding of reference conditions and the development of monitoring tools indicating a measure of departure from reference state. However, water quality monitoring in Canada has mostly been based on traditional chemical measurements, and water agencies have been seeking new approaches allowing for a more adequate monitoring of aquatic ecosystem integrity. Efforts have been made (especially in Ontario and Quebec) to promote a macro-invertebrate biomonitoring network for evaluating aquatic ecosystem conditions. However, although macro-invertebrates are excellent general indicators of habitat and water quality at the watershed scale, macro-invertebrate monitoring alone cannot always provide a reliable indication of eutrophication and is not as sensitive to water quality fluctuations as diatom assemblages are.

Inspired by the success of routine diatom-based monitoring in certain European countries, a similar approach has been suggested for assessing stream biological integrity in Eastern Canada. The Eastern Canadian Diatom Index (IDEC: *Indice Diatomées de l'Est du Canada*) was developed almost 10 years ago to monitor the biological integrity of streams and rivers in Eastern Canada. The IDEC gained popularity in Eastern Canada and has been used successfully to evaluate the biological status of more than 1500 diatom assemblages over the past 10 years. The experience gained on diatom-based monitoring over the years as well as the large number of samples added to the database led to the modelling of a new version of the IDEC in 2012.

This presentation will explain the steps to the creation of the IDEC, its evolution over the years, and will also expose our reflections on 10 years of diatom-based development, persuasion, and ongoing challenges.

## A MULTIFACETED AND INTEGRATED APPROACH TO THE STUDY OF MARINE PLANKTONIC DIATOMS

Marina Montresor

Stazione Zoologica Anton Dohrn

Diatoms represent the most abundant phytoplankton group at the LTER MareChiara in the Bay of Naples (Mediterranean Sea); they dominate during the spring and autumn blooms and produce minor blooms during summer stratification. Detection of microalgal distribution patterns relies on the biological units (species, populations). Results of our research uncovered cryptic diversity in all genera examined: *Skeletonema*, *Pseudo-nitzschia*, *Chaetoceros* and *Leptocylindrus*. These findings challenged our capability to monitor species with standard techniques, demonstrating the need for molecular tools such as clone libraries, microarrays, metagenomic approaches. On the other hand, detection of cryptic diversity clarified temporal and spatial patterns in morphospecies. Analysis of environmental samples using clone libraries of partial LSU rDNA confirmed distinct seasonal patterns in different *Pseudo-nitzschia* species. In *Skeletonema*, analysis of global isolates showed species-specific distribution ranges, challenging the assumption that phytoplankton species lack biogeographic structure. This broad diversity calls for integrated approaches towards delineating species, including assessment of functional differences. Screening of *Pseudo-nitzschia multistriata* population structure with microsatellites showed marked intraspecific diversity and provided evidence that genetically different metapopulations coexist in sympatry. Moreover, microsatellite profiles in culture vary over time, suggesting that the high genetic diversity depends on rapid accumulation of clonal mutations and genetic recombination during sexual reproduction.

Life cycle traits of microalgae also play an important role in regulating the dynamics of natural populations. Many diatoms at LTER-MC form resting stages, but interactions between vegetative and resting stages are complex and links between the onset of species-specific blooms and the germination of resting stages is difficult to assess. In species that do not form resting stages, gradual reduction and sudden increases in cell sizes shed light on timing for asexual and sexual phases. Analysis of cell size distribution patterns of *Pseudo-nitzschia multistriata* coupled with modeling approaches showed that the length of life cycle – from the initial cells to the smallest cell size - lasts about 4 years; if sexual reproduction does not take place, the population is condemned to local extinction.

Population dynamics represents the interplay between the organisms' complex life histories and their environment. The 'observation' of natural populations over time and space, the coupling with experimental approaches, and a deeper understanding of the organism biology – now possible with genomic approaches –allows progress in elucidating their complex dynamics.

## LIFE IN A GLASSHOUSE: FROM CELL WALL MORPHOGENESIS TO NANOTECHNOLOGICAL APPLICATIONS

Nicole Poulsen

B Cube: Center of Innovative Competence for Molecular Bioengineering, TU Dresden

The diatom cell wall structure is a species-specific characteristic demonstrating that its morphogenesis is genetically encoded. Understanding the molecular mechanisms by which a single cell executes the morphogenetic program for the formation of an inorganic material (biomineralization) is not only a fascinating biological problem, but also of great interest for nanomaterials science and technology. Recently, diatom genome sequencing projects have provided the opportunity to identify the entire set of proteins involved in silica formation. Although subsequent transcriptomics and proteomics analyses provided hundreds of potential candidate proteins they have failed to identify already known silica forming proteins. This has raised concerns regarding the specificity and suitability of such high-throughput methods for studying diatom silica formation. In this presentation I will describe the results that we have obtained using an entirely different approach. The genome database of the model diatom *Thalassiosira pseudonana* was screened *in silico* for proteins containing domains that mimic the amino acid compositions (rather than sequence) of silica forming proteins. The screen retrieved the known silica forming proteins in addition to 86 other proteins, of which most did not exhibit sequence homology to any proteins from other organisms. Among these diatom-specific proteins are six proteins, which we denoted cingulins, that exhibit unique and highly repetitive amino acid sequence motifs. Subsequent *in vivo* functional analysis of cingulins resulted in the identification of a previously unknown yet apparently general principle for diatom silica morphogenesis. In the final part of my talk I will demonstrate how the emerging insight into the molecular mechanism of diatom silica formation has enabled the development of an entirely biological method for the production of nanopatterned silica with desired catalytic activities.

## METAGENOMICS: FROM PARTS LISTS TO ECOLOGY

Jeroen Raes

VIB Department of Structural Biology

Meta-omics (metagenomics, metatranscriptomics, metaproteomics) are powerful tools for the analysis of the (unculturable fraction of) microbial communities. Because of its complexity, meta-omics data has required the development of novel computational analysis tools to determine the functional and phylogenetic composition of the sampled community (Raes et al., *Curr Opin Microbiol* 2007).

However, to go from a metagenomic 'parts list' (i.e. a bag of genes) to an initial understanding of the ecosystem structure and functioning, current tools are not sufficient (Raes & Bork, *Nat Rev Microbiol* 2009). I will present a range of approaches to analyze metagenomes, infer ecological parameters, extract species interaction and competition relationships, interpret metabolic changes and identify biologically relevant features from meta-omics data with applications in diverse environments. I will specifically focus on data coming from the Tara oceans expedition, integrating multi-omics approaches with detailed oceanographic measurements from viruses to fish larvae.

## TRADITIONAL TAXONOMY IN THE MOLECULAR AGE: CONTROVERSIES OR SYNERGIES?

Rosa Trobajo

Aquatic Ecosystems, Institute for Food and Agricultural Research and Technology (IRTA)

A sound species taxonomy of diatoms is of paramount importance, among other reasons because everything else – ecology, biogeography, evolution, stratigraphy, palaeoecology, biomonitoring, biotechnology, etc (all of which are rapidly expanding) – is underpinned by our ability to identify species and link data together via species names. The introduction of molecular data into diatom taxonomy has led to much discussion and even some antagonism. At their most extreme, opposing attitudes could perhaps be caricatured as (1) morphology is unreliable, subjective and should be abandoned as a source of data and replaced by modern molecular techniques; versus (2) the molecular approach is full of fake promises about improvements, and all it has done so far is reveal high genetic diversity whose relevance (if any) is unknown. However, increasingly there are examples that show that multiple sources of data (e.g. morphological and molecular but also physiological, reproductive behaviour, etc) provide different but complementary insights into species delimitation and identification. To illustrate this, I will mainly use our work on some *Nitzschia* sect. *Lanceolatae* species, but I will also make reference to other diatoms from time to time. *Nitzschia* is a large genus, notorious for its taxonomic difficulty, and members of the sect. *Lanceolatae* are particularly challenging, because most have small cells and delicate structure, and because they offer few diagnostic characters. However, these difficulties need to be overcome, for instance because the *Lanceolatae* are of great importance from the (palaeo-) ecological point of view: they occur frequently and abundantly in all kinds of waters, and several of them are considered to be indicators of particular environmental conditions. I will explain how we have used culture experiments to investigate 1) how phenotypic plasticity (e.g. in relation to salinity) affects the reliability of morphological characters for taxonomy, 2) morphological changes associated with the life cycle, 3) reproductive biology, and 4) ecophysiology, and I will then explore how these results can be integrated with molecular phylogenetic data. Finally, I will provide a brief evaluation of the current status of DNA-based barcoding in diatoms and its potential and limitations in future characterizations of diatom communities.

## LIFE ON THE EDGE: HOW INTERTIDAL BENTHIC DIATOMS THRIVE IN COASTAL ENVIRONMENTS AND WHY WE SHOULD CARE'

Graham J. C. Underwood

School of Biological Sciences, University of Essex

Golden brown patches of colour on the surface of intertidal mudflats and sand flats are the signs of abundant populations of microphytobenthic diatoms. Species-rich and physiologically versatile, these intertidal assemblages nicely illustrate some of the key advantages of living in biofilms. Biofilms are three dimensional structures, with cells held within a matrix of sediment particles and extracellular polymeric substances (EPS). Irradiance levels at the biofilm's surface can be extremely high, and different species of microphytobenthic diatom show distinct migratory behaviour, in response to light gradients, and use vertical migration within the biofilm as a mechanism of photoacclimation, combined with physiological responses to deal with light stress and desiccation. Changing light and nutrient conditions also mediate patterns of production of different EPS fractions within biofilms, and EPS serves both a protective and nutritional function for benthic diatoms. Diatom species composition is also influenced by nutrients, spatial distribution and environmental factors such as anoxia and disturbance, and has the potential to feedback to effect biofilm properties. All these factors result in an interplay between species richness, abundance and ecosystem functioning. High rates of production, nutrient uptake and oxygen production mean that estuarine biofilms play an important role in the biogeochemistry and ecology of shallow estuarine and coastal waters. Quantifying and understanding diatom biofilm processes is a necessary component for good management of important coastal ecosystems.



## DIATOM DISTRIBUTIONS IN SPACE AND TIME – A CASE STUDY FROM THE POLAR REGIONS

Elie Verleyen

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The composition, diversity and distribution of present-day biota are shaped by the geological, tectonic and climatic past, which resulted in the distinct biogeographical realms we observe in the terrestrial biosphere. Here I summarize our recent studies on the biogeography of polar freshwater diatoms. Antarctic lake-dwelling diatom communities are impoverished and imbalanced in comparison with Arctic communities, and characterized by high levels of endemism. Molecular data on ubiquitous morphospecies that are widespread in the Antarctic region are starting to reveal substantial hidden diversity, with distinct Antarctic and sub-Antarctic lineages. This suggests that estimates based on the morphospecies concept are conservative and may strongly underestimate the observed levels of endemism. The Antarctic communities are further characterized by the absence of key functional groups such as planktonic taxa, a general paucity of globally successful genera, and an overrepresentation of terrestrial lineages. Comparison of contemporary Antarctic floras with fossil Miocene assemblages points to high rates of local extinction during glacial maxima, in combination with radiations and the selective survival of aerophilic taxa in glacial refugia. We also observed strong bioregionalisation patterns within the Antarctic Realm, which are highly concordant with the three main biogeographical regions traditionally recognized in plants and animals, namely Sub-Antarctica, Maritime Antarctica and Continental Antarctica. Within Continental Antarctica, the observed biogeographic provincialism is likely related to differences in the glacial history of the ice-free regions. Sediment records spanning the Late Quaternary period indicate that lake districts which escaped complete glacial overriding during the Last Glacial Maximum (LGM) hold a relict diatom flora, composed of Antarctic endemics and ubiquitous taxa that inhabit cold environments elsewhere. By contrast, in regions that were completely overridden by the East Antarctic Ice Sheet, the diatom communities are composed of aerophilic Antarctic endemics and ubiquitous taxa. These taxa were probably derived from a local diatom pool that was able to survive in local terrestrial nunataks. This is confirmed by a time-constrained molecular phylogeny of the aerophilic diatom *Pinnularia borealis* which suggests that the Antarctic lineage diverged 7.8 (2-15) Ma ago, and hence before the onset of Pleistocene glacial-interglacial cycles. In addition, there is evidence for large-scale extinctions during the LGM of species currently thriving in Sub-Antarctic habitats but which were present in Continental Antarctica during the warmer Eemian interglacial.



**ORAL  
PRESENTATIONS**



## LARGE-SCALE MORPHOLOGICAL CONVERGENCE COINCIDES WITH REPLICATE RADIATIONS OF DIATOMS INTO FRESHWATERS

Andrew J. Alverson

Universtiy of Arkansas, Department of Biological Sciences

Salinity represents a substantial barrier to the distribution of diatoms and other algae. A phylogeny of the Thalassiosirales revealed multiple independent freshwater colonization events, two of which led to major evolutionary radiations. These two lineages share a number of independently derived phenotypes, including thickened costae, marginal chambers, and several strutted process characters. Comparative analyses show a strong correlation between the evolution of these traits and their occurrence in freshwater taxa, providing compelling evidence that the convergent traits confer some adaptive value to life in freshwaters.

## APPLICATION OF NEW FLUORESCENCE-TAGGED AMINES AND POLYMERS IN STUDY OF DIATOM PHYSIOLOGY

Vadim V. Annenkov, Tatjana N. Basharina & Elena N. Danilovtseva

Limnological Institute of the Siberian Branch of the Russian Academy of Sciences

We present the results of the growth of the fresh-water diatom *Synedra acus* subsp. *radians* (Kützing) Skabichevskii in the presence of new fluorescence-tagged amines containing one or two tertiary amine moieties and high-molecular polymers with carboxylic, amine and imidazole groups. The following observations and conclusions have been made:

- Poly(acrylic acid) (PAA) bearing fluorescence groups (one group *per* chain) was found in silica frustules after *S. acus* growth in the presence of PAA. Non-tagged PAA was also detected in the frustules after removing of organic components of the cells. Such neutral polymer as poly(1-vinylimidazole) and positive charged poly(vinyl amine) are not included into the frustules. We consider this behaviour of PAA is explained with (macro)pinocytosis hypothesis [Vrieling E. G. et al. PNAS 104:10441]. The most probable cytoplasmic form of silicon is slightly condensed silicic acid [Annenkov V.V. et al. Biomacromolecules 12:1772] and PAA is similar with poly(silicic acid) in acidity, so we can suppose that during some stages of silicon assimilation the diatom cell does not discriminate these substances and PAA penetrates into growing valves.

- Fluorescence-tagged short-chain amines penetrate into the diatom cell and stain new frustules similar to the known isotrackerers PDMPO and HCK-123 [J. Desclés et al. New Phytol. 177:822]. Formation of dyed submicron cytoplasmic vesicles was observed on the first stage of new valve growth. These vesicles disappear after the formation of a two-dimensional valve, which takes 5–10 min for individual cells. Further formation of the three-dimensional valve structure takes 10–12 h, following which cell division occurs. We speculate that the small cytoplasmic vesicles observed are related to silicic acid assimilation via a (macro)pinocytosis mechanism which could take place on the first stage of the valve formation.

- Fluorescence-tagged short-chain amines are useful substances for monitoring processes in diatoms. We have found that the introduction of the dyes into a synchronized culture under silicon starvation is a good way to estimate fraction of cells stopped in G<sub>2</sub> phase (synthesis of new valves) – these cells contain stainable vesicles as opposed to good synchronized cells. The ability of new dyes to stain SDV (Silicon Deposition Vesicle) was used to monitor splitting of diatom frustules under the action of terahertz laser irradiation. We have found that this irradiation allows to remove silicon valves from the organic membrane without deterioration of chloroplasts and SDV.

The study was partially supported by Project # 11-04-00707-a of the Russian Foundation for Basic Research.

## CELL-STATE TRANSITIONS IN THE DIATOM *THALASSIOSIRA PSEUDONANA* DURING DIURNAL GROWTH

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<sup>1</sup>Institute for Systems Biology

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Diatoms are important primary producers in marine ecosystems whose success depends on the regulation of their physiological states under changing environments. We have conducted a genome-wide survey of the diatom *Thalassiosira pseudonana* during growth at two moderate CO<sub>2</sub> levels. This reveals large, reproducible cell-state transitions between four principal conditions: I) exponential and nutrient replete, II) stationary and nutrient depleted, III) dark and IV) light phases of growth. Here we present a gene-level characterization of these physiological modes, including patterns of differential expression in metabolism, nutrient assimilation, and multiple levels of cellular regulation (transcription factors, chromatin modifying enzymes, and signaling and sensory proteins). The co-expression of thousands of known and unknown genes of under specific physiological and environmental contexts provides clues into their functions and biological regulation. This is important for the diatom clade, in which most of the genes that are predicted and expressed are of unknown function. Physiological states and transitions that are consistent at both ambient and elevated  $p\text{CO}_2$  are relevant to diatom ecophysiology in a changing climate, and will facilitate further annotation, hypothesis generation, and the interpretation of data collected in the field.

## TESTING THE GENERIC LIMITS OF THE BIDDULPHIACEAE (BACILLARIOPHYCEAE): REVISITING ROSS & SIMS (1971) WITH MOLECULAR DATA

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The ocellate and pseudocellate diatoms in the Biddulphiaceae are distinctive and have a rich fossil history, making them common components of marine coring studies and good candidates for molecular dating work. Also, these diatoms are important to understanding the phylogeny of the diatoms as a whole, since the distinction between the araphid pennate and multipolar centric diatoms has become blurred by the increased use of molecular markers. However, the convoluted taxonomic history of these groups has the potential to disrupt both types of studies, as many taxa still have multiple generic designations that are commonly used in the literature. In 1971, Ross and Sims used scanning electron microscopy to examine valve characters of several ocellate and pseudocellate diatoms and came up with a scheme of morphological characters that could define these genera. In this study, we used molecular characters (nuclear-encoded SSU rRNA and plastid-encoded *rbcl* and *psbC*) to test if Ross and Sims' morphological characters are synapomorphic with respect to the diatom molecular phylogeny. While some morphological characters do indeed appear to be synapomorphic, others do not.



## SPATIO-TEMPORAL DYNAMICS OF MICROPHYTOBENTOS IN THE TAGUS ESTUARY (PORTUGAL) DETECTED BY SPATIAL REMOTE SENSING

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Benthic microalgae can make a substantial contribution to the food web in coastal ecosystems, in particular diatoms, which dominate photo-autotrophic assemblages in intertidal mudflats. They form dense biofilms in the most superficial sediment layers and contribute to their stabilization. Many studies investigated the spatio-temporal dynamics of these biofilms at microscale (below 1 m<sup>2</sup>) using coring techniques to sample surfaces often smaller than 20 cm<sup>2</sup>. However, the validity of microscale sampling protocols used to infer spatial structures at macroscale or to analyse temporal patterns of microphytobenthos biomass is questionable (Spilmont *et al.* 2011).

In this study, we analyzed spatio-temporal variations of benthic diatom biofilms at the macroscale of the Tagus estuary (Portugal), one of the largest estuarine systems in western Europe (38°44'N, 9°08'W), using SPOT multispectral satellite images. The intertidal flats (mean tidal amplitude of 2.6 m) are dominated by muddy sediment and represent 128 km<sup>2</sup>. SPOT HRV sensor is characterized by a low spectral resolution (3 broad spectral bands of *ca.* 100 nm) but a high spatial resolution with pixel size ranging from 10x10 m to 20x20 m. The Normalized Difference Vegetation Index (NDVI) involving red and near infrared (NIR) bands:  $NDVI = (NIR - red) / (NIR + red)$ , was used to discriminate benthic microalgae from the other benthic photoautotrophs: angiosperms of the saltmarshes in the upper intertidal or macroalgae attached to dead oyster shells in the lower intertidal. The spatial distribution of microphytobenthos showed that the main assemblages were detected in the upper intertidal flats (+3 to +5 m above the lowest astronomical tide) with a decrease toward the lowest bathymetric levels. The temporal dynamic was analysed with 5 images covering a seasonal cycle. NDVI time-series revealed a striking seasonal variation, with high NDVI values in winter and low NDVI values in summer. This result is the opposite of the pattern detected in north European estuaries with MODIS satellite images (van der Wal *et al.* 2010). A significant empirical relationship was obtained between an extensive microphytobenthos biomass dataset collected in the top millimetres (2-5 mm) of the sediment during winter *in situ* campaigns and NDVI values retrieved from a synchronous satellite image. This relationship was used to obtain a biomass distribution map (mg chlorophyll *a* .m<sup>2</sup>) that was compared with other microphytobenthos biomass data measured in the Tagus estuary.

## PHOTOPROTECTION CAPACITY DIFFERS AMONG MICROPHYTOBENTHIC DIATOMS INHABITING INTERTIDAL MUDFLATS: POSSIBLE CONSEQUENCES ON THEIR SPATIAL DISTRIBUTION RELATED TO THE LIGHT ENVIRONMENT

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Coastal ecosystems are one of the most productive areas in the world. Their productivity is mainly supported by the microphytobenthos (MPB) which inhabits estuarine intertidal mudflats. MPB is mainly dominated by diatoms that can divide in three main functional forms: 1) motile through the sediments/biofilm-forming at the surface; mainly in muddy mudflats, 2) attached to the sediments (stalked and adnate); mainly in sandy mudflats, 3) tycho planktonic (they need to spend part of their life cycle resuspended in the water column) in all habitats. The light environment in intertidal mudflats is strongly variable so that MPB can experience very extreme light climates in terms of intensity and light fluctuations (amplitude, frequency). Diatoms have evolved physiological processes in order to acclimate to the light environment and especially to resist to excess stressful light conditions. Among these so-called photoprotective processes, the photosystem II electron cycle (PSII CET), the non-photochemical quenching of chlorophyll fluorescence (NPQ) and the associated xanthophyll cycle (XC) are believed to be the most important ones. The two aims of this study were to gain insight 1) on the potential photoprotective ability of the three functional forms of MPB diatoms, 2) on their ability to resist to a light stress and to maintain photosynthesis performances under such conditions. We performed a screening on 15 species (7 motile, 3 adnate, 3 stalked, 2 tycho planktonic) isolated from different ecosystems (Atlantic coast and North Sea). We measured the PSII CET, NPQ and the XC extent and kinetics by performing light curves of different intensities up to full sunlight ( $2000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ ). We especially observed that attached diatoms show a higher NPQ as well as a higher PS II CET capacity independent of stalked and adnate forms. In contrast, the motile tycho planktonic (grown in benthic mode) forms showed a lower PS II CET and NPQ. Interestingly, the NPQ ability is not directly related with the extent of the XC. These results will be discussed as regards to the MPB ability/inability for motility through the sediment and the physical positioning of the diatom cells at an optimum level of light for photosynthetic production, i.e. are the motile forms showing a lower physiological photoprotection (PSII CET, NPQ, XC) because they can exert behavioural photoprotection (so-called 'migration') in contrast to the attached forms ?

## THE ROLE OF DIATOM COMMUNITIES IN THE QUALITY EVALUATION AND ECOLOGICAL CHARACTERIZATION OF A REGULATED MEDITERRANEAN STREAM

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In 2005, a study was conducted on the diatom communities of the R.Mucone, a regulated Mediterranean stream in a mountainous part of Calabria (S.Italy). Diatoms were sampled in 4 locations situated at increasing distances from the dam which forms Lake Cecita and in other 3 locations along unregulated tributaries. Three sampling campaigns were undertaken, in the course of which aquatic chemical-physical factors and stream morphometric characteristics. Physical substrate was characterized, and several water chemistry variables were analyzed in all stations.

A total of 97 taxa were identified. The most abundant were *Achnanthydium minutissimum* (23.5% of identified individuals), *Cocconeis euglypta* (9.6%), *Cocconeis pseudolineata* (7.2%), *A.pyrenaicum* (6.9%), *Fragilaria crotonensis* (3.9%), *Navicula gregaria* (3.8%) and a less well known species, *Adlafia suchlandtii* (3.6%).

A Detrended Correspondence Analysis (DCA) on the taxonomic data of the 15 most abundant taxa preliminarily allowed to distinguish different groups of samples: a first group, with a certain degree of internal differentiation, which included the first 3 stations along the R.Mucone downstream of the dam and a group including the samples taken in the final station along the R.Mucone and in three reference sites situated along the tributaries. A Cluster Analysis (CA) and a Multi-Response Permutation Procedure (MRPP) also confirmed the segregation of these groups of samples (and sites). Several diatom-based water quality indices were calculated.

The mentioned groups of stations showed differences in community structure. In particular, in those downstream of the dam the most abundant species was *A.minutissimum* (33.9% of individuals identified), *C.euglypta* (15.5%) and *F.crotonensis* (8.6%), while in the group of reference sites including the most downstream station of the R.Mucone the 3 most abundant species were *A.minutissimum* (15.3% of individuals identified in this group), *C.pseudolineata* (12.6%) and *A.pyrenaicum*. (12.3%). *A.suchlandtii* was found only in the group of reference stations. Several species showed strong differences in average abundance between segregated groups of samples.

The differences between the groups of samples can be explained primarily by the presence of the dam and its influence on the hydrology downstream. The final station along the R.Mucone appeared to be more similar to the reference sites, indicating a significant degree of recovery, thanks to the increasing distance from the dam and to the effect of the tributaries.

The results proved the existence of differences among sites ascribable to the effects of the dam. Diatoms, with other ecological and environmental variables, proved to be a useful tool in the evaluation of the effects of stream regulation.

## CAN DIGITAL IMAGING FLOW CYTOMETRY REPLACE MICROSCOPE MEASUREMENTS OF DIATOM CELL DIMENSIONS?

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Size analysis of diatom populations is a potentially powerful tool for understanding diatom life histories, population dynamics and phylogenetic relationships. However, measuring cell dimensions on a light microscope is a time consuming process. An alternative technique has been developed using digital flow cytometry on a FlowCAM<sup>®</sup> (Fluid Imaging Technologies, Inc.) to capture hundreds, or even thousands, of images of a chosen taxon from a single sample in a matter of minutes. Up to thirty morphological measures may be quantified through post-processing of the high resolution images. We evaluated FlowCAM size measurements, comparing them against measurements from a light microscope. We found good agreement between measurement of apical cell length in species with elongated, straight valves, including small *Achnantheidium minutissimum* (11-21  $\mu\text{m}$ ) and large *Didymosphenia geminata* (87-137  $\mu\text{m}$ ) forms; however, a taxon with curved cells, *Hannaea baicalensis* (37-96  $\mu\text{m}$ ), showed differences of  $\sim 4 \mu\text{m}$  between the two methods. Discrepancies appear to be influenced by the choice of feret or geodesic measurement for asymmetric cells. *Using examples from the three test species, both the benefits and limitations of this new technology will be discussed.* The increased speed of data acquisition through use of imaging flow cytometers like the FlowCAM is an essential step for advancing studies of diatom populations.

## MORPHOLOGY AND TAXONOMY OF FOUR SPECIES OF THE MARINE PLANKTONIC DIATOM GENUS *BACTERIASTRUM* (BACILLARIOPHYTA) FROM THE ADRIATIC SEA

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The multipolar centric diatom genus *Bacteriastrum* Shadbolt (Chaetocerotaceae) abounds in the phytoplankton of warm and temperate seas. The genus comprises c. 12 - 15 valid species, belonging to two sections, *Isomorpha*, and *Sagittata*, respectively characterized by having the setae of the terminal valves morphologically similar or different from those of the posterior terminal valve. Since the number of valid taxa is still debated, a re-examination of the species is presently needed as well as the verification of the characters supporting the interspecies distinction and the very circumscription of the genus. In the study, four colonial species from the Adriatic Sea, *B. furcatum* Shadbolt, *B. mediterraneum* Pavillard, *B. hyalinum* Lauder and *B. jadrantum* Godrijan, Maric et Pfannkuchen, were investigated using both cultures and natural samples. The species were studied in detail with light and electron microscopy and compared in terms of the general morphology, ultrastructure, and molecular phylogeny. Phylogenetic analysis of 28S (LSU) sequences resulted in two clades, placing together *B. furcatum* and *B. hyalinum* as one clade and *B. jadrantum* and *B. mediterraneum* on the other one. EM observations showed that these pairs of species share some ultrastructural features. For instance, *B. hyalinum* and *B. furcatum* share T-shaped spines ornamenting the twisted setae. However, all four species share the same fine ornamentation of the valve and girdle bands. Species-specific traits were encountered as well. *Bacteriastrum jadrantum* possesses smooth setae (i.e., without spines) originating inside the valve margin, while the intercalary cells of *B. hyalinum* possess small Y-shaped siliceous projections on the fused part of the setae. Results of SEM observations provided the fine morphological details of the ornamentation of *B. hyalinum* resting spores. The surface of the primary valve is covered with numerous spines, simple or often branching dichotomously at their end and the secondary valve is smooth bearing the single marginal ring of poroids. Our results, based on a limited number of species, confirmed that in the genus *Bacteriastrum* the general shape of the cells and mode of colony formation are more informative on the level of species distinction than the ultrastructural characters.

## USE OF “SPECIES SENSITIVITY DISTRIBUTION” FOR HERBICIDES TOXICITY ASSESSMENT ON BENTHIC DIATOM ASSEMBLAGES

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Herbicide presence in lake littoral zones is often characterized by a higher diversity of molecules and higher concentrations than in pelagic zones. The tolerance threshold of benthic diatoms, which represent a great part of the fixed biomass, to herbicides is not well known. With the view of assessing the ecological risk of herbicides for aquatic microorganisms, some models as Species Sensitivity Distribution (SSD) have been developed on phytoplanktonic species of the pelagic zone. Our study is aimed to determine if SSD models are adapted to describe the sensitivity of benthic diatoms to herbicides. In this view, we assessed the sensitivity of 11 benthic diatoms species to 5 herbicides (diuron, isoproturon, terbutryn, atrazin and metolachlor).

First, we constructed a database of sensitivity thresholds for each herbicide and each diatom species through 96h monospecific bioassays based on growth inhibition. From dose-response curves, an effective concentration that inhibits 50% of growth (EC50) was extrapolated for each dose-response curve. EC50 values showed a great variability of sensitivity among diatom species for a same herbicide and between all of them. Then, for each herbicide, a SSD curve was built with EC50 values of each diatom species (SSD-EC50) describing the variation of sensitivity of diatoms. Different groups of diatoms have been defined according to their tolerance level. This observation was more blatant for photosystem II (PSII) inhibitors. For this mode of action, four strains (*Nitzschia palea*, *Craticula accommoda*, *Gomphonema parvulum*, *Eolimna minima*) were always the most resistant out of the 11 tested species. This tolerance could be explained by two life-history traits: the trophic mode and the ecological guild. Indeed, N-heterotroph and motile guild species seemed to be more resistant to PSII inhibitors than N-autotroph and other profile guild species.

## AN INTEGRATIVE ANALYSIS OF LIGHT RESPONSES IN *PHAEODACTYLUM TRICORNUTUM*

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Light-associated processes are among the most fundamental to photosynthetic algae. Microalgae such as diatoms experience large, rapid and unpredictable fluctuations in light intensity and spectral quality. The molecular mechanisms behind sensing and responding to these environmental changes and how they are connected to the observed physiological responses are still poorly known.

We have, through a series of experiments, investigated different aspects of the light response in *Phaeodactylum tricornutum*. Transcriptional profiling using full-genome microarrays, metabolic analyses, and variable fluorescence technique were used to generate an integrative view of the molecular and physiological responses through several time series measurements.

1. Low light acclimated cultures were transferred to high light conditions
2. Dark-grown (48h) cultures were re-exposed to light
3. A diel time series was made over a complete day-night cycle

Results from the individual experiments are presented. We will also combine the three datasets in order to reveal more complex connections underlying responses to light.

## DIATOM TAXONOMIC CONSISTENCY: RESOURCES AND APPROACHES AT THE ACADEMY OF NATURAL SCIENCES, PHILADELPHIA, USA

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The ANSP Phycology Section has several resources available for diatom researchers to help promote taxonomic consistency. Three of the most useful are the current set of taxa names (2011 ANSP / NAWQA / EPA), the algae image database and website, and the NADED database with diatom counts and site information. These three are integrated with each other and with a counting program (Tabulator) and a program for calculating metrics (Phyco-Aide).

The Phycology Section is part of the Patrick Center for Environmental Research, and works closely with the Diatom Herbarium (Marina Potapova, Curator). The Phycology Section had its origins in the Limnology Department, which Ruth Patrick started in 1947. Beginning about 1994, the Phycology Section began storing diatom counts and related data in the North American Diatom Ecological Database (NADED). Section staff and collaborators have added diatom data to NADED from many projects throughout the US, including those national in scope such as the USGS National Water-Quality Assessment Program (NAWQA) and EPA's National Aquatic Resource Surveys (NARS). NADED now contains more than 25,000 diatom counts (and more than 16,000 soft-algae counts) from over 10,000 sites in the U.S. These records can be searched to find distribution data for individual taxa and to find accession numbers for the corresponding more than 47,000 slides archived in the ANSP Diatom Herbarium.

The 2011 ANSP/NAWQA/EPA set of taxa names is an update of previous versions and is the product of much work by many diatomists at several institutions. It includes recent synonyms for 2,000 diatom taxa (>1715 described) and more than 1100 soft algae. This set of names and its predecessors have been used in analysis of algal samples for many state and federal projects, including NAWQA, NARS, and the diatom component of the NEOTOMA paleoecology database. Agencies have supported this effort with the goal of providing a common and consistent set of names that can be used in projects they fund so that resulting datasets can be more effectively compared and combined. Files of names can be downloaded at: <http://diatom.ansp.org/nawqa/Taxalist.aspx>. Each name is associated with a unique NADED ID. Names are intended to be consistent with those used in the "Diatoms of the United States" website and equivalent in form to those in the California Academy Catalogue of Diatom Names.

The Algae Image Database and website ([http://diatom.ansp.org/algae\\_image/](http://diatom.ansp.org/algae_image/)) document many taxa in the sets of taxa names, particularly undescribed and less common taxa.



## EFFECT OF LIGHT ON THE ACCUMULATION OF FREE AMINO ACIDS IN THE MARINE DIATOM SKELETONEMA COSTATUM RESTING CELLS DURING GERMINATION

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The influence of light on nitrogen uptake and the accumulation of free amino acids in *Skeletonema costatum* resting cells during germination, but before cell division, were studied. Nitrogen (N) uptake occurred prior to cell division when cells were placed in the light. Carbon fixation might be taking place simultaneously due to the increasing free amino acid concentration. Different rates of N uptake were measured after 3h depending on irradiance to the rejuvenating cells, and the results showed a large increase at 225 and 300  $\mu\text{mol quanta m}^{-2} \text{ s}^{-1}$  after 12h. The rapid and increasing N uptake at high light enabled *S. costatum* to rebuild and reorganize its cell contents quickly and to dominate subsequent vegetative growth. Compared to those in the logarithmic phase and stationary phase, much higher concentrations of amino acids glycine (Gly) and histidine (His) were detected in resting cells. The amino acid His appeared to be a storage compound in *S. costatum* resting cells. Furthermore, alanine(Ala), proline (Pro), Gly and His in *S. costatum* resting cells could be associated with the survival of resting cells in unfavorable environments, such as anoxic, cold, dark and heavy metal stress, and with the following recovery process. Compared to glutamine at 12h and 36h, glutamate had the highest  $^{15}\text{N}$  labeling enrichment at 3h, 6h, 24, 30h and 48h, showing a diel variation of N status during germination. The  $^{15}\text{N}$  labeled urea was detected at 36h, compared to the urea cycle intermediates, ornithine and citrulline at 30h, indicating that urea was produced as an N reservoir due to the higher nitrogen uptake in the dark, and the urea cycle was involved in *S. costatum* resting cell germination. However, in resting cell germination, Pro could be produced by other activity, other than the urea cycle.

This work was funded by the Natural Science Foundation of China under contract No: 40906058 and the Natural Science Foundation of Fujian Province of China under contract No: 2011J01279

## THE IMPORTANCE OF USING CORRECT DESCRIPTIVE TERMINOLOGY AND AN INTRODUCTION TO DIATERM – DEVELOPING A REVISED GLOSSARY OF TERMINOLOGY FOR DIATOMISTS.

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Over the course of time all disciplines develop their own specialised vocabulary to describe observations and concepts, the study of diatoms being no exception. Whereas some descriptive conventions have changed, e.g. primary and secondary or side and face, versus girdle and valve view, others have been retained more or less unchanged, e.g. striae, raphe, valve, frustule. By the mid 20<sup>th</sup> century, despite linguistic variations, the terms in use for light microscopical descriptions of cleaned diatoms were largely consistent and readily understood across languages. However, with the development of electron microscopy and the discovery of another scale of variation in diatoms, many new terms were introduced to describe ultrastructural features. It also became clear that apparently similar LM features could have contrasting ultrastructure.

Since, despite the development of molecular techniques, morphological characters continue to underpin diatom taxonomy and systematics, and inferences about relationships are made on the basis of shared (usually ultrastructural) characters, it is critical that terminology is applied precisely and consistently. Inaccurate terminology not only produces confusion but can also lead to incorrect inferences of relationships.

Diaterm is a recent international initiative to review existing diatom terminology and produce a revised, highly illustrated glossary that can be used by all diatomists. It builds on previous terminology publications but will also review and evaluate all subsequently published terms, in order to develop a rational and practical descriptive vocabulary. Any rejection of duplicate or inappropriate terminology, as well as the introduction of new terms, will be justified and explained.

## THE SPINES OF CORETHRON; TYING UP LOOSE ENDS

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The magnificent spines of the marine diatom genus *Corethron* have been acknowledged as unique for some time yet the complex relationship of the three different types of spine in each cell when held beneath the cylinder of the cingulum has remained difficult to establish. Now, new information has emerged from old data to reveal a situation even more complex than was thought but which allows us to understand how the diatom ties up its loose ends.

## HOLOCENE VARIABILITY OF THE NORTH AMERICAN MONSOON: DIATOM-BASED EVIDENCE FROM LAKE SEDIMENTS IN WESTERN MEXICO.

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We present a multi-proxy sediment record from Laguna de Juanacatlán, Jalisco (20°37'N, 104°44'W), in the western fringes of the Trans-Mexican Volcanic Belt. The lake lies in the core of the North American Monsoon region, with more than 80% of precipitation occurring between May and October. Whilst the Pacific Ocean is the dominant influence, moisture originating from the Gulf of Mexico / Atlantic is also important. This unique sedimentary archive provides an opportunity to investigate long term changes in monsoon intensity during the Holocene. High-resolution X-ray fluorescence (XRF) analysis, magnetic susceptibility and organic geochemistry are combined with diatom species data and oxygen isotope analysis of diatom silica to provide a multi-proxy reconstruction of climate variability.

Laguna de Juanacatlán lies at c. 2000 m a.s.l. and has a maximum depth of c. 28 m. Periodic monitoring has revealed that the lake is consistently thermally and chemically stratified. Its waters are of calcium-magnesium-bicarbonate composition, with a pH of c. 8.5 and electrical conductivity of 105  $\mu\text{S cm}^{-1}$ . Lying in a remote, mountainous area, the lake has experienced minimal human impact, meaning that the climatic signal should not be obscured by anthropogenic disturbance.

A 7.8m composite sequence of finely laminated sediments provides an annually resolved record spanning the last 6000 cal yrs BP. Laminations are composed of alternating minerogenic and organic-rich diatomaceous layers. Downcore diatom assemblages are dominated by *Discostella stelligera*, *Aulacoseira granulata* (+ var. *angustissima*), punctuated by brief periods of dominance by *Nitzschia palea* at 1000 cal yrs BP and 3200 cal yrs BP, reflecting increased nutrient concentrations. *Synedra* spp. become more important after 1000 cal yrs BP, being replaced by *Fragilaria crotonensis* in the most recent sediments. These taxa are also found in significant numbers between 5000 and 6000 cal yrs BP. Diatom species changes are best explained in terms of fluctuations in nutrient and light availability rather than ionic composition or concentration. Diatom-based oxygen isotope values are higher between c. 1600 and 6000 cal yrs BP, oscillating between 29 and 32 ‰, whilst during the last 1600 years, variability increases along with a shift to generally lower values. This is interpreted as a trend towards wetter conditions but with greater variability in monsoon intensity.

## EXPLORING THE MOLECULAR BASIS OF RESPONSES TO LIGHT IN MARINE DIATOMS

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Light is a source of energy for photosynthesis and a major source of information from the environment. As on land, both irradiance and light quality change drastically in different marine habitats. Spectral quality also varies with depth due to the absorption properties of water, with blue light prevailing at increasing depths. Recent progress in genomics and environmental metagenomics has revealed that differences in the light field have led to a variety of evolutionary adaptations and to previously unsuspected types of phototrophy in aquatic microorganisms, which are without parallel in terrestrial systems. These discoveries highlight the fact that the light-driven processes are still largely unknown in marine organisms and we expect many mechanisms remain to be discovered.

The major focus of our research is in understanding the biology of the diatoms. We believe that these prominent algae must have developed sophisticated strategies (physiological, biochemical and behavioral) for responding to environmental light variations. We are therefore exploiting novel genetic tools and genomic information to try to decipher light sensing and acclimation mechanisms, still largely uncharacterized at molecular level. In particular, we have undertaken a comprehensive characterization of the diatom photoreceptors identified in the genomes by studying their spectral properties, their signaling pathways and their function *in vivo*. The structural and functional characterization of a Cryptochrome/Photolyase Family member (CPF1) isolated from the diatom *P. tricornutum* has revealed that PtCPF1 is a novel and interesting member of this family because it displays a dual activity: a (6-4) photolyase activity, likely of functional relevance for cell survival following UV irradiation, and a photoreceptor activity, controlling gene expression and possibly circadian regulated processes. We now also focus on possible red light signal, by characterizing a Red/Far Red Phytochrome photoreceptor identified in the genome. Its down-regulation by RNA interference induces the formation of cell chains and aggregates, possibly through altered photoprotection and/or neighbour perception. These data raise novel hypotheses about the role of photoreceptors in controlling growth and life strategies in the oceans.

## DIATOM COMMUNITIES AND WATER QUALITY OF SHORELINE ROCK POOLS IN THE NORTH AMERICAN GREAT LAKES

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Rock pools are prominent features along much of of Lake Superior's shoreline and serve as critical habitat for several amphibian and plant species of concern. These unique habitats are model ecological systems with simple food webs, where organisms must balance reproductive risks and resource use with pool permanence, daily physical extremes, and predation. Threats to rock pools and organisms that depend on them include climate warming, atmospheric deposition, recreation, invasives, and potential for shipping spills. In spite of these threats and dependence by species of concern, there are few baseline studies of the physical, chemical, and biodiversity characteristics of rock pools. Shoreline pools are delimited into two zones. Splash pools are located closer to the lake and have hydrology and permanence that is strongly controlled by wave inundation. Lichen pools, so-called because of the predominance of lichens on surrounding rock, are located above the wave and ice scour zone, and are often larger, more permanent, and have hydrology more strongly controlled by precipitation, runoff, and groundwater. We intensively sampled 35 pools in Great Lakes national parks during 2010. Field measures included size, depth, temperature, pH, conductivity, and DO. Water quality samples were taken for analysis of total and dissolved nutrients, DOC, DIC, chlorophyll *a*, anions, cations, and metals. Diatom samples were collected at each pool from comparable microhabitats. Water quality and diatom communities in rock pools were strongly separated across lichen and splash zones. There is little difference in water chemistry between splash pools and Lake Superior waters except for nitrate-nitrite (higher in Superior). Lichen pools are more productive systems compared to either splash pools or Lake Superior with greater nutrients (except nitrate), DOC, and chlorophyll-*a*. Splash pools can be characterized as low productivity, oligotrophic systems compared to lichen pools, which are mesotrophic and possibly N-limited. Diatom communities in pools have been characterized and comprise several floral elements including Lake Superior species, a soft water flora, N-fixers, and rare taxa. This baseline ecological study of diatom communities and water chemistry of shoreline pools establishes a framework for monitoring these sensitive systems, and provides guidance for developing remediation targets with which to respond to current and potential threats from invasives, warming, atmospheric deposition, and shipping spills.

## HOW CLEAN IS CLEAN? A DIATOM-BASED PALEOLIMNOLOGICAL RECONSTRUCTION OF REFERENCE CONDITIONS IN LAKES OF NEW JERSEY, USA

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Diatom species were analyzed in 78 sediment core samples from lakes in New Jersey (NJ), USA. These samples are sediment surface samples taken from core tops (0-0.5 cm interval = present-day, or modern samples) and core bottoms that represent past, or pre-disturbance conditions if the core was of sufficient length to reach this time period. The sediment surface samples were combined with a subset of US EPA's National Lake Assessment ([www.epa.gov/owow/lakes/lakesurvey/](http://www.epa.gov/owow/lakes/lakesurvey/)) samples to derive transfer functions for total phosphorus and total nitrogen using WA-PLS techniques. The transfer functions were used to reconstruct nutrient levels in core bottom samples and to quantify natural, pre-disturbance conditions and assess the current degree of cultural impairment. This study provides information that is critical to establish criteria for reference conditions and help lake managers set realistic targets for lake conservation and recovery.

## THE ROLE OF PHOTOACCLIMATION IN BENTHIC DIATOM PHOTOBEHAVIOUR

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In tidal estuaries, microphytobenthic communities, mainly composed of diatoms, are major primary producers, with crucial ecological role. This high productivity is achieved despite living in extreme and ever changing environment. However, most of the diatom benthic species are motile, having the capability to move in and out of the sediment photic zone. This opens the possibility that these microalgae, could actively choose their exposition to light according to the status of their physiological photoprotective mechanisms (photoacclimation). In this work we tested the hypothesis that the migratory photobehaviour of benthic diatoms is conditioned by long term photoacclimation status. The photoaccumulation patterns of cells of the motile benthic diatom *Navicula* sp. isolated from the microphytobenthic community and grown in two contrasting light regimes (20 and 300  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ) were studied. Diatoms were exposed to a horizontal wide light gradient (0-1600  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ) in a custom-built photoaccumulation chamber, which allowed the cells to freely move and their light distribution was recorded after 3 hours of incubation. A simple transmission ratio index (T750/T675) was used to calculate biomass, using a spectroradiometer to quantify the transmitted light at 675nm (T675) and at 750nm (T750). The photophysiological status of the cultures was accessed using Pulse Amplitude Modulation (PAM) fluorometry, by measuring light-response curves (LCs) of the relative electron transport rate (rETR) of photosystem II (PSII) and the maximum quantum yield of PSII ( $F_v/F_m$ ). The results showed a photoaccumulation pattern evidencing a clear avoidance of extreme low or high light levels, with a maximum around 65  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for the low light-acclimated cells and 104  $\mu\text{mol m}^{-2} \text{s}^{-1}$  for the high light-acclimated cells. We found a strong relationship between the photoaccumulation curves and the photophysiology parameters of these cultures, which suggests that the use of motility to select the optimal light exposure depends on cell's photoacclimation status.



**STEROL BIOSYNTHESIS IN *P. TRICORNUTUM***

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Diatoms represent one of the more successful phytoplankton population and have a considerable influence on global climate and marine environment. Their evolutionary success is linked to their peculiar and flexible metabolism, which is also of great interest for industrial applications and sustainable production of bioenergy. To deepen our understanding and exploit the metabolic resources of diatoms, we recently developed DiatomCyc ([www.diatomcyc.org](http://www.diatomcyc.org)), the online database on *Phaeodactylum tricorutum*'s metabolism. By using it as a laboratory tool, we are currently studying important lipid-related biochemical pathways, such as the sterol biosynthesis in *P. tricorutum*. The *in silico* reconstruction of this pathway, unknown in diatoms, resembles a mix between those occurring in plants and fungi. Additionally, the apparent lack of a conventional squalene monooxygenase, key enzyme of the pathway and well conserved among eukaryotes, suggests that in diatoms the pathway might have evolved differently. By using specific chemical inhibitors, gene silencing, genetic complementation in yeast and GC-MS analysis we are investigating gene functions and differential intracellular sterols accumulation. Preliminary results confirmed the correctness of both *in silico* and manually curated predictions reported in DiatomCyc. In addition, we observed a connection between the metabolism of sterols and the accumulation of triacylglycerols (TAGs) in *P. tricorutum*, which makes the regulation of this pathway an attractive target for metabolic engineering.

## DIATOMCYC

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Diatoms have played a crucial role in the global ecosystem for millions of years. To explore these organisms for biotechnological applications like sustainable production of fuels, pharmaceuticals, health foods, materials relevant to nanotechnology as well as for pollution mitigation and carbon dioxide abatement we developed DiatomCyc, a detailed pathway/genome database of *Phaeodactylum tricornutum* using a genome-wide approach. DiatomCyc contains 286 pathways with 1719 metabolic reactions and 1613 assigned enzymes, spanning both the central and parts of the secondary metabolism of *P. tricornutum*. Central metabolic pathways, such as those of carbohydrates, amino acids and fatty acids, are present in the database. The discovery of a functional Entner–Doudoroff pathway, an ancient alternative for the glycolytic Embden–Meyerhof–Parnas pathway, uncommon in eukaryotes indicate that the used approach to re-construct the pathway/genome database is valuable. DiatomCyc is accessible online (<http://www.diatomcyc.org>), and offers a range of software tools for the visualization and analysis of metabolic networks and ‘omics’ data. We anticipate that DiatomCyc will be key to gaining further understanding of diatom metabolism and, ultimately, will feed metabolic engineering strategies for the industrial valorization of diatoms.

## FLYING DIATOMS - A KEY TO THE PATH AND ORIGIN OF A DUST STORM

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Particles of a dust storm hitting Asuncion, Paraguay on 04.04.2009 were investigated. The dust was rich in amorphous silica. Microscopic analysis showed, it was remains and fragments of silica secreted by diatoms, sponges, as well as sedges, reeds, and grasses.

The diatoms are well preserved and practically unbroken, and in most cases occurred still combined to frustules. In chain-building species the frustules occurred still connected to chains.

All diatoms were freshwater diatoms. The majority of taxa among the diatoms belong to the genera *Pinnularia* and *Eunotia*. The most common species were *P. latevittata* Cleve, *P. major* Ehrb., *P. microstauron* (Ehrb.) Cleve, *P. divergens*, *P. streptoraphe* var. *parva* Krammer, and *E. zygodon*, Ehrb., *E. glacialis*, *E. didyma* Grun. var. *didyma*. Other characteristic but more rarely present species are *Rhopalodia rupestris* var., *Stauroneis phoenicenteron*, different species of the genus *Nitzschia*, *Staurosira* cf. *pinnata*, *Staurosira* sp., *Frustulia rhomboides*, *Diploneis* sp., *Encynonema* sp., *Epithemia* sp., *Diadismis* sp., *Caloneis* sp., and a few frustules and chains of one species of the genus *Aulacosira* sp..

Some of the diatom species are characterized by large frustules, e.g., the relatively common *Pinnularia latevittata* (160 - 230 µm), *P. major* (>300 µm), and *Eunotia glacialis* (170 – 230 µm) are most prominent because of their size. Other large but more rare frustules were those of *Surirella rumrichorum* (>300 µm) and species of the genus *Nitzschia*.

The diatom association is characteristic for the neotropics and neosubtropics of South America, an oligotrophic, silica-rich shallow water body of low electrolytic level. This together with the lack of minerogenic detritus and abundance of plant detritus suggests a large swamp area as the source.

Such swamp lands occur in the lowlands along the Rio Paraguay. At the end of the very dry summer 2008/9 these swamps had fallen dry, and the area had fallen victim to wild fires. A storm created by the convergence of a cold front with warm tropical air created a storm, which picked up these burnt swamp relicts.

## GENOME SEQUENCING AND MUTAGENESIS IN THE PLANKTONIC DIATOM *PSEUDO-NITZSCHIA MULTISTRIATA*

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*Pseudo-nitzschia multistriata* is a planktonic diatom that regularly blooms in the Gulf of Naples (Mediterranean Sea). It is a toxin-producing species, as a number of strains have been shown to produce domoic acid, although it has never been linked to harmful events. *P. multistriata* has a typical size reduction-restitution life cycle and a heterothallic mating system. The ecology, life cycle and population genetics of this species have been studied intensively in the past years. Because of its ecological relevance and because of its life cycle characteristics, we propose to establish *P. multistriata* as a genetic model that could be used to study gene function in a planktonic organism through classical genetic approaches. To show that the genetics of this species can be controlled, we initiated a breeding program and produced a pedigree with four generations. We defined the minimal generation time, verified Mendelian inheritance of microsatellites markers and started a project to sequence the genome of *P. multistriata*. Initial results produced using the Illumina/Solexa platform, indicate that the genome is AT-rich and has a size of about 100 Mb. Further rounds of sequencing are ongoing to refine the assembly.

In addition, we are generating transcriptomes from strains of opposite mating types above and below the sexual size threshold and from sexual stages. These experiments, designed to explore the molecular mechanisms regulating the life cycle and sexual reproduction in this species, will reveal the genetic bases of the mating system and at the same time will aid in the annotation of the *P. multistriata* genome.

Finally, we are using chemical and UV mutagenesis to generate stable mutants and are designing a forward genetic screen which will involve identification of mutants for domoic acid or fatty acid production. Availability of the genome sequence will be exploited to identify the genes responsible for phenotypes of interest, either by positional cloning or by genome resequencing.

Through this project, we aim to consolidate *P. multistriata* as a model organism for molecular, genetic and biotechnological studies, complementing existing diatom models.

## INVESTIGATION OF THE MATING PATHWAY IN THE DIATOM *SEMINAVIS ROBUSTA*

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The aim of this study is to identify and functionally characterize the chemical signals employed by pennate diatoms during their mating process, using the benthic marine species *Seminavis robusta* as model organism. Based on physiological experiments, a metabolomics approach was applied to compare extracellular metabolite profiles from sexually active cultures and inactive controls using liquid chromatography coupled to mass spectrometry. By means of multivariate statistics, possible candidate signals that are unique for sexually active cultures could be identified. Structure elucidation of the attraction pheromone is based on high-resolution mass spectrometry, nuclear magnetic resonance spectroscopy and circular dichroism spectroscopy. In bioassay experiments, it was confirmed that synthetic pheromone induces similar responses compared to the natural pheromone. It is also shown that the mating and signaling process is controlled by light. The methodological approach developed here was successful in discovering the first pheromones in diatoms. Our results are a first important step towards the elucidation of the complex signaling pathways employed by pennate diatoms to ensure a cost-efficient mating process.

## SPATIAL VARIABILITY OF THE DIATOM COMMUNITY OF LAKE WIVENHOE, AN AUSTRALIAN FRESHWATER RESERVOIR

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Australia, in particular South East Queensland, relies on reservoirs to store freshwater to supply both drinking water and water for irrigation purposes. In addition, Australia has low annual rainfall and a high loss of surface water due to evaporation. There is a need to develop robust indicators of reservoir health to better inform management of these systems, especially in times of drought. Reservoirs have unique hydrology and can be separated into three zones, lacustrine, transition and riverine. Seventy six species of diatoms were identified over 18 sites throughout the reservoir, with only 11 species showing abundances over 1% at more than three sites. This study shows that the composition of the diatom community from 18 sites within Lake Wivenhoe is strongly correlated with the three zones of a reservoir. Historical water quality data was also found to strongly correlate with the three zones of the reservoir. The variability of the diatom community between sites can be attributed to a combination of the hydrological regime and water quality of each site. Diatom community and water quality zonation in the reservoir matched remarkably well. The main water quality parameters that separate each site (secchi, ammonia, true colour, pH, conductivity and  $\text{No}_x$ ) could be expected to drive the differences in the diatom community. From this work, it can be determined that the diatom community does change spatially and by collecting a sediment diatom sample from each zone of the reservoir, the diatom community will be adequately captured. This information can be used for future work in developing a diatom index for South East Queensland reservoirs, leading to efficiency in the sampling methodology.

## THE BLUE DIATOMS FROM THE GENUS *HASLEA*: AN OVERVIEW

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The study of the blue diatoms from the genus *Haslea* is an arcane topic, due to investigations being restricted to the only species *Haslea ostrearia* (Gaillon/ Bory) Simonsen. This diatom, first observed in the early 19<sup>th</sup> century, has the peculiar ability to produce a non-photosynthetic blue pigment, the so-called marennine. This pigment's name refers to the French region of Marennes-Oléron, a place of intense oyster farming. In the ponds used for oysters' fattening, the releasing of marennine can turn water's color to green. By filtering the seawater, oysters fix marennine onto their gills, turning them green, a phenomenon which results in an increase in the bivalve's price.

During the last 5 years, several new species of the genus *Haslea*, all able to produce blue pigments, have been discovered around the world. The molecular phylogenies obtained using ITS1-5.8S-ITS2, partial *cox1* and *rbcl* markers have shown that blue diatoms belong to a single cluster of species, sometimes clearly separated from some non-pigmented species of the same genus, as evidenced by the *rbcl* marker.

For *H. ostrearia* and two of the species newly described, sexual reproduction has been studied. This, alongside with the preliminary molecular characterization, led to the investigation of the mitochondrial inheritance during auxosporulation in *H. ostrearia*, which proved to be uniparental. The diatom *H. ostrearia* has been revealed an interesting model, both because of its uncommon blue pigment's synthetic pathway and of its dioecious character, and a genomic approach has been considered.

In addition, diatom's blue pigments displayed several biological activities, including antibacterial, antifungal, allelopathic, antiviral, antiproliferative, antioxidant properties, leading us to hypothesize putative valorizations.

## LATE MIOCENE DIATOM FLORA FROM DEEP HOLE CENTRAL-1 (THE BERING SEA)

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Geological Institute of Russian Academy of Sciences

Data obtained on fossil diatoms from deep Hole Central-1 first drilled on the Chukhi shelf of the Bering Sea within the East Anadyr Depression is presented. The 14-m-thick stratigraphic interval (609.5-623.5 meters below the sea floor) contains more than 70 diatom taxa in core samples collected from tuffaceous-diatomite sequence.

In general, the diatom flora is composed by boreal and subarctic species typical of high to middle latitudes. Except for two samples, it is dominated by marine taxa, in the first place, *Odontella aurita*, *Paralia sulcata*, and *Ikebea tenuis*. Determining a precise age for diatom flora is difficult owing to the absence of some biostratigraphically significant and age-diagnostic marker marine planktic taxa, first of all, from genera *Denticulopsis* and *Neodenticula* characteristic of the North Pacific Cenozoic diatom zones. The most biochronologically important found taxa are *Pyxidicula zabelinae*, *Ikebea tenuis*, and *Cosmiodiscus insignis*. Their co-occurrence indicates the late Miocene age with an age range of about 7.9 to 5.5 Ma, i.e. from the upper part of the Neogene *Thalassionema schraderi* Zone to the top of subzone "a" of *Neodenticula kamtschatica* Zone.

Overall, diatom assemblages from the Hole Central-1 are persistently dominated and subdominated by marine neritic-planktic and sublittoral taxa (including *Paralia sulcata*, *Odontella aurita*, *Chaetoceros* spores, *Actinoptychus senarius*, *Actinocyclus octonarius*, and *Stephanopyxis* spp.) that are accompanied by benthic and nonmarine forms. The presence of the Neogene cool-water species *Pyxidicula zabelinae*, *Cosmiodiscus insignis* and *Porosira punctata* typical of coastal environments of the Bering Sea and northwest Pacific is documented. Oceanic and open-sea planktic elements are very rare or essentially absent. Flora lacks typically warm-water species, but diatoms associated with sea ice are also not found. Such features of taxonomic composition and structure imply deposition in productive, relatively cold and shallow water of marine shelf. Two documented stratigraphic levels which possess rare diatoms practically lacking marine forms and dominated by nonmarine taxa may indicate either the episodes of environments caused by considerable shoaling of shelf zone and sedimentation under the influence of the river flow. On the other hand, possible erosion of sediments at that time also cannot be excluded.

The study was partially supported by the Russian Foundation for Basic Research, project no. 12-05-00192.



## SPATIAL SCALING OF DIATOM DIVERSITY IN DUTCH PEATLANDS: COMPARISON OF THREE ECOLOGICALLY CONTRASTING SYSTEMS.

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Diatoms are widely used in ecological monitoring because of their high sensitivity to environmental disturbances, but knowledge on the spatial patterns in diversity is scarce. In general, the similarity in species composition between ecological communities is typically decreasing with increasing distance. For macroorganisms, this phenomenon is often quantified using distance decay relationships and species area relationships (SAR) and is often explained by the occurrence of environmental gradients and the spatially limited dispersal capacity of the species involved. In recent years, a debate arose whether microorganisms such as diatoms exhibit similar patterns. Available studies show that generalizations across all unicellular organisms studied so far are unwarranted as they exhibit wide variability.

The present study attempts to identify the mechanisms that generate diatom distribution patterns according to chemical and hydromorphological gradients by the comparison of three peatland areas in The Netherlands: Wormer and Jisperveld, Oostzanerveld and Naardermeer.

The sampling scheme follows a spatially explicit design along six kilometers sampling tracks. Distance decay relationships were analyzed using Bray Curtis similarity indexes to quantify variation in beta diversity and Mantel tests to determine significant relationships between community variability and environmental or geographical distance. Beta diversity was partitioned in spatial and environmental components and the origin of communities dissimilarities were analyzed through canonical redundancy analysis (RDA).

Preliminary results suggest that decay of community similarity was driven primarily by environmental gradients rather than geographic distance, as is expected for organisms with high dispersal abilities. However, strong environmental heterogeneity could produce steeper species – area relationships providing diatoms with very high turnover rates, even comparable to turnover rates of macroorganisms. Thus benthic diatoms exhibit spatially predictable patterns leading to estimates of the total community composition dissimilarities and diatom diversity over wide areas.

The extrapolation of spatial patterns of diatom communities is expected to provide ecological background for the design of an optimal sampling strategy, number of sampling locations and replicates for biological monitoring.

## CELEBRATING 100 YEARS OF THE FRITSCH COLLECTION OF ALGAL ILLUSTRATIONS

Elizabeth Y. Haworth

Freshwater Biological Association

Professor F E Fritsch began to systematically file published figures of freshwater algae by species, on foolscap sheets, according to his very basic classification, and then alphabetically by genera and species for ease. In 2012 we celebrate the Centenary of this unique Collection and welcome all users by phone, email, letter or personal visit.

Fritsch's Collection came to Dr J W G Lund at the Freshwater Biological Association after his death in 1954 and it is continuously updated. There are now over 100,000 sheets and several million algal entries, with an author index of c. 25,000 references. It is used to check on species identification and morphology, the original publication and the subsequent varieties. Where records are sufficiently numerous they also provide information on the global locations.

All figures and text are labelled with author and date, and cross-referenced to the original publication, many of which are in the FBA's own library. A recent study suggests that the Fritsch Collection contains about 70% of the original illustrations and/or the diagnoses, i.e. the type information.

Illustrations are entered as published (including errors in some cases, especially in authority citation!) and changes are occasionally made. Cross-references are frequently noted. We are currently checking authorities and identifying types and duplicate naming in the desmid section which has been part of the pilot study of digitization to provide searchable internet access. Part of the author index is already searchable at [www.fritschalgae.info](http://www.fritschalgae.info)

## THE LAST 150,000 YEARS IN THE NORDIC SEAS: SEA SURFACE TEMPERATURE AND SEA ICE RECONSTRUCTIONS BASED ON DIATOMS AND ORGANIC PROXIES

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The overall goal of the presented study is a reconstruction of the exchange of Polar water with Atlantic surface water in the Nordic seas (Faroe Islands and Svalbard margin). Long-term changes in summer sea surface temperatures, palaeoproductivity and sea ice distribution in relation to natural variations in climate, focusing the past 150,000 years, are investigated. A combination of the results of the most commonly used marine biological proxies, diatoms and foraminifera, together with reconstructions of the relatively newly implemented biomarker IP25 is used to solve the general problem of extended gaps in marine records aiming reconstructions of palaeoproductivity and palaeoceanography in the polar- and Nordic seas. These gaps very often arise out of a poor preservation and a high degree of dissolution of both diatom frustules and foraminifera shells. Therefore, the presented study is based on diatom species determinations, quantitative temperatures and sea ice distribution calculated by transfer functions together with geochemical measurements of marine biomarkers (e.g., IP25), plus determination of paleoproductivity of diatoms and silica. Special focus is put on the Eemian interglacial and the Holocene.

Preliminary results of an ongoing multiproxy investigation (e.g.: quantitative diatom analysis, foraminifera analysis, multi-sensor core logging, XRF- colour and mineralogical scanning - heavy minerals, X-ray, TOC and  $\delta^{13}\text{C}$  measurements, IP25) of sediment core JM11-FI-19PC are presented. The core has a length of ~11 m and was recovered from the Northern Faroe slope at 1179 m water depth during a scientific cruise with R-V "Jan Mayen" in April/May 2011. According to the measurements of the magnetic susceptibility, sediments up to Marine Isotope Stage 5 were successfully recovered.

## CONTROL OF LIGHT-DEPENDENT CELL CYCLE ONSET BY THE DIATOM-SPECIFIC CYCLIN *DSCYC2*

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Cell division in photosynthetic organisms is tightly regulated by light. Although light-dependency of cell cycle onset has been well characterized in various phototrophs, little is known about the cellular signaling cascades connecting light perception with cell cycle activation and progression. Here, we demonstrate that the *Phaeodactylum tricornutum* diatom-specific cyclin 2 (*dsCYC2*) displays a transcriptional peak only minutes after light exposure, long before the onset of cell division. In accordance with a role for *dsCYC2* in controlling a light-dependent cell cycle checkpoint, *dsCYC2* silencing decreases the cell division rate due to a prolongation of the G1-to-S phase transition. Interestingly, transcriptional induction of *dsCYC2* is triggered by blue light in a fluence rate-dependent manner, hinting at a photoreceptor-mediated regulation of *dsCYC2*. Using yeast-one-hybrid screening we were able to identify a blue light photoreceptor of the aureochrome family as an interactor and activator of the *dsCYC2* promoter sequence. We are currently further investigating the light-regulated transcription of *dsCYC2* and control of cell cycle onset by analyzing the effect of silencing of this photoreceptor on the expression of *dsCYC2* and other cell cycle regulators. The functional characterization of a cyclin whose transcription is controlled directly by light and whose activity connects light signaling with cell cycle progression contributes significantly to our understanding of the molecular mechanisms that underlie light-dependent cell cycle onset in diatoms and possibly other eukaryotes.

## A MOLECULAR APPROACH TO THE TAXONOMY OF *COCCONEIS PLACENTULA* SENSU LATO

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Taxa from the freshwater *Cocconeis placentula* s.l. are difficult to differentiate by morphological features. These monoraphid diatoms show the same morphology of their raphid valve (raphe-sternum valve), only the araphid valve (sternum valve) and the first girdle bands of the valves (valvocopula) possess features which up to now have been used for differentiating the taxa. These taxa – be it as species or infraspecific varieties include – among many others – *C. placentula* var. *placentula* Ehrenberg (1838), *C. limbata* Ehrenberg (1840), *C. lineata* Ehrenberg (1849), *C. euglypta* Ehrenberg (1854), *C. placentula* var. *pseudolineata* Geitler (1927), *C. placentula* var. *tenuistriata* Geitler (1932), *C. placentula* var. *euglyptoides* Geitler (1958).

Geitler (1927, 1932, 1958, 1982) was able to differentiate his taxa with the help of cultures, investigating their mating, noting especially the morphology of their auxospores. Since his descriptions, two of his taxa were rediscovered in springs and raised to species rank: *C. pseudolineata* (Geitler) Lange-Bertalot and *C. euglyptoides* (Geitler) Lange-Bertalot (Werum & Lange-Bertalot 2004). Monnier et al (2007) are able to differentiate *C. lineata* and *C. euglypta* by their morphology and their ecology, claiming that they are good indicators of water quality with *C. lineata* preferring oligotrophic habitats and *C. euglypta* habitats with higher conductivities. Also, the recent compendium for identifying species of Central Europe for water quality purposes, show differentiating features for *C. lineata* and *C. euglypta* (Hoffmann et al. 2011) but these characters are different from the concept of Monnier et al (2007), Jahn et al. (2009) and Romero & Jahn (in prep.).

12 monoclonal strains of fresh to brackish water *Cocconeis* specimen were established from mixed samples and from very different habitats and served for morphological and molecular analyses. Besides our standard DNA-Barcode 18SV4 (Zimmermann et al. 2011) further DNA-markers (e.g. *cox1*, *rbcL*, 5.8S+ITS) were tested and analyzed for their efficiency to resolve taxonomical questions.

## DIATOM LIFE CYCLES: COUNTING THE COSTS.

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The number of known species of diatoms has increased from 50 in the early Cretaceous to more than 100,000 at present. One adaptation that has contributed to this increase in diversity is the cycle of size decline and size regeneration (Diatom Sex Clock), which is used to time life cycles longer than one year. However, there are only a very few species where we have a full understanding of the quantitative impact (i.e. 'cost'). Part of the reason for this is that sexual reproduction has evolved in such a way that it does not usually interfere with the increase in cell numbers by asexual reproduction. As a result it is often overlooked or considered unimportant in studies of populations in natural environments. In most other plant and animal groups, the significance of recruitment, mortality, and length of the life cycle are fundamental to understanding population dynamics and evolution. Results will be presented from a range of species, including long-term studies of *Aulacoseira* and *Hannaea* populations in Lake Baikal, to show how the impact of sexual reproduction can be quantified and put into a context of other related factors (such as size decline, nutrient limitation, grazing, sedimentation, etc.) over the length of the life cycle.

## MORPHOLOGICAL VARIATION WITHIN SALINE AND FRESHWATER STRAINS OF *FISTULIFERA SAPROPHILA*

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*Fistulifera saprophila* has been reported from highly enriched rivers of Europe and North America as well as from coastal waters and inland saline lentic waters. The species has been studied as a possible biofuels candidate for many years, and was one of the species of greatest promise in the biofuels effort led by the Aquatic Species Program of the Solar Energy Research Institute (SERI) in the mid-1980's. In recent biofuels-from-algae work in our lab it has again been demonstrated to be a species of promise. The work conducted in the 1980's was based on populations isolated from coastal oceanic waters (lagoons, estuaries, wetlands), inland saline lakes, and other saline lentic habitats. The strains examined in this project had relatively high salinity tolerance (optimum growth in water of 25-40 mS•cm<sup>-1</sup> conductivity). The morphology of 14 of these strains was documented in a series of TEM micrographs taken at the time but never reported in the literature. Recent isolations of *Fistulifera saprophila* were made from freshwater rivers in Ohio and Texas. The purpose of this study was to examine all of these populations with intent to answer the question whether the saline-habitat populations could be the same species as the freshwater populations. The two recent freshwater populations were compared to the one surviving (SERI strain) in SEM and TEM. All strains showed high morphological similarity. Ultrastructural details of the fistula, striae, raphe, axial area and valve margin are identical. The width of the valve exclusive of the marginal extensions differs slightly between saline populations (1.4-2.4 µm) and freshwater populations (2.3-3.4 µm), the striae counts, while overlapping, differ slightly (saline populations 65-80 in 10 µm, freshwater populations 46-70 in 10 µm). The difference in physiology and morphology indicate genetic separation between saline and freshwater populations, but at present it is not possible to separate these populations clearly into separate species. We conclude that more evidence (physiological, molecular sequence) is needed to demonstrate that the saline and fresh populations represent separate monophyletic species.

## OBSERVATIONS ON MIDDLE EOCENE MARINE DIATOMS FROM THE CENTRAL ARCTIC OCEAN

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Middle Eocene biosiliceous sediments containing well-preserved diatoms, silicoflagellates, ebridians, actiniscids and archaeomonads, were obtained from the Central Arctic Ocean during the Arctic Coring Expedition (ACEX; Integrated Ocean Drilling Program Leg 302) in summer 2004. Although a number of papers have already documented the diatom resting stages in these deep-sea drilling samples, new scanning electron microscope (SEM) observations on some of the remaining taxa are presented here.

For instance, the samples contain well-preserved specimens of *Corethron* with spines still articulated in their sockets, as well as hook spines lying nearby. The hook spines are like tiny parasols, totally unlike those of modern taxa, and probably represent the oldest known example of hook spines in the fossil record. The ACEX specimens are compared with two similar taxa from Mors, Jutland (Early Eocene); *C. penicillus* (Grunow) Fenner, which is currently the oldest known species in the genus, and *Pseudopyxilla harrensis* Fenner, which we believe also belongs in *Corethron*. The data suggests that *P. harrensis* (and the ACEX specimens) may be the vegetative stage of *C. penicillus*, with the type specimen representing the resting stage. In the Mors sample, the resting stage usually occurs as a doublet, with opposing valves characterized by spines that are fused to the valve (i.e. there are no sockets), whilst the other valves are domed and spineless. The two valves are joined by a long single, perforated girdle band, identical to that found in the vegetative stage. However, so far we have not found any *Corethron* resting stages in the ACEX material.

The ACEX samples also contain a number of quadrangular centric diatoms that appear to be closely related to *Triceratium basilicum* Brun, which was originally described from Kuznetsk, Russia (Middle Eocene). SEM observations on the ACEX material and light microscope observations on the type slide, including the type specimen, have revealed at least three distinct morphotypes, and suggest that *T. basilicum* belongs in *Trinacria*.

We have also found several examples of transition cells, which bear valves of both the vegetative and resting stages (e.g. of *Pseudostictodiscus* and *Pseudopyxilla*). These specimens will prove invaluable when reconstructing the life cycles of extinct taxa and documenting the synonymy of each species.



## IDENTIFYING HOMOLOGY IN MORPHOLOGICAL SUBCOMPONENTS OF FULTOPORTULA

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The fultoportulae is considered a homologous structure, and has been the preferred feature for diagnosing membership and shared ancestry among species in the Thalassiosirales. Since the structure's discovery, numerous species have been moved from other centric orders to the Thalassiosirales transforming it from a small genus and species poor group to the largest centric diatom order. Phylogenetic reconstructions utilizing molecular data support the order's monophyly and the fultoportulae's derivation in the group. Systematic studies considering relationships among Thalassiosirales species have used morphological components of fultoportula as putative homologies for reconstructing evolutionary relationships. Many of these features have withstood tests of homology, and relationships reconstructed using morphological features have been validated with analyses utilizing multiple genes as the basis for phylogenetic reconstruction. These homologous morphological components of the fultoportula have utility in diagnosing monophyletic subgroups within the Thalassiosirales, but are rarely considered or illustrated in investigations describing and comparing species. Even when fultoportula morphologies are compared among species, discussions are limited to comparisons coarse comparisons of non-homologous features such as satellite pore number. In this presentation, we review the fultoportulae's morphological composition and clarify terminology for these morphological subcomponents. These features are then discussed in context of the current understanding of evolutionary relationships among Thalassiosirales species. Structures with utility as diagnostic features are highlighted and information is provided concerning how these features should be illustrated in species descriptions and comparisons. Additionally, morphometric patterns in distribution of valve face and marginal fultoportulae are discussed and suggestions are presented concerning how these patterns should be documented in morphologically focused investigations.

## DETECTING TOXIN CONTAMINATION IN STREAMS: COMBINING DIFFERENT FORMS OF DIATOM VALVE MALFORMATION AND CHANGES IN DIATOM BIOLOGICAL TRAITS FOR A SCREENING METHOD

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Ongoing work on the development of new metric using benthic diatoms to assess heavy metal or pesticide pollution in Swedish streams and lakes gave the preliminary result that the amount of amount of diatom valve malformations cannot be used as the only tool separating impacted from non-impacted sites. Instead, new focus was placed on the strength and the character of the malformations (like different forms of deformed valve outlines or changes in striation patterns) and on diatom biological traits as alternative bioindicators to detect disturbances of a natural environment. Diatom biological traits have earlier been defined as life-forms (for example benthic, mobile, tube forming), size classes and ecological guilds (living in the same kind of environment, for example prostrate, high-profile). Indeed, a combination of changes of the amount, strength and form of diatom valve malformation and a change in the biological traits gave the best assessment of heavy metal or pesticide pollution in Swedish streams.

## NEW BIOMONITORING APPROACHES BASED ON NEXT GENERATION SEQUENCING: A TEST FOR FRESHWATER DIATOM COMMUNITIES

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The EU member states are required to assess rivers quality with diatoms. Species composition and abundance must be defined. As microscopic identification requires time and expertise, the development of a new tool for fast and accurate identification is a major challenge for routine biomonitoring of aquatic ecosystems.

We evaluated the capacity of Next Generation Sequencing to assess the composition of environmental diatom samples. We pyrosequenced 3 mock environmental communities made of known diatom strains (30) with 3 markers (18S, rbcL, cox1) in order to establish their species inventories. Read libraries obtained from pyrosequencing were compared to reference databases (laboratory and GenBank diatom sequences) using metaMatch software to establish the inventories. Their accuracy was tested by comparison to real compositions.

18S sequences have a low resolution. Cox1 seems to have the largest resolving power, but insufficient reference database. Inventories obtained from rbcL are closely related to real inventories. RbcL combines a high resolving power with large reference databases, and coupled to pyrosequencing, could serve as a molecular tool for quick and easy identification of diatom species in environmental samples.

## ORIGIN AND HISTORICAL DEVELOPMENT OF FRESHWATER FOSSIL DIATOMS OF THE ORDERS AULACOSEIRALES, THALASSIOSIRALES, STEPHANODISCALES, AND COSCINODISCALES, FROM ASIA VERSUS WESTERN USA: SINGLE OR PARALLEL EVENTS?

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The origin and development of fossil freshwater diatom communities from the Asia and Western USA have been documented for the last 70+ years, and within the last 20 years our understanding of the taxonomic diversity of the floras has increased dramatically. There are similarities in the timing, taxa and sequence of community development of the floras on both sides of the Pacific, and the similarities between them have long been recognized. In this presentation we describe the late Cretaceous origin and development through to the Pleistocene of the floras from eastern and southeastern Asia and western North America, and examine similarities and differences between the two regions. Twenty-five genera from these groups are known from the Cenozoic freshwater sediments within Asia, while the representatives of sixteen genera were reported from the Cenozoic freshwater deposits of North America. The freshwater extinct centric genera of *Concentrodiscus*, *Mesodictyopsis*, *Tertiariopsis*, *Stephanopsis*, *Cyclostephanopsis*, *Thalassiobeckia*, *Ectodictyon*, *Pseudoaulacosira*, *Undatodiscus* and *Lobodiscus* are known, at present, only from Asia, and the extinct genus *Eoseira* is known only from North America. While fossil *Thalassiosira* species were not found, some fossil species of the new genus *Spicaticribra* were observed both from North America and Asia. We explore the ideas of whether invasions of representatives of marine lineages have happened separately several times, leading to independent floras in Asia and western North America, or whether the invasions of each lineage have happened once, followed by subsequent dispersal in one or both directions. We document the taxa found in each region, their origin (and in some cases extinction) as well as relationships to other taxa within their Orders.

## USE OF PASSIVE SAMPLERS EXTRACTS FOR TOXICITY ASSESSMENT OF ENVIRONMENTAL REALISTIC MIXTURE OF PESTICIDES ON NATURAL BIOFILMS COMMUNITIES

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The objective of this study was to evaluate the potential use of passive samplers extracts in order to highlight effects of representative pesticide mixtures on natural biofilm communities.

Chronic and acute impacts of pesticides in mixture were evaluated using POCIS (Polar Organic Chemical Integrative Sampler) extracts on natural biofilm communities. Biofilms were exposed to POCIS extracts or placed in clean water in order to model different levels of toxic pressure in relation with a realistic pesticide mixture directly isolated from the field.

After 13 days of exposure, a significant decrease of diatoms density on upstream and downstream biofilms exposed to PE compared to non exposed biofilms was observed; moreover DW and AFDM were lower for downstream biofilms exposed to PE compared to non exposed biofilms. Taxonomic analyses revealed impacts of mixture of pesticides on diatom composition. These results indicated that the mixture of pesticides has an impact on growth of the biofilms and particularly on diatom density evolution and on structural composition diatom communities.

Acute toxicity tests at day 13 on downstream biofilms revealed a significant higher tolerance for biofilm exposed to PE compared to non exposed biofilms.

Moreover a decrease of tolerance of downstream biofilms from day 0 to day 13 was observed for both treatments; with  $51 \pm 13$  and  $43 \pm 2\%$  of inhibition at day 13 compared to  $25 \pm 3\%$  of inhibition at day 0 in the highest concentration (d0). This global decrease of tolerance to PE with time could be explained by differences between channel and field conditions (light intensity, PE composition different from real river exposure due to POCIS selectivity, pesticides concentrations lower in channels than in river,...)

Nevertheless, tolerance of downstream biofilms at day 13 still be much more higher than tolerance of biofilms originated from upstream site (significant inhibition of  $F_v/F_m$  for d0, d1, d2 and d3 for upstream biofilms).

Our results underline impacts of pesticides in mixture on growth (DW, AFDM and diatoms density), diatom composition, and community tolerance of a natural biofilm. For downstream biofilm, in the one hand, removing the toxic pressure stimulates global biofilm growth but in the other hand it leads to a decrease of biofilms tolerance. A global decrease of tolerance over time for downstream biofilms was observed; nevertheless without reaching the tolerance levels of upstream biofilms.

This study highlights the potential use of passive sampler extracts combined with acute toxicity tests in order to evaluate effects of more realistic pesticide mixtures on natural biofilms communities, and then the future and likely applications of such approaches for ecological risk assessment.

## BACTERIAL COMMUNITIES HELP SURVIVALS OF DIATOM POPULATIONS FROM VIRAL ATTACKS

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A number of field studies and laboratory experiments showed that diatom dynamics in nature are affected by various environmental factors (e.g., water temperature, salinity, light, nutrients, and water movement). During the last two decades, several studies reported that viral impacts are also important for disintegrations of phytoplankton bloom population. In bacterial free culture experiments, diatoms are completely crashed due to viral infections. The diatom populations in natural environments, however, are not completely killed by viruses and continuously grow under the viral pressure. There may be several strategies for the host population to reduce the viral impacts. In this study, we tried to analyze the survival mechanism of diatoms post viral infections, using a host–virus culture system, marine planktonic diatom *Chaetoceros tenuissimus* Meunier (Bacillariophyceae, Centrales) and its single-stranded RNA virus CtenRNAV, especially from the view point of bacterial affects.

In axenic conditions, *C. tenuissimus* cells were almost crashed due to viral inoculations and a regrowth of the population was not observed. The host populations inoculated with marine bacterial community also showed the rapid decreases of the cell concentration. After 7 days of post viral infection, however, a few cells survived and the population showed regrowth under a high viral concentration environment. This indicated that the some functions of the bacterial community or species might help for *C. tenuissimus* survivals from viral infections. The bacterial community was isolated from the same sea water used for the *C. tenuissimus* isolations. We prepared 96 bacterial isolates and added each of them to the host-virus system. The results showed that any bacterial isolates induces the survival of the host population. Under epi-fluorescent microscopy using SYBR-Gold staining, many bacteria were observed onto the *C. tenuissimus* cell surfaces, and which might be significantly related to the survival. In nature, many environmental factors and host strategies may affect the survival of the diatom populations from viral attacks. The host cell associated bacteria might be one of these factors, but the mechanisms are still unknown. Now, we are trying to clarify the bacterial functions affected to the host survival mechanism, by using physiological, molecular biological and cell biological analyses.

## CULTURE MEDIUM OPTIMIZATION AND LIPID PROFILING OF *CYLINDROTHECA*, A LIPID- AND POLYUNSATURATED FATTY ACID-RICH PENNATE DIATOM AND POTENTIAL SOURCE OF EICOSAPENTAENOIC ACID

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*Cylindrotheca*, an epipellic benthic pennate diatom, holds promise as a nutraceutical source and may be useful for aquaculture. Experiments were done on two *Cylindrotheca* species, *Cylindrotheca fusiformis* (UTEX 2084) and *C. closterium*, which was isolated from seawater collected offshore from Visakhapatnam, India. *C. closterium* was identified through microscopy and rDNA typing. Type and concentration of nutrient components in the culture medium that promoted best growth and highest lipid accumulation were identified. Lipid content was gravimetrically estimated. For relative comparison of the effects of different culture media on lipid content, we made estimations through rapid *in situ* screening method using Nile red staining and spectrofluorimetry. The fatty acid profile of lipid was obtained through gas chromatography-mass spectroscopy. Nualgi, a commercially available micronutrient ready-mix with elements adsorbed as nanoparticles on a modified silica sol, was found to significantly boost growth in both *Cylindrotheca* species when used *in lieu* of a conventional micronutrient mix prepared from eight compounds. Among the three nitrogen sources tested – sodium nitrate (NaNO<sub>3</sub>), urea, and ammonium chloride (NH<sub>4</sub>Cl) – best growth of *C. fusiformis* occurred on nitrate and urea, while NH<sub>4</sub>Cl was best for *C. closterium*. Lipid productivity was much higher in cultures supplied with NH<sub>4</sub>Cl for both *Cylindrotheca* species and compensated for lower biomass in *C. fusiformis*. Mixotrophy with glycerol or sodium acetate resulted in no significant increase in growth over photoautotrophy. Both *Cylindrotheca* species were lipid rich; lipid constituted 18–27% of dry biomass in the medium with NaNO<sub>3</sub>. Among total fatty acids, polyunsaturated fatty acids constituted <40%, eicosapentaenoic acid 25%, and arachidonic acid ~8% and ~4% in *C. fusiformis* and *C. closterium*, respectively. NH<sub>4</sub>Cl, phosphate, and Nualgi micronutrient ready-mix in concentrations optimal for each strain contribute to a good culture medium for *Cylindrotheca*.

## IMMISCIBILITY OF DIATOM FLORAS IN LAKE BAIKAL: SCIENTIFIC PHENOMENON OR FANTASY?

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The species composition of the diatom assemblages in Lake Baikal are changing geographically and spatially. Diatom assemblages are different along the shoreline of Lake Baikal and with increasing water depth (Flower et al. 2002, Flower 2005). Differences in species composition of various organismic groups between Lake Baikal shallow water area (inhabited by widespread Holarctic taxa) and deep water ones that are dominated by endemic species is known as the immiscibility theory. It was first observed and recognized by Dybowsky (1912) who studied Lake Baikal fish. Later this phenomenon was discussed by e.g. Wereschtschagin (1935, 1940), Kozhov (1962), Timoshkin (2001) for other organisms. Presented results are based on examination of samples from deeper water part of Lake Baikal, especially from Ushkaniy Islands where the sampling site depth exceeded 50 m, and from the Southern, shallow water part of Lake Baikal. Indeed, in the shallow areas we have observed numerous Holarctic species characterized by broad geographic distribution. In contrast, assemblages of deeper water are significantly different and include numerous rare and endemic taxa. In the context of the species inhabiting shallow waters of Lake Baikal, the question arises about their identity. Are they really identical with Holarctic/Palearctic species? To explore this problem, a culture collection with a few hundreds of strains from Lake Baikal has been established, and molecular biology methods has been applied to establish their relationship. Using the large scale research based on morphological and molecular methods, the similarity of Baikal diatoms with taxa from other areas of Eurasia is discussed. We hope that our findings will be important for recognizing this problem and will further studies on this topic.

This work was supported by the Russian President Foundation (MK-5681.2012.4) and Polish Ministry of Science and High Education (N304017840).



## REFERENCE LIBRARY OF DNA-BARCODED DIATOMS – A USE CASE FOR PUBLISHING DATA VIA THE GBIF DATABASE ALGATERRA

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The Global Biodiversity Information Facility (<http://data.gbif.org>) is a global network of 57 countries and 47 organizations providing standardised observation and specimen data via the Internet to give open access to our knowledge of biodiversity. Thus GBIF fits to the needs of taxonomy and monitoring. Currently more than 367 million records provided by 406 data publishers are available via the data portals of GBIF (<http://data.gbif.org/occurrences>) and BioCASE ([www.biocase.org](http://www.biocase.org)).

GBIF-D *Plants, Algae and Protists* ([www.gbif.de](http://www.gbif.de)), funded by the Federal Ministry of Education and Research (BMBF, grant 01 LI 1001 A-F), is engaged in extending the quantity of data and datasets of German providers and in solving data quality issues. Aim of this initiative is to raise the usability of those data for taxonomy.

The database *AlgaTerra* ([www.algaterra.org](http://www.algaterra.org)) is providing research data on diatoms to GBIF for more than five years. The *AlgaTerra* Information System for micro algal biodiversity comprises type information, specimen data as well as images, videos, and molecular data for terrestrial and limnic micro algae. One focal area of *AlgaTerra* is the publication of images from diatom field observations as well as specimen and type data of African, American, and European diatoms. In 2012 *AlgaTerra* is subject to changes in its data architecture and lay out. *AlgaTerra* moves from a Berlin Model database to the Common Data Model (CDM) and will migrate to the EDIT Platform for Cybertaxonomy. This will result in a better integration of multimedia items and related observation and specimen data.

As a repository of primary biodiversity data *AlgaTerra* publishes voucher images of research projects, such as of diatom DNA barcoding. Advantages of publishing these images on the Internet are: (i) accessibility of these data independently from the publication, (ii) publication of several images is not limited by available space in a printed journal, (iii) flexibility because additional images can be added after publication, (iv) images can be analysed easily in other contexts. Our aim is to link valve morphology to molecular data of specific strains/taxa. These *AlgaTerra* records are cross-referenced with research papers (e.g. Zimmermann et al. 2011, Barcoding diatoms: evaluation of the V4 subregion on the 18S rRNA gene, including new primers and protocols. – *Organisms Diversity & Evolution* 11: 173-192, and [http://www.gbif.de/botany/barcoding\\_3](http://www.gbif.de/botany/barcoding_3)).

## PARMALES: AN INSIGHT INTO THE ORIGIN AND EVOLUTIONARY SUCCESS OF DIATOMS

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The order Parmales (Heterokonta) is a small marine phytoplankton group, 2 to 5 μm in diameter, with a silicified cell wall composed of several plates. Parmales is widely distributed in the world's oceans, from polar to subtropical regions, and is often abundant in polar and subpolar waters. Based on similarities in cell wall structure, Parmales has been proposed as a close relative of diatoms and may play a key role in answering questions on the origin of their silica cell wall and early evolution, which have not yet been clearly established. However, we still have very little biological information on Parmales, due to the lack of laboratory investigations. Recently, with the aid of a fluorescent silicon tracer PDMPO, we isolated the first ever culture of Parmales from the coastal Oyashio region of Japan in the Western Subarctic Pacific. SEM, TEM, molecular phylogenetics and photosynthetic pigments analyses of this culture indicated that Parmales was within the bolidophycean clade of autotrophic naked flagellates and a sister group of diatoms. It can be hypothesized that parmalean and bolidophycean algae (or their common ancestor) have a life cycle that switches between silicified non-flagellated and naked flagellate stages. This hypothetical life cycle would be similar to centric diatoms, which have a vegetative stage that switches to production of naked flagellated male gametes (sperms) for sexual reproduction. Understanding how the life cycle of such a hypothetical ancestor of Parmales and Bolidophyceae relates to that of diatoms is a key question to understand the early evolution of diatoms. We present our ongoing study of the evolutionary close relationship between Parmales, Bolidophyceae and diatoms using ecological, physiological, genomic and biogeochemical approaches.

## METABOLIC INSERTION OF ORGANOALKOXYSILANES INTO THE FRUSTULE OF THE DIATOM *THALASSIOSIRA WEISSFLOGII*

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Functionalization of cleaned non-living diatoms with silanes *via* reaction with the free hydroxyl groups on the frustule surface has been employed to successfully introduce amino-<sup>[1,2,3,4]</sup>, mercapto-<sup>[5,6]</sup> and vinyl-<sup>[6]</sup> groups. These moieties serve as an anchor for further reactions or attachment of biomolecules to the diatom. Modification of the living diatom *via* metabolic insertion of silanes and organo-alkoxysilanes remains under explored. It was hypothesised that a solution of tetramethylorthosilicate (TMOS) and 3-mercaptopropyltrimethoxysilane (MPTMS) will provide an alternative source of silica for the growth of the diatom *T. weissflogii* enabling metabolic insertion of an organo-alkoxysilane into the diatom frustule.

Synchronised *T. weissflogii* cultures were inoculated in enriched artificial seawater. Hydrolysed TMOS/MPTMS was added to the culture at the time of inoculation and at 48 hour intervals. Cultures were harvested at 192 hours post inoculation. Control cultures were prepared using sodium metasilicate (NaSiO<sub>3</sub>). Diatoms were cleaned by successive washes in hydrochloric acid, de-ionised water and methanol. The chemical composition of the cleaned diatom was determined using <sup>29</sup>Si-NMR and energy dispersive X-ray analysis (EDX). The architecture of the diatom was characterised and quantified using transmission electron microscopy (TEM), scanning electron microscopy (SEM) and atomic force microscopy (AFM).

EDX analysis confirmed the presence of sulphur in the TMOS/MPTMS modified diatom. <sup>29</sup>Si-NMR analysis confirmed the presence of an organic moiety extending from the Si backbone of the TMOS/MPTMS modified diatom skeleton. The gross morphology of the TMOS/MPTMS modified diatom is unaltered. AFM analysis revealed that the distance between ribs in both a radial and a rotational direction is decreased in the TMOS/MPTMS modified diatom compared to NaSiO<sub>3</sub> diatom. There is significant reduction in the pore dimensions of width, length, perimeter and area in TMOS/MPTMS modified diatoms compared to NaSiO<sub>3</sub>.

This is the first study to demonstrate that organo-alkoxysilanes can be used as a source of silica for the growth of the diatom *T. weissflogii*. The possibility to use the living diatom following modification of the chemistry of the frustule is an exciting area of research and requires further investigation.

This material is based upon works supported by the Science Foundation Ireland under Grant No. [07/SRC/B1163] and MICINN (MAT2009-10214 and PET2008-0168-01).

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## NEW METHODS TO EVALUATE THE RELATIVE EFFICIENCY OF VERTICAL MIGRATION AND PHYSIOLOGICAL MECHANISMS AGAINST PHOTOINHIBITION IN MICROPHYTOBENTHOS

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The capacity of estuarine microphytobenthos (MPB) to withstand the variable and extreme conditions of the intertidal environment, prone to cause photoinhibition of the photosynthetic apparatus, has been attributed to particularly efficient photoprotection mechanisms. However, little is known regarding its actual photoprotection capacity or the mechanisms responsible for the protecting against photoinhibition.

The general objective of our experiments was to evaluate the relative contribution of the photoprotection provided by migration relatively to the overall photoprotective capacity of MPB biofilms and, more specifically, to determine the relative efficiency of the main physiological processes: xanthophyll cycle, antioxidant defenses and D1 protein repair.

A new experimental protocol was developed, combining (i) chlorophyll fluorescence imaging, for the simultaneous measurement of replicates and experimental treatments; (ii) inhibitors for the vertical migration (latrunculin A-Lat A), the xanthophyll cycle (dithiothreitol-DTT) and the D1 protein repair (lincomycin); (iii) recovery kinetics analysis of photosynthetic activity during light stress-recovery experiments.

Our first results showed a high photoprotective capacity with photoinhibition rates remaining below 25%. The contribution of the vertical migration and the xanthophyll cycle to overall photoprotection varied between sampling periods but reached only ca. 20%. This suggests the participation of other photoprotective mechanisms which are currently under study.

FIRST REPORT OF A 'PLASTID-TO-NUCLEUS RETROGRADE SIGNALING MECHANISM' IN DIATOMS: EVIDENCE THAT THE REDOX STATE OF THE PLASTOQUINONE POOL TRIGGERS THE PHOTOPROTECTIVE RESPONSE IN *PHAEODACTYLUM TRICORNUTUM*

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Diatoms possess a pronounced capacity for a photoprotection mechanism called Non Photochemical Fluorescence Quenching (NPQ), which helps them to populate habitats exhibiting light fluctuations regularly punctuated by high light intensity exposures. The most prominent part of NPQ is the energy dependent fluorescence quenching, qE. Three main actors of qE in diatoms can be distinguished: 1) the build-up of a transthylakoidal proton gradient in the light, 2) the conversion of the xanthophylls diadinoxanthin (Dd) to diatoxanthin (Dt) depending on the magnitude of the proton gradient, and 3) the presence of specific nuclear-encoded antenna proteins, called Lhcx. We used *P. tricornutum* as a model organism to investigate its acclimation to different light climates with respect to the expression of the four *lhcx* genes and the amount of Dd+Dt. Some *lhcx* genes were markedly up-regulated in response to high light (HL) illumination and the amount of Dd+Dt was concomitantly increased. Both correlated with a pronounced rise of qE. By blocking the conversion of Dd to Dt with DTT or by inhibiting the translation of *lhcx* genes with cycloheximide, the amplification of qE was stopped and the diatom suffered stronger photoinhibition during HL exposure. By applying DCMU and DBMIB to artificially modify the redox state of the plastoquinone (PQ) pool we achieved to stop the Dd+Dt synthesis under HL as well as to stimulate their synthesis under low light (LL) conditions, respectively. However, it appears that the regulation of Dd+Dt content was not exerted on the gene level, but probably via enzyme activation in the chloroplast. In contrast, a selective expression induction of some of the *lhcx* genes under LL conditions by an artificially reduced PQ pool (with DBMIB) was reached. These results underline the central role of the redox state of the PQ pool in acclimation responses to HL in diatoms. Moreover, they bring strong evidence for a signaling cascade from the chloroplast, via the redox state of the electron transport chain, to the nucleus, as previously described in green microalgae and land plants. This is the first report for the existence of a so-called 'plastid-to-nucleus retrograde signaling mechanism' in an organism with secondary endosymbiosis derived chloroplasts.

## EFFECT OF SALINITY CHANGES ON THE MORPHOLOGY AND THE CELLULAR PROCESSES OF DIATOMS

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The composition of the phytoplankton community and the morphology of the silica frustule of diatoms (i.e., pore diameter and thickness of the silica cell wall) fluctuate depending on environmental conditions. Here we focus on the impact of salinity changes in marine environments (i.e., associated to climate change and/or desalination) on the morphology and cellular processes of diatoms. Our project combines ecological, chemical and molecular expertise to understand the impact of excess evaporation and/or brine discharge on the growth, morphology and silica uptake of diatoms. As part of this project, five diatom species: *Cylindrotheca sp.*, *Entomoneis sp.*, *Skeletonema sp.*, *Striatella sp.* and *Amphora sp.* were isolated from the waters of the Gulf St Vincent (South Australia) and have been established as mono-specific cultures in our laboratory. Salinity trials on the growth indicated a species-specific response to salinity changes. In order to obtain a better understanding of the species-specific adaptation to salinity changes, *Striatella sp.* (which adapt very poorly to salinity change) and *Cylindrotheca sp.* (which grows very well in high salinity) were studied for changes in morphology and biosilicification. <sup>29</sup>Si cross polarisation-magic angle spinning (CP-MAS) nuclear magnetic resonance (NMR) analysis showed that the Q<sub>4</sub>/Q<sub>3</sub> ratio of the <sup>29</sup>Si spectra was higher in *Cylindrotheca sp.* cells grown at 50 psu compared to those grown in 36 psu. This indicated the existence of intracellular pools of silica which were in a more condensed form and potentially helps in diatom adaptation to higher salinity conditions. *Striatella sp.* (which grows poorly at 45 psu) showed reduced silica content compared to when cultured at 36 psu. Results from NMR spectroscopy, scanning electron micrographs and expression of silicon transporter genes, which play important role in biosilicification, will be discussed in relation to the growth of these species in different salinities.

## A NEW SPECIES OF *ORTHOSEIRA* WITH AN ENIGMATIC DISTRIBUTION.

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While studying algal biodiversity on the relatively remote volcanic archipelago of Hawaii in the northern Pacific Ocean (Lowe & Kociolek) and Ile Amsterdam in the southern Indian Ocean (Van de Vijver), we independently discovered that lava tubes present unique isolated microhabitats that appear to support several new species of algae. So far, a few new species have already been described (Lowe & Sherwood 2010, Lowe et al. 2009) whereas several others await a formal description (Van de Vijver & Cox, unpublished results). In this case, a new species of the centric diatom genus *Orthoseira* Thwaites was independently observed on both geographic locations. Based on light and scanning electron microscopy observations, the two populations from Hawaii and Ile Amsterdam are morphologically almost indistinguishable from each other with similar size ranges, stria densities, carinoportulae number and morphology and of the large linking spines. The enigma arises from the fact that these two populations are from rare habitats on oceanic islands separated by 14,550 kilometers on opposite sides of the equator. Recent research on diatom biogeography leads us to expect that these two populations are most probably a clear example of cryptic diversity and hence represent two species but lacking molecular data, we currently cannot currently separate them.

## UNEXPECTED HIGH DIVERSITY IN *CHAETOCEROS*

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*Chaetoceros* is one of the largest marine diatom genera with more than 400 described species. It is ecologically important due to a cosmopolitan distribution of the genus and a frequent and abundant occurrence of many species; sometimes even in bloom proportions. Species identification is often problematic, due to species being similar and intermediate forms occurring. Looking into the diversity of *Chaetoceros* we focused on a few sections, e.g. on the section *Diocladia*, more commonly known as the “lorenzianus” group, comprising *C. lorenzianus*, *C. decipiens* and *C. mitra*. The two former species are reported occurring from tropical waters to polar areas, the latter restricted to cold waters. Species delineation is extremely problematic, with morphological characters used for delineation having changed over time. Based on detailed morphological studies, studies of life cycle stages and analyses on molecular data of cultures isolated from Arctic, temperate and tropical areas, we found a diversity being >100 % larger than previously described. Characters for differentiating the species in the “lorenzianus” group will be discussed, as well as the distribution of the species. Resting spores proved to be an important taxonomic character. Considering the unexpected high diversity in a group of globally distributed, frequently recorded and characteristic group of species, one can only speculate on the diversity hidden in the diatoms as a group.



## DYNAMICS IN DIATOM POPULATION ABUNDANCE AND ACTIVITY INFERRED FROM STABLE ISOTOPE LABELING OF PHOSPHOLIPIDS FATTY ACID BIOMARKERS IN THE DUTCH WADDEN SEA

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Marine diatoms are major players in carbon sequestration; it is therefore important to understand how planktonic diatom communities change with fluctuations of environmental parameters. To explore community changes, we used <sup>13</sup>C stable isotope labeling of phospholipids derived fatty acids (PLFA). Fatty acids are ubiquitous in living organisms, and due to their biological specificity, they can be used as biomarkers for marine primary producers and as tracers of organic carbon in estuarine food web. ). Planktonic diatom communities were studied in the Dutch Wadden Sea at different temporal scales (tidal and seasonal: February, March, May and September 2010) and also spatial scale, 5 different locations were sampled. Objectives of this study were (1) to follow changes in natural diatoms abundance and activity resulting from changes in environmental parameters, and (2) to characterize quality food resource for higher trophic level. In general, results showed that diatom was a major and most productive group in the entire phytoplankton community. Overall, no tidal effects were observed in diatoms populations. Spatial and seasonal variability in environmental parameters suggested a major role of temperature and had an impact on the activity of enzymes PLFA desaturase and elongatase. Increase of abundance and C incorporation are found in diatoms through the year.

## THE LIMITS TO RARITY IN DIATOMS AND THE INTERPRETATION OF ABSENCE

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Recent statistical approaches indicate that dispersal is a significant constraint on the distribution of freshwater diatoms, supporting the idea that unspecified species may be endemic to particular regions for spatio-temporal, rather than ecological reasons. However, in biogeographical analyses, we often want to know, and sometimes claim that we do know, about the distribution and origin of *specified* individual species, in which case we need to demonstrate where a species is present, but also – and critically – where it is absent.

It is sometimes feasible to record every individual of a rare vertebrate, and it is now highly doubtful whether any native angiosperm remains unrecorded in the UK, implying effectively complete survey, even though most individual plants have not been checked. A complete census of all the individuals in a natural diatom community, on the other hand, even in a puddle or on a single cobble in a stream, is clearly impossible. It cannot be done either by microscopical observation or molecular survey. How can we get equivalent assurance for diatoms as for vertebrates or angiosperms and be confident that information about the distribution of a particular species is useful? What does it mean if a species was not recorded in a sample? Does it mean that the species, though present, was misidentified, or correctly identified within a different taxonomic paradigm? Or that too few cells were examined to reveal it? Or that it was truly absent? Clearly, it is only the last of these possibilities that provides biologically interesting information. And does absence from a *particular* sample indicate that the species is absent from the area where the sample was obtained? If the sample location or method had been slightly different, would the species have been found, i.e. were we looking in the right place? And if we had returned a some months later, would the species still have been absent, even if no gross change in conditions had occurred meanwhile?

The answer to identification problems is more care, standardization, and vouchers. Assuming that this has been done, there remains the more difficult problem of knowing whether our sampling is ‘fit-for-purpose’, minimizing the uncertainties outlined above. I will explore this problem via the curious example of *Sellaphora gregoryana*, ‘appearing’ and ‘disappearing’ at its type locality in Loch Leven, Scotland; analogies with the UK angiosperm flora; limits to rarity set by the diatom life cycle; and N American diatometer data.

**REDOX REGULATION OF PLASTID METABOLISM BY THIOREDOXIN IN THE MARINE DIATOM *PHAEODACTYLUM TRICORNUTUM***

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Thioredoxin (Trx), a major regulator of photosynthetic fixations of inorganic carbon and nitrogen in plant chloroplasts, was thought to play little role in controlling the Calvin cycle in marine diatoms. Nevertheless diatoms possess a set of subtypes of chloroplastic Trxs. In the present study we examined influences of redox state and the involvement of Trxs on activities of two pyrenoidal carbonic anhydrases, PtCA1 and PtCA2, in the marine diatom *Phaeodactylum tricornutum* which are presumably a critical part of CO<sub>2</sub>-concentrating mechanism (CCM). The recombinant mature PtCA1 and 2 (mPtCA1 and 2) completely lost their enzymatic activity when oxidized by 50 μM CuCl<sub>2</sub> whereas DTT activated CAs in a concentration-dependent manner. The maximum activity of mPtCAs in the presence of 6 mM reduced DTT were significantly stimulated by the addition of 10 μM Trxs from *Arabidopsis thaliana* (AtTrx-f2 and -m2) and 5 μM of Trxs from *P. tricornutum* (PtTrxF and M). Kinetic analyses of mPtCA activation by Trxs in the presence of 6 mM DTT revealed that the maximum mPtCA1 activity was enhanced about 3 fold in the presence of Trx whereas mPtCA2 was only weakly activated by Trxs, and that half saturation concentration of Trxs were significantly lower in PtTrxs compared to AtTrxs. Site-directed mutagenesis of potential disulfide-forming cysteines in mPtCA1 and mPtCA2 resulted in a lack of oxidative inactivation of both mPtCAs. These results reveal the first direct evidence of a target of plastidic Trxs in diatoms, indicating that Trxs may participate in the redox control of inorganic-carbon flow in the pyrenoid, a focal point of the CO<sub>2</sub>-concentrating mechanism.

## ENHANCING LIPID PRODUCTION OF *PHAEODACTYLUM TRICORNUTUM* THROUGH METABOLIC ENGINEERING

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Diatoms hold much promise for the production of lipids and other metabolites. We apply a functional genomics approach using the model diatom *Phaeodactylum tricorutum* in order to identify the key regulator genes that drive lipid synthesis. To this end we are using RNA-seq to sequence the transcriptome of *P. tricorutum* during stress conditions and comparing these transcriptome profiles with non-lipid producing conditions. In this way we map the metabolic reprogramming that cells undergo during the switch from growth towards carbon storage and in particular lipid production. Since we are chiefly interested in the regulators involved in this reprogramming and, to a lesser degree, in the enzymes actually effectuating this change, we are performing our analysis during the first 24 hours of these lipid producing conditions when lipid productivity is only starting. In the second phase of the project we will attempt to activate this program during conditions that favor exponential growth using genetic engineering. Preliminary transcriptome analysis using cDNA-AFLP shows that 2-5% of genes respond to investigated stress conditions. Furthermore, metabolic analysis shows that there are quantitative changes detectable in chrysolaminaran and lipid storage products. These changes appear to be coupled to cell cycle progression. We have also shown for the first time that *P. tricorutum* lipid productivity can indeed be enhanced by overexpression of endogenous genes.

## DIATOMS CHALLENGE TO CHANGE PEOPLE'S AWARENESS ABOUT RIVERINE ENVIRONMENT WITH AID OF AN INTERNATIONAL WEB-BASED EDUCATIONAL SYSTEM

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The United Nations has designated water sanitation and safety as one of millennium goals and has emphasized the role of international cooperative efforts in achieving this goal. Promoting awareness to the nations about riverine environments is one approach to this goal and education has the potential to actualize it. We have developed Web-based multilingual educational teaching aides in 17 languages for achieving this aim and compiled them into "DiatomProject" Webpage. The contents are composed of "SimRiver" a Web-based program simulator that uses diatoms to enhance the understanding of the relationship between human activity and water quality, the streaming videos introducing basic knowledge of diatoms, visual tools informing circumstances of various water environments in different areas and in different time frames.

Effectiveness of the system was assessed in classes using 6 languages in 5 countries. Before and after the classroom activity students described answer for the same question "What thought do you have about river today?" The descriptions were treated with text mining software, followed by correspondence analysis (CA). In comparison of the pre- and post-tests, Japan and Korea showed similar shift of student groups, in which their awareness was orientated toward improvement of riverine environment. In Indonesia and two Indian language areas, they were rather orientated to SimRiver program and diatom itself. Thai students showed intermediate shift between eastern and southern Asian countries. The difference of the group positions in CA may be caused by difference of the river pollution levels and social systems. Although orientations of the thoughts were different, learners' awareness about river environments was promoted in all study areas.

## RECENT DISCOVERIES OF UNUSUAL SILICOFLAGELLATE DOUBLE SKELETONS, WITH EVOLUTIONARY AND TAXONOMIC IMPLICATIONS

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Double skeletons of silicoflagellates have been observed previously to consist of two skeletons with the corners of each being in close alignment, and general symmetry across the basal ring. All observed Cenozoic silicoflagellate double skeletons conform to this corner-to-corner configuration. Our recent discoveries of double skeletons from Santonian and Campanian (Upper Cretaceous) marine sediments of the northern Canada Arctic Margin show a different configuration where each skeleton is rotated along the basal plane with respect to the sibling. For *Corbisema*, the double skeleton configuration is similar in general pattern to the star-of-David. Similar rotated configurations are observed for specimens of Cretaceous *Schulzyocha* and *Vallacerta*. Thus, known double skeletons of the Cretaceous and Cenozoic exhibit separate and distinct patterns of symmetry alignment across the basal plane.

The recent discovery of a late Paleocene or early Eocene double skeleton specimen of *Corbisema hastata* with the star-of-David configuration suggests that three-sided silicoflagellates of both the corner-to-corner and star-of-David patterns occur in the early Cenozoic. We have previously documented the possibility of two divergent lineages of corbisemid silicoflagellates derived from *Cornua* in sediments from the Santonian of Devon Island. Here, we provide further evidence of this divergence in three-sided silicoflagellates. At present, representatives of these groups can only be distinguished from one another as double skeletons. The group with corner-to-corner double skeletons led to most or all of the known silicoflagellate genera of the Cenozoic. However, the discovery of a star-of-David type double skeleton of *Dictyocha medusa* from the late Eocene of DSDP Site 280 suggests that a distinct group of four-sided silicoflagellates may have evolved from three-sided star-of-David group. This conclusion would complicate the interpretation of early Cenozoic silicoflagellate evolution and taxonomy.

## EPILITHIC DIATOMS FROM WEST GREENLAND LAKES ACROSS A CLIMATIC GRADIENT: IMPLICATIONS FOR PALAEOCLIMATE RECONSTRUCTIONS

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The widest ice-free land mass in Greenland around Kangerlussuaq straddles a climate gradient which influences the physical properties of the region's lakes. Coastal lakes have a shorter ice-free season than those inland because snow cover is more extensive and summers are cooler. Lakes close to the ice sheet are strongly influenced by katabatic winds from the ice sheet which also prolong the ice-covered period relative to the inland lakes. Epilithic diatom samples were taken from 18 lakes in the inland, coastal and ice sheet areas of this region to investigate the role that variability in length of ice-free season has on rock-dwelling diatom assemblages. We hypothesized that epilithic diatom communities in lakes close to the coast and ice sheet would be less diverse with simpler growth forms because of the shorter growth season. However, diatom diversity proved to be lowest in the inland lakes, suggesting that longer growth seasons favoured the success of a smaller range of more competitive diatoms (*Cymbella microcephala* and *Nitzschia fonticola*). Rock scrapes from coastal lakes and ice sheet lakes with shorter ice-free seasons were dominated by colonial diatoms such as *Staurosira pinnata* and *Tabellaria flocculosa*. Comparison of epilithon communities with diatoms from two 2000-year lake sediment core records showed that epilithic diatoms comprised a small (<20%) proportion of the sedimentary diatom assemblages. However, the 'Little Ice Age' cooling in this area was associated with an increase in the relative abundance of epilithic diatoms that are now associated with coastal and ice sheet sites. Therefore, investigation of diatom communities associated with the marginal rocks of these arctic lakes might assist palaeoenvironmental interpretations and provide information about likely shifts in lake phytobenthos communities with future warming.

## DNA-BASED SPECIES DELIMITATION IN DIATOMS

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Species are basic units in studies of biodiversity, systematics, biogeography and ecology. We all accept the idea that species represent fundamental units of biology. Because there has been a great deal of disagreement with regards to the criteria used to delimit species this has led to a proliferation of different species concepts. Among the diatoms the morphological species concept is that most commonly used. However, more and more cryptic species have been revealed and many of these cryptic species are supported by a biological species concept through breeding experiments. The phylogenetic species concept has revealed many paraphyletic taxa, which some workers are reluctant to accept. However in nearly every case where paraphyletic taxa have been discovered, good morphological genera and species would have to be disregarded to accept that the phylogenetic species must be monophyletic. By treating species as separately evolving metapopulation lineages formal analyses of species boundaries become possible. Using metapopulations as the evolving unit, it is possible to demonstrate that evolution proceeds from polyphyly to paraphyly to monophyly. I present a number of case studies in diatoms at the class and generic level to show how DNA-taxonomy can provide new insights into morphological evolution, biogeography and speciation of diatoms and how the recognition of paraphyletic taxa is unavoidable because evolution is not a static process.



## MIDTAL MICROARRAYS FOR THE DETECTION OF HARMFUL ALGAE: A COMMERCIAL REALITY AND $\mu$ AQUA NOT FAR BEHIND FOR DIATOMS IN ASSESSING WATER QUALITY

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In the EU FP7 project MIDTAL (microarrays for the detection of toxic algae), we targeted rapid species identification using rRNA genes to generate “barcodes” specific for probe design to recognize species or even strains. A toxin-based microarray includes antibody reactions to specific toxins produced by these microalgae because even when cell numbers are low, toxins can be present and can accumulate in the shellfish. Microarrays are the state of the art technology in molecular biology for the processing of bulk samples for detection of target RNA/DNA sequence. Probes for *Pseudo-nitzschia* were particularly challenging because of the low variation in the LSU making it impossible to design probes for only one species. The project is finished, with a published manual and patents applied for the microarray and commercial contacts are established to make a kit, with estimated costs of about 40€ per sample with two pseudo-replicates.  $\mu$ AQUA is another EU FP7 project that involves making microarrays for freshwater pathogens (bacteria, viruses, cyanobacteria and their toxins). Diatoms are included in this project as a measure of water quality.

## MICROPHYTOBENTHOS PRODUCTIVITY ESTIMATION USING HYPERSPECTRAL REMOTE-SENSING: FROM SPECIES TO THE ECOSYSTEM LEVEL.

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The estimation of primary production at the ecosystem level is a real challenge. It is crucial to understand ecosystem functioning in the context of monitoring, management and protection, as well as biogeochemical cycling. Primary productivity models are typically build using limited observations and sampling, covering at best several 100 km<sup>2</sup> for canopy and less then 10 km<sup>2</sup> for phytoplankton or microphytobenthos due to the difficulty to reach the ecosystems they inhabit. The results can be extrapolated to the entire ecosystem using a geostatistic approaches, but small areas are often not sufficient for a robust extrapolation (Forster and Kromkamp 2006). In this context, remote sensing, using airborne and space borne sensors, provides a simpler alternative for studying and mapping microorganism communities and their productivity. The main objective of this study is to develop the first primary productivity model for intertidal mudflat microphytobenthos using hyperspectral remote sensing data only.

Primary productivity models require, as input parameters 1) a biomass estimation (e.g. Chl *a* concentration), 2) the absorbed fraction of the photosynthetic available radiation (PAR), and 3) an efficiency parameter that describes the photophysiological response of organisms to PAR. The later is most often estimated by PAM-fluorimetry, a rapid and non-invasive technique. However, to reach our objective, the three parameters must be estimated by hyperspectral remote sensing data. Currently, remote sensing allows estimation of microphytobenthos biomass and PAR absorption (absorption cross section) using the quantitative transfer model (MPBOM), developed by Kazemipour et al. (2011). The estimation of an efficiency function that describes the photophysiological response of organisms to PAR is the challenge that remains to be taken up. The aim of this experimental study is calibrate hyperspectral data with fluorescence measurements using two diatom strains known for their different photophysiology: *Navicula phyllepta* (epipelagic) and *Plagiogrammopsis vanheurckii* (thycopelagic). Following the first attempt carried out by Jesus et al. (2008), our objective is to retrieve absorption features at specific wavelengths from hyperspectral remote sensing data, and to develop radiometric indices based on reflectance changes in absorbance due to xanthophyll cycle pigments diadinoxanthin (DD) and diatoxanthin (DT). These indices were further linked to the differential photophysiological response of the two strains as illustrated by the non-photochemical quenching of fluorescence (NPQ) and the photosystemII quantum efficiency ( $\phi$ PSII).

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## EXAMINATION OF TYPE MATERIAL OF ARAPHID DIATOMS (BACILLARIOPHYTA) NOMENCLATURALLY ASSOCIATED TO *FRAGILARIA*, *STAUROSIRA* OR *PSEUDOSTAUROSIRA*: A LONG HISTORY OF SPECIES CONCEPT DRIFT

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The study of type material in diatom research is important to help define the original concept of taxa, especially at the genus and lower taxonomic ranks. Analysis of type material and of additional geographically spread populations using light (LM) and electron microscopy (EM) becomes crucial in establishing boundaries for a taxon, which could then lead to its better identification in field collected samples, thus improving the accuracy of – for example– environmental assessments. This thorough characterization of taxa would allow the recognition of morphological and ecological variants that can then be proposed as new, thus making the description of diatom diversity a more accurate process. Freshwater araphid diatoms are an important group in benthic and planktonic habitats in rivers and lakes. Their contribution to total biomass can be considerable under certain ecological situations and many species are key players in food webs due to their high productivity. Despite this relevance, the identity and ecology of most araphid species is not well defined especially because many are originally only known from a single drawing; furthermore, the great majority lacks LM and EM data gathered from type material. Misinterpretation of iconotypes by subsequent authors led to considerable species concept drift so that now many frequently reported taxa differ radically from the original concept. The present study includes detailed LM and scanning EM information from the type material of several araphid taxa (*Fragilaria brevistriata* Grunow in Van Heurck, *Odontidium harrisonii* Roper, *Odontidium harrisonii* var. *beta* W. Smith, *Odontidium mutabile* W. Smith, *Odontidium parasiticum* W. Smith and *Odontidium tabellaria* W. Smith. This detailed information is also presented for geographically distinct populations of these taxa in Europe and elsewhere. Finally, an assessment of the morphological variability of these species is presented as an approximation of their variability in nature. It is recommended that more studies of this type should be performed in order to provide a better foundation for the identification of araphids during applied diatom studies.

## DIATOM IMMIGRATION DRIVES BIOFILM RECOVERY AFTER CHRONIC COPPER EXPOSURE

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We investigated the impact of immigration on diatom community recovery after chronic exposure to copper in laboratory microcosms.

We examined the recovery trajectories of copper-contaminated biofilms after reducing copper stress and with or without facilitated connectivity to unimpaired communities. The biofilms mixed with unimpaired communities went back to a "control" community structure within 6 weeks, with differential recovery patterns depending on the endpoint considered (i.e. 2 weeks for relative abundances of diatom species but 6 weeks for total diatom biomass). In contrast, no recovery was observed in the communities placed under water control conditions without external immigrants.

These results suggest that immigration has prominent effects on the recovery of quantitative and qualitative characteristics compared to unconnected biofilms.

## MOLECULAR SIGNATURES ALONG THE GROWTH CURVE OF A DIATOM UNDER DIFFERENT CO<sub>2</sub> CONDITIONS

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Diatoms are important primary producers that play an important role in the marine carbon cycle and over geological times have influenced global climate. The success of diatoms in global oceans relies on a combination of traits, including their molecular physiology allowing their adaptation and evolution to different environments. We have conducted genome-wide transcription profiling of the model diatom *Thalassiosira pseudonana* during a growth curve at two CO<sub>2</sub> levels, present day and a doubling CO<sub>2</sub> level reflecting the projected scenario for the 21<sup>st</sup> century. The growth reveals large, reproducible cell-state transitions between two conditions: a) exponential and nutrient replete, b) stationary and nutrient depleted, phases of growth. In this presentation we will focus on the analyses of metabolic modes associated with the cells states under the two CO<sub>2</sub> conditions. This work demonstrates the power of a system biology approach to understanding the mechanisms and regulation during growth of a model diatom under different conditions.

## RAPID OCEANIC CHANGES AT THE YOUNGER DRYAS TERMINATION INFERRED FROM DIATOM ANALYSIS OFFSHORE NEWFOUNDLAND

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The coastal waters of eastern Newfoundland form an ideal site to study past variability in the southwestern sector of the North Atlantic Subpolar Gyre. At the meeting point of the cold Labrador Current flowing south and the North Atlantic Current bringing warm, saline waters northward, marine sediment cores from this area are used to infer large scale shifts in ocean current regimes during the last deglaciation. Although in this region several records of the Younger Dryas – Holocene transition are available from the terrestrial realm, marine records spanning this interval at high resolution are so far rare. In this study we present results from a multi-proxy reconstruction of oceanic conditions and sea ice variability at the onset of the Holocene.

During a 2007 research cruise of RV ‘A. Ioffe’, a sediment gravity core (AI07-14G) was taken from 239 m water depth in Placentia Bay off the south coast of Newfoundland. Based on 6 radiocarbon dates, the 510 cm core spans the age interval from 12.9 to 9.9 cal. kyrs. BP and changes in the core show a very good correlation with both nearby terrestrial and Greenland ice core records. With an average accumulation rate of 5.7 years/cm, the core provides a high resolution record of the transition from the Younger Dryas stadial into the Holocene. After X-ray fluorescence (XRF) core scanning, the core was subsampled and analyzed for diatoms, benthic foraminifera, grain size distribution, calcium carbonate content, total organic carbon content, and the geochemical diatom sea ice proxy IP<sub>25</sub>.

The transition from the Younger Dryas into the warmer Holocene is clearly reflected in the record as a sudden increase in productivity of both foraminifera and diatoms, with a relative increase in warmer water diatom species, and is further characterized by a steep rise in both calcium and organic carbon content. Based on the calcium record from the XRF core scan, the entire transition took place in only 55 years. The presence of sea ice, inferred from the IP<sub>25</sub> analysis, is greatly reduced after the Younger Dryas termination, although the concentrations of “classic” sea ice diatom species do not show any significant change. The abrupt transition is followed by a 1000-year interval of stable conditions in the early Holocene after which further warming picks up in the youngest section of the core.

## REVISION OF THE GENUS *MASTOGLOIA*, SECTION SULCATAE THROUGH ELECTRON MICROSCOPY

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The genus *Mastogloia* Thwaites ex Smith includes a number of species living as epiphytes on seaweeds and seagrasses. The genus is highly speciose and quantitatively significant in benthic communities. It has a tropical to temperate worldwide distribution and is one of the largest diatom genera.

*Mastogloia* is the only diatom genus characterized by a specialized valvocopula that develops into a series of hollow chambers or partecta attached to each other to form a well-developed partectal ring running along the inner side of the girdle band. Hustedt (1933) has divided this genus into 11 sections of which one comprising the freshwater species.

The section Sulcatae has distinct external valve features including a variously shaped, elongated median depression between the raphe-sternum and the valve margin, siliceous outgrowths (i.e., conopeum, pseudoconopeum, ribs and ridges) and deflected or sinuous raphe branches. Internally, the valve face shows a lateral sterna and partecta with different size and shape.

In this study, we present new ultrastructural details on the frustules of 11 species of *Mastogloia*, section Sulcatae: *M. baldjikiana* Grunow, *M. borneensis* Hustedt, *M. hustedtii* Meister, *M. mediterranea* Hustedt, *M. umbra* Paddock & Kemp, *M. canni* Kemp & Paddock, *M. depressa* Hustedt, *M. exilis* Hustedt, *M. jelineckii* (Grunow) Grunow, *M. macdonaldii* Greville, *M. pisciculus* Cleve, including three new taxa (*M. neoborneensis* Pennesi & Totti, *M. oculoides* Pennesi & Poulin and *M. sergiana* Pennesi & Poulin). Specimens were collected from seagrasses and seaweeds in tropical (Siladen Island, Celebes Sea, Indonesia and Phú Bài, China Sea, Vietnam), subtropical (Sharm el-Sheikh, Red Sea, Egypt) and temperate (Patmos Island, Aegean Sea, Greece) regions. We propose a revision of the Hustedt's Sulcatae section by dividing it in two subgroups: (1) one with a median depressions on the external valve surface between the raphe-sternum and the margin, and variably developed siliceous outgrowths (i.e., conopeum and pseudoconopeum) covering the depressions to various degrees; (2) the other lacking a developed conopeum or pseudoconopeum which covers the median depression.

## SPECIES DISTRIBUTION MODELING OF MARINE PELAGIC DIATOMS

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Species distribution models (SDMs) aim at predicting the potential distribution of a species using statistical or machine learning approaches by combining geo-referenced taxon occurrence data and layers of environmental parameters. During the last two decades, the methodology became a standard approach in biogeography as well as conservation and climate change science, though with a strong bias towards terrestrial organisms. Marine organisms are clearly underrepresented and there is little experience with the applicability of SDMs for planktonic organisms. This study evaluates the performance of species distribution modeling methods for marine pelagic diatoms. Occurrence data are mainly harvested from the GBIF network and other public resources. Environmental parameters include sea surface temperature, pH value, salinity, radiation, sea ice coverage and nutrient concentrations. The main modeling method used is maximum entropy. First results of this study will be presented to give an overview to the current availability of data records, a selection of environmental parameters and model evaluation by the example of a few key diatom taxa of the Southern Ocean.

In summary, the resulting potential distribution maps of the models agree well with species distributions expected based on background knowledge. Especially abiotic variables, like sea surface temperature and nutrients (nitrate, phosphate and silicate) are excellent explanatory variables. Our experiments clearly show that SDM methods are suitable to model the geographic distribution of pelagic diatoms. In a next step, we will project niche models on expected future environmental conditions for different IPCC scenarios to explore potential biogeographic shifts in response to climate change.



## USING NULL MODELS TO TEST HYPOTHESES ABOUT THE DRIVERS OF FRESHWATER DIATOM DIVERSITY AND DISTRIBUTION.

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Ecologists have long emphasized that well-designed null models are essential to minimizing the risk of misinterpreting ecological patterns. Consequently, null models are now commonplace in the broader community ecology, biogeography, and biodiversity literature, and ecologists are more cognizant of the pitfalls of interpreting observational data at face value. Has research on diatom ecology, distribution, and diversity witnessed similar advances? Or are important insights potentially being missed owing to an over-reliance on traditional, correlative analyses?

I first provide a brief review of how null models have been used in diatom research, and by drawing parallels with research on macro-organisms, I highlight when and where their application could prove beneficial. Then, using large calibration datasets from North America, I show how null models can reveal intriguing sub-patterns within broader diversity patterns that would otherwise go unnoticed. For example, research on macroorganisms has shown that widespread and rare taxa can contribute to diversity patterns in dramatically different ways, with widespread species accounting for most of the variation in richness. Similar patterns are observed among diatoms: widespread taxa largely account for the observed unimodal relationship between local (lake-scale) taxonomic richness and pH. However, null models of pH tolerance that account for variation in occupancy rate reveal strikingly different patterns, including an intriguing peak in the richness of pH “specialists” that coincides with the pH of the oceans: 8.2. Additional null models show that this pattern is itself highly unlikely to have arisen by chance. Although null models are certainly not a panacea, I argue that their appropriate application could be as beneficial and revolutionary to diatom research as it has been for research in the broader ecology realm.

## RECENT AND PAST DIVERSITY AND ONGOING ENVIRONMENTAL CHANGE WITHIN CENTRAL EUROPEAN OMBROTROPHIC WETLANDS

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The peat bogs in the Jizerské hory and in the Jeseníky Mts. (crossborder area among Czech Republic, Poland, and Germany), represent unique ecosystems within Central Europe, characterized by ombrotrophic conditions, a very low pH and an unaffected water regime.

Both regions, 150 km distant mountain ridges in the Sudeten Mts., differ in their air contamination and deposition loads, especially that which was supplied to them in the past. The depositional load was high, particularly at the end of the 1980's; and only recently have the first symptoms of environment recovery been observed.

Permanent sampling plots were established in both areas in 1991 - 1993, in order to monitor the environmental changes. Diatoms, desmids, testate amoebae, bryophytes, and vascular plants were sampled recently at 59 permanent plots. At the same set of plots, water-chemistry and water-level dynamics had been monitored three times a year during 15 years before the species-data sampling. Furthermore, bryophyte herbarium specimens were used for studies on the past diatom diversity. We tested the species-environment relationships by Canonical correspondence analyses with Monte Carlo permutation tests and by the Mantel tests.

A total of 47 diatom species occurred at 59 sites within 13 ombrotrophic mires along the Czech-Polish-German border areas. Both regions do not differ in diatom species richness, and the two-group pattern along Axis 1 in NMDS ordination analysis do not correspond with the regional distribution. The inter-site variation in diatom assemblages is lower than the intra-site variation. The combination of three environmental variables (conductivity, pH, and height above water table - HWT) appears to be able to explain the diatom distribution patterns.

While all taxonomic groups responded to a natural gradient of water level, only short-lived and well-dispersing diatoms and testate amoebae responded to the pH/calcium gradient which has appeared recently in one region because of aerial liming. Single measurements of environmental parameters were sufficient to describe this pattern.

We confirmed that long-lived organisms do not respond as rapidly to changing environment to indicate reliably either ongoing environmental change or restoration of natural conditions. On the other hand, response of well-dispersing short-lived organisms differs by taxonomic group and may be obscured by more complex relationship to environment. Bioindication systems should therefore include more groups of organisms. Herbarized bryophytes has been successfully used to assess subrecent diatom diversity in mires sensu „paleolimnology without a core“.

## WHAT'S *MELOSIRA ARCTICA* DOING IN WELLINGTON CHANNEL, NUNAVUT, CANADA?

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First-year ice sampling at 21 stations located in the Canadian Arctic Archipelago around Cornwallis Island, Nunavut, Canada in May 2012 revealed not only rich algal communities in the lower ice layers but a significant sub-ice community, observed only once, at a nearshore station in Wellington Channel. This Arctic sub-ice community was dominated by the well-known filamentous colonial centric diatom, *Melosira arctica* Dickie, and occurred in cm long mucilage-rich strands. This widespread taxon across the entire Arctic regions has been often reported, however, associated epiphytes are poorly known. These epiphytes were commonly observed and consisted mainly of two pennate diatoms, *Synedropsis hyperborea* (Grunow) Hasle, Medlin & Syvertsen and *Pseudogomphonema arcticum* (Grunow) Medlin, with occasional occurrence of small solitary cells of *Chaetoceros* less than 10 µm in size. Interestingly these epiphytic pennate diatoms form small tuft colonies themselves adhering to the cell wall of *M. arctica*. In addition to these epiphytes, some solitary pennate diatom cells, namely *Entomoneis paludosa* var. *hyperborea* (Grunow) Poulin & Cardinal, *Haslea crucigeroides* (Hustedt) Simonsen, *Nitzschia longissima* (Brébisson ex Kützing) Grunow and *Pleurosigma stuxbergii* Cleve & Grunow, were observed crawling over the colonial curtain formed by *M. arctica*. Bottom ice and sub-ice habitats pose different challenges and selective pressures to the diatom species colonizing these environments. Suggestions for why *Melosira* assemblages can become dominant under certain conditions will be offered.

## CALIBRATING DIATOM INDICATORS: HOW MANY SAMPLES ARE ENOUGH?

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Diatom-based transfer functions have become popular indicators of aquatic condition for monitoring and paleolimnology programs. Assuming rigorous sampling of the environmental gradient, it is expected that training sets with more samples will be “better.” I.e., they will provide better definition of environmental and diatom assemblage conditions in the region of interest, and as a result a model based on a larger sample set should provide more reliable inferences of condition. The substantial effort involved in developing a training set necessitates that an optimal sample size be estimated using model performance criteria.

Three large, diatom-based training sets were investigated to determine optimal sample sizes for inference models. The sample sets included (1) assemblages from Great Lakes coastlines, (2) phytoplankton from the pelagic Great Lakes and (3) surface sediment assemblages from Minnesota lakes. Diatom-based weighted average models to infer nutrient concentrations were developed for each training set. Training set sample sizes ranging from 10 to the maximum number of samples were created through random sample selection, and performance of each model was evaluated. For each model iteration, diatom-inferred (DI) nutrient data were related to stressor data (e.g., adjacent agricultural or urban development) to characterize the ability of each model to track human activities. The relationships between model performance parameters (DI-stressor correlations and model  $r^2$ , error and bias) and sample size were used to determine the minimum sample size needed to optimize models for each region. Depending on the training set, at least 40-80 samples were needed to capture the variation in diatom assemblages and environmental conditions to such a degree that non-analogue situations should be rare, and so should provide an unambiguous result if the model was applied to any sample assemblage from the region. It is recommended that one exercises caution when dealing with smaller training sets unless there is certainty that the selected samples reflect the regional variability in diatom assemblages and environmental conditions. Further, we advise that our findings for minimum required sample size may not necessarily extend to other regions and environmental variables. We encourage training set users to employ a similar evaluation to determine whether they have effectively sampled their region of interest.

## MILLENNIAL-SCALE VARIABILITY OF MARINE DIATOM PALEOPRODUCTIVITY DURING THE LAST 50 KA: AN EQUATOR-TO-SUBTROPICS COMPARISON ALONG WESTERN AFRICA

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Coastal upwelling accounts for ca. 50% of the global export production in eastern boundary current systems. In these areas, diatoms deliver a substantial part of the ocean's primary productivity. Although productivity variations in coastal areas are usually attributed to wind stress-forced changes in upwelling intensity, the dynamics of primary production along the western African coast is less straightforward due to the complex atmospheric and hydrographic settings. In this study, we compare high-resolution marine diatom records encompassing the last 50 ka, which were generated at three hemipelagic sites drilled along the western African coast between 20°N and 25°S. The studied cores are: GeoB3606-1 (SE Atlantic, off Namibia), GeoB4905 (Guinea Basin), and GeoB7926-2 (NE Atlantic, off Mauritania). Though the three sites are coastal in location, the processes and mechanisms behind the preserved signal differ. This is clearly mirrored in both the variations of total diatom concentration and the qualitative composition of the community at each core site. While the inflow of silica-rich waters of Southern Ocean origin played a significant role off Namibia, the precipitation-controlled riverine input of dissolved silica was decisive in the Guinea Basin. Off northwestern Africa, changes in wind intensity, the subsequent upwelling of dissolved silica and the seaward extension of the chlorophyll filament were responsible for diatom production. The implications of our observations for the late Quaternary productivity and the nutrient dynamics from low-latitude ocean areas, the possible effect of abrupt climate changes as well as interhemispheric teleconnections at both Milankovitch and sub-Milankovitch time-scales are discussed.

## A CULTURE-BASED STUDY OF *COCCONEIS LINEATA* EHRENBERG AND *COCCONEIS EUGLYPTA* EHRENBERG (BACILLARIOPHYTA): MORPHOLOGY, TYPIFICATION, AND BARCODING

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The nominate varieties of the monoraphid diatoms *Cocconeis lineata* Ehrenberg and *Cocconeis euglypta* Ehrenberg are typified. Discrepancies from common concepts in Ehrenberg's types, habitats and descriptions were discovered, which might affect our current understanding of both *Cocconeis* species. Lectotypes of both taxa are preserved at the Ehrenberg Collection (Museum für Naturkunde, Berlin, Germany). The lectotype of *C. lineata* is an original, poor-detailed drawing, which shows an ovoid sternum valve with two-to-three rows of apical striae. The lectotype of *C. euglypta*, which is contained in a mica, shows a unique, broadly elliptical sternum valve with an apical zigzagging pattern of four to five striae on each hemivalve. This is roughly consistent with the current concept of *C. lineata* and *C. euglypta* and with their usage during the last 160 years. To ensure stabilization of names and current concepts for these two taxa, culture-based epitypes of *C. lineata* and *C. euglypta* are presented. For both taxa, light and electron microscopy observations, morphometric data and the barcoding from several clones collected from European and Eastern Asian localities are presented and discussed. The morphology and the morphometry of the sternum valves reveal the most significant differences. The morphometry-based differentiation is partially supported by the molecular information. The usefulness of a multiparameter approach on the identification and characterization of diatom taxa at the species levels is discussed.

## SPATIAL DISTRIBUTION OF EPIPHYTIC DIATOMS IN RELATION TO ENVIRONMENTAL FACTORS IN THE LAKE LADOGA

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In this study, epiphytic diatom assemblages were assessed in the Lake Ladoga, Northwestern Russia, to determine which environmental variables best explained spatial distributions of diatom taxa, and whether SPI (Specific Polluosensitivity Index) and BDI (Biological Diatom Index) could be applicable for water quality assessment in the Lake Ladoga. Diatom samples were collected from emergent macrophytes (mainly reeds) at 35 wetland sites in 2006 and 2010. Canonical correspondence analysis (CCA) revealed two major ecological gradients. The first was an eutrophication gradient integrating total phosphorus (TP) concentration and electric conductivity. The second was a latitudinal gradient related to water temperature and geomorphology of shoreline, which separated open wetlands of north-eastern and western coasts from more protected wetlands of shallower southern coast. CCA divided all the sites into three groups along the eutrophication gradient: small group of hypertrophic sites (mean TP 143  $\mu\text{g l}^{-1}$ ) and two large groups of eutrophic (mean TP 55  $\mu\text{g l}^{-1}$ ) and oligo-mesotrophic (mean TP 24  $\mu\text{g l}^{-1}$ ) sites. Each group was characterized by specific diatom assemblage. Hypertrophic group consisted of riverine wetlands proximal to the major inflow river Volkhov. *Cocconeis placentula* var. *euglypta*, *Ctenophora pulchella* and *Navicula veneta* were identified by the indicator value method as the most characteristic taxa of this group. The two large groups were further divided into subgroups along the latitudinal gradient. Mesotrophic sites of the southern coast were characterized by *Brachysira neoexilis*, *Encyonopsis subminuta* and *Eunotia intermedia*, while oligo-mesotrophic sites of the northern coast were indicated by *Achnantheidium lineare*, *Diatoma moniliformis* and *Fragilaria capucina* var. *mesolepta*. For eutrophic group, *Eunotia bilunaris*, *Ulnaria ulna* and *Placoneis clementis* were among indicator species of the southern coast, whereas *Aulacoseira ambigua*, *Ulnaria ulna* var. *acus* and *Gomphonema olivaceum* characterized the north-eastern and western coasts. Relative abundance of motile and low profile diatom taxa significantly correlated with latitude suggesting that distributional patterns of these functional groups were driven by changes in hydrogeomorphic features of coastal wetlands. The diatom indices SPI and BDI classified the wetland groups defined by CCA as having an excellent and good status and, thereby, overestimated water quality in nutrient-rich waters. Despite the fact that both indices were significantly correlated with TP concentration, they failed to differentiate between the group of eutrophic sites of the north-eastern and western coast and the group of oligo-mesotrophic sites. These results indicate the need for development of the regional biomonitoring system on the base of a diatom phosphorus inference model.

## TIME AND LIGHT DEPENDENT CHANGES OF EXPRESSION LEVELS OF CALVIN CYCLE GENES IN *PHAEODACTYLUM TRICORNUTUM*

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We are investigating light and time depending regulation of the Calvin Cycle in diatoms, namely the model organism *Phaeodactylum tricornutum*. The Calvin Cycle in diatoms is clearly regulated via different mechanisms than in higher plants or green algae. While light induced redox-regulation of the Calvin Cycle is of central importance in higher plants, the capacity for such redox-regulation is generally reduced in diatoms.

As this elaborate control at the enzymatic level is seemingly missing, we investigated the possibility of increased control at the transcriptional and protein level for in higher plants redox-regulated Calvin Cycle genes. We determined transcript and protein levels over time under two different light conditions to identify diurnal effects and their dependency to light. We were able to identify a surprisingly strong transcriptional regulation for both the phosphoribulokinase (PRK) (light modulated diurnal) and plastidic glyceraldehydes-3-phosphate dehydrogenase (GAP C1) (primarily light independent diurnal), which are exceeding any relative changes which are known for higher plants under similar light and time conditions by far. Most of the other investigated Calvin Cycle genes show a light modulated diurnal control of weak to moderate magnitude.

Investigation of protein abundances via western blot analysis mostly confirms these results. In total the expression levels of the Calvin Cycle enzymes are increased after light exposure, which is consistent to expectations as light provides the energy needed for carbon fixation. Our investigations emphasise the importance of regulatory mechanisms via expression levels for the diatom Calvin Cycle.



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## AN ASTONISHING CELL EXPANSION DURING AUXOSPORULATION IN THE ARAPHID PENNATE DIATOM *PSEUDOSTAUROSIRA TRAINORII*

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We observed auxospore expansion and perizonium structure of an araphid pennate diatom *Pseudostaurosira trainorii*, in which we recently demonstrated control of sexualization via pheromones. This species is heterothallic, with clear differentiation into female and male clones; auxosporulation does not require contact between female and male gametangia. Immediately after the fertilization of a sessile egg by a motile male gamete, the zygote secretes mucilaginous substances and during auxospore (zygote) development, the mucilage envelope expands as the auxospore elongates, until the length of the auxospore exceeds c. 5 times that of the mother cell. Then the auxospore expands further, extending beyond the mucilage envelope at both ends. The degree of expansion is astonishingly high, the auxospore finally being more than c. 20 times longer than the mother cell. Because of the cell length, the chain-like colonies formed by post-auxospore cells of *P. trainorii* look superficially like *Fragilaria*, although ultrastructurally they differ, e.g. in the type of pore field, and in the presence (*Fragilaria*) or absence (*P. trainorii*) of labiate processes. Because of this similarity at the LM level, it would not be surprising if large-celled populations of *P. trainorii* have sometimes been misidentified or described as species of *Fragilaria* by previous taxonomists.

The perizonium of *P. trainorii* is delicate but is of the most common type, i.e. comprising aligned open transverse bands, with the widest one at the centre, and longitudinal bands lying underneath the suture of the transverse ones. No incunabular structures, e.g. strips, scales or caps, were detected, even after thorough observation.

## INFLUENCE OF STREAM HEADWATER FLOW BIOTOPES ON WITHIN-REACH VARIABILITY IN BENTHIC DIATOM ASSEMBLAGES

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Benthic biofilms in headwater streams play a critical role in river ecosystems, for example through the fixing, cycling and retention of nutrients and energy. However, headwater streams are also spatially and temporally dynamic systems. This dynamism extends to benthic biofilms, potentially confounding relationships between biofilms and in-stream or broader catchment characteristics such as land use. One approach to constraining and understanding this dynamism involves drawing on the concept of physical biotopes: flow-defined units of in-stream habitat that are hypothesised to strongly influence lotic biodiversity and function. This meso-scale approach to analysis is important for enhanced understanding of benthic ecology. In this paper, emphasis is placed on benthic diatom communities as indicators of system integrity due to their sensitivity to a range of water quality conditions and response to physical habitat characteristics. This study tests the hypothesis that distinct diatom communities are associated with physical biotopes, and that these relationships persist between seasons making physical biotopes a useful component of ecological assessment tools. Our research draws on the catchment of the River Eden, Cumbria, UK, a DEFRA Demonstration Test Catchment. The research addresses the spatial variability of headwater benthic diatom communities in terms of both their structure and function, and the physicochemical parameters<sup>2</sup> that drive this variability.

In this paper we present preliminary data concerning the structural and functional attributes of benthic biofilms, including community composition and benthic chlorophyll *a*, at the physical biotope scale. The study focuses on a 10 km<sup>2</sup> sub-catchment, Morland Beck, part of the River Eden and assesses within-reach variability in the benthic diatom community. Our results suggest that riffles are areas of higher productivity and lower biodiversity compared to pools. It is thought that the intermediate disturbance hypothesis is likely to underpin the observed productivity-diversity relationship for this headwater system with near-bed scouring re-setting benthic communities. The Trophic Diatom Index demonstrated little variation among biotopes within the reach, but does discriminate between three headwater catchments. The TDI is an effective biomonitoring tool which encapsulates within-reach headwater variability. This study, provides an assessment of the use of specific physical biotopes for biomonitoring, integrating physical and chemical parameters and explores their effects on benthic structure, process and function.

**PINNULARIA BOREALIS: DISENTANGLING THE EVOLUTIONARY HISTORY OF A TERRESTRIAL DIATOM USING GENETICS, FOSSILS AND ECOPHYSIOLOGICAL DATA.**

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*Pinnularia borealis* Ehrenberg is a terrestrial diatom, commonly found in moist soils and temporary freshwater habitats all over the world, including the (sub)Antarctic. Given the widespread (pseudo)cryptic species diversity in diatoms and the restricted distribution patterns of many (sub)Antarctic diatoms revealed by dedicated morphological studies, we asked whether *Pinnularia borealis* is indeed a single species and started reconstructing the evolutionary history of this cosmopolitan diatom. We observed that, unlike freshwater diatoms, resting cells of *P. borealis* are tolerant for at least short periods of desiccation, suggesting that airborne dispersal is possible which may limit opportunities for allopatric speciation. Nevertheless, molecular phylogenies based on the plastid gene *rbcL* and the nuclear 28S rDNA (D1-D3 region) revealed that *P. borealis* consists of multiple lineages, including a distinct continental Antarctic lineage. Ongoing work includes the addition of isolates from the maritime Antarctic and the (sub)Antarctic Marion Island. A molecular clock for *Pinnularia* estimates the age of *P. borealis* at 35.8 (30-47) million years (Ma), and the continental Antarctic lineage at 7.8 (2-15) Ma. Compared to the lineages from non-polar regions, the continental Antarctic lineage of *P. borealis* has a lower optimal growth temperature and upper lethal temperature, indicating niche differentiation. The distinct molecular lineages, the old age of these lineages and the (partial) thermal niche differentiation suggest that long-distance dispersal is not common enough in *P. borealis* to prevent speciation, despite the desiccation tolerance of the resting cells, and indicate that besides freshwater diatoms, also terrestrial diatoms are not as ubiquitous as previously thought.

## HISTORICAL DISTRIBUTION AND MODERN INVASIONS: *DIDYMOSPHENIA GEMINATA* IN THE ROCKY MOUNTAINS OF THE WESTERN US

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Nuisance blooms of the diatom *Didymosphenia geminata* occur in streams and rivers of the Rocky Mountains. However, questions remain concerning the degree to which blooms are natural events, or whether they are in response to recent environmental change and spread of invasive genotypes. Although *D. geminata* is known from lakes, the species is most often recorded in flowing waters where it reaches its greatest biomass. Determining the history of diatoms in streams and rivers, however, is more problematic than in lakes, as streams are high flow systems that typically do not leave a continuous sedimentary record that can be interpreted. In sites where streams and rivers flow into lakes, records of historical change in river systems may be archived in lake sediments. For example, the relative abundance of stream diatoms (*Hannaea arcus* and *Meridion circulare*) found in lake sediments was used to reconstruct historical river discharge in the high arctic. Because *D. geminata* reaches its greatest abundance in streams and rivers, the concentration of cells in lake sediments is directly related to the concentration of cells in stream inflows. We examined lake sediments to reconstruct the history of abundance of *D. geminata* in Beauty Lake, Montana over the past approximate 14,000 years. It is estimated that Beauty Lake was deglaciated at close to 14,000 ybp, yet our results show that the peak in concentration occurred nearly 10,000 ybp. This result is important because it demonstrates that 1) *D. geminata* formed large populations in a newly deglaciated site within a relatively short period of time, 2) the large populations were present absent human transport, and 3) maximum cell concentrations show a strong relationship to the period of warm, dry climate in the region. The historical record is informative for understanding the expansion of *D. geminata* from its presumed Siberian origin, the relationship to modern nuisance blooms and recommendations for appropriate management response.

CROSSING THE FRESHWATER → BRACKISH-WATER/MARINE BOUNDARY: NEW INSIGHTS, SUCH AS MORPHOLOGY, BIOGEOGRAPHY AND MOLECULAR DATA, FROM THALASSIOSIRACEAE.

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Diatoms likely originated in the Mesozoic, however there is still little information on the initial habitats of diatoms. Commonly accepted hypothesis proposed diatoms crossed a uni-directional barrier, with the most probable colonization events proceeding from marine→freshwaters. It was suggested that genera in diatoms are stenohaline and the euryhalinity-effect can be caused by not well resolved taxonomy; in a few species a modern 'leakage' from brackish-water→freshwater was suggested. Now the euryhalinity in diatoms is reported not only as an artefact of classification, but as a real phenomenon with examples in raphid, araphid and non-pennate diatoms. Further, three independent re-colonisations of the marine habitat, two of which led to speciation events are suggested from molecular data (in Stephanodiscaceae-Thalassiosiraceae). From fossil evidences a relatively recent colonisation event from freshwater→brackish water/marine is proposed; within a linkage selected as of freshwater origin in traditionally marine Thalassiosiraceae (see *Conticribra* Stachura-Suchoples & Williams; Stachura-Suchoples & Williams, 2009).

Thus, this study presents the concept of the genus *Conticribra* (Thalassiosiraceae) and further focuses on several modern, euryhaline species in Thalassiosiraceae raising the question on possible recent colonisation event through the salinity gradient and its direction.

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## DELINEATING FORENSIC PALEOECOLOGY AS A SUB-DISCIPLINE; CSI DIATOMS?

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Forensic science employs the scientific method in its approach to the collection of data that are subsequently presented in a court of law. Paleoecological research frequently uncovers criminal and/or civil violations of environmental laws, i.e. illegal filling in of wetlands or dumping of toxic material. In many cases, these abuses trigger the paleoecological study to determine the impact of humans at a specific site. Paleoecology is not necessarily Forensic Paleoecology. The manner in which the researcher approaches the data, both in terms of the logic process used to interpret data classes and the type of data classes used can vary between studies either destined for the courtroom or those for publication. Researchers, with a law enforcement background, can combine examination of sediment cores with forensic investigations and interviews to determine changes in the cores and obtain direct evidence of criminal conduct for presentation in court. The standard for valid data collection is the same, regardless of the overarching question. An example of such an investigation at Polecat Bay in Mobile, Alabama will be presented.

## HAT ARE HUMAN IMPACTS ON DIATOM BIODIVERSITY?

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The United States Environmental Protection Agency conducted a nationwide assessment of the ecological conditions of lakes using a probabilistic sampling design of 1000 lakes. In the National Lakes Assessment, the United States Environmental Protection Agency determined that 47% of US lakes were in good condition, 27% in fair condition, and 23% in poor condition. Substantial changes in species composition and decreases in native species diversity were observed along the human disturbance gradient. Shifts in species composition were largely from taxa adapted to low nutrient concentrations to taxa requiring high nutrient concentrations for growth. The number of taxa in 500 valve counts decreased from approximately 50 to 30 along the human disturbance gradient indicated by total phosphorus (TP) concentration. Native taxa were identified using indicator species analysis as species characteristic of minimally disturbed (“reference”) sites. The percentage of native taxa decreased from approximately 40% to less than 10% in counts along the human disturbance gradient indicated by TP. Thus, in the 27% of US lakes that were deemed in fair condition, only half of the expected native taxa were observed. In the 23% of the lakes deemed in poor condition, only 25% of the expected number of native taxa was observed. Realizing that 500 valve counts identify a relatively small proportion of rare taxa in samples, we estimated the proportion of rare taxa as the proportion of all taxa that occurred at 1% of sites or fewer. Seventy percent of the 2264 diatom taxa observed in the National Lakes Assessment was considered rare.

On one hand, we could argue that there is little chance that we are losing taxa with such a large number of surface waters in the US as well as the large number of refugia and cells in each water body. On the other hand, we could argue that we have to be losing large numbers of diatom taxa as a result of human disturbance because of the large numbers of waters, the large proportion of rare taxa, and large proportion of native taxa lost with disturbance. The absence of many relatively common native taxa in examinations of large numbers of lakes that have been disturbed by human activities also indicates that we are losing diatom taxa. Given the possibility of great loss in diatom biodiversity and great uncertainty in our estimates of loss, there is great risk that human activities cause major loss of diatom biodiversity.

## INTER PHYTOPLANKTON COMPOSITION IN OFFSHORE WATERS OF SOUTH ADRIATIC

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Data on phytoplankton in open waters of the South Adriatic in winter period are very inadequate because the established offshore sampling station was not always reachable due to bad weather conditions during winter.

The South Adriatic has an important role in the Mediterranean deep circulation and it is considered a major source of Adriatic dense water (AdDW). The flow of the warm Levantine intermediate water (LIW) with a high salinity and the Ionian surface water (ISV) into the Adriatic is greater in winter but varies year-to-year due to climatic oscillations that occur from the Atlantic to the Southeast Mediterranean. Deep convection takes place in the winter period, when saltier water entering from the Ionian Sea is exposed to episodes of cold, dry northerly winds. Cooling of the surface waters results in mixing with deeper water masses, and causes transport of nutrients from the deep reservoir to the surface, thus making them available for primary producers.

Water masses entering from the Ionian Sea were detected by measuring salinity, temperature and density. Phytoplankton composition, structure and abundance were investigated with light microscope in two winter samplings at station p-1200 situated in the open waters of the South Adriatic (60 km southwest of Dubrovnik) in 2011 and 2012. One hundred and fourteen taxa of microphytoplankton were identified. Diatoms dominated microphytoplankton samples.



## HIDDEN GENETIC DIVERSITY IN DIATOMS FROM TROPICAL LAKE SEDIMENTS IN KENYA

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This study provides insights into the morphological and genetic diversity in diatoms occurring in surface and core sediments from tropical lakes in Kenya. We developed a genetic survey technique specific for diatoms utilizing a short region (76 bp) of the ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene as genetic barcode. Our analyses (i) validated the use of rbcL as a barcoding marker for diatoms, applied to sediment samples, (ii) showed a significant correlation between the results obtained by morphological and molecular data, (iii) indicated temporal variation in diatom assemblages (on the inter- and intra-specific level) in a short sediment core from Lake Naivasha and (iv) showed spatial genetic variation in ecologically different habitats.

(iii) Diatom assemblages from a short core from Lake Naivasha show a drastic shift over the last 200 years, as littoral species (e.g. *Navicula*) are replaced by more planktonic ones (e.g. *Aulacoseira*). Within that same period, we detected periodic changes in the respective frequencies of distinct haplotype groups of *Navicula*, which coincide with wet and dry periods of Lake Naivasha between 1820 and 1938 AD.

(iv) Diatom sequences from surface sediments indicated distinct *Navicula* haplotypes occurring in either saline-alkaline or freshwater lakes assuming genetic diversity behind similar morphotypes.

Our genetic analyses on recent and historical lake sediments revealed inter- and intra-specific variation in diatoms, which is partially hidden behind single morphotypes. The occurrence of particular genetic lineages is probably correlated to environmental factors.

## LHC-LIKE SUPERFAMILY PROTEINS IN DIATOMS

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The light harvesting complex-like (LHC-like) superfamily of proteins comprises different families of chlorophyll binding proteins with one to four transmembrane helices. We identified members of this superfamily in the genomes of the diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* and found homologues of one helix protein 1 - like (OHP1-like), one helix protein 2 (OHP2) and stress enhanced Protein (SEP, two transmembrane helices) to be encoded on the nuclear genomes of the investigated diatoms. In addition, we found so far uncharacterised three helix LHC-like proteins. Phylogenetic analyses revealed that these proteins are not related to the three helix ELIPS (early light induced proteins) found in green algae and higher plants. Instead they form a distinct protein family that is exclusively found in red algae and algae with secondary plastids of red algal origin. Via presequence analyses and fusions with the green fluorescent protein (GFP) we found out that these proteins are plastid targeted in diatoms. Transcription patterns of the three helix LHC-like genes resemble those of FCP genes rather than those of ELIPS in higher plants as shown by quantitative reverse transcription PCR. This indicates that they might have a different function in diatoms than ELIPS in higher plants. Also the investigated OHP1-like, OHP2 and SEP genes showed different transcription patterns compared to their respective plant homologues. Taken together our results show that LHC-like genes in diatoms are distributed and transcribed in a different way than in green algae and plants, which might reflect the differences in high light protection between these groups.

## THE DIATOM PHYLOGENY AS INFERRED FROM NSSU AND 6 CHLOROPLAST GENES

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We have sequenced the nuclear SSU and 6 chloroplast genes for over 200 diatoms and *Bolidomonas*, yielding an aligned dataset of about 10,000 nucleotides. Here we report on our preliminary analyses of this dataset, and give an overview of the diatom phylogeny. We specifically test the placement of several diatoms of interest, including species used as models in genomic studies (*Cyclotella nana* and *Phaeodactylum tricorutum*), and diatoms whose placement in the diatom phylogeny has been particularly problematic or controversial (e.g., *Attheya*, *Striatella*). We find increasing support that the so-called radial centrics are not monophyletic, but rather are a grade. Monophyly of the so-called polar diatoms remains equivocal. We discuss the implications of this tree for the diatom classification and diatom origin. Previous inferences about the Ur-diatom as a small, perhaps siliceous flagellate, have assumed that the outgroup represented the ancestral morphology. Making that same inference by optimizing characters on our phylogeny (and on other molecular phylogenies), yields a very different view, suggesting that the Ur-diatom may have been highly elongate and perhaps even filamentous.

MORPHOLOGICAL AND PHYSIOLOGICAL EFFECTS IN *PROBOSCIA ALATA* (BACILLARIOPHYCEAE) GROWN UNDER DIFFERENT LIGHT AND CO<sub>2</sub> CONDITIONS OF THE MODERN SOUTHERN OCEAN.

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The combined effects of different light and aqueous CO<sub>2</sub> conditions were assessed for the Southern Ocean diatom *Proboscia alata* (Brightwell) Sundström in laboratory experiments. Selected culture conditions (light and CO<sub>2(aq)</sub>) were representative for the natural ranges in the modern Southern Ocean. Light conditions were 40 (low) and 240 (high) μmol photons·m<sup>-2</sup>·s<sup>-1</sup>. The three CO<sub>2(aq)</sub> conditions ranged from 8 to 34 μmol·kg<sup>-1</sup> CO<sub>2(aq)</sub> (equivalent to a pCO<sub>2</sub> from 137 to 598 μatm, respectively). Clear morphological changes were induced by these different CO<sub>2(aq)</sub> conditions. Cells in low [CO<sub>2(aq)</sub>] formed spirals, while many cells in high [CO<sub>2(aq)</sub>] disintegrated. Cell size and volume were significantly affected by the different CO<sub>2(aq)</sub> concentrations. Increasing CO<sub>2(aq)</sub> concentrations led to an increase in particulate organic carbon concentrations per cell in the high light cultures, with exactly the opposite happening in the low light cultures. However, other parameters measured were not influenced by the range of CO<sub>2(aq)</sub> treatments. This included growth rates, chlorophyll a concentration and photosynthetic yield (F<sub>v</sub>/F<sub>m</sub>). Different light treatments had a large effect on nutrient uptake. High light conditions caused an increased nutrient uptake rate compared to cells grown in low light conditions. Light and CO<sub>2</sub> conditions co-determined in various ways the response of *P. alata* to changing environmental conditions. Overall *P. alata* appeared to be well adapted to the natural variability in light availability and CO<sub>2(aq)</sub> concentration of the modern Southern Ocean. Nevertheless, our results showed that *P. alata* is susceptible to future changes in inorganic carbon concentrations in the Southern Ocean.

## PENNATE DIATOM VIRUSES ARE SIMILAR TO CENTRIC DIATOM VIRUSES

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Diatoms constitute one of the major lineages of photosynthetic eukaryotes on earth. Their contribution to global biogeochemical cycling is significant and accounts for a large part of marine primary production. Based on morphological features, diatom species are composed of 2 groups: centric and pennate. Marine virus studies have revealed the significance of viruses to host population dynamics. Diatom viruses are also considered to be important for the understanding of the impact of diatoms on biogeochemical cycling. So far, isolations of several viruses infecting centric diatoms, *Rhizosolenia* and *Chaetoceros*, have been reported. However, viruses have not been isolated from pennate species. Here, we report isolations and characterizations of 2 distinct pennate diatom viruses, AglaRNAV and TnitDNAV infecting *Asterionellopsis glacialis* (Castracane) Round and *Thalassionema nitzschioides* (Grunow) Mereschkowsky, respectively. AglaRNAV is a lytic virus and its infection is species specific. The virions are icosahedral in shape and 31 nm in diameter, and randomly accumulated in its host cell cytoplasm. The genome of AglaRNAV is a single-stranded RNA (ssRNA) of 8842 nt encoding two open reading frames (ORFs), putative replication-related proteins and capsid proteins. Phylogenetic analysis based on the deduced amino acid sequence of the RNA-dependent RNA polymerase domains strongly supported the monophyly of AglaRNAV and centric diatom ssRNA viruses with a bootstrap value of 99%. The phylogenetic tree also showed the possibility that AglaRNAV is an ancestor of centric diatom ssRNA viruses. The relationship between diatoms and ssRNA viruses might be originated from an ssRNA virus infecting a pennate diatom species. The infection of TnitDNAV is lytic, and the viral particles are icosahedral in shape and 35 nm in diameter. The virions are accumulated in the host cell nucleus with a crystalline array formation. The genome of TnitDNAV is a closed circular single-stranded DNA (ssDNA) of 5573 nt encoding at least two ORFs. Phylogenetic analysis based on a putative replication-related protein showed that TnitDNAV is most similar to CdebDNAV infecting *Chaetoceros debilis*, of which genome is ssDNA but its structure is unknown. The studies for pennate diatom viruses are just started. To elucidate the ecological and evolutionary relationships between centric and pennate viruses, further isolations of diatom viruses are necessary in future studies.

## POLYMORPHISM IN *ALVEOLOPHORA* SPECIES (AULACOSEIRACEAE) FROM FOSSIL DEPOSITS IN WESTERN NORTH AMERICA

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*Alveolophora* Moisseeva & Nevretdinova was first described from the Russian Far East by Moisseeva & Nevretdinova (1990). They applied an illegitimate (later homonym) name *Melosira areolata* Moisseeva (Moisseeva 1971) as the basionym for type species. According to the International Code of Botanical Nomenclature (Art. 6.3, Art. 10.1; McNeill et al. 2006), the generic name *Alveolophora* was legitimately and validly published even though the type species name was illegitimate. In this case, we consider *Miosira* Krammer, Lange-Bertalot & Shiller as a synonym of *Alveolophora*.

*Alveolophora* as currently understood is entirely extinct, but it existed from the Late Eocene until the end of the Miocene. There are seven species known from the Russian Far East (Moisseeva & Nevretdinova 1990; Khursevich 1994, Kozyrenko *et al.* 2008) and Lake Baikal (Khursevich & Fedenya 2006), as well as from Europe (Krammer *et al.* 1997) (see Khursevich & Kociolek 2012). Only one species *A. jouseana* (Moisseeva) Moisseeva was reported from western North America by Bradbury (1986).

We present three new species of *Alveolophora* - *A. bradburyi* Usoltseva, Kociolek & Khursevich sp. nov., nom. prov., *A. nevadica* Usoltseva, Kociolek & Khursevich sp. nov. nom. prov. and *A. americana* Usoltseva, Kociolek & Khursevich sp. nom. prov. described from late Miocene age 9.5-11.8 ma in the western United States. Despite many reports of *A. jouseana* from diatomites in North America, we have been unable to verify the presence of this species, first described from Russia, in sediments of North America.

These species expressed a high degree of polymorphism (Kociolek & Stoermer 2011) in several valve features. For example, within individual species (*A. bradburyi*) areolae may be present across the valve face, restricted to the margins, widely scattered or lacking. Valve shape may be round, to ovoid to nearly linear in *A. americana*. Tubes of the rimoportulae of *A. bradburyi* can be quite long or short. In all three species the ribs may be longitudinal and/or transverse creating an anastomosing network, and be thin, thick or, in rare cases such as *A. bradburyi*, absent all together. Such polymorphism (either in the number of features or in the variation within any individual feature) is not known in the species described from Asia.

Before this study there were no *Alveolophora* species described with oval valves. *A. americana* is similar to elliptic and oval *Aulacoseira* from early Miocene sediments of Yamato Rise, Japan Sea (Usoltseva & Tsoy 2010) and Japan (Tanaka *et al.* 2008).

## WHAT CONSTITUTES A STIGMA? A REVIEW OF ISOLATED PORE STRUCTURES IN RAPID DIATOMS

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The presence of isolated pores near the central raphe endings has long been known for several raphid genera such as *Cymbella*, *Didymosphenia*, *Gomphonema* and *Luticola*. Since its original designation in 1894 by Cleve for “the peculiar punctum or pore” in some *Cymbella* species, the term ‘stigma’ has been used to refer to any isolated pore near the central area, although the ultrastructure of such pores varies. Recently, several other names have been proposed, such as ‘fistula’ (in *Fistulifera*) or ‘buciniportula’ in *Olifantiella*) for particular types of isolated pore, and it is clear that a revision of the terminology for such structures is needed.

Using high resolution scanning and transmission electron microscopy, the structure and diversity of isolated pores in a range of raphid diatom genera have been investigated. The results of the observations indicate that two types of isolated pore can be recognised, unoccluded and occluded, although there are sub-types within each group.

Unoccluded pores are found in genera of the Cymbellales (*Cymbella*, *Brebissonia*, *Didymosphenia*, *Gomphonema*, *Oricymba*, *Placoneis*) and in *Geissleria*. These isolated pores are characterized by an unoccluded internal foramen with a rounded to slit-like external opening. The most complex type is found in *Cymbella* and *Didymosphenia*, whereas in other genera the isolated pore is reduced to a simple pore through the valve wall. Therefore, we propose to restrict the use of the term ‘stigma’ to the complex type as found in *Cymbella*, and ‘stigmoid’ for the simpler type, as found in *Gomphonema*.

Internally occluded isolated pores can be found in the marine, brackish or terrestrial genera, *Fistulifera*, *Labellicula*, *Luticola*, *Olifantiella*, *Parlibellus*, and *Proschkinia*. In *Fistulifera*, *Labellicula* and *Proschkinia*, the isolated pore has an internal domed hymenate occlusion, whereas *Luticola* and *Olifantiella* have an internally collared hymenate occlusion. The published terms fistula and buciniportula apply respectively. *Parlibellus* has an isolated pore that splits into two internally, for which the term cuniculus was coined and can be retained. This study is part of the DIATERM working group programme, which is collating and revising the terminology used to describe the morphology of the diatom valve.

## GENOTYPIC DIVERSITY AND DIFFERENTIATION AMONG BENTHIC FRESHWATER DIATOM POPULATIONS AS REVEALED BY MICROSATELLITE ANALYSIS

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Microsatellite-based studies of natural populations provide insight into the genetic and genotypic diversity within populations and patterns of gene flow between them at different spatial scales. In diatoms and other microalgae, such studies are almost exclusively confined to the marine phytoplankton. Patterns for freshwater microalgae can be expected to be different due to the typically highly fragmented and more short-lived nature of their habitat. We developed 6 microsatellite loci for the epiphytic freshwater diatom *Eunotia bilunaris* "robust" and used these to investigate genotypic diversity and differentiation between *E. bilunaris* "robust" populations at different spatial scales, from ponds within the same pond system, over the same region, to between regions on a Western European scale. For this, 340 isolates from 9 populations were obtained, their species identity confirmed using valve morphology combined with *rbcL* sequences, and their microsatellite allelic composition determined. Analyses show that while in some populations there was a very high genotypic diversity with every strain belonging to a different genotype, in others one or a few genotype(s) made up half of the population. Population differentiation was low between ponds from the same pond system or for a single population sampled twice ( $F_{st} < 0.1$ ) but could already be very high ( $F_{st} > 0.2$ ) for populations from the same region, located some tens of kilometers from each other. These results will be compared with a similar dataset for the benthic freshwater diatom *Sellaphora capitata* (356 isolates from 8 Scottish populations and one Belgian) using 7 microsatellite loci, allowing to test the generality of findings in *Eunotia*. Finally, possible causes for the observed large population differentiation will be discussed.



*DREPANOTHECA* SCHRADER, *ANAULUS* EHRENB. AND *EUNOTOGRAMMA* WEISSE: PHYLOGENETICS RELATIONSHIPS OF SOME 'NON-ARAPHID', 'NON-CENTRIC' DIATOMS WITH BOTH FOSSIL AND LIVING RELATIVES

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The genus *Drepanotheca* Schrader was first described for a handful of specimens found in the Oamaru fossil deposits, New Zealand. It included two species: *Drepanotheca macra* Schrader and *Drepanotheca bivittata* (Grun. et Pant.) Schrader. Its primary characters were a pair of transapical cross-members and the structure of the striae, which are more or less absent from the valve centre, scattered towards the margins – Schrader also mentioned the valves shape as crescent-like (“Schalen sichelförmig”). *Drepanotheca bivittata* was based on *Eunotogramma bivittata* Grun. et Pant., specimens from Miocene deposits in Felső-Esztergály (*Horné Strháre*) and Szent-Péter (*Pôtor*) in Slovakia, Central Europe. Schrader concluded that *Drepanotheca* was best considered a pennate diatom because it lacked a raphe and placed it among the ‘araphid’ diatoms in Araphideae, family Fragilariaceae. Schrader’s primary source of comparison for species in *Drepanotheca* was the ‘centric’ diatom genus *Eunotogramma* Weisse, as these too have characteristic valves with transapical bars. *Eunotogramma* has been placed in and associated with Biddulphiaceae, a bilaterally symmetrical group of ‘centric’ diatoms. In the order ‘Biddulphiées’, Van Heurck, for example, included eight genera of which *Anaulus* Ehrenb. was but one, its one species, *A. debilis* (Grun. in Van Heurck) Van Heurck, was assigned to the sub-genus (‘sous-generé’) *Eunotogramma* based on Van Heurck’s earlier view that *debilis* was a species of *Eunotogramma*. *A. debilis* also has many valves with cross-members, similar to that seen in *E. laevis*, placed at one time in *Anaulus*, but now considered the type of *Eunotogramma*. In short, it has been, and still is, difficult to determine the relationships of species currently in *Drepanotheca*, *Anaulus* and *Eunotogramma* and place them in any larger group (family, order, and so on). We will offer some suggestions to the following questions with respect to *Drepanotheca*:

How is *Drepanotheca* defined?

What species are to be included, assuming it can be defined?

How is it related to *Anaulus* and *Eunotogramma*?

Does it really contain ‘living fossils’?

This presentation will offer evidence to support our views with illustrations of valves from fossil material of the three genera, and offer some ideas as to how best classify these enigmatic taxa, particularly *Drepanotheca*.

## ICE DIATOMS - THREE CASE STUDIES ON EFFECTS OF ELEVATED CO<sub>2</sub> AND TEMPERATURE

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Sea ice algae, dominated by pennate diatoms, are a vital part of the primary production in ice-covered Polar Regions and provide a substantial carbon source for higher trophic levels. Simultaneously, as atmospheric CO<sub>2</sub> increases, sea surface temperature rises due to global warming. This has resulted in decreased summer ice cover and loss of multi-year ice. Thus, there is an urgent need to study the sea-ice communities and their role in the ecosystem and carbon cycling. In three different experiments, we studied the effects of elevated *p*CO<sub>2</sub> and temperature on pennate diatoms isolated from Arctic and Antarctic sea ice. In the first experiment (Expt A), we investigated the response of *Navicula directa* (isolated from the Svalbard area) to elevated *p*CO<sub>2</sub> (960 ppm) in combination with temperature increase of 4°C. In the second experiment (Expt B), we used a similar set-up but we studied the response of *Nitzschia* sp. (isolated from Amundsen Sea area). The third experiment (Expt C) was performed to test physiological responses to a temperature range (5 levels from -2 to +12°C) on *Nitzschia* sp. For *N. directa* (Expt A), no interaction effects of temperature and *p*CO<sub>2</sub> were found. Temperature alone had significant effects on growth rate and photosynthetic activity ( $F_v/F_m$ ). Interestingly, at elevated *p*CO<sub>2</sub> the growth rate was 5% lower ( $p < 0.05$ ) compared to ambient concentrations. For *Nitzschia* sp. (Expt B), a significant interaction effect for growth rate was observed. Growth rates were only promoted by increased *p*CO<sub>2</sub> when temperature was increased from -1.8 to 2.5°C. In general, temperature had a stronger effect where e.g. primary productivity and photosynthetic activity increased when exposed for 2.5°C compared to -1.8°C. Of the 5 levels tested (Expt C), the optimal growth temperature was 5°C, and highest  $F_v/F_m$  was reported at 8°C. No oxidative stress (lipid peroxidation) was observed until 12°C. These experiments have a mechanistic approach and we can only speculate on what could happen in a future polar ocean / ice habitat. However, elevated temperature stimulated photosynthesis and growth within the temperature window for the studied species, but responses to elevated *p*CO<sub>2</sub> may be more taxa-specific.

## MELOSIRA VARIANS – A TRUE COSMOPOLITAN FRESHWATER DIATOM SPECIES OF LOW MOLECULAR AND MORPHOLOGICAL DIVERSITY?

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Knowledge about cosmopolitanism versus endemism of widespread diatom species remains rather limited, due to a lack of analyses of the intraspecific morphological and genetic variation as well as biogeographical patterns of single species. Here we studied the molecular and morphological variation of the 'radial centric' freshwater diatom *Melosira varians* C.AGARDH (Bacillariophyceae) based on 13 strains from its global distribution range. Colonies of *M. varians* have been isolated of lakes and rivers from Faroe Islands, Spain, Germany, European and Asian Russia, Vietnam, Korea, and South Africa. Genetic differentiation among strains was investigated using the *rbcl* pp, the 18S V4 marker and the ITS1-5.8S-ITS2 rDNA region. Quantitative and qualitative morphological characters among strains were compared statistically. It was furthermore determined whether the populations of the taxon demonstrate any correlation between geographic, morphological or genetic distance. The data support some nuclear genetic diversity and a high conformity on the chloroplast level. The findings furthermore indicate that *M. varians* is a true cosmopolitan freshwater species with a stronger biogeographic separation between eastern and western populations in Eurasia and lesser North-South variation among populations from Faroe Islands, Europe and South Africa. Furthermore, a recognized morphodeme or DNA barcode (18S V4, resp. *rbcl* pp) of *M. varians* can be used as a proxy for the species in phylogenetic and biodiversity assessments as well as in biomonitoring of freshwater quality.

## DEVELOPMENT OF MICROPHYTOBENTHIC COMMUNITIES ON HARD ARTIFICIAL SUBSTRATA IN THE BRACKISH WATERS OF THE GULF OF GDAŃSK

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Studies on the early stages of development of microphytobenthic communities on hard artificial substrata were conducted in the Gulf of Gdańsk between November 2008 and October 2009. Experimental panels for both flora and fauna analyses were exposed above the seabed for one month at 3.5 m depth in two sites with different habitats. The colonization process (i.e. settlement of micro- and macroalgae) was examined for three subsamples on each occasion and analysis included the taxonomic richness, number of organisms and biomass in 1 cm<sup>2</sup>.

Altogether 56 taxa of microalgae and 5 of macroalgae were identified. Most of the taxa represented were diatoms, both adnate (mostly *Cocconeis* spp.) and erect growth forms (e.g. *Rhoicosphenia abbreviata* and representatives of *Fragilaria*, *Gomphonema* and *Licmophora*). Free-living motile forms (e.g. *Bacillaria paxilifer*, tube-dwelling *Berkeleya rutilans*, *Navicula ramossimsima*) were also well represented. The Cyanobacteria (e.g. *Dolichospermum flos-aquae*, *Lyngbya* sp., *Merismopedia* sp., *Microcystis* sp., *Oscillatoria* sp., *Spirulina* sp.) was the second most important group of microalgae in terms of richness. Macroalgae were observed rarely and mostly as very young growth forms. Microalgae were dominated both in numbers and biomass by *Cocconeis pediculus*, *Fragilaria fasciculata* and *Licmophora* sp. whereas macroalgae by Pheophyta from *Ectocarpacae* family.

No differences in structure of communities and colonization process were observed between the sites. Canonical analysis suggested that the most important factors influencing the development of microphytobenthic communities were associated with seasonally-variable environmental variables such as temperature, light and water dynamics. Pioneer communities were found mostly in autumn and winter. In warmer periods the succession was much faster and communities were dominated by more specialised and light competitive species. Changes in composition and structure of studied assemblages generally followed patterns described in the literature; however, the importance of accidental events (i.e. not recognised as life requirements) was also demonstrated.

## NEXT-GENERATION SEQUENCING (NGS) METHODS IN ENVIRONMENTAL BARCODING – A PROGRESS REPORT ON BIOMONITORING OF BENTHIC RIVER DIATOMS.

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DNA barcoding is a tool that uses a short, standard portion of DNA to identify organisms based on a shared database of DNA sequences. In diatoms, a consensus on an appropriate DNA barcode has not yet been reached, and several markers are still in discussion (e.g. nuclear 18S rRNA, 28S rRNA, 5.8S rRNA + ITS2, mitochondrial *cox1*, plastid *rbcl*). But recent findings in the protist community are favouring the V4 region on the 18S rRNA gene at least as a pre-barcode for biodiversity assessments.

DNA barcoding is especially interesting in regard to high throughput methodology (e.g. NGS) used in the analysis of environmental samples. Therefore we have established a standard procedure for DNA barcoding in diatoms to develop a standardised identification tool to serve routine water quality assessments using the 18S V4 region (Zimmermann et al. 2011; [http://www.gbif.de/botany/barcoding\\_3](http://www.gbif.de/botany/barcoding_3)).

Here we demonstrate the feasibility of using the V4 barcode in a 454-pyrosequencing approach for species-level analysis of benthic freshwater diatoms commonly used for biomonitoring. Although considerable effort is still needed until NGS tools will be commonly applied to identify species from environmental samples, our results indicate the potential of an environmental barcoding approach for biomonitoring programs.

To optimise environmental barcoding with the V4 marker for water quality assessment we chose the river system Neisse/Oder (Czech Republic/Germany) as study area. The diatom flora of the Neisse/Oder is well known and is representative for a typical central European river system. This enables us to compare the findings of the NGS as well as the morphological analysis of the same samples to evaluate the accuracy of the DNA barcoding approach.

This applied part needs as a fundament a liable reference database, which is established at the BGBM, which will provide the basis for a DNA based identification system. To build the reference database DNA barcodes generated via Sanger sequencing of specific diatom taxa (cultivated in unialgal cultures from the environmental samples) are linked to light-/scanning electron microscope-photographed vouchers simultaneously. This database contains up to now sequences of 550 diatom strains. It is constantly updated and will be freely accessible through *AlgaTerra* ([www.algaterra.org](http://www.algaterra.org)).



**POSTER  
PRESENTATIONS**





## FOSSIL *PROBOSCIA* SPP. FROM LATE CRETACEOUS TO EARLY OLIGOCENE SEDIMENTS

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Observations on samples from deep-sea cores and terrestrial outcrops of marine origin have revealed the presence of a rich diversity of diatoms assignable to the genus *Proboscia*. Although most of these can be regarded as new species, some of them were previously described as species of *Rhizosolenia*, *Riedelia* and *Clavícula*.

Many Late Cretaceous forms are heavily silicified, with thick longitudinal ridges separated by multiple rows of pores, a tip bearing 1-3 large spinulae and a distinct longitudinal slit (external portion of the rimoportula). Paleocene forms closely resemble those in the Late Cretaceous, although often with a smaller tip, however, Eocene and Early Oligocene forms exhibit a wide range of morphologies, including probosces lacking ridges and tips bearing a ring of small spinulae. Complete basal portions or basal fragments bearing claspers have not been found yet in any of these old sediment samples.

Given the strong curvature, twisted nature and length (>100 µm) of some probosces, it is possible that these species did not possess claspers. However, modern species often have shorter, less-silicified valves in the spring season, so these long, heavily-silicified fossil probosces may represent 'winter' valves. In one Middle Eocene sample from the Central Arctic a monospecific mat of fossilized probosces was observed, suggesting a similar ecology to modern mat-forming *Rhizosolenia* spp. The change in morphology (e.g. loss of ridges, smaller spinulae, shorter probosces) in forms from the Greenhouse to Icehouse suggests that *Proboscia*, like many other diatom genera, reacted to oceanographic and climatic changes across the Eocene-Oligocene boundary.

## MORPHOLOGICAL STUDY OF THE MARINE DIATOM *BERKELEYA CAPENSIS* GIFFEN (BACILLARIOPHYCEAE)

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The genus *Berkeleya* was first proposed by Greville in 1827. Since then Cox (1975) established that the genus *Berkeleya* has indistinctive boundary with regards to their morphological characteristics: forming mucilage tubes, having linear to linear-lanceolate valves, a distance of the central nodule, parallel striae in the central area, radial striae in the apical area and a mature cingulum. *B. capensis* was described by Giffen in 1970, but has been reported only once since then. This species was collected from Cape Town in South Africa. Valves are linear to linear-lanceolate with obtusely rounded ends, 20-36 µm long, 5-6 µm broad. Raphe branches are about one third of the length of the valve, central pores somewhat enlarged, terminal pores distant from the ends of the valve. The central area is absent. In girdle view, short pseudosepta are seen at the ends of the valve. 2-3 girdle segments are observed, punctate along the margins.

The materials used in this study were collected from a stipe of *Eisenia bicyclis* in Japan. Valves are linear to linear-lanceolate with obtusely rounded ends, 12-22 µm long, 3-4 µm broad. Central pores are enlarged. Terminal fissures are hooked, meeting a transverse furrow near the valve margin and producing a T-shape. Internal raphe endings terminate in helictoglossae at the poles, while the central endings are hooked to the same side. Valve mantle produced slight pseudoseptae at either pole. The valvocopula bears one transverse row of round poroids. These characters are different from the genus *Berkeleya*, based on pseudosepta, internal central endings and external raphe endings. The genus *Cuneolus* Giffen is classified in the family *Rhoicospheniaceae*. Valves are slightly heteropolar and lanceolate. Raphe-slits are unequal in length. But we find similar characters between the genus *Cuneolus* and *B. capensis*. Therefore, the aim of this study was to verify the difference among the genus *Berkeleya* and *B. capensis*, and reassign *B. capensis* to the other group.

## VIABILITY ASSESSMENT OF DIATOM ASSEMBLAGES IN BALLAST WATER

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Determination of organism viability is instrumental in answering ecological or physiological questions associated with the survival and succession of phytoplankton. Algae communities, particularly diatoms, are monitored and used as ecological indicators of water quality health. For decades aquatic invasive species, including many algal taxa, have been introduced and established through the discharge of commercial ships' ballast water. These organisms threaten ecological stability, diversity and abundance of native taxa as well as maritime commerce. The success of management systems and ability of ships to meet ballast water discharge standards are adjudicated by the number of viable organisms in treated water. Using land-based and ship-board test facilities, the Great Ships Initiative (GSI – [www.greatshipsinitiative.org](http://www.greatshipsinitiative.org)), is evaluating candidate shipboard treatment systems for their ability to prevent the introduction of freshwater nuisance species.

We present a process of ambient assemblage concentration, staining with fluorescein diacetate (FDA), and microscopic observation as a reliable and efficient method to assess densities of viable freshwater algae. The selected method provides a strong foundation for live/dead algal enumeration of ambient assemblages, particularly diatoms, in ballast water treatment assessments. This process may also be used or adapted for any field of research that examines a broad taxonomic range of autotrophic and heterotrophic plankton.

## PURIFICATION AND CHARACTERISATION OF AN UNUSUAL DNA GLYCOSYLASE IN DIATOMS

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A large number of DNA repair enzymes ensure that damage to the DNA by spontaneous mutations, radiation and chemical mutagens is rapidly and efficiently repaired. DNA glycosylases recognise damaged bases in the DNA and removes these as part of the base excision repair (BER) pathway. Two classes of DNA glycosylases are Uracil N-glycosylase (UNG), which recognises uracil, and Nei-like glycosylase (NEIL), which recognises oxidised bases.

A database search for UNG genes in the sequenced genomes of two diatoms, *Thalassiosira pseudonana* and *Phaeodactylum tricornutum*, identified two UNG orthologues. One of these genes encodes a DNA glycosylase with a novel domain organisation, where a C-terminal UNG domain has been fused to an N-terminal NEIL-like domain. This glycosylase, termed Dual DNA Glycosylase (DDG), appears to be conserved in, and is unique for diatoms.

DDG from *P. tricornutum* was cloned as full-length DDG and the single NEIL and UNG domains into the expression vector pBADM-30, which contains both a histidine tag and a glutathion S-transferase (GST) tag. Recombinant proteins expressed in *E. coli* were purified by the use of both histidine tag and GST tag affinity columns, and verified by MALDI-TOF analysis. Enzyme activity assays using the purified fusion proteins showed that PtDDG carries both UNG and NEIL activity. The activity optima of PtDDG was also analysed with regard to pH, temperature and NaCl concentration.

## Evaluation of the Trophic Diatom Index for assessing water quality in the Nišava River, Southern Serbia

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The main objective of this paper is to report the results of using Trophic Diatom Index to estimate water quality in the Nišava River, southern Serbia. Assessing water quality using diatoms as bioindicators is done for the first time in the Nišava River. Monthly samples from stones were collected from May 2008 to May 2009, according to Kelly et al. 2001. Samples were collected from 11 sites along the Nišava River, and one at Jerma River and Temska River, tributaries of the Nišava River. Physical and chemical factors (water temperature, pH, conductivity, ammonium ion, nitrates, nitrites, silica and phosphates) are measured at each sampling site. Diatom samples were cleaned using chemical agents, mounted on permanent slides, identified and counted. In total 194 diatom taxa were identified. The most species-rich genera are *Navicula* (25), *Nitzschia* (17) and *Gomphonema* (13), while the other 56 genera are presented with one or more species. Detailed floristic analysis of benthic diatom flora was not conducted earlier on these rivers either. The structure of diatom assemblage was used to calculate TDI. TDI showed a statistically significant correlation with phosphates, nitrates and ammonium ion. These correlations are generally weak and positive. There is no noticeable progressive growth or decline of any properties. Growth or decline of TDI from one site to the other, follows the same trend of phosphates, nitrates and ammonium ion. Our results show that major pollution points along the Nišava River are sites that are located downstream of major cities (Dimitrovgrad, Pitor and Niš). This work presents the baseline for future research and offers preliminary results of critical pollution points in the Nišava River.

## THE ACTION OF TERAHERTZ LASER IRRADIATION ON DIATOM FRUSTULES

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The main feature of diatoms is the presence of a silica exoskeleton and a lot of biochemical and molecular biology studies are focused on the elucidation of the biosilica synthesis mechanism. A diatom cell uses a special organelle SDV (Silica Deposition Vesicle) to obtain elements of new diatom frustules. This organelle is relatively large, visible in light microscopy but there are no reliable data concerning the structure of its membrane and its contents. The silica exoskeleton is the main obstacle in a soft destruction of diatom cells and the isolation of non-destroyed SDVs. Silica frustules are highly resistant to mechanical and chemical actions, so searching for new approaches in order to remove silica frustules from the diatom cells is an actual task.

Laser ablation using a free electron laser (FEL) is a novel method for soft destruction of supramolecular systems stabilized by hydrogen bonds [A. K. Petrov et al. Nucl. Instrum. Methods Phys. Res., Sect. A. 575:68]. The advantage of ablation with FEL consists in correspondence between laser frequencies (40-80 cm<sup>-1</sup>) and vibrations of hydrogen bonds, and so the destruction of covalent bonds is unlikely. The softness of the ablation technique has been confirmed by the retention of enzymatic activity of horseradish peroxidase and PCR-proven integrity of lambda phage DNA, plasmid pUC18DNA after all manipulations.

We have found that the action of terahertz laser irradiation on the fresh-water diatom *Synedra acus* subsp. *radians* (Kützing) Skabichevskii results in the splitting of diatom frustules. The most effective was an irradiation of a wave length of 100 μm which is close to the diatom length. It seems as silica valves are torn away from the cell membrane and the whole cell contents remains in a non-destroyed inner membrane. Chloroplasts and SDVs do not change their shape after the valve removing.

The study was partially supported by the Siberian Branch of the Russian Academy of Sciences (project #39).

## ACTIN: A KEY PLAYER FOR LOCOMOTION

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Edgar and Pickett/Heaps (1984) proposed a mechanism for pennate diatom gliding, involving polysaccharide/proteoglycans fibers, which attach to the substratum while they remain connected through the plasmamembrane via myosin bridges with one of the two prominent actin cables that run parallel to the raphe. Force is generated by the actin/myosin system, i.e., as myosin molecules move along the actin cable, the entire cell becomes displaced against the mucilage threads as long as they remain firmly attached to the substratum. The molecular details of the mechanism, however, are not well understood, though it has been proven, that the actin cytoskeleton is an essential component for gliding. It is known from diverse organisms, that actin is required for the establishment and maintenance of cell polarity and morphology. For instance, it is required for intracellular trafficking, endocytosis, as well as cytokinesis. Despite its importance, up to date very little is known about the actin cytoskeleton in Diatoms. We are using the large freshwater diatom *Craticula cuspidata* to visualize the intracellular processes while the cells are engaged in gliding. We have established an immunofluorescence protocol for this non-model organism, which allows us to visualize the actin- as well as the microtubule cytoskeleton of *C. cuspidata*. To analyze the internal structure of the endomembrane system and cell organelles, vital staining procedures with fluorescent dyes have been successfully established, the resulting images complement electron microscopic observations. We identified and sequenced two different actin genes from *C. cuspidata* and analyzed their primary structure in comparison with actin sequences known from other diatoms.

## INFLUENCE OF THE LIGHT CLIMATE (QUANTITY AND QUALITY) ON THE COUPLING BETWEEN MIGRATION, PHOTOSYNTHESIS AND CELL DIVISION IN MICROPHYTOBENTHIC DIATOMS

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Coastal ecosystems are one of the most productive areas in the world. Their productivity is mainly supported by the microphytobenthos (MPB) which inhabits estuarine intertidal mudflats. MPB in muddy sediments is dominated by motile forms ('epipellic') diatoms which can exert vertical migration as a function of the tidal and diurnal cycles, and of the light climate. It is commonly accepted that photosynthesis occurs during emersion and the cellular division occurs during the dark periods of immersion. The specific aims of this experiment were 1) to decipher how the light environment influences the MPB migration and its coupling with photosynthesis, 2) to better understand the coupling between MPB migration and the cell cycle. In a first step, we achieved to preserve the MPB migration rhythm in laboratory conditions in freshly collected mud. Migration measurements have been carried out over more than 1 year on muddy sediments (Aiguillon Bay, Atlantic coast) in order to integrate seasonal/tidal cycle influence. It was continuously recorded for sequential periods of 24 h with an Imaging-PAM fluorometer. The chlorophyll fluorescence was used as a proxy for the MPB biomass at the surface of the mud. Different light intensities (from 10 to 320  $\mu\text{mol photon}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$ ) and qualities (blue, green, red, white) were tested. MPB photosynthetic parameters and pigment content were punctually recorded during migration. As a comparison, the same measurements were performed on sandy sediments (Bourgneuf Bay, Atlantic coast) which MPB is less motile. We showed that the amplitude of diurnal migration is influenced by the intensity and the quality of light. It increases with the light intensity until a threshold; 130  $\mu\text{mol photon}\cdot\text{s}^{-1}\cdot\text{m}^{-2}$  being an optimum. Migration is positively influenced by blue and green radiations, even at low intensity, and even in sandy sediments. In parallel, lens tissues were used to harvest the diatom cells at the surface and in the sediments (0.25 cm depth). The DNA content of the cells was analyzed by flow cytometry in order to measure the stage of the cell cycle. Different diatom populations at different stages of their cell cycle were discriminated; they correspond to different size classes of epipellic diatoms (i.e. 'small, medium and large'). We showed that diatom cells divide when they are in the sediments (i.e. during immersion), as expected. Based on these data, further experiments will explore the direct coupling, and its dynamics, between light (intensity/quality) migration, photosynthesis and the cell cycle of MPB diatoms.



## DIATOM ASSEMBLAGES IN THE SPRINGS OF THE MARITIME ALPS NATURAL PARK (NW ITALY)

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In 2008-2010, the diatom communities of 60 springs of the Maritime Alps Natural Park, in the Piemonte Region (NW Italy) were studied. The area with an extension of over 28,000 ha is situated in the upper part of the basin of the R.Gesso and borders with the French Mercantour national Park. The study was coordinated by ARPA with the collaboration and financial support of the Maritime Alps Natural Park. Single springs, situated between 1000 and 2400 m a.s.l., occur in areas characterized by different geological substrates. This area is important for biodiversity, due to its particular position (in the Alps but close to the Mediterranean Sea). Springs are vulnerable habitats and also islands of biodiversity from which the colonization of the surrounding territory occurs. Diatoms are an often underestimated component of biodiversity and of the ecological functions of aquatic environments.

Diatom samples were taken once in each spring, following standard procedures. Over 24,000 individuals (about 400 for each sample) were taxonomically identified in the 60 springs. All samples yielded a list of 226 taxa while single spring maximum richness was 52 taxa.

Considering all 60 springs, the most abundant taxa were *Achnantheidium minutissimum* (36.8% of identified individuals), *Diatoma mesodon* (17.3%), *Planothidium lanceolatum* (5.0%), *Fragilaria arcus* (4.0%), *Achnanthes linearis* (3.5%), *Encyonema minutum* (3.1%), *Denticula tenuis* (1.5%), *Encyonema silesiacum* (1.4%), *Cocconeis placentula* var. *lineata* (1.3%), *Achnantheidium pyrenaicum* (1.0%) and *Cocconeis pseudolineata* (1.0%).

The most frequently occurring species (regardless of abundance) were *A.minutissimum* (57 samples), *D.mesodon* (53), *A.linearis* (50), *E.minutum* (44), *E.silesiacum* (34), *P.lanceolatum* (33) and *Amphora pediculus* (25).

Several taxa sampled in this study are considered to be rare or were found for the first time in the area. Moreover, a characteristic morphotype perhaps of *Diatoma hyemalis* or *D. mesodon* (but morphologically distinguishable from the typical forms of both species) was present in several springs.

The taxa found in this study were included in the database of the ATBI (All Taxa Biodiversity Inventories) promoted by the European Distributed Institute of Taxonomy (EDIT) in the Alpi Marittime and Mercantour nature reserves.

A number of diatom water quality and trophic indices were calculated for all samples.

This study supplied a preliminary inventory of the diatom taxa of the area's springs. Further investigations on diatoms, other taxonomic groups and water chemistry and hydrology of the springs are needed in order to improve the conservation and management of these habitats, which are sentinels of environmental change.

## THE TYPE MATERIAL OF *EUNOTIA ARCUOIDES* FOGED

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The type material of *Eunotia arcuoides* Foged housed at the ANSP Diatom Herbarium is investigated with light and electron microscopy. *E. arcuoides* is a poorly known species that shares a number of morphological characters with a few *Eunotia* taxa known from alkaline waters. Among other characters of this species, we describe an unusual “epithemoid” or “amphoroid” shape of the frustule and a unique round helictoglossa. *E. arcuoides* has equally wide dorsal and ventral parts of the mantle, and valve faces in recently formed frustules are parallel. The girdle bands are, however, wider in their dorsal parts compared to ventral parts, so that the cells become progressively more amphoroid as they grow and more girdle bands are added.

## SPATIO-TEMPORAL DIVERSITY OF MICROPHYTOBENTHOS IN THE LOIRE ESTUARY

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This study investigates the spatio-temporal variability of benthic microalgal assemblages in a macrotidal estuary of the European Atlantic coast. Intertidal sediments are colonized by photosynthetic microorganisms grouped under the generic term of microphytobenthos. They form transient biofilms at the sediment surface at low tide and play two major roles, representing up to 50% of the total primary production of estuarine waters and contributing to the stabilization of sediments by exopolysaccharide secretion. However, the spatio-temporal variations of the microphytobenthos biofilm composition has received much less attention in estuaries compared to coastal areas. In this work, we studied the spatio-temporal variability of biofilms specific diversity, collected on mudflats in the poly- (16 to 30 psu) and mesohaline (5 to 15 psu) regions of the Loire Estuary, France, every month from January 2011 to January 2012. The top surface 2-mm sediment was collected using the contact core method. After the removal of the microalgae from the sediment particles using the LUDOX separation technique, the identification and enumeration were performed using light microscopy coupled with scanning electron microscopy.

Observations in light microscopy revealed that the major part of the microalgal taxa belonged to diatoms, while chlorophytes and euglenids were only observed during summer. Forty diatom taxa have been identified so far, belonging to the Coscinodiscophyceae, Fragilariophyceae and Bacillariophyceae. The greatest species richness was recorded in the mesohaline mudflats, whereas in the polyhaline region, the surface sediments had the lowest diversity, with a marked decreasing gradient toward the mouth of the estuary. Dominant taxa throughout this study are epipelagic microphytobenthic diatoms (moving cells) with a size <30 µm. This study also confirmed the occurrence of typical mesohaline diatom taxa, such as *Navicula spartinetensis* Sullivan et Reimer, while other marine taxa have not been recorded from adjacent coastal environment, e.g., *Navicula phyllepta* Kützing. An important proportion of small centric pelagic diatoms, such as *Thalassiosira decipiens* (Grunow) Jørgensen, *Odontella aurita* (Lyngbye) Agardh or *Cyclotella meneghiniana* Kützing, were commonly observed for all sites, as a result of strong deposition of suspended particles over the mudflats.

## COMPOSITION OF THE EPILITHIC DIATOM FLORA FROM A SUBTROPICAL RIVER, SOUTHERN BRAZIL

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The diatom biodiversity investigations in lotic system from Southern Brazil have been carried out mainly on the plankton. Epilithic diatoms are considered more useful for analysis of environmental conditions in the rivers due to their sessile lifestyle, short life cycles and efficient responses to changes occurred in the environment. The knowledge of the composition of this community is therefore necessary for increasing the accuracy of the taxonomical basis of the water quality indices proposed regionally, like the BWQI (Biological Water Quality Index). This study aims to present the flora composition of the Pardinho river, located between coordinates 53°28'48" to 53°30'49" W and 29°14'10" to 29°47'32" S, from 719 to 17 m altitude, in a subtropical region where the average yearly precipitation is 1,500 mm, and the rainfall is distributed along the year. The samplings were carried out seasonally from August 2001 to July 2002, in five sites along the Pardinho river. The material was scrubbed off the upper surfaces of submerged pebbles of 10-15 cm of diameter using a toothbrush. Samples were fixed with formalin, oxidized and cleaned to observations in light (LM) and scanning electronic microscopes (SEM). The epilithic diatom flora of Pardinho River was represented by 97 taxa, which were distributed among 22 families and 41 genera. The most representative families were *Naviculaceae* (14 taxa), *Gomphonemataceae* (11 taxa) and *Bacillariaceae* (10 taxa). The richest genera in species were *Gomphonema*, *Navicula*, *Eunotia* and *Nitzschia*. Amongst the taxa studied, *Encyonema sprechmanii*, *Fallacia omissa*, *Gomphonema lujanense*, *G. lippertii*, *G. affine* var. *rhombicum* and *Luticola aequatorialis* are new records for the subtropical region of Brazil. News species of *Nupela* and *Surirella* are proposed. 238 LM and SEM photomicrographs are presented.

## EXPLORING THE POSSIBILITIES OF COLLECTION-BASED ECOSYSTEM RESEARCH AT THE HUSTEDT CENTRE

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Hustedt Diatom Study Centre, Polar Biological Oceanography, Alfred Wegener Institute for Polar and Marine Research

The Hustedt Diatom Study Centre, founded around Friedrich Hustedt's diatom collection in 1965, has been part of the Alfred Wegener Institute for Polar and Marine Research since 1986. Our activities in the last two years have focused on exploring possibilities of involving the collection in ecosystem research centered on Southern Ocean diatoms, a traditional group of focus for the AWI. We identified three main, connected disciplines which can benefit from the double embedding into a taxonomic collection and into an Earth systems science research institute: digital taxonomy, biogeography and biodiversity informatics. Besides their inherent connections, these disciplines are also increasingly in contact through their shared interest and activities aimed at archival, exchange and re-use of primary biodiversity data. We initiated projects using primary biodiversity data in parallel with ones providing such, in an attempt to augment traditional ways in which the collection is being used and to experiment with improving the communication between taxonomy and its 'user disciplines'. The poster presents an overview of our activities, including establishment of a digital taxonomic repository about planktonic diatoms of the Southern Ocean, progress towards data provision to the GBIF network, using species occurrence data in a species distribution modeling project and initial experiments towards automating digitization of microscopic slides and downstream use of these images. The mid-term goal of these activities is to extend the current information infrastructure of the Hustedt Centre into a digital biodiversity research facility useful for a researcher community beyond taxonomists, while keeping and extending traditional taxonomic roles like deposition of types.

## ATTACHED INTERTIDAL DIATOMS HAVE STRONGER PHOTOPROTECTIVE CAPACITY COMPARED TO MOTILE DIATOMS.

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Despite being exposed to a highly fluctuating light climate, intertidal sediments belong to the most productive ecosystems on Earth. The main primary producers in this habitat are diatoms which can be divided in different functional groups [attached, motile/biofilm-forming, (tycho) planktonic]. It is hypothesized that large motile diatoms (epipelon) can migrate within a vertical light gradient to the most optimal light climate whereas smaller attached forms (epipsammon) must be able to cope with a fluctuating light climate using mainly physiological mechanisms. In this study we compared the response of the motile diatom *Seminavis robusta* and the attached diatom *Opephora* sp. to different light intensities using non-sequential light curves. After each light step we measured the main photoprotective mechanisms [non-photochemical quenching of fluorescence (NPQ), and associated xanthophyll cycle and the PSII electron cycle (PSII CET)] using PAM fluorometry, HPLC and a flash O<sub>2</sub> electrode. *Opephora* sp. showed a higher capacity for both NPQ and PSII CET, which might be an adaptation related to its incapability to migrate away from oversaturating light conditions.

## A NOVEL TYPE OF COLONY FORMATION IN MARINE PLANKTONIC DIATOMS REVEALED BY ATOMIC FORCE MICROSCOPY

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Diatoms have evolved a variety of colonial life forms in which cells are connected by organic threads, mucilage pads or silicate structures. In this study, we provide the first description of a novel strategy of colony formation among marine planktonic diatoms. *Bacteriastrum jadrantum* is a chain-forming centric diatom in which sibling cells are enclosed within the optically transparent organic matrix instead of fusing of silica between setae as it is the case in all other members of the family Chaetocerotaceae. This cell jacket structure was detected by staining procedures (Alcian Blue and Coomassie Brilliant Blue G), which showed that the polysaccharides are predominant matrix constituents and revealed that the jacket reaches the span of the setae. The scanning electron microscopy (SEM) observations showed distinguishable fibrillar network firmly associated with cells. Using atomic force microscopy (AFM), we were able to visualise and characterise the cell jacket structure at molecular resolution. At nanoscale resolution, the cell jacket appears as a cross-linked fibrillar network organised into a recognisable structure. It is composed of non-evenly distributed high density domains (patches) surrounded and interconnected by thicker fibrils. Inside the patches are the hexagonally shaped pores decreasing in size from the patch edge towards the center. The large pore openings found between the patches are formed by the robust branching fibrils and these represent the backbone of the fibrillar network. Such structure is porous, but at the same time highly flexible and mechanically stable. We conclude that the *Bacteriastrum* polysaccharide jacket represents an essential part of the cell, as such specific and unique patterns have never been found in self-assembled polysaccharide gel networks, which are usually encountered in the marine environment.

## SEASONAL DYNAMICS OF MARINE DIATOMS IN AN AREA PERIODICALLY INFLUENCED BY SUBMARINE SPRINGS AND RIVER INFLOW (MALI STON BAY, SOUTH-EASTERN ADRIATIC)

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Fine vertical and temporal phytoplankton resolution, based on physico-chemical properties and small herbivorous zooplankton, has been observed in Mali Ston Bay which is traditionally an area of shellfish farming. The bay is influenced by the freshwater income from the submarine springs (vruljas) in the inner part and from the Neretva River at the outer part; together with precipitation these are significant sources of nutrients in the bay. An extensive year-through research was conducted during 2002. Diatoms dominated microphytoplankton abundance mostly in winter and autumn, positively correlated with  $\text{NO}_3$ ,  $\text{NO}_2$  and  $\text{PO}_4$  and negatively with temperature and  $\text{NH}_4$ . Their development was not silicate-limited. The most abundant taxa were *Chaetoceros affinis* ( $1.5 \times 10^4$  cells  $\text{L}^{-1}$ ) in late March, *Chaetoceros socialis* ( $1.0 \times 10^4$  cells  $\text{L}^{-1}$ ) in late April and *Chaetoceros* spp. ( $3 \times 10^4$  cells  $\text{L}^{-1}$ ) in mid May. *Thalassionema nitzschioides* and various undetermined pennate diatoms were the most frequent Bacillariophyta during the year. The abundances of the diatoms were controlled by herbivorous zooplankton grazing ('top-down' control) more than is the case for other groups of microphytoplankton. The results showed a significant difference in environmental conditions, as well as in the annual phytoplankton succession and community structure, as compared with studies carried out more than 20 years ago in this area. Diatoms that were previously identified as the dominant group of phytoplankton, in terms of both number of taxa and abundance, are replaced by dinoflagellates.



## VARIATIONS ON THE MACDONALD-PFITZER THEME: MATHEMATICAL MODELS AND A PALEOLIMNOLOGICAL RECORD

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In D. Jewson's (1992) variation on the classic MacDonald-Pfitzer model, the increment of size reduction for diatom offspring decreases as a linear function of parental cell size. The consequences of this for the distribution of cell sizes in diatom populations were investigated using analytical and numerical models and the results applied to a detailed paleolimnological record. In the analytical model, a recurrence equation was developed describing the relationship between parental cell size ( $l_n$ ) and size of the smaller offspring cell ( $l_{n+1}$ ) using parameters of minimum cell length, maximum cell length and initial increment of decrease. A closed form solution to the recurrence equation was found, which was an affine function and invertible. The inverse function describes the relationship between cell size-class number ( $n$ ) and measurable cell size ( $l$ ). These functions were used to model patterns of cell size distributions in single strain and multi-strain populations, with the assumption that the underlying size-class distribution in single-strain populations is inherently binomial, and in multi-strain populations can be treated as Gaussian. These models were then applied to a 70-year annual-resolution paleolimnological record of a centric diatom population from the varved sediments of Foy Lake, Montana, USA. Three hundred valves from each year were measured, a total of over 21,000 valves, ranging in diameter from 14 to 44 micrometers, with modal diameter of 21 and mean diameter of 22.15 micrometers. Five hundred initial valves from 33 to 45 micrometers in diameter were also observed throughout the record. Single and multiple normal (Gaussian) and log-normal (Galton) distributions were fitted numerically to the data, both directly and following transformation by the model. For most years, the data were best described by single Gaussian distributions following transformation. This result agrees with the life-cycle length and frequency of significant sexual reproduction indicated by the record of initial valves, and supports the predictions of Jewson's variation on the classic MacDonald-Pfitzer theory.

## ON THE MORPHOLOGY AND PHYLOGENY OF THE DIATOM GENERA *RHIZOLENIA*, *PROBOSCIA* AND *PSEUDOLENIA* FROM THAILAND

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The marine planktonic diatom genera *Rhizolenia*, *Proboscia*, and *Pseudolenia* were studied with respect to morphology and phylogeny. The samples came from seven locations in marine coastal waters of Thailand in the four provinces, Rayong, Chonburi, Chumphon and Phuket. The samples were collected in the period October 2008 to January 2011. Fifty strains were established in culture and used for morphological and phylogenetic analyses, and complemented with morphological studies of field material. The morphological studies were done using light microscopy, scanning and transmission electron microscopy. Ten species and two varieties of *Rizosolenia* were identified. The morphological diversity of *R. setigera* was high and did not agree with preliminary molecular data. *Pseudolenia calcaravis* was also found and the detailed studies indicate this to be a species complex. *Proboscia* was represented by *P. indica* and a new *P. siamensis* species. Culturing and molecular analyses were often difficult or unsuccessful, but in spite of the problems it was successful for several taxa. The results on the diversity within selected the genera will be discussed, based on the results of the phylogenetic and morphological studies

## SEM-STUDY ON *CYCLOTELLA OPERCULATA* (C.AGARDH) KÜTZING (CENTRALES, BACILLARIOPHYCEAE)

Tsang-Pi Chang

Biobasis

Scanning electronic microscopy (SEM) was used to improve the classification of *Cyclotella operculata*. This first centric diatom species was often incorrectly classified in the past due to observations based on light microscopy (LM). Therefore, the present author reexamined Agardh's original material with SEM and found *C. operculata* showing variable valve morphology. Various patterns appeared on the external surface with smooth or roughly structured faces decorated with one punctum or 3-5 depressions (s. Chang 1999, Chang & Chang-Schneider 2010). The valves with one punctum on the rough or smooth valve face have been considered as the type of *C. operculata*. The one with rough valve face was determined as *C. kuetzingiana* or else in the past and the other one with a smooth valve face was once determined by Hustedt (1930) as *C. comta* var. *unipunctata*. One other diatom with few depressions on the valve face was identified as *C. ocellata* (s. Houk et al. 2010). However, all these species have shown a similar pattern on the internal valve faces. It is argued that all variables in Agardh's original material should be classified as one species in different growth stages and thus emphasized that a good classification of *C. operculata* and similar species should be based on examining both valve faces with SEM.

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## A STUDY ON THE TYPE MATERIAL OF CYCLOTELLA KUETZINGIANA THWAITES (CENTRALES, BACILLARIOPHYCEAE)

Tsang-Pi Chang

Biobasis

This study refers to the identification of *Cyclotella kuetzingiana* and argues that it is imperative to use type material in order to come to a correct classification. The studied species has a long history of misleading. Originally, Thwaites (1848) described this diatom with type material based on two sources, i.e. two diatom species could be included. The type material, stored in the Natural History Museum in London, was analyzed for the available study. The present author examined both samples and found *C. meneghiniana* Kützing (1844) in the first sample from Thwaites' collected material in "Shirehampton near Bristol" and *C. kuetzingiana* in the second sample on a glass slide, prepared by Wm. Smith with his 1851 collection from Wareham. The latter seems to be unsuitable as type material due to a later collection. Therefore, he tried to find *C. kuetzingiana* in Smith's prepared slides in other institutions (cf. Smith 1853 & 1856) and argued his searching as a complementary determination. In this study, other prospects concerning the classification are also given.

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## SHAPE ANALYSIS IN THE 3D DIMENSION: ARE CENTRIC DIATOMS FINALLY SHOWING SOME CHARACTER

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Quantitative shape analysis is a well established technique that has been utilized on diatom taxa with morphometric data collected via the light and scanning electron microscope. Results of these investigations have identified shape groups potentially reflecting multiple evolutionary lineages for specimens identified by a single species name. Utility of this technique has been restricted to pennate species. This limitation is a result of the minimized shape variations exhibited by centric diatom taxa in the X and Y axes and the inability of light and electron microscopes to capture quantitative Z axis data. Atomic force microscopy (AFM) allows accurate measurement of Z axis topologies at micro and nano scale resolutions. In this investigation, Z axis topographic data captured via the AFM is used to compare species in the *Stephanodiscus niagarae* complex. Data for the longest diameter transect for individual specimens were converted into a complex polynomial equations. Equations were developed for a minimum of 10 specimens per species. Species investigated included *S. niagarae*, *S. remerii*, and *S. superiorensis*. Intra and inter specific variations in polynomial equations were compared statistically to identify this techniques success in distinguishing between these closely related lineages.

## EPIPHYTIC DIATOM ASSEMBLAGES ON A SUBTROPICAL MANGROVE (*AVICENNIA MARINA*) AND ITS RELATION TO WATER QUALITY

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The temporal and spatial distribution of epiphytic diatom assemblages on a subtropical mangrove (*Avicennia marina*) has been investigated in the estuary of the Zhangjiang River (23°55'N, 117° 27'E) and in a natural mangrove reserve in Futian, Shenzhen Bay (22°32'N, 114°04'E) in southern China. Samples were collected seasonally (spring [April], summer [July], autumn [October] and winter [January]). A total of 101 epiphytic diatom species and varieties belonging to 28 genera were identified. Dominant species included *Nitzschia fasciculata*, *Hydrosera whompensis*, *Nitzschia brevissima*, *Denticula tenuis* and *Achnanthes javanica* var. *subcontracta*. Further analysis using canonical correspondence analysis revealed that diatom assemblages could be closely related to environmental variables such as salinity and nutrients resources of nitrogen and phosphate. Epiphytic diatom assemblages on mangrove roots would be a good indicator for the evaluation of water quality in mangroves.

This work was funded by the Natural Science Foundation of China under contract No: 40906058 and the Natural Science Foundation of Fujian Province of China under contract No: 2011J01279

## NUCLEOTIDE TRANSPORTERS IN DIATOM PLASTIDS

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Genome sequencing projects revealed that in diatoms, nucleotide *de novo* biosynthesis takes place in the cytosol. Nucleotides are essential metabolites for a variety of processes in the plastids; hence there is a constant demand for nucleotide import.

Two plastidic nucleotide transport proteins (NTTs) have been characterized in diatoms. The combined activities of these two plastidic NTTs facilitate net import of the complete set of nucleotides and their corresponding desoxy-forms. This function is different to the role of NTTs known in plant plastids, which solely counter-exchange ATP with ADP+Pi to provide energy to the organelle.

Unlike plant genomes, which usually encode not more than two NTT isoforms, the genomes of the diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricornutum* harbor several distantly related *ntt* genes (8 in *T. pseudonana* and 6 in *P. tricornutum*).

The already characterized diatom NTTs (NTT1 and NTT2) are most likely targeted to the innermost plastid membrane, based on their pre-sequence structure and on GFP-fusion experiments. Interestingly, not all NTTs possess typical plastid targeting pre-sequences.

When compared to plant plastids (with two membranes), metabolite transport into diatom plastids is more complex due to the presence of two additional membranes surrounding the organelle.

The unusually high number of NTTs in diatoms might result from the complex plastid structure as well as from the need to transport newly synthesized nucleotides from the cytosol into the stroma.

## DIATOMS OF THE GENUS *GOMPHONEMA* EHRENBERG FROM LAKE GLUBOKOE (MOSCOW AREA, EUROPEAN RUSSIA) WITH SOME NOTES ON THE MORPHOLOGY OF STRIAE

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The mesotrophic Lake Glubokoe is situated at 90 km to the west from Moscow and represents one of the most antropogenically undisturbed water bodies of the Moscow Area. During the study of the recent (r) and fossil (f) diatom flora of the lake, 25 species belonging to the genus *Gomphonema* Ehrenberg were found: *G. acuminatum* Ehrenberg 1832 (r, f), *G. acutiusculum* (O. Müller) Cleve-Euler 1955 sensu auct. (f), *G. angusticephalum* Reichardt et Lange-Bertalot 1999 (f), *G. auritum* A. Braun ex Kützing 1849 (r), *G. bavaricum* Reichardt et Lange-Bertalot 1991 (f), *G. brebissonii* Kützing 1849 (r, f), *G. capitatum* Ehrenberg 1838 (f), *G. clavatum* Ehrenberg 1838 (r, f), *G. aff. cymbelliclinum* Reichardt et Lange-Bertalot 1999 (r, f), *G. cf. geisslerae* Reichardt et Lange-Bertalot 1997 (r), *G. gracile* Ehrenberg 1838 (r, f), *G. hebridense* Gregory 1854 (r, f), *G. italicum* Kützing 1844 (r, f), *G. jadvigiae* Lange-Bertalot et Reichardt 1996 (f), *G. jergackianum* Reichardt 2009 (r, f), *G. lateripunctatum* Reichardt et Lange-Bertalot 1991 (f), *G. micropus* Kützing 1844 (f), *G. minusculum* Krasske 1932 (r, f), *G. parvulum* (Kützing) Kützing 1849 (r, f), *G. sarcophagus* Gregory 1856 (f), *G. cf. turgidum* Ehrenberg 1854 (r), *G. utae* Lange-Bertalot et Reichardt 1999 (r), *G. vibrio* Ehrenberg 1843 (f), *G. sp. 1* (f), *G. sp. 2* (f). Comparison of our data with published lists of the Lake Glubokoe diatoms is problematic due to the drastic changes in the taxonomy of genus *Gomphonema* in the last two decades and to the absence of images and descriptions in the majority of previous publications. Presumably 15 species are new for the lake flora. The morphology of all reported taxa was documented with light microscope (LM). Scanning electron microscope (SEM) images were taken for only 13 species. Based on the ultrastructure, the studied species may be divided into four distinct groups according to the structure of their striae. Generally, the striae in *Gomphonema* are composed of two parts: (1) proximal (psp), extending from the raphe sternum to the advalvar region of the valve mantle, laying in a groove (alveolus) on the internal valve surface, and (2) distal (dsp), restricted to the valve mantle and flushing with the internal mantle surface. In the first group (*G. auritum*, *G. gracile*, *G. parvulum*) psp and dsp are slightly differentiated, e.i. they are indiscernible in LM, areolae in dsp bear volate occlusions. Struts on the internal side of vimines in psp occur only in this group. In the second group (*G. acuminatum*, *G. angusticephalum*, *G. italicum*) psp and dsp are clearly differentiated: they have different appearance in LM, areolae openings in the dsp are round with no or only a rudimentary flap-like volae. In the third group (*G. cf. geisslerae*) striae parts are strongly differentiated, but the dsp is composed of a single areola. Fourth group (*G. minusculum*) is characterised by the fully reduced dsp, so each stria is composed only of alveolate psp. The first character state may be considered plesiomorphic because of the presence of infraviminal struts in other genera of the Cymbellales (species of *Encyonema*, *Cymbella*, *Cymbopleura*, *Placoneis*).



## DIATOM COMMUNITIES FROM ARCTIC DRIFT AND LAND-FAST ICE AS REVEALED BY HIGH-THROUGHPUT PYROSEQUENCING

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Ice-associated diatoms and other large protists have been studied extensively using microscopy. However, little information on the genetic diversity of these eukaryotic cells is available. We applied high-throughput tag sequencing of the V4 region of the 18S rRNA gene to investigate the eukaryotic microbiome of drift and land-fast ice. Ice cores were collected at 6 stations from March to early May 2008 as part of the International Polar Year-Circumpolar Flaw Lead (CFL) system study, in the Canadian Beaufort Sea. The 0-3 cm (bottom) and 3-10 cm (top) horizons were sampled for all cores, as well as the ice-water interface (sub-ice) at one station. All horizon ice communities were very diverse and the diatom sequences in the assemblages included many known sea ice genera. Raphid pennate diatoms (Bacillariophyceae) were by far the most common sequences in all samples, except one where a bloom of the centric diatom *Melosira* was observed. Sequences matching a previously recovered environmental clone, Baltic Sea ice diatom clone 8-90, were the largest contributors to the pennates and were the only taxa shared by all of the samples, regardless of ice type or horizon. Fewer sequences of Mediophyceae (primarily *Thalassiosira*) and Fragilariophyceae were recovered. We also show that the ice-associated communities living within drift and land-fast ice harbored taxonomically distinct communities, with higher occurrences of sequences of *Pseudogomphonema* and *Nitzschia* spp. in the land-fast ice. In addition, *Pleurosigma* and *Entomoneis* spp. were found suspended at the ice-water interface of drift ice. We compare these results with those obtained from traditional microscopy and discuss them in the context of the changing Arctic sea ice cover.

MORPHOLOGY AND ULTRASTRUCTURE OF THE PALEOCENE *AULACOSEIRA GALLICA* (EHRENBERG 1854, LAUBY 1910) *COMB.NOV.* FROM THE BITUMINOUS SCHISTS IN THE MAAR OF MENAT (PUY-DE-DÔME, AUVERGNE)

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Since the beginning of the XIXth century, the lacustrine sediments of Menat have been subject to numerous paleontological studies on leaves, insects, fishes ... These sediments are the result of an explosion crater situated over a volcanic pipe.

In 1898, Renault and Roche highlighted the microscopic constitution of the bituminous schists of Menat, which contained, among others, numerous diatoms. However, Héribaud argued in 1902 that there were no diatoms in sediments. In 1910, a list of 112 diatoms was published by Lauby. In 1929, Cayeux claimed that this pond was constituted of 3 types of rocks. One of them consisting essentially of diatoms and spicules of sponges. In 1934, Dangeard discussed about the difficulty in finding this rock.

Indeed, none of the tens of samples taken from Menat in July 2008 during the paleontology field camp organized by the Rhinopolis association displayed the presence of diatoms. On the other hand, the 48,80 m deep core, made by the BRGM in June 2008, allowed to find diatoms in 19 out of the 49 samples.

According to valve morphology, *Melosira gallica* (Ehr.) Lauby belongs to the genus *Aulacoseira* Thwaites, hence we propose its formal transfer into this genus. The morphology of this species is documented by light and scanning electron micrographs.

A new study (unpublished data 2012) of isotopic carbon organic matter ( $\delta^{13}\text{C}_{\text{org}}$ ) indicates a Selandian/Thanetian age (Upper Paleocene). This would mean that the freshwater diatoms of Menat are the oldest in France.

## APPLICATIONS OF DIATOMS IN ARCHEOLOGY

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This paper provides an overview of a number of applications of diatoms in archeological science. Diatoms are microscopic unicellular algae that form a tiny bipartite, robust skeleton of biogenic silica. The geographic distribution of diatoms – they occur from the poles towards the tropical regions – depends on the environmental conditions of their habitats. Their ecological variability and the fact that diatom shells have a high preservation potential make diatoms superb indicators of environmental change in modern and past times. Diatoms are also increasingly used in archeological sciences in a number of applications. Diatoms from archeological deposits may for example provide valuable information on local or regional environmental conditions such as salinity, trophic level and disturbance. Or diatoms extracted from pottery shards may tell something about the provenance of the studied artifacts. A number of examples (pottery material provenance, paleo-salinity, depositional environment reconstruction) is provided illustrating the value of diatoms for setting archeological material in a broader site and landscape context.

## CHANGES IN BENTHIC DIATOM ASSEMBLAGES AND THEIR RELATIONSHIP WITH ENVIRONMENTAL VARIABLES IN A RIVER OF CENTRAL CHILE

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In Chile the use of diatom communities as water quality indicator is not common yet. A study to establish the diversity and structure of benthic diatom assemblages and their relationship with environmental variables was carried out at Maule River (VII Region, Chile). Samples for physical, chemical and biological analysis were collected from sites along the river covering zones with different land uses to obtain the major spatial variability on environmental conditions. The environmental measured were: temperature, pH, total phosphorous, total nitrogen, suspended solids and turbidity. The diatom samples were collected from submerged stones, and for getting the algal material were scraped off the upper surfaces of the stones. The biological (composition and abundance of each taxa), and physical-chemical data were analyzed through the multivariate ordination. According to specific physical and chemical parameters, the best water quality was recorded by the sampling sites located near to mountain. The obtained results showed an increase in the concentration of those parameters which are related with the level of nutrients in the water (phosphorus and nitrogen) and those related with the material from the basin (solid suspended and turbidity), from the upper part towards the lower part of the river, been the total phosphorous, suspended solids and turbidity the most important variables into the data set. The diatom assemblages also showed significant changes in their composition between the different sampling sites of the river. Some of the dominant species in the river were: *Staurosira construens*, *Staurosirella pinnata*, *Pseudostaurosira brevistriata*, *Fragilaria vaucheriae*, *Achnantheidium minutissimum*, *Bacillaria paxillifer*, *Nitzschia fonticola* and *Ulnaria ulna*, among others. Finally, it is possible to indicate that the water quality observed in the river would be principally depending on the environmental conditions surrounding the sampling site.

Research funded by FONDECYT N° 11100160

## SYNTHESIS AND PROPERTIES OF NEW FLUORESCENCE DYES FOR *IN VIVO* STAINING OF DIATOMS

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Methylated oligopropylamines (MOPA) had been found in diatoms as a part of modified proteins (silaffines [M. Sumper et al. J. Mater. Chem. 14:2059]) and in a free state. It is evident that MOPA take part in silicon metabolism but the exact physiological role of these compounds is not clear. Recently we have elaborated a stepwise method for MOPA synthesis [V.V. Annenkov et al. Arkivoc 2009 xiii:116-130] and this work is devoted to fluorescence derivatives based on MOPA. We have obtained the following new dyes for *in vivo* staining of diatom cells:

- Compounds based on amines with 2 or 3 nitrogen atoms: H-[NMe-(CH<sub>2</sub>)<sub>3</sub>-NMe]-R and H-[NMe-(CH<sub>2</sub>)<sub>3</sub>-NMe-(CH<sub>2</sub>)<sub>3</sub>-NMe]-R where R = H, Me. 7-nitrobenzo-2-oxa-1,3-diazole (NBD), rhodamine B, rhodamine 101 and fluorescein residuals were used as fluorescence groups. NBD was introduced into MOPA molecules using 4-chloro derivative, rhodamines and fluorescein were converted into chloranhydride form before the reaction with amines. Substances bearing one or two fluorescence groups have been obtained, their structure and purity were confirmed with HPLS, HRMS and NMR. Fluorescent properties of the new dyes were studied in water solution depending on pH and association of the tagged MOPA was found at high pH values and in the case of molecules with two fluorescence groups. This association decreases emission of the fluorophores and changes their spectra.
- High-molecular polymers of various charge of the main chain. By the reaction of poly(acryloyl chloride) with NH-containing tagged MOPA or fluoresceine, tagged poly(acrylic acid) was obtained. Positive charged polymers were prepared by modification of poly(vinyl amine) with NBD-Cl or with chloranhydride derivatives of rhodamines or fluorescein. Copolymers of 1-vinylimidazole with vinylamine were used to obtain non-charged (at neutral pH values) tagged polymers. These copolymers were prepared by copolymerization of 1-vinylimidazole with N-vinylformamide followed with alkali hydrolysis of the amide units. The absence of free fluorescence dye in the tagged polymers was confirmed with size-exclusion chromatography.

The obtained fluorescent dyes were used in cultivation experiments with fresh-water diatoms and in synthesis of new fluorescence materials from diatom frustules and by *in vitro* condensation of silicic acid in the presence of tagged MOPA.

The study was partially supported by Project # 11-04-00707-a of the Russian Foundation for Basic Research.

## 'PSEUDOPODIAL' ACTIVITY OF THE GAMETE SURFACE IN ARAPHID PENNATE DIATOMS

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Some araphid pennate diatoms such as *Synedra* (*Ulnaria*) or *Tabularia* being nonmotile and sessile in their vegetative phase, nonetheless involve heterothally, and male gametangia release their gametes into surrounding water. Successful fertilization depends thus on reaching a gamete of the opposite mating type. Any locomotion organelles were unknown in pennate diatom gametes until quite recently. Specific cell surface structures that induce gamete movement and thereby promote syngamy were found in three araphid pennate diatoms, *Tabularia fasciculata*, *T. tabulata* (Davidovich et al. 2011), and *Pseudostaurosira trainorii* (Sato et al. 2011). Slender cytoplasmic projections, resembling pseudopodia arise at the surface; they elongate over a relatively short period of time, then retract and "disappear". Formation and retraction of cytoplasmic projections coincide with gamete movement in shifting manner (spinning, milling about, or moving in a chaotic fashion). This forces gamete migration on relatively long distance. Here we present evidence that yet another araphid pennate diatom, freshwater *Ulnaria ulna* (Nitzsch) P. Compere (= *Synedra ulna* (Nitzsch) Ehrenberg) reveals considerable activity of gamete surface. The general manner of projection expansion and retraction in *U. ulna* resemble those of three other mentioned species, while differ in minor details. Pseudopodia in *Ulnaria* are shorter and do not provoke as vigorous activity as that of gametes in *Tabularia*. The longest pseudopodia were found in *T. tabulata*. *Pseudostaurosira trainorii* extruded not only "threads" but also relatively big blobs. As in other species, formation of cytoplasmic projection in *U. ulna* was accompanied with change of gamete form; from spherical to angular. Finding of similar modes of male gamete motility in so distantly related araphid species suggests the existence of other diatoms which gametes may possess dynamic pseudopodium-like surface structures. The last are poorly investigated but undoubtedly important for the process of sexual reproduction. The evolutionary origin of these structures is yet to be understood.

## PHOTONIC VALVE ARCHITECTURE OF *COSCINODISCUS WAILESII*: A NOVEL APPROACH BASED ON DIGITAL HOLOGRAPHY

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One of the most versatile photonic architecture present in nature is definitely given by diatom frustules. Valves are characterized by hierarchical, complex and quasi-ordered patterns of pores (areolae and poroids) whose dimensions can range from nanometer to micrometer scale. Several studies suggest that these patterns are able to play an active role in light manipulation and exploitation, possibly affecting the photosynthetic process. The impressive similarity of diatom areolae with the pores of artificial photonic crystals enforces these hypotheses. Among radial centric diatoms, *Coscinodiscus wailesii* Gran & Angst 1931 has been the object of recent studies which have demonstrated the ability of its valves to confine coherent radiation coming from a laser in a spot a few microns and that this confinement effect takes place also for non-coherent radiation, most likely due to in-phase superposition of the diffracted waves arising from the pores of the frustule itself.

In this work we report the use of Digital Holography (DH) as a novel approach to the characterization of coherent light transmitted by a single valve of *C. wailesii*. This technique is not only able to three-dimensionally define the valve microstructures but also allows us to better reconstruct the light propagation through the valve depth and cytoplasmatic volume giving precise information about its intensity and phase variations during the interaction with sub-micrometric valve pores. Furthermore, compared with other photonic techniques, DH enables to separate and discriminate the diffraction contribution due to the valve edges from that coming from the pores patterns. Our results on light manipulation ability of *C. wailesii* valves, correlated with ongoing *in vivo* studies on monochromatic light mediated plastid response in cell culture, could be useful to determine the effect of frustule architecture on diatom photobiology.

## MASS CULTURE OF DIATOMS FOR FUN AND PROFIT

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Algal biomass production is an emerging for profit industry with target algal strains produced in kilogram quantities for biofuels, nutraceuticals, and animal feed supplements. While research is ongoing with taxa grown in these commercial settings, topics are typically focused on enhancing growth rates while minimizing nutrient inputs and enhancing the biochemical composition of the biomass produced. Application of these results to the broader scientific community is often limited. Diversity among these target strains is often limited as the number of governmentally approved taxa is limited to a few dozen species. The expense of building an algal bioreactor often bars use in a laboratory settings. We present protocols for establishing and maintaining large batch diatoms cultures along with the applications and experimental utility of these cultures. The protocols and results were produced a small (~200L) low cost (~500€) laboratory scale bioreactor. Both marine and freshwater strains were produced at the grams per week scale. Products from the bioreactors have had broad utility in laboratory environments. These have included use as a) calibration materials for  $^{18}\text{O}/^{16}\text{O}$  ratios in isotopic analysis, b) a tracer material in paleolimnological analysis, and c) as tracer particles in flow dynamic investigations. Experimentation conducted with large culture volumes has considered the rate of size diminution in single cell isolates and the degree of genetic diversification in single cell isolates.



## TETRACYCLUS-SPECIES FROM MIOCENE DEPOSIT OF KHANKA LAKE (RUSSIA, PRIMORYE)

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The research of A.P. Jousé and A.I. Moiseeva was devoted to the history of the development of the diatomaceous flora of Khanka Lake from the Miocene epoch till our days. The following species of the genus *Tetracyclus* Ralfs were described by these authors: *T. lacustris* var. *elongates* Hustedt, *T. ellipticus* (Ehrenberg) Grunow, *T. jimboi* (Pantocsek) Jousé, *T. polygibbum* (Pantocsek) Jousé, *T. aff. rupestris* (Braun) Grunow, *T. circulus* Jousé, *T. ellipticus* var. *clypeus* (Ehrenberg) Hustedt, *T. ellipticus* var. *lancea* f. *lancea* Hustedt, *T. ellipticus* var. *lancea* f. *subrostrata* Hustedt, *T. ellipticus* var. *latissimus* Hustedt, *T. lacustris* var. *strumosus* (Ehrenberg) Hustedt.

As the previous investigations were conducted by means of light microscopy (LM) it was necessary to review the systematic position of the above-mentioned taxa and to study scanning electron microscopy (SEM) the morphology of valves taking into consideration the modern views of valve ultrastructure and taxonomic changes.

In this area we pointed out the following species of genus *Tetracyclus*: *Tetracyclus* sp., *T. ellipticus*, *T. polygibbum*, *T. glans* (Ehrenberg) Mills, *T. excentricum* (Ehrenberg) Williams, *T. lancea* (Ehrenberg) Peragallo, *T. radius* Saito-Kato, Hayashi & Tanimura. In our previous study we identified (Dubrovina *et al.*, 2011) the *Tetracyclus* sp. as *T. clypeus* (Ehrenberg) Li, and the previous investigators (Jousé, 1953; Moiseeva, 1971) defined it as *T. ellipticus* var. *clypeus*. However more detailed investigation of the valve ultrastructure showed significant differences from the type material (Williams, 1989).

It was shown that *Tetracyclus* sp. is similar to *T. clypeus* by its circular shape of its valves, the deep mantle of valve, the numbers and situation of the primary transapical ribs, but it differs by the absence of secondary ribs at the polar regions, the nonuniform density of the striae (it forms a rare row in the central part of a valve, but rows at the polar regions are compact), the numbers and position of the rimoportula and the great variety in the length of sternum.

The absence of the secondary ribs at the poles is one of the characteristic features of other circular species *T. subdivisium* Williams & Li, *T. subclypeus* Li & Williams, *T. excentricum* (Ehr.) Williams and *T. divisium* (Ehr.) Li. But *Tetracyclus* sp. also differs from the above-mentioned species by the deep mantle of the valve, the nonuniform density of the striae, the different position and construction of the ribs and the number and position of the rimoportula.

Taking the above-mentioned features as a ground we presuppose that the above-mentioned species *Tetracyclus* sp. is a new species.

## A NEW SPECIES OF *PLATESSA* LANGE-BERTALOT FROM THE NORTHEASTERN UNITED STATES

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A new species of *Platessa* is described from modern and subfossil lake sediments from the Northeastern United States. This species is placed in the genus *Platessa* based on biseriate striae and absence of terminal raphe fissures. The new species is easily distinguishable from other *Platessa* species by its valve shape and the presence of a depression in the central area of the rapheless valve. None of the other *Platessa* species reported so far have such a depression. The new species was found only in soft-water lakes.

## DIATOMS AS A PROXY IN RECONSTRUCTING THE HOLOCENE ENVIRONMENTAL CHANGES IN THE PATAGONIAN COAST, ARGENTINA

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Southern South America is an important area for research about environmental and climatic changes from the Southern Hemisphere. However, the information about fossil diatoms is scarce. The study of diatom assemblages from Mid to Late Holocene sequences from coastal areas of Patagonia, Argentina allowed reconstructing their evolution in relation to salinity and tidal changes. The study area includes two estuaries from Northern Patagonia: the Negro River (40° 57' S; 62° 49' W) and the Chubut River (43° 22' S; 65° 03' W). Two sequences were analyzed from the estuary of the Negro River (1.5 km and 10 km from the inlet); another from the estuary of the Chubut River (0.40 km from the inlet). The diatom content of modern sediment samples along the rivers were studied in order to achieve ecological information useful as modern analogous for Patagonian coast. The analyses included 22 modern samples and 86 fossil samples belonging to the three cores in order to interpret the environmental changes in terms of salinity and depth. Diatom zones were characterized by constrained incremental sum of squares cluster analysis (single linkage, Euclidean distance) in the fossil sequences. Fossil and modern diatom assemblages were compared with Detrended Correspondence Analysis (DCA). Diatom analyses allowed interpreting the evolution of Negro river estuary during the last 2,000 years BP suggesting a shallow vegetated brackish/freshwater environment that evolved gradually into marine conditions (tidal channel) and at last into a marsh. At the inlet of the Chubut River, a marine coastal environment (estuary) is inferred at 4,376±69 <sup>14</sup>C years BP evolving into a salt marsh about 672±39 <sup>14</sup>C years BP. Considering the strong influence of salinity changes on diatom assemblages, tidal effects are the main controlling factor for the composition and distribution of diatoms along these mesotidal estuaries from Patagonia. The knowledge of palaeoenvironmental conditions derived from fossil diatom assemblages is very important to infer changes induced by man in coastal areas and can be used as reference levels for the assessment of recent coastal changes (dredging, harbour construction, flood control, pollution).

## LIVING WELL WITH A SCRAMBLED METABOLISM: CO<sub>2</sub> FIXATION AND CARBOHYDRATE PATHWAYS IN DIATOMS

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Diatoms are responsible for up to 20% of the global carbon fixation, while the mechanisms of CO<sub>2</sub> fixation in these organisms are still unclear. Diatoms have evolved by secondary endosymbiosis, which apparently led to subcellular reorganization of metabolic pathways within the organisms. Studying the genome of the diatom *Phaeodactylum tricornutum* we have identified a number of genes for enzymes which are important for operating a C<sub>4</sub>-like pathway including several carboxylases. However, we could not yet identify plastid localized decarboxylases. Such enzymes would be important for a putative carbon concentrating mechanism (CCM) by releasing CO<sub>2</sub> from malate or oxaloacetic acid in close proximity to RubisCO. The only decarboxylases identified so far apparently are targeted to the mitochondrial matrix which is separated from the plastid stroma (and thus the RubisCO) by six membranes. Therefore it is unclear yet whether *P. tricornutum* might operate either a biochemical or a biophysical CCM or a combination of both.

To study individual enzymes of the photosynthetic carbon fixation, we are using a “reverse genetics” approach including silencing of genes which are involved in the biochemical CCM via RNAi. Mutants have been designed with sense-antisense constructs for silencing phosphoenolpyruvate carboxylases (PEPC1 & PEPC2), phosphoenolpyruvate carboxykinase (PEPCK), pyruvate carboxylases (PYC1 & PYC2) and NAD malic enzyme (ME1). To control expression of the silencing constructs we have decided to use a nitrate reductase (NR) promoter which can be switched on and off depending on the nitrogen source in the medium. The obtained mutants will be tested for silencing efficiency by Western Blots. Physiological investigations will be conducted under e.g. different CO<sub>2</sub> and light conditions to learn more about their adaptation abilities due to future environmental changes.

## SPATIAL PERIPHYTIC DIATOMS BETWEEN TWO SUMMERS IN A LARGE SUBTROPICAL SHALLOW LAKE

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Diatom communities are commonly used as indicators for environmental disturbance. Both diatom growth and the composition of the diatom community reflect the organisms' sensitivity to environmental changes. Their sessile habit and inability to migrate away from adverse conditions make them reliable proxies for their environmental surroundings and limnological conditions. In this study, we provide the responses of diatom community in two summers in Mangueira Lake, which is a large shallow oligo-mesotrophic system under continuous wind influence. The lake is placed in the Taim Hydrological System (THS), southern Brazil, site 7 of the Long Term Ecological Research of the Brazilian network (LTER=PELD –CNPq). Samplings were carried out in 2006 and 2008 in three distant sampling points (North, Center and South). Biofilms were scraped from natural substrata and we provide quantitative analyses. Seventeen physical and chemical variables of water were measured in each sample resulting in Principal Components Analysis (PCA), which explained 93.9% of the variance in the first two axes, showing spatial and temporal variation. The South was characterized by high transparency. The main descriptive variables of the summer of 2006 were turbidity, total inorganic carbon and SRP, and in 2008 were silica, temperature and total organic carbon. Diatoms as in richness and abundance were the most representative group in total periphyton (average 50%). These have specialized structures for attachment to substrata and competition for resources. *Gomphonema gracile*, *Epithemia turgida* and *G. parvulum* had expressive contribution for total periphytic algae biovolume. Diversity was high, except in South/06 (1.96 bits.ind<sup>-1</sup>) with dominance of *Achnantheidium minutissimum* (59% of total density). Difference ( $p < 0.02$ ) between densities and community composition in Center/06 and North/06, revealed spatial distribution. North/06 showed lower biomass, with abundance of *G. parvulum* that tolerates high turbidity and higher concentration of phosphorus. Temporal distribution between 2006 and 2008 pointed to higher density of *Punctastriata lancettula* in the North/08 ( $p < 0.007$ ) and increased of total densities especially *E. turgida*, *A. minutissimum* in the South/08 ( $p < 0.04$ ) that may have occur in response to availability of silica and higher temperatures. We also performed a Two-way Cluster Analysis were the primary clustering separate North/08, South/06 and South/08 were the most similar, and Center can show characteristics of both.

## BLOOM OF *THALASSIOSIRA* SP. IN THE SOUTHWESTERN ATLANTIC OCEAN: A NEW SPECIES?

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The Southwestern Atlantic Ocean is recognized as one of the most productive areas in the world and supports a very rich fishery. In order to study the biodiversity and seasonal dynamics of phytoplankton in Argentinean continental shelf and slope waters, a cooperative research Project has been established (GEF- Patagonia, Argentina). As part of this project, both qualitative and quantitative samples collected during spring 2005, were examined using light and scanning electron microscopy. An extensive bloom of a small *Thalassiosira* sp. was observed along shelf waters from 39-48°S, reaching concentrations up to  $4.5 \times 10^6$  cells l<sup>-1</sup> and representing more than 90% of total phytoplankton abundance. The observed specimens are small (8.5-12.0 µm), solitaires, rectangular in girdle view, and with numerous discoid chloroplasts. The valve face shows a single strutted process slightly subcentral and others in one ring located between the valve face and mantle (3-4 per valve). The strutted processes are externally surrounded at their base by areolae with robust walls, and internally have four satellite pores and long tubular extensions. One labiate process is situated adjacent to the subcentral strutted process. The areolae pattern shows tendency toward fasciculation (20-23 in 10 µm), sometimes with radial ribs. The cingulum is composed of numerous copulae with vertical rows of pores and a valvocopula with a similar ornamentation. The morphological features of these specimens are compared with other related taxa like *Thalassiosira* (*Shionodiscus*) *bioculatus* var. *exiguus*, *T. bioculatus* var. *raripora*, *T. oceanica*, *T. oestrupii*, *T. perpussilla*, *T. rosulata* and *T. spinulata*.

We acknowledge funding from the GEF-PNUD ARG 02/18 and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) PIP 112-200801-01734.

## ANTARCTIC DIATOM COLLECTION. CATALOGUE OF THE TAXA ERECTED BY J. FRENGUELLI

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One of the main topics that have gained consensus within the scientific community is the assessment of biodiversity as the basis for a sustainable management. In this sense, during last years the importance of historical records and the utility of scientific collections have been revalued.

In many scientific meetings has been raised the need to make known those properly organized collections. A list of the most prominent diatom collections in the world has been already elaborated but only those existing in Mexico, Brazil and Venezuela have been included for Latin America.

In this framework and considering the relevance of Dr J. Frenguelli's Diatom Collection, housed at the Museo de La Plata, the 38 taxa of Antarctic diatoms erected by Frenguelli, which have not been published according to the rules of the ICBN, are being analyzed. The aim is to produce an illustrated catalogue of the type materials of the specific and infraspecific taxa. The text will be both in Spanish and in English.

We present here results obtained so far, which include the survey of the preservation status of the microscope slides, a list of the taxa recorded for each sample, a synopsis of the environmental characteristics of the sampled sites and the migration of all related information to a computer database.

Further work in progress includes the obtaining of digital light microscopy microphotographs of the taxa and the translation of the original diagnosis into Latin. This catalogue will revalue Frenguelli's Collection within the biological collections and, at the same time, will facilitate its consultation for the international community of diatomists.

Acknowledgements: Proyecto UNLP N542; PIP 01734 CONICET

## TAXONOMY AND ECOLOGY OF FRESHWATER DIATOMS OF THE SENEGAL RIVER

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Five years ago, a new international collaboration between Senegal, France Belgium and Luxemburg was started aiming to install a water quality monitoring based on freshwater diatoms for the Senegal River in the northern part of Senegal. With a length of more than 1800 km and a catchment of almost 350.000 km<sup>2</sup>, the Senegal River, forming the border between Senegal and Mali and Mauretania, is the seventh largest river in Africa. During a sampling campaign in 2007, the entire river was sampled from its entry in Senegal near the eastern town of Kidira up to its mouth at the Atlantic Ocean near Saint-Louis. More than 100 samples were taken. For each site, samples were taken for both physico-chemical and diatom analyses. In a first stage, the taxonomy of the observed diatom flora will be revised and the different taxa will be morphologically and ecologically characterized. This will result in the creation of a methodological identification guide facilitating routine water quality monitoring in the future.

The poster presents the different habitat aspects of the Senegal River, the results of the first sampling campaign and the wide diversity of the observed diatom flora. A very diverse and species-rich diatom flora composed of both typical cosmopolitan and African taxa have been found. Several species of both groups are illustrated using both light and scanning electron microscopy. Typical (tropical) cosmopolitan taxa include *Diadesmis confervacea* Kützing, *Lemnicola hungarica* (Grunow) Round & Basson and *Achnantheidium catenatum* (Bílý & Marvan) Lange-Bertalot. The African flora is mainly dominated by taxa belonging to the genera *Nitzschia* and *Navicula* s.l. Especially within the genera *Placoneis*, *Navicula* and *Sellaphora* a large number of species have been observed with typical taxa such as *Navicula densa* Husted and *Navicula nyassensis* O. Müller. It is clear that several of these taxa will need a taxonomic revision.



## GEOTACTIC BEHAVIOUR IN BENTHIC DIATOMS

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Vertical migration by benthic diatoms is a well-known phenomenon, occurring in intertidal and permanently submerged sediment-inhabiting microphytobenthos. It is known to be partially endogenously-driven, as cell movements can be observed in the absence of external stimuli like light, temperature or water cover. This vertical migration of diatoms under constant conditions has often been attributed to geotactic orientation, although this hypothesis was never experimentally demonstrated. This study was set out to test the geotactic nature of the vertical migratory behaviour of benthic diatoms, by using an experimental setup designed to distinguish surface-oriented from geotactic movements.

The variation of surface microalgal biomass during migratory cycles was compared in sediment samples kept with their surface facing upwards (surface-oriented movements coinciding with geotactic movements; controls) and with the surface facing sideways or downwards (surface-oriented movements not coinciding with geotactic movements). Samples were maintained in complete darkness, in custom-made sealed chambers designed to avoid any contact between the sediment and the atmospheric air and the formation of gradients near the surface. Surface biomass was monitored non-intrusively using pulse amplitude modulating (PAM) fluorometry, by measuring dark-level fluorescence.

The results showed a clear effect of sample orientation in relation to the gravitational stimulus. In the controls, a marked biphasic increase-decrease pattern in surface biomass was observed, with the formation of a marked biomass peak (ca. 4-fold variation). Contrastingly, in samples positioned sideways or facing downwards a comparable peaking in surface biomass was never observed, and biomass levels increased only slowly during the all duration of the experiment. These results indicate that the diatom migration towards the sediment surface is mostly determined by geotaxis and not due to the following of other environmental cues, confirming the hypothesis that in diatom-dominated biofilms upward migration is started by an endogenous, negative geotactic behaviour.

## DIATOM MONITORING PROGRAMS IN SURFACE WATERS IN FLANDERS (BELGIUM) FOR THE WATER FRAMEWORK DIRECTIVE

Wim Gabriels, [Gaby Verhaegen](#) & Lidia Anokhina

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To meet the requirements of the European Water Framework Directive (WFD), a good ecological status should be reached in all natural surface water bodies by 2015, and a good ecological potential in all artificial or heavily modified surface water bodies. For this purpose, member states establish monitoring programs for evaluating the ecological status or potential of their water bodies, based on those biological quality elements that are specified by the WFD for each surface water category. According to the WFD, the assessment method used by the member states to evaluate the ecological status or potential should be expressed as an ecological quality ratio (EQR) that can take values between zero and one, with high ecological status represented by values close to one and bad ecological status by values close to zero. The EQR range is divided into five quality classes.

The member states establish three monitoring programs. Surveillance monitoring is carried out at least once every six years for a sufficient number of surface water bodies to provide an assessment of the overall surface water status. Operational monitoring is undertaken at least once every three years to establish the status of those water bodies identified as being at risk of failing to meet their environmental objectives. Investigative monitoring is carried out in case the reason for any exceedance is unknown.

Monitoring of diatoms (one of the relevant biological quality elements for the surface water categories 'rivers' and 'lakes') consists of routine sampling and sample preparation, followed by identification and counting of the valves and EQR calculation.

This poster provides an overview of the current progress in implementing the diatom monitoring program for the WFD in Flanders (Belgium) by the Flemish Environment Agency (VMM).

In the Flemish region, the first samplings for the WFD were carried out in 2007. Approximately 1400 samples were taken in the period 2007-2010.

After sample preparation, 500 valves are identified at species level and counted. Subsequently, the taxonomic lists can be integrated into an electronic database to enable EQR calculation. Until now approximately 1500 species were found.

A new diatom-based assessment method for Flanders, called PISIAD (Proportions of Impact-Sensitive and Impact-Associated Diatoms; Hendrickx & Denys, 2005), has been developed by the Flemish Institute for Nature and Forest Research.

To ensure that quality classes based on assessment methods of different member states are comparable, international comparison is necessary. At the European level, intercalibration exercises were organised to ensure that the two highest quality class boundaries are comparable. Results of these intercalibration exercises were already endorsed by the European Commission, including PISIAD class boundaries for most types of Flemish rivers. Furthermore, bilateral comparisons with France, the Netherlands and with the Belgian regions Wallonia and Brussels Capital take place regularly in order to compare results obtained by samplings carried out at the same location.

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## MORPHOLOGY AND OCCURRENCE OF A MARINE EPIZOIC DIATOM *FALCULA HYALINA* FROM WATERS OF FUJIAN AND GUANGDONG, CHINA

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*Falcula* M.Voigt was first described by Voigt in 1961 and five species have been reported so far. However, the genus *Falcula* has never been reported in domestic literatures in China except that Voigt (1961) mentioned in his paper about the description of the new species *F. paracelsiana* whose sample was collected from Paracel and Hainan Islands in the South China Sea. *Falcula hyalina* Takano was first observed as an epizoic diatom in a marine copepod *Acartia steueri* by Takano (1983) in the Japanese sea area and was found to be a widespread epizoic species in temperate and subtropical inshore waters of the Indian, Pacific, Atlantic oceans and the Gulf of Mexico.

In a study of the diatom species composition in mariculture waters in Quanzhou City (N24°56', E118°36'), Fujian Province, and in mangrove waters in Futian Mangrove Conservation Zone (N22°32', E114°03'-05'), Shenzhen, Guangdong Province, China, *F. hyalina*, was observed using light and electron microscope which was first found for its occurrence in China. This species occurs in waters while tends to attach to marine copepods. The taxonomic characteristics, ecological habit and distribution of *F. hyalina* were described in the present paper and the LM and EM photographs of the species were also presented.

This work was supported by National Natural Science Foundation of China (Grant No. 41076079) and Public Science and Technology Research Funds Projects of Ocean (Grant Nos. 201205031-07, 201005015-5).

## NANTES CULTURE COLLECTION: MICROPHYTOBENTHOS BIODIVERSITY FOR RESEARCH AND DEVELOPMENT ADVANCES

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Estuaries and coastal areas are amongst the most productive marine ecosystems on Earth. They are particularly valuable as habitats and feeding grounds for many organisms (birds, shellfish, demersal fish, invertebrates), which often support local economies. However, these ecosystems face strong anthropogenic pressure caused by intensive exploitation. In these ecosystems one of the major components responsible for primary production is the microphytobenthos, microscopic algae that inhabit intertidal sediments and forms dense biofilms at the sediment surface. Microphytobenthos provides an important energy source for the estuarine food web, has a central role for carbon flow in coastal sediments, and contributes to sediment stabilization. In spite of this key role in the functioning of coastal ecosystems, only few studies deal with microphytobenthos ecology, biology and physiology, and even less with microphytobenthos as a source for useful metabolites. Indeed, most microalgae studied are planktonic, rarely diatoms, almost never benthic, whereas the global microalgal diversity is largely dominated by diatoms, and microphytobenthos remains a 'secret garden' with a great potential in research and development.

The **Nantes Culture Collection** (NCC) is dedicated to the preservation, the storage and the assessment of microalgae, mostly being benthic species. NCC is referenced in the '*World Data Center for Microorganisms*' as 'NCC WDCM 856' since 2002, and it is the world's only collection hosting mainly benthic diatom strains. With 40 genus and more than 300 strains, including *ca.* 100 'blue diatoms', NCC is a real conservatory of microphytobenthos diversity mainly originating from the French Atlantic coast.

Currently, the NCC offers services as the distribution of strains and related products (culture medium, concentrate, lyophilisate) to academics and companies, either in the context of scientific program or for sale.

For further information, please contact Vona Méléder, the scientific curator of the NCC : [vona.meleder@univ-nantes.fr](mailto:vona.meleder@univ-nantes.fr)

## MULTISPECTRAL SATELLITE REMOTE-SENSING OF MICROPHYTOBENTHOS CHLOROPHYLL A CONCENTRATION USING MERIS

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Multispectral ocean color satellite remote-sensing have proven extremely useful to monitor phytoplankton structure and functioning in the coastal ocean over a continuum of space and time scales relevant to ecosystems studies. Among available sensors for the 2002-2012 decade, the MEdium Resolution Imaging Spectrometer (MERIS), being characterized by a set of 15 narrow bands in the spectral range from 412 to 900 nm and by a ground resolution of 300 m in every band, shows the greatest promises to study the spatio-temporal dynamics of benthic microalgae biofilms covering intertidal sediments. The potential of MERIS to retrieve biogeochemical information on microphytobenthos is investigated here. A library of *in-situ* and synthetic hyperspectral reflectance data will be used to determine the most suitable reflectance indice to indirectly measure microphytobenthos chlorophyll *a* concentration using MERIS data. Preliminary examples of applications will be shown in Bourgneuf Bay (France) and other intertidal zones of the European coast.

## THE INFLUENCE OF NUTRIENT LIMITATION ON ANTIOXIDANT ACTIVITY AND UV-SUSCEPTIBILITY OF *PHAEODACTYLUM TRICORNUTUM*

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The influence of nutrient limitation on C:N:P ratios, carotenoid content and lipid formation as well as on overall biomass composition is well-described in literature. On the other hand, much less is known about the influence of medium composition on the antioxidant content of microalgae. In previous research, we found that *Phaeodactylum tricornutum* is a microalgal species with moderate to high antioxidant capacity. However, it was observed that samples from the same species, but from different origin, showed significantly different antioxidant activity. Therefore, in this study, we focussed on the effect of nitrogen or phosphorus limitation on total antioxidant activity, phenolic content as well as carotenoid content. Moreover, the effect of the applied nutrient limitation on the susceptibility of *P.tricornutum* to short-term UV exposure was monitored by measuring the decline in quantum yield as well as its recovery after UV-exposure. In accordance with our previous research, the antioxidant activity was more related to the total phenolic content than to the carotenoid content of the algal biomass. The data further showed that nutrient limitation did not only result in reduced growth and functioning of the photosynthetic apparatus, but also lower levels of phenolics, carotenoids and antioxidant activity were found. Nitrogen limitation was found to have a larger impact than phosphorus limitation. Finally, it was found that the susceptibility of the photosynthetic apparatus towards UV-light exposure was higher under nutrient limited conditions than in nutrient replete conditions. Nitrogen limited cultures were the most UV-sensitive since recovery of the photosynthetic apparatus after UV-exposure was lower than in the other cultures. In conclusion, our results indicate that the physiological changes induced by phosphorus limitation have a different impact on antioxidant response and UV-stress than nitrogen limitation.

## OPTICAL DENSITY AS A TOOL FOR MEASURING DIATOM GROWTH IN VIVO

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In our study we investigate the possibility of applying optical density (OD) for diatom growth measurement. There are several techniques for estimating diatom abundances, however, for chain-forming diatoms; the most commonly applied techniques are manual cell counting and chlorophyll *a* absorbance. Both of these methods require time consuming counting or preparation of the samples and also loss of sample volume.

OD (600nm) is commonly used for estimating bacterial and yeast growth cultured in microtiter plates. This method allows for easy measurements of large sample sizes, as well as *in vivo* measurements.

We have tested the application of OD as an alternative method to estimate diatom growth. For this, OD measurements and traditional manual cell counting was compared for three diatom species, the chain-forming *Skeletonema marinoi* and the two solitary species, *Cylindrotheca fusiformis* and *Ditylum brightwellii*. The results from the two types of estimation methods show significant correlation for all of the species. We report on adjustments (relative to bacterial and yeast culture) and experimental setting for further maximizing the efficiency of OD as a measurement method for estimating growth rate of diatoms, with additional focus on *S.marinoi*. Our anticipation is to be able use OD as a more effective way of measuring diatom growth, especially in situations when dealing with larger number of replicated samples for performing *in vivo* experiments.

## THE GENUS *PUNCTASTRIATA* AND THE COMPLEX *STAUROSIRELLA PINNATA* (ARAPHIDS, BACILLARIOPHYCEAE) FROM LACUSTRINE SEDIMENTS IN NORTHWESTERN PATAGONIA, ARGENTINA

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Araphid diatoms are frequent components of planktonic and benthic communities of lakes and rivers. These diatoms are particularly abundant in high-latitude, alpine environments, especially in post-glacial lacustrine sediments. The high morphological variability within this diatom group together with their usual small cell size make accurate identifications difficult at the light microscopy level. Consequently, there are uncertainties regarding the distributional and ecological ranges of many taxa based on current literature reports, restricting the usefulness of the group as ecological indicators for water quality assessments and paleoenvironmental reconstructions.

During the last three decades the use of electron microscopy has allowed the description of several new taxa and the detailed study of type materials. However, these studies have been focused mainly on materials collected in the northern hemisphere, while similar studies in other regions such as South America remain relatively scarce.

As part of a study aimed at reconstructing the main past environmental changes in different late Quaternary forest ecotones from the northern Patagonian Andes, the diatom assemblages of a 10 m sediment core recovered from Lake Torta (39°06'S, 71°21'W) are being analyzed. Light and scanning electron microscopy studies have revealed that along most of the sediment sequence, diatom assemblages are dominated by araphids in the genera *Staurosirella*, *Staurosira*, *Pseudostaurosira*, *Pseudostaurosiropsis* and *Punctastriata*, the former two exhibiting the highest species richness.

This presentation documents the genus *Punctastriata* and the *Staurosirella pinnata* (Ehrenberg) Williams & Round complex represented by at least 15 different morphotypes in the core, many of them probably new taxa to science. Since the identity of *S. pinnata* is not well defined in the literature, type material for this species was analyzed using light microscopy. These data, together with a critical analysis of the original illustrations by Ehrenberg show that *S. pinnata* is not an araphid, but rather a diatom in close association with *Denticula* Kützing.

This study evidences that detailed morphological studies are needed to improve the use of diatoms as proxies for reconstructing paleoecological changes and that the revision of type material is a key task for accurate identification of morphologically similar taxa.

Acknowledgments: Carlos E. Wetzel and Luc Ector collaborated with the analysis of the type material for *Staurosirella pinnata* and coauthored the resulting manuscript, presently under revision.



## TAXONOMIC COMPOSITION OF BENTHIC DIATOMS (BACILLARIOPHYTA) FROM TWO MARINE LAKES ON THE ISLAND OF MLJET (ADRIATIC SEA, CROATIA): PRELIMINARY RESULTS

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The marine lakes, Malo and Veliko Jezero ("Little" and "Big" Lakes), are one of the first established marine reserves in the world. In fact, they are two karstic basins on the island of Mljet which are filled by the holocene ingression of the Adriatic Sea. The lakes with the surrounding area have been protected under the category of the National Park since 1960. The aim of the study was to determine the species composition and abundance of benthic diatoms in two marine lakes. Knowledge of the diatom community structure in the marine protected areas is important for marine ecology and conservation biology. Samples were taken from two stations (Malo jezero - 42° 46' 58" N 17° 20' 56" E at 1 m depth; Veliko jezero - 42° 46' 57" N 17° 21' 06" E at 8 m depth) during April 2011. Samples were fixed in a 4% formalin solution and prepared for investigation by cleaning frustules using the method described by Hustedt (1930). Examination and identification of benthic diatoms were carried out using the oil immersion lens (100 x) of the Microstar binocular microscope, AO Scientific Instruments. Samples were also studied by means of scanning electron microscopy. Photomicrographs were taken for the majority of taxa. Altogether, 96 taxa included in 33 genera were identified. Among them, 60 taxa were found in Veliko jezero and 59 in Malo jezero. Sørensen similarity index of 37% indicated that diatom community of two lakes was highly distinct. The largest number of taxa was found within the genera *Mastogloia* (11), *Amphora* (10), *Diploneis* (10) and *Nitzschia* (7). This work reports the first records of benthic diatoms in the lakes. The unique situation of marine lakes within a karstic system made it possible to get valuable new information about Adriatic sea diatoms.

## USE OF PENNATE DIATOMS AS TEMPLATES FOR NANOTEXTURATION OF ELASTIC POLYMERS

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Nanotextured materials represent a very exciting subject of investigation in the field of material science due to the astonishing variety of morphology and physical-chemical properties. However, fabrication of assemblies reaching nanosize ordered features is particularly challenging. Man-made materials are therefore in strong competition with natural nanostructured materials, among which, diatoms play a significant role. Their siliceous frustules provide a unique natural source of three-dimensional nanostructured patterns with a considerable potential in different scientific fields and applications in nanotechnologies. Therefore diatoms have recently attracted attention of material engineers as promising templates for biomimetic fabrication. Among several biomimetic methods such as sol-gel technique or physical vapor deposition used for various natural templates, imprint lithography was recently applied to fabricate nanotextured polymers or metals based on diatom morphology. In the present work, we tested the reproducibility and preciseness of a soft lithographic technique using elastic polymer polydimethylsiloxane (PDMS Sylgard 184<sup>®</sup>) that was previously successfully used to replicate the micro- and nanoscale patterns from centric diatoms. In this approach, diatoms are immobilized on a glass surface coated with a UV-curable polymer and the diatom pattern is consequently transferred onto an elastic polymer producing a negative replica. The trials were focused on the optimisation of different parameters (pressure, temperature, ratio of curing agent and elastomer) at different steps (preparation of the master, PDMS molding and curing) of the molding process using freshwater pennate diatoms (e.g. *Encyonema silesiacum*, *Fragilaria* spp.). The results indicate that optimised parameters of the molding process allow replicating features of 50 nm. Such polymeric replicas with nano-scaled pattern can be potentially used in many different scientific fields; the possible applications include optical elements, nanofabrications, biosensing device components and nanoreactors. However, our study revealed some limitations regarding the size and morphology of the species used. Compared to previous works, the method seems to provide better results for large centric diatom species than for pennates that appeared rather fragile to resist the particular steps of the cast molding.

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## COMPARATIVE STUDY OF TYPES OF SEVERAL FRESHWATER *NITZSCHIA* OF THE SECTION DISSIPATAE WITH A DESCRIPTION OF A NEW SPECIES

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Morphological examination of type materials of four different diatom species belonging to the section Dissipatae was performed in order to reveal the identity of a new *Nitzschia* species discovered in European freshwaters. Type materials of *Nitzschia bavarica* Hustedt, *N. dissipata* (Kützing) Rabenhorst, *N. media* Hantzsch and *N. rectiformis* Hustedt were studied using light and electron microscopy and their morphology was compared with the new *Nitzschia* species. Except for *N. dissipata*, all findings about species' morphology are in line with previously published data. All examined taxa have very similar morphology, their valves are linear to lanceolate, with distinct, parallel, irregularly spaced fibulae and transapical uniseriate striae difficult to distinguish under light microscopy, uniseriate transapical striae consisting of small round areolae occluded by hymenes externally, prominent raphe lacking an interruption in the centre, terminal raphe fissures hooked to the same side and a distinct conopeum visible also in a light microscope. On the other hand the interspecies differences are rather difficult to define and the most reliable features to separate the taxa are the valve shape, the length/width ratio and the striae density. The new species can be clearly distinguished by the robust valves with subcapitate ends and prominent and very irregular fibulae. So far, it was observed in several small alkaline oligotrophic rivers in France, Luxembourg and Slovakia indicating wide distribution in European freshwaters.

## KAMCHATKA, EASTERN RUSSIA: FINDINGS FROM DIATOMS IN MID- TO LATE HOLOCENE LAKE SEDIMENTS

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The presented study comprises an investigation of fossil freshwater diatom assemblages, used as palaeoecological indicators of climate-related past terrestrial environmental changes from faunal and floral remains in lake sediments during the Holocene. A combination of taxonomical and geochemical (stable oxygen isotope composition of the siliceous diatom cell walls ( $\delta^{18}\text{O}_{\text{diatom}}$ )) methods was applied for the first time on lake-sediment records from Kamchatka, Eastern Russia.

The studied lakes were selected to cover different environmental boundary conditions. They comprised a hydrologically closed seepage lake (innominate) at a moderate elevation of about 280 m above sea level next to an open through-flow lake (Two-Yurts Lake) in central Kamchatka and an open through-flow lake at an elevation of almost 500 m above sea level (Lake Sokoch) in south Kamchatka.

The composition of the diatom assemblages differs between the three studied lakes, dependent on several parameters, such as water temperature and the geochemical character of the host water, stratification of the water column, turbulence, trophic, or the amount of available nutrients.

The closed innominate seepage lake situated on the eastern slope of the Central Kamchatka Mountain Chain reveals lowest numbers of diatoms per gram sediment (biological productivity) most likely due to its lacking inflows. Diatom assemblages document changes in palaeolimnology related to ontogenetic changes of the depositional environment.

Two-Yurts Lake reveals highest biological productivity (diatoms), due to a steady input of nutrients and diluted silica by its inflows, additionally supported by frequent occurring strong turbulences, enabling for a reworking and hence recycling of deposited nutrients/silica from the lake bottom surface sediments.

Lake Sokoch shows less intense biological productivity, most likely caused by minor nutrient and diluted silica input into the lake and a geographical setting which is less exposed to fall winds than Two-Yurts Lake is.

## DIVERSITY OF PLANKTONIC DIATOMS IN KUWAIT TERRITORIAL WATERS – ARABIAN GULF

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The diversity of the major planktonic diatoms along the coast of Kuwait has been studied for 12 years (Jan 2000-Jan 2012) and their taxonomic accounts have been given. The phytoplankton population was represented mainly by diatoms, constituting more than 90 % of the total population. Among the dominant diatom genera, blooms of *Pseudo-nitzschia seriata* and *Pseudo-nitzschia pungens* were observed every year during September. The overall dominant species, present throughout the whole year, were *Chaetoceros curvisetus*, *Ch. pseudocurvisetus*, *Rhizosolenia*.

A detailed account of 19 genera and 100 species of diatoms will be presented.

## A NEW SPECIES, A NEW COMBINATION AND RELATED SPECIES IN *HYDROSERA*.

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We found several differently shaped diatoms belonging to the genus *Hydrosera* attached to *Bostrychia simpliciuscula* Harvey ex J. Agardh (Rhodophyta) from southern islands in Japan. We observed these specimens using light microscopy and scanning electron microscopy. As a result, we decided to describe a new species, *H. stellata*, and make a new combination, *H. hexagona* (Hustedt) comb. nov.

*Hydrosera stellata* sp. nov. was collected from a freshwater stream in Iriomote-jima island, which is located in the southernmost part of Japan. Valves are five-angled, about 200 µm in diameter in valve view, and rectangular in girdle view. The cells form chains by mucilage pads secreted from pseudocelli. The valve face is flat, but bears numerous tiny projections, except in the central area. Internally, the valve has a conspicuous rimoportula with S-shaped lips, which lies near the base of one of the indentations separating the angular projections; it opens to the external surface as a slit. The pseudosepta extend across the bases of the five projections. Internally the valve surface is covered with scale-like plates with pores, which are a characteristic feature of this genus that has previously been overlooked.

*Hydrosera hexagona* (Hustedt) comb. nov. was collected from Amami-oshima island. The valves are six-angled and 90–104 µm in diameter; again, the cells form chains. The poles are rounded and bear pseudocelli and the pseudosepta extend across the bases of the six projections. In LM valves appear sometimes lack one or two pseudocelli and pseudosepta. This diversity is caused by variation in the size of the pseudocelli (sometimes very small or perhaps absent altogether) and in the degree of development of one or more of the pseudosepta (which are sometimes so undeveloped that they are only detectable in SEM as plain strips internally). A rimoportula with S-shaped lips is located near the base of one of the indentations separating the angular projections, or sometimes near the valve centre. The small area with three cavities (pores) and a short ridge characteristic of *H. triquetra* Wallich is absent in *hexagona* valves; therefore we made a new combination from *H. triquetra* var. *hexagona*. Scale-like plates were also found in this taxon.

In addition to the two taxa mentioned above, we will also discuss related species.

## PHYTOPLANKTON COMMUNITY STRUCTURE DURING BLOOMS OF *NOCTILUCA SCINTILLANS* (MACARTNEY) KOFOID & SWEZY IN SOUTH ADRIATIC OPEN SEA WATERS

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The phytoplankton community structure was investigated during a mass occurrence of the mixotrophic dinoflagellate *Noctiluca scintillans* in the open sea of the South Adriatic. This study was conducted in April 2009 at three stations (P-100, P-300, P-1200) along a transversal transect from the coast to the Southern Adriatic Pit. The abundance of *N. scintillans* was determined using a Nansen net (250  $\mu\text{m}$  mesh, 1 m in diameter and 255 cm in length). Water samples for the analysis of chlorophyll *a* concentrations and phytoplankton community were taken by 5-L Niskin bottles at standard oceanographic depths from surface to 100 m. The abundance of *N. scintillans* was exceptionally high (93 ind.  $\text{m}^{-3}$ ) in the upper 50 m and this has already been linked to hydroclimatic changes in the East Mediterranean in last two decades. At the same time, the average of the chlorophyll *a* concentration for 0-50 m layer was 0.11  $\text{mg m}^{-3}$ . The phytoplankton community was dominated by dinoflagellates and coccolithophorids. Among diatoms, only small naviculoid cells (15-25  $\mu\text{m}$ ) were prominent. Only taxa of genera *Diploneis*, *Cyclotella*, *Cocconeis*, *Navicula*, *Thalassiosira*, *Proboscia*, *Thalassionema*, *Licmophora* and *Biddulphia* were occurred.

## SAND, DINGOS AND DIATOMS

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Fraser Island, the largest sand island in the world located few kilometres off the coast of southeast Queensland, Australia is a world heritage area. Known as K'gari (paradise) by the aboriginals who inhabited the island for 5 000 years, the sand island was formed over one million years of movement of sand by the oceanic waves laid on the continental shelf. It is 122 km long and 5-25 km wide covering 160,000 ha. In spite of logging and sandmining of the bygone years, the landscape is dotted with pristine rainforests, bush lands and crystal clear lakes and streams. This paper deals with the lakes and streams in transition and the diatom flora of the Island.

Amidst the giant trees and the interplay of shifting sand, wind and water, the wandering much maligned dingos, and the ever increasing tourists, the biologists still find this a paradise of discoveries.

As part of the project, diatom flora of Australia, fresh water diatoms of Fraser Island have been investigated.

The water quality of the lakes and streams was mostly pristine with a pH ranging from 5-6 and low Electrical conductivity. The deep tannin-coloured water in the streams emerging from the rainforest moving through the reddish sandy sediment painting puzzling patterns do harbour a unique diatom flora different from the mainland of Queensland, Australia.

The crystal clear water in the freshwater lakes hides the vast abundance of diatoms

The diatoms in the lakes truly reflect the water quality. Some of the lakes are steadily encroached by shifting sand from the sea, whereas the rest of the lakes remain oligotrophic and fresh. The discovery of several interesting forms of *Frustulia*, *Eunophora*, *Eunotia*, *Surirella* and *Navicula* are discussed in the context of moving sand engulfing some lakes and increasing number of tourists. The wide variation in the distribution pattern of *Eunophora* which is found only in Poona Lake, the closest part of the mainland is discussed with ultrastructural studies.



## A NEW DIATOM INTERMEDIATE IN CHARACTERS BETWEEN *PODOSIRA* AND *HYALODISCUS* FROM A TEMPORARY FRESH WATER LAKE IN WESTERN AUSTRALIA

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Many wetlands in the Swan coastal plain Western Australia have been affected by declining rainfall in recent years. Many of the groundwater dependent wetlands have become temporary with prolonged periods of desiccation and short periods of inundation. During a palaeoecological investigation of such a lake - Yeal Lake in 2011, a diatom taxon combining the characters of the marine diatoms *Podosira* and *Hyalodiscus* were found in abundance. The objective of this paper is to describe the ultra-structural characters of this diatom.

The fresh cell is furnished with disc-shaped chloroplasts and occurs in colonies of clusters. The cell is lens-shaped with a thick cell wall, forming one of the dominant diatoms soon after inundation during the winter rains. The highly thickened cell walls display the typical striae pattern of *Hyalodiscus* in the inner surface with a well-defined central area (umbilicus) and period striae in undulate rows reaching the margin with shorter striae in between. The striate zone occupies almost half of the radius. With scattered undeveloped rimoportulae.

The external surface of the valve face and mantle is characterised by striae with tiny pores compactly arranged in criss-cross manner without any central area –somewhat similar to external surface of *podosira*. The cross section of the cell wall indicates the pattern of silicification changes halfway from regularly arranged tubular chambers to criss-cross chambers towards the external surface. While a layer of silica seems to form the central area on the internal surface. No such layer is formed on the external surface. Thus a single valve shows the structure of *Hyalodiscus* in the inner surface and that of *Podosira* on the outer surface. This diatom tends to be present in abundance in Yeal lake for the past 3000years according our palaeoecological studies. The significance of the species in the context of declining rain fall and the taxonomic status of the diatom should initiate some interesting discussion

## USE OF HAND-HELD MULTISENSOR SYSTEMS IN PHYTOPLANKTON STUDIES OF A JAPANESE LAKE

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Over the last 15 years we have studied the physico-chemical conditions of lakes, ponds, lagoons and hot springs using relatively cheap hand-held instruments, however, recently two multisensor machines have been purchased; the U-22XD (Horiba, Ltd.) and the AAQ170-RINKO (JFE ALEC Co., Ltd). The U-22XD has a 30-m cable and provides simultaneous data on 10 parameters, with a built-in memory function for data logging. In contrast, the AAQ170-RINKO relays data on 8 parameters back to a computer in real time. With its titanium external body, a 100-m cable and weighing about 2 kg, this instrument will hopefully prove to be very versatile in phytoplankton studies in water bodies that are shallow or have restricted access (e.g. marine lakes in jungles), or in coral reef areas where the water depth at drop offs can suddenly plunge to 80 m.

Recently we have tested both instruments in Itabashi-numa, a freshwater lake in Kenminno-mori, a prefectural park near Yamagata. Many of the 17 lakes and ponds in this park are believed to have been formed more than 80,000 years ago by debris flows, following the catastrophic collapse of Mount Shirataka. Itabashi-numa covers an area about 0.7 x 0.15 km, and has a maximum water depth of <10 m. In winter the surface waters are completely frozen, with several large ice cracks forming across the entire lake. Water column profiles of various parameters at a number of stations in the lake are presented, and clearly show (as expected) that in the early summer the temperature, pH and dissolved oxygen decrease with depth, whilst the turbidity increases. The AAQ170-RINKO can also be used as a towed instrument and record changes in parameters in transects across the lake surface. Several examples of these transects are presented, one across the lake width and the other traversing the entire lake length. Thus, we believe that such instruments will be useful in documenting the lake or marine environment, and in providing support data for phytoplankton studies.

Surveys of the spring net phytoplankton have shown that Itabashi-numa is normally dominated by synurophytes, notably *Mallomonas* (6 spp.) and *Synura* (2 spp.), while the planktonic diatom genera *Aulacoseira* and *Cyclotella* are also common.

## MORPHOLOGY AND ECOLOGY OF A NEW *GOMPHONEMA* SPECIES FROM WEST AND NORTH EUROPE AND RE-EXAMINATION OF *GOMPHONEMA EXILISSIMUM*

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The correct identification of taxa in the *Gomphonema parvulum* Kütz. complex is often difficult, but critical if false conclusions for pure and applied ecological and taxonomic studies are to be avoided. Two *Gomphonema* species with similar morphology but different ecology to *G. parvulum* were investigated. The type material and a recently collected population of *Gomphonema exilissimum* (Grunow) Lange-Bert. & E. Reichardt from Scotland are documented using light (LM) and scanning electron microscopy (SEM). The other species is new to science and similar in LM and SEM to *G. exilissimum*. *Gomphonema cymbelliclinum* E. Reichardt & Lange-Bert. apud Reichardt is also similar in valve outline but not closely related. In contrast to these taxa the new species appears to be typical of acidic and low alkalinity waters. It is often common and abundant in western and northern European freshwaters. Its variability is demonstrated using populations from Finland, France, Ireland, Norway, Sweden, and the U.K. Ecological data with respect to species associations and water chemistry spanning seven years of observations in a river catchment in Wales are presented for the new *Gomphonema* species.

## TELLING TIME WITH DIATOMS: CORROBORATING PHYLOGENETIC RELATIONSHIPS AND OCCURRENCE IN THE FOSSIL RECORD WITH MOLECULAR DATA

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Diatom taxa are frequently used as biostratigraphic indicators in marine and freshwater sediments. Prior to the development of molecular clock techniques, stratigraphic distribution of species was the only data set providing temporal information. Corroboration of first occurrence observations for lineages in the paleontological record could not then be independently verified other than observationally. Despite this, robust hypotheses for the stratigraphic ranges of numerous species and genera were developed. These distributions were refined and supported via stratigraphic observations of species lineages from disparate biogeographic localities. Among these are distributions for Thalassiosirales genera and species in marine and freshwater habitats. Oceanic cores have yielded data allowing the construction of a detailed species chronology for *Thalassiosira* species from the Oligocene to present. Freshwater diatomites containing Thalassiosirales species in Asia and the western North America have facilitated the development of a chronological sequence for the divergence of freshwater genera beginning in the late Miocene. The order of generic diversification in marine and freshwater Thalassiosirales lineages has largely been corroborated by phylogenetic investigations utilizing both morphological and molecular data sets. The timing of these divergences suggested by the fossil record have not, however, been the subject of scrutiny by molecular clock techniques. Recently, freshwater Thalassiosiroid genera with Miocene and Pliocene origins were reported from Eocene sediments. This observation potentially disrupts preexisting hypotheses concerning Thalassiosirales distributions in both marine and freshwater sediments. Sequence data (SSU, psbC, LSU, rbcL) is used to calculate molecular clock estimates of Thalassiosirales divergence events using calibration points from multiple stratigraphic horizons. This temporal hypothesis refutes the Eocene observations and identifies divergences events supporting the phylogenetic hypotheses for generic occurrence and proposed stratigraphies for oceanic and freshwater diatomites from Asia and western North America.

## DYNAMICS OF *CHAETOCEROS TENUISSIMUS* AND ITS VIRUSES IN HIROSHIMA BAY, JAPAN

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Since the findings of diatom viruses, it has been suggested that the viral agents are important for diatom dynamics in nature as well as traditional environmental factors, such as e.g. water temperature, salinity, light, nutrients, and water movement. Their relationships in nature, while, are not well understood, because the data based on field research are rarely accumulated. So far, over ten diatom virus species are isolated and characterized. They were divided into two groups based on genome type, single-stranded DNA and single-stranded ssRNA. Among them, CtenDNAV and CtenRNAV share a same host species, *Chaetoceros tenuissimus* Meunier (Bacillariophyceae, Centrales). In this study, we conducted a field survey to analyze the relationship between dynamics of *C. tenuissimus* and its viruses in Hiroshima Bay, Japan, from April 2010 to Dec 2011 and tried to identify the species of viruses detected during the research.

*C. tenuissimus* cell numbers were measured by real-time PCR methods, and viral abundances were determined by MPN method using two different host strains, *C. tenuissimus* strain 2-10 and 2-6. These strains have different viral susceptibilities, i.e. the former is susceptible to both CtenRNAV and CtenDNAV, but the later only to CtenDNAV. Viral isolates were established from each MPN culture plate.

Blooms of *C. tenuissimus* were observed from July to September every year and the maximum were as high as  $\sim 10^4$  cells ml<sup>-1</sup>. Specific increases of the viruses detected using the two different host strains were also observed during those bloom periods, which indicated the viruses affected the dynamics of the bloom population. Each of the viruses, however, showed individual dynamic patterns during the bloom periods. For example in 2001, the increases of the viruses detected with the host strain 2-10 (2-10 type viruses) were observed simultaneously at the temporal decreasing of the host cell numbers during the early and mid bloom periods. The viruses detected with the host strain 2-6 (2-6 type viruses) were gradually increased from mid bloom periods and reached the maximum at just before the host bloom disintegrations. Preliminary analysis revealed that the genome type of the most 2-10 type viruses seemed to be RNA and that of the 2-6 type viruses were DNA. Then, our hypothesis is as following; in early- to mid-bloom periods, the host strains susceptible to RNA viruses were dominant in the host population, and they were attacked by their infective viruses. The strains which are resistant to RNA viruses but susceptible to DNA viruses were gradually increased among the population from the mid bloom periods, which are caused the increases of the DNA viruses from mid to late bloom periods.

## FOSSILS AND DIATOM PHYLOGENY: CAN AMBIGUOUS PHYLOGENETIC RELATIONSHIPS BE RESOLVED WITH ONE KIND OF DATA?

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Two years ago we offered some comments on fossil evidence in relation to diatom classification and phylogeny (David Williams & J. Patrick Kociolek, 2010. Towards a comprehensive diatom classification and phylogeny (Bacillariophyta), *Plant Ecology and Evolution* 143 (3): 265–270, 2010, doi:10.5091/plecevo.2010.401). In this poster we extend that dialogue to some specific points, particularly those relating to the ancient basal “centric” diatoms as revealed by recently published molecular analyses. Our aim is to document lineages that have known fossil representatives and offer commentary on the relation between sampling, extinction and accuracy in determining relationship from limited amounts of evidence (molecules, in particular). Examples will be drawn from Lithodesmiales, Ardissoniales and other selected ‘araphid’ groups. We will discuss whether we can “sequence our way” to resolving ambiguous relationships of some of these groups, and what role fossil evidence may (uniquely) provide in resolving these relationships.

## THE FRESHWATER DIATOM FLORA FROM TWO CONTRASTING ANTARCTIC LOCALITIES

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It is widely accepted that climate change will have significant effects on polar ecosystems. Because of their (bio-)geographically interesting location, the study of Antarctic environments currently receives considerable attention. Diatoms (Bacillariophyta) form one of the principal algal components of the freshwater and terrestrial ecosystems of the Antarctic Region.

The current research project focuses on the taxonomy and ecological characterisation of aquatic diatom communities of two islands, located close to the Antarctic Peninsula: James Ross Island and Livingston Island. Although located close to each other, their geographical position on different sides of the Antarctic Peninsula makes them interesting localities to explore the differences in the environmental impact on their diatom communities.

The present poster discusses the results of the study of the diatom communities living in various freshwater habitats such as lakes and seepage areas in relation to ecological factors determining their composition and diversity. In this study, more than 150 diatom taxa have been identified during the survey. Until recently, most of the non-marine Antarctic diatom species were believed to have a cosmopolitan nature, mainly due to the use of non-appropriate taxonomic literature. A detailed revision based on scanning electron microscopy observations was therefore necessary to clarify the taxonomic position of most taxa resulting in the description of more than 20 new taxa such as *Eolimna jamesrossensis* Kopalová & Van de Vijver, *Luticola truncata* Kopalová & Van de Vijver and *Luticola tomsui* Kopalová (Kopalová et al. 2009, 2011)

### References:

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## FOUR NEW SMALL-CELLED NAVICULOID TAXA FROM THE MARITIME ANTARCTIC REGION

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The Maritime Antarctic Region comprises the Antarctic Peninsula and several adjacent archipelagos and islands, such as the South Shetland Islands. In 2008, a detailed taxonomic and ecological study of the limno-terrestrial diatom flora of Livingston Island (South Shetland Islands) and James Ross Island was begun to improve our understanding of the biodiversity and biogeography of the Antarctic diatom flora. The revision of several genera, such as *Pinnularia*, *Luticola* and *Hantzschia* has already resulted in the description of more than 20 new species with a restricted Antarctic distribution. During the survey, several unknown, small-celled naviculoid taxa were also found. Based on detailed scanning electron microscopy, these four taxa could not be identified using the current Antarctic literature and will be described as new to science.

*Adlafia* spA is characterized by its small dimensions, and separated from similar taxa, such as *A. muscora*, *A. suchlandtii* or *A. minuscula*, by its valve outline, which lacks subrostrate or capitate apices, and by its striation density. *Chamaeapinnularia* spA shows some resemblance to *C. australomediocris* but can be separated by the lack of a well-defined fascia, smaller valve dimensions and a more narrowly-lanceolate outline. *Mayamaea* spA is related to *M. agrestis* but has less acutely rounded apices, clearly curved striae and larger central area due to the presence of shorter central striae.

The taxonomic position of *Microcostatus* spA is rather unclear as this taxon possesses several features that conflict with its position within the genus, such as the presence of a conopeum, and striae composed of one large elongated areola. However, the structure of the raphe, axial area, valve outline and valve dimensions justify its position within *Microcostatus*.

All four new taxa are illustrated using both LM and SEM. Details of their morphology and ecology are included, and their relationships to the most similar taxa discussed.



## ASSESSMENT OF SPECIES DIVERSITY AND DISTRIBUTION OF DIATOMS IN HIGH ALTITUDE AQUATIC ECOSYSTEM OF TRANS HIMALAYA, INDIA

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We studied the species diversity and distribution of diatoms in high altitude aquatic ecosystems of the Indian Trans Himalayan region. We sampled three lakes, namely Tsomoriri (Ramsar site), Pangong Tso and Tsokar Lake, representing lentic ecosystems, and four different second to third order streams and the Indus River, representing lotic ecosystems. The sampling was carried out during the months of August-September and involved collecting periphyton diatom samples by filtering the surface water and collecting phytobenthos by scrapping the submerged boulders. Thin surface of coastal sandy layer was collected in absence of boulders. We investigated 19 physico-chemical variables to examine how species respond to a range of environmental factors. We recorded 193 species from the collected water samples from the lakes and rivers and streams. Diatom valves which could not be identified were not recorded. Our study shows that streams had the highest species richness (103 sp) compared to rivers (93 sp) and lakes (95 sp). Density of diatoms was the highest in streams and the lowest in lakes. Among the lakes, the brackish lake Tsomoriri (salinity  $0.6 \pm 0.43$  ppt) had high species richness compared to Pangong Lake (salinity  $6.4 \pm 0.15$  ppt) and Tsokar Lake (salinity  $55 \pm 2.9$  ppt).

We performed ordination analysis using canonical correspondence analysis (CCA) to explain the variation within diatom data, with four explanatory environment variables, i.e. dissolved oxygen (DO), total hardness, pH and temperature. The first two axes explained 29.5% of the variance in the species data. The diatom distribution in the lotic ecosystems was mainly driven by DO, considered to be comparatively low at high altitudes. The dominant taxa found in the lotic ecosystems were: *Achnantheidium spp*, *Gomphonema spp* and *Encyonema spp*. The dominant taxa observed in the lentic ecosystems were *Cyclotella spp* and *Cocconeis spp* in Tsomoriri Lake, *Surirella spp* in Pangong Lake and *Stauroneis spp* in Tsokar Lake. Every aquatic habitat is important and needs preservation in order to know more about ability of diatoms in harsh conditions. More in-depth studies are needed to understand the drivers controlling the diversity and distribution of diatom species in the lentic and lotic ecosystems. We raise the question whether the low biodiversity in these endorheic lakes is due to high salinity? We suggest that more emphasis needs to be laid on water chemistry and geomorphology of these aquatic ecosystems for understanding diatom diversity and distribution.

## MORPHOLOGICAL STUDY OF SIX MARINE DIATOM SPECIES RELATED TO THE GENUS *FALLACIA*

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Diatoms in the genus *Fallacia* are important components of intertidal benthic diatom flora, especially epipsammic and epipelagic species. However, because of the difficulties of isolation and observation of many small diatoms belong to the genus *Fallacia*, the taxonomy is problematic and confusing. There still more species never been described. In this study, six species related to the genus *Fallacia* were collected and isolated from intertidal and river mouth sediments around Tokyo Bay. Morphology of *Fallacia forcipata* var. *densestriata*, *F. tenera*, *Fallacia* sp.1, *Fallacia* sp.2, *Fallacia* sp.3 and *Navicula* cf. *dissipata* were observed and compared in detail. All six species possess the common characteristics of one basically H-shaped plastid, lateral sterna, porous conopeum, hooked external terminal fissures, internal central raphe ends deflated to one side, and diminutive helictoglossae located on the two terminals. The major differences among each species are of the shape and size of conopeum, connection structure extending from valve margin, the outline of valve face and the arrangement of longitudinal parts composed of areola. In addition, the central area also forms a large elliptical area occupying about 1/3~1/2 the valve width with “ghost striae”. However this feature was not stable in culture. The length of striae between hyaline lateral area and the punctate striae were also variable. The possibility of those characteristics used as the delimitations need further observation.

## IDENTIFICATION OF PROTEINS INVOLVED IN DIATOM BIO-SILICIFICATION PROCESS BASED ON THE COMPARATIVE PROTEOMIC APPROACH

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The ability of utilizing silica acid to bio-silicification is one of the most important life features of marine diatoms, as they can generate their highly ornamented silica cell walls (frustules). This ability is thought to be contributed to the diatom productivity in the oceans and the main procedure of the biogeochemical cycling of silicon. However molecular detail of this biological process is poorly understood. Therefore, unraveling the proteins involved in frustules biogenesis will facilitate our understanding of the molecular process involved in this important feature of diatom. In this study, a comparative proteomic approach was performed on the cells of a centric diatom *Thalassiosira pseudonana* and a pennate diatom *Pseudo-nitzschia multiseriata*. The different expression patterns of proteins connected with silicon metabolism were determined during the silicified cell walls formation based on the 2-DE profiling compare. Furthermore, some proteins which are involved in silica uptake and bio-silicification were identified by mass spectrometry. The possible functions of the identified proteins were discussed. The results improve our understanding of the molecular mechanism of the silicon cell wall formation, and also enhance our understanding of the important role of diatom in silicon biogeochemical cycling.

This work was supported by Fujian Province Science Fund for Distinguished University Young Scholars (Grant No. JA10001) and the National Natural Science Foundation of China (Grant No. 41076080, 40676082).

## MALDI-TOF MS ANALYSIS OF THE EXTRACELLULAR POLYSACCHARIDE RELEASED BY *THALASSIOSIRA PSEUDONANA* (BACILLARIOPHYTA)

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The extracellular polysaccharides (ECPS) released by diatoms have been proved to have significant roles in marine ecosystems and possess various potential applications. In this study, the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) technology was used in the structural analysis of ECPS released by *Thalassiosira pseudonana* (Bacillariophyta). Three different de-protein methods, sevag method, trichloroacetic acid method (TCA) and enzymolysis method, were applied to purify ECPS and compared. The results suggested that TCA was the best de-protein method among three methods for MALDI-TOF MS investigation due to its high ECPS yield, protein removal ability and reliable MALDI-TOF MS fingerprint. The degree of polymerisation (d.p. profiles), the molecular weight of the ECPS and the distribution pattern of the polymers with different molecular mass were described respectively based on the MALDI-TOF MS spectra. The work represents the whole-level conformation of ECPS released by the diatom and has improved our knowledge about structural characterization of ECPS.

This work was supported by National Natural Science Foundation of China (Grant No. 40876079)

TAXONOMIC COMMENTS ON THE VARIETY *TRYBLIONELLA MARGINULATA* VAR. *DIDYMA* WITH THE VALIDATION OF A NEW FORM AND THE EMENDATION AND TRANSFER OF ANOTHER FORM TO GENUS *TRYBLIONELLA* (BACILLARIOPHYCEAE)

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On the basis of morphological data seen under the light microscope two forms originally included within the genus *Nitzschia* and under the same variety *Nitzschia marginulata* var. *didyma* are taxonomically revised, i.e. (i) the form *Nitzschia marginulata* var. *didyma* f. *elongata* Hustedt 1921 in A. Schmidt *et al.* 1874, fig. 331/1, *nomen nudum*, is validated herein as *Tryblionella marginulata* var. *didyma* f. *elongata* (Hustedt) ex Louvrou & Economou-Amilli f. *nov.*, and the holotype and an isotype are designated, and (ii) the form *Nitzschia marginulata* var. *didyma* f. *parva* Grunow in Van Heurck 1881, 58/15, is emended and transferred to the recently reinstated (Round *et al.* 1990) genus *Tryblionella* as *Tryblionella marginulata* var. *didyma* f. *parva* (Grunow) Louvrou & Economou-Amilli *comb. nov.* Both forms have been found in periphytic marine samples of Milos Island representing new records for the Greek diatom flora.

## ATOMIC FORCE MICROSCOPY (AFM) APPLICATION TO DIATOM STUDIES: REVIEW AND PERSPECTIVES

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Scanning Probe Microscopy (SPM) is a type of microscopy that produces images of surfaces using a physical probe that scans the object. One of the noteworthy types of SPM discovered in 1986 is Atomic Force Microscopy (AFM), which can image both conductive and non-conductive samples and is currently applied in various environments (air, liquid and vacuum) and types of materials (soft samples such as polymers and biological samples and hard surfaces of inert materials). Three modes of AFM were reviewed in this work: contact, non-contact and tapping modes, but the tapping mode was highlighted as the best for biological samples, because it enables the topography of soft and adhesive trails to be imaged in their natural hydrated state.

Diatoms, unicellular microalgae that make a composite cell wall of silica and organic material, are a group of organisms that offer great opportunities for AFM studies. These algae are recognized as useful model organisms to understand biomineralization. Thus application of AFM to the study of diatoms is good to 1) study biosilica formation, 2) the ultrastructural characterization of the diatom frustule, 3) to determine the micromechanical properties of the frustule and 4) the characterization of the extracellular polymeric substances (EPS) of the frustule and their adhesion properties. All of these observations can be made at a resolution scaled in nanometers. In the present study, previous work on these 4 topics has been reviewed. From a taxonomical point of view, new data about *Amphipleura pellucida* Kützing is presented, showing topographical images of diatom frustules, putting in evidence the small pores of this taxon. They are at the limit of the light microscope's resolution. These AFM images enable the comparison of pore size and height, as well as pore-to-pore distances. Thus, without the need of sample coating, AFM allows detecting small differences in surface morphology providing new insights into diatom taxonomy and future applied technologies.

## MIMMICKING PYRITIC MINES CONDITIONS IN THE LABORATORY: $\text{Fe}/\text{SO}_4^{2-}$ CHRONIC EXPOSURE AND $\text{Cu}/\text{Zn}$ ACUTE TOXICITY TO FLUVIAL PERIPHYTON IN ACIDIC AND CONTROL CONDITIONS

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Several studies have highlighted the biofilm sensitivity to a large group of toxicants. Heavy metals are one of the most common inorganic pollutants in aquatic ecosystems. Most of the heavy metals in aquatic ecosystems (e.g. Cu, Mn, Fe, Zn) are essential micronutrients for algal growth. However, at high concentrations and at acidic pH these elements can be toxic to algae as well as to other aquatic organisms. In the present study, a system of indoor channels was colonized with fluvial biofilms to study the chronic effects of Fe,  $\text{SO}_4^{2-}$  and acidity and the contribution of chronic exposure to community tolerance to toxic metals by short-term Cu and Zn toxicity experiments.

Biofilms were subjected to four different treatments during 8 weeks: treatment 1: 1 mg Fe/L + 700 mg  $\text{SO}_4^{2-}$ /L at pH of 8.1-8.3; treatment 2: 0.01 mg Fe/L + 300 mg  $\text{SO}_4^{2-}$ /L at pH of 8.1-8.3; treatment 3: 0.01 mg Fe/L + 300 mg  $\text{SO}_4^{2-}$ /L at pH of 3.6-3.9; treatment 4: 1mg Fe/L + 700 mg  $\text{SO}_4^{2-}$ /L, at pH of 3.6-3.9. These treatments aimed to mimic the water conditions of the surrounding streams of Aljustrel mining area (Alentejo, Southwest of Portugal).

Short-term exposure of biofilms to Cu and Zn (24h exposure) took place after the indoor channel system experiment had finished, in vials containing the water of the respective treatment and increasing concentrations of Zn and Cu (100-8000  $\mu\text{g}$  Zn/L and 15-1200  $\mu\text{g}$  Cu/L). Disturbances in the ecosystem would first lead to physiological and biochemical changes within the biofilm that can evolve in community changes if perturbations maintain. To evaluate these changes, the endpoints AFDW (Ash-Free-Dry-Weight), Chl*a* (Chlorophyll *a*), diatom taxonomy, PhytoPAM (Pulse Amplitude Modulated) fluorometry parameters ( $F_0$ ,  $Y_{\text{max}}$ ,  $Y_{\text{eff}}$ ), enzymatic activities [catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and glutathione reductase (GR)] and non-enzimatic [GSH (total glutathione) and PC (total phytochelatins)] were analysed.

The main question was if pH and/or  $\text{Fe}+\text{SO}_4$  might ameliorate metal effects. Based on the results it seems that acidic pH affects CAT and GR and reduces metal toxicity in terms of low reduction in biomass ( $F_0$ ) and photosynthetic capacity ( $Y_{\text{max}}$ ) which may explain why acidic areas (as pyritic mines) with several metals in high concentrations have algal growth with typical acidic diatoms as *Pinnularia aljustrellica* found in high abundances. In addition, Fe is also affecting metal toxicity and GR. Based on  $Y_{\text{eff}}$  at the highest Cu/Zn concentration, higher inhibition is observed with Fe than without.

## THE GENUS *CRATICULA* IN IOWA WETLANDS (USA)

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This paper discusses the taxonomy, distribution, and abundance of diatoms belonging to the genus *Craticula* from Iowa wetland mitigation sites. Twelve sites created to replace wetland habitat lost to highway development were studied and compared with three natural wetland control sites in the same agricultural region. Samples were collected during April, June, August, and October 2005 from these 15 shallow alkaline hard-water wetlands that vary in age from 1 year to over 1000 years. The distribution and abundance of diatom taxa in prepared slides of these samples are being compared with taxa of other protists and micro-invertebrates observed in wet mounts at the time of collection. *Craticula* taxa occur in greater numbers (13 known and 4 undescribed) and abundance (>5% in five of 88 samples) in some of these wetlands than previously reported in the Midwestern United States. These specimens provide useful information for taxonomic re-consideration.



## DIATOM COMMUNITY DYNAMICS ACROSS ECOREGIONS IN GEORGIA, US

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Diatom community dynamics (e.g., species abundance and richness) are controlled by climate, habitat, human activities and species interactions. Few studies address primary producers' responses to multiple factors simultaneously, while accounting for species morphologies and ecological preferences. Within the U.S., the State of Georgia is divided into seven ecoregions: Southwestern Appalachian Plateau, Valley & Ridge, Blue Ridge, Piedmont, Interior Plateau, Southeastern Plains and Southern Coastal Plains. The objectives of the study were to predict water quality of aquatic habitats throughout the state based on diatom species composition and to compare algal communities between the ecoregions. 155 samples from wadeable streams and rivers were enumerated following standard protocols; in addition data from published river and stream surveys was utilized when collection and processing protocols were deemed comparable with the survey. Nutrient data was provided by the state agencies or measured in the field. Algal communities were dominated by diatoms in 97% of the sites. More than 500 diatom species were reported with varied abundances and distributions within the State. Measurable differences within community indices varied primarily due to habitat structure between ecoregions. Taxa with higher than 10% abundance occurring in at least 3 ecoregions were compared with published literature and taxonomic evaluation and ecological inferences were reported. The northern ecoregions of Georgia had low diatom species density and were dominated by *Achnantheidium minutissimum*. Piedmont communities were dominated by several *Achnantheidium* species, together with taxa from *Eunotia*, *Gomphonema*, *Frustulia* and *Pinnularia*. Rivers and streams located in the Piedmont ecoregion had the highest diatom densities, total abundances varied across sites potentially without density compensation. Taxonomic diversity of freshwater, brackish and marine diatoms was highest at the Coastal areas with higher nutrients due to anthropogenic influence, no droughts and presence of multiple habitats.

## MORPHOLOGICAL AND TAXONOMICAL STUDIES OF SMALL PANDURIFORM *DIPLONEIS* FROM MARINE IN SOUTHERN JAPAN.

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We found small panduriform-shaped diatoms belonging to the genus *Diploneis*, collected from Iriomote-jima, southern islands in Japan. We observed these specimens using light microscopy and scanning electron microscopy.

As a result, we identified several of them as *D. gravelleana* Hagerstein, and *D. interrupta* var. *caffra* Giffen (*D. caffra* Witkowski.) whereas others (*Diploneis* sp.) could at present not be identified.

*D. gravelleana* Hagerst was described from San Juan Bay in Puerto Rico by R. Hagerstein in 1938. The valves are panduriform-shaped, 16-18  $\mu\text{m}$  in length, 7-8  $\mu\text{m}$  in breadth, striae 14-15 in 10  $\mu\text{m}$ . The fine structure of the areolate striae reveals many series of areolae which are occluded by cribra. The external interstriae are smooth.

*D. interrupta* var. *caffra* Giffen (*D. caffra* Witkowski) was described from Kowie River, Port Alfred, Eastern Cape Province in South Africa by Giffen in 1970. Valves are 18-20  $\mu\text{m}$  in length, 7-8  $\mu\text{m}$  in breadth with striae 12-15 in 10  $\mu\text{m}$ . The fine structure of the areolate striae shows that they are composed of slits with foramen lips and occluded internally by cribra.

Other species with small panduriform-shaped valves could not be identified in this study. These species showed three to five series of areolae which are occluded by cribra.

In addition to the taxa mentioned above, we will also discuss some related species.

## MORPHOLOGY OF THE MARINE BENTHIC DIATOM GENUS *HYALOSIRA* KÜTZING IN JAPAN

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The genus *Hyalosira* belonging to the order Striatellales was established by Kützing (1844) and is composed of 5 species. They have been reported from various parts of the world. The main morphological features of the genus are the follows: 1) zig-zag or ribbon-like colonies, 2) numerous copulae, 3) internal costae, and 4) septa at one side of valve. But the description of this genus is indistinct because of few morphological researches until now.

In the present study, the fine structures of the frustules were observed using light and scanning electron microscopies (SEM) on *H. interrupta*, *H. tropicalis*, *Hyalosira* sp. 1 and *Hyalosira* sp. 2 which were collected from the several coasts of Japan.

These taxa can be distinguished from each other by 1) shape and size of valves, 2) length of septum and 3) density of striae. Because some species which have short or no septum are similar in girdle views, their taxonomy are confusable. However in SEM, the following fine structures seem to be very useful for the classification in this genus, 1) shape and size of areolae, 2) number of rimoportulae, and 3) row of areolae on copulae. Therefore, these taxa can be divided into two main categories. *H. interrupta* and *Hyalosira* sp. 1 have septum extending to the center of the copulae and two rimoportulae near the apices per valve. *H. tropicalis* and *Hyalosira* sp. 2 have short or no septum and a rimoportula per valve.

## MORPHOLOGICAL AND TAXONOMICAL STUDIES OF *HYDROSERA COMPRESSA* WALLICH.

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*Hydrosera compressa* Wallich was described from Gangetic Sunderbunds, 1858 by G. C. Wallich. He collected them from a soft mossy stratum upon submerged algae or tree stems. We found the species attached to *Bostrychia flagellifera* Post (Rhodophyta) from Iriomotejima (Iriomote island) in southern Japan. We observed the specimens using light microscopy and scanning electron microscopy.

The cells form short chains (two to four cells) by mucilage pads secreted from pseudocelli and attached to the thallus of *B. flagellifera*. The valves are divided into three compartments by two septa thrown across it internally. Valves have three compartments that are inflated; the central one being the largest. About 60 µm in length in valve view, and rectangular in girdle view. The valve face is flat, but bears numerous tiny projections. The valve has a conspicuous rimoportula with S-shaped lips similar to other *Hydrosera*, which lies near the center of the valve, it opens to the external surface as a slit. The pseudosepta extend across the bases of the two parts. Internally the valve surface is covered with scale-like plates with pores, which are a characteristic feature of the genus that has previously been overlooked.

We will also discuss the geographical distribution of this species in Japan and *B. flagellifera* relationships.

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## REVISITING THE TAXONOMY OF THE GENUS *LEPTOCYLINDRUS* CLEVE (BACILLARIOPHYCEAE) IN THE GULF OF NAPLES USING MORPHOLOGICAL, MOLECULAR AND PHYSIOLOGICAL APPROACHES

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*Leptocylindrus* is a radial centric diatom genus within which two species, *L. danicus* and *L. minimus*, are reported as abundant in coastal waters worldwide. In the Gulf of Naples, *L. danicus* is found all year round, with major blooms in summer, while *L. minimus* recurs in autumn. However the diversity of the genus has not been investigated in detail so far. We analysed 85 strains from the Gulf of Naples and strain CCMP 1856 from the Atlantic US coast using nuclear rDNA (18S, D1/D2 region of the 28S and ITS), the plastid 16S rDNA, the large subunit of the ribulose biphosphate carboxylase gene (*rbcL*) and the photosystem II gene, *psbC*. Morphology of the strains was investigated using light and electron microscopy. The strains grouped into five clades, suggesting the existence of five distinct species. Two of these matched the description of *L. danicus* Cleve, which typically has a subcentral pore on the valve and forms spiny resting spores following sexual reproduction. Genetically, they were nearest sisters and they showed subtle morphological differences; one of these is described as a new species. The taxon in another clade was previously reported as *L. danicus* var. *apora*, differing from the nominal species because it lacks the pore in the valve and does not form resting spores. This taxon is raised to the species status. A fourth clade consisted of a species new to science, which had distinctively convex valves. Finally, the strains in the basal clade matched the description of *L. belgicus* Meunier, so far considered as synonym of *L. minimus* Gran. However, based on marked ultrastructural differences, a new genus is erected for it. The actual *Leptocylindrus minimus* was not found in the Gulf of Naples, but its 18S sequence from Genbank differed from those of all our strains. The five *Leptocylindrus* species found in the Gulf of Naples showed different seasonal distributions, though with overlaps. *Leptocylindrus danicus* and *L. aporus* showed opposite seasonal distributions. The former occurred year round except during summer, rarely attaining high concentrations, whereas the latter was found from the summer throughout mid-autumn, producing intense blooms in summer. Physiological experiments showed that the two species responded differently to lower temperature (12°C) but had similar performances at higher temperatures (19 and 26 °C). The study demonstrates that taxa occurring year-round, i.e., under markedly different environmental conditions, may in fact consist of genetically, morphologically and physiologically distinct species.

INITIAL CELLS OF A FRESHWATER DIATOM *FRUSTULIA CRASSINERVIA* (BRÉBISSON) LANGE-BERTALOT & KRAMMER

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During an ongoing biodiversity inventory in the “Vepssky forest” Natural Park a sample of foam from a water surface was collected (Lerinskoe Lake, 60°08'03.3"N, 035°04'01.3"E). The sample contained a few *Frustulia* species, one of which, *F. crassinervia* (Brebisson) Lange-Bertalot & Krammer, was caught during sexual reproduction. Newly formed initial cells, partially covered by the perizonium, were measured. They range in size from 62 to 65 µm long and from 12 to 14 µm wide. The initial thecae, as normal, had a modified morphology. Unlike vegetative cells, they were narrow, linear to linear-lanceolate, often with wavy and uneven margins, ends were not protracted, rostrare or subrostrate. The helictoglossae were long, narrow, and linear. The proximal raphe fissures were widely separated, ranging from 6.2 to 8 µm. However, as well as vegetative cells, initial cells had central and terminal raphe ends with T-shaped fissures and striae around the terminal nodules. Since initial cells of pennate diatoms are not seen very often in nature, their descriptions from different localities maybe of interest. A curious fact about this finding is that *Frustulia* reproduced near the water surface, though it is a benthic and epiphytic species.

## MORPHOLOGICAL DIVERSITY WITHIN THE *ACHNANTHIDIUM MINUTISSIMUM* SPECIES COMPLEX

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During a survey of benthic diatoms sampled in watercourses from Mainland Portugal, several *Achnantheidium* populations morphologically ascribable to the *A. minutissimum* complex have been found. They clearly belong to the genus *Achnantheidium* on the basis of the valve outline, radiate striae, uniseriate, wider spaced striae in the centre of the valve and cells shallow-V-shaped in girdle view. The raphe valve has a straight central raphe hardly expanding at the centre and a row of elongated areolae in the mantle. Within the genus *Achnantheidium* these morphotypes belong to the complex of *A. minutissimum* (Kützing) Czarnecki, due to their straight terminal raphe fissures, in opposition to the species with terminal raphe fissures clearly deflected on the same side of the valve.

Since these individuals were abundant in numerous sites, especially in the Centre and North of Portugal, a more detailed examination was performed by means of light (LM) and scanning electron microscopy (SEM). Furthermore, the examination of the environmental characteristics of the sites where they have been sampled allowed us to gather sufficient information on the ecological preferences of the different taxa.

The main features that allow the separation of these taxa among each other and from all the other known *Achnantheidium* species are the different valve outline and dimensions, the size and shape of the central area, the apices shape and the density of the striae throughout the valve and near the apices.

The LM and SEM analyses of the type materials of *Achnantheidium microcephalum* Kützing, *Achnanthes minutissima* Kützing and *A. minutissima* var. *cryptocephala* Grunow in Van Heurck have been done in order to help to clarify the taxonomy of this group. A comparison with the literature on *Achnantheidium minutissimum* species complex was performed, but the new Portuguese *Achnantheidium* taxa present a set of distinct morphological and ecological features that separate them well from all other similar species. Furthermore, two of these taxa have already been recorded from French watercourses with similar environmental characteristics.

## USE OF DIATOM ECOLOGICAL GUILDS AS INDICATORS OF LAND USE IN HEADWATER STREAMS

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Changes in diatom assemblages in headwater streams differing in the degree of land use in Luxembourg were evaluated with respect to the applicability and sensitivity of diatom ecological guilds as an assessment tool. With the objective of simulating the effect of global warming, two pairs of headwater streams differing significantly in their watershed land use (mostly forested or agricultural) were sampled along their longitudinal gradient. In order to maximize the differences in the land use and to eliminate the influence of other environmental parameters, the selected streams had similar geology, granulometry, mineralization and catchment size and basically represented either forested unimpacted sites with no human alterations or open impacted sites without riparian vegetation. The results were evaluated in terms of diatom composition, diatom indices and diatom ecological guilds. Diatom ecological guilds were assigned to all diatom taxa reaching sufficient abundance in the sample according to their growth form: low-profile, high-profile and motile guilds.

The statistical analysis showed that unimpacted forested sites were mostly dominated by the low profile diatom guild indicating high physical disturbance and limited access to light and to nutrients whilst the diatoms at impacted sites showed much higher variability with a significant proportion of high profile or motile guilds. The ecological guilds were significantly correlated with temperature, conductivity, oxygen, phosphates, nitrites and ammonium. The low profile and motile guilds were furthermore significantly correlated with the Specific Polluosensitivity Index (IPS), which also clearly differentiated between the shaded and unshaded sites.

This trend was also confirmed by evaluating the community structure. Multivariate analyses indicated that the diatom community structure at unshaded sites was much more influenced by seasonal changes and varied along the longitudinal gradient whilst these factors appeared much less relevant at shaded sites. This is probably due to the high natural disturbance of unimpacted sites, with low light and nutrient availability that affects diatom composition regardless of season or stream order, whilst at impacted sites the physical disturbance is low due to high accessibility to resources such as nutrients and light.

These results show that ecological guilds prove useful as an additional tool to reflect changes in freshwater aquatic environments related to anthropogenic influence such as land use.



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A NEW NAVICULOID DIATOM GENUS *MORENEIS* GEN. NOV.  
(BACILLARIOPHYCEAE) FROM SAND FLATS IN KOREA

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A new diatom genus *Moreneis* has been described from the Yellow Sea sand flats on the west coast of Korea. The new genus is characterized by a unique combination of morphological characteristics including the shape of the plastids. The valve morphology resembles other genera belonging to Lyrellaceae, within which we place this genus. In terms of areolae structure *Moreneis* resembles *Petroneis* and *Placoneis*, however, it differs from both genera with respect to the raphe system and plastid shape. Cells of *Moreneis* spp. have a single large plastid appressed to the girdle of the secondary side of the valve, with two lobes extended toward the primary side of the valve. Furthermore, the unique feature of *Moreneis* frustules is the raphe, which has both external central and apical endings bent in opposite directions. Four taxa were differentiated and describe as new for science. However, based on our findings, several established species from *Navicula* should also be formally transferred to *Moreneis*, including *N. alpha* Cleve, *N. besarensis* Giffen, *N. epsilon* Cleve, *N. menaiana* Hendey, *N. polae* Heiden, and *N. quadri-undulata* F. Meister. Analysis of published data revealed that species belonging to *Moreneis* are numerous in tropical marine littoral waters, and in moderate climate zones, especially in the western Pacific, with only a few species occurring in the Mediterranean and Atlantic.

## A NULL MODEL ANALYSIS DEBUNKS WIDELY-CITED EVIDENCE OF DISPERSAL LIMITATION AMONG FRESHWATER DIATOMS

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Are diatoms ubiquitously dispersed? Or, like larger organisms, do diatoms only disperse over limited geographic extents? Unequivocal answers to these questions remain elusive, primarily because direct measurements of diatom dispersal are scarce. In 2006, Telford et al. published a study in *Science* purporting to provide indirect evidence against the controversial “ubiquitous dispersal hypothesis”. Their article has garnered widespread attention: it has been cited almost 100 times (including articles published within *Diatom Research*), and citations continue to accumulate rapidly. Here, I use a traditional null model analysis – involving a randomization procedure with the original datasets – to first show that the patterns documented by Telford et al. were expected entirely by chance, and therefore do not constitute evidence of dispersal limitation. Then, drawing upon large calibration datasets from North America and Europe, in conjunction with null model analyses of pH tolerance, I provide preliminary evidence in support of the hypothesis that taxa adapted to the most common pH environments are the least dispersal-limited.

## REGIONAL VARIATION OF DIATOM ASSEMBLAGES IN COLOMBIAN RIVERS: A FIRST APPROACH

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In tropical areas, knowledge about the autecology of diatoms and spacial variations along environmental gradients of diatom assemblages are scarce. Recent studies held in diferent regions of Colombia evidenced a high diatom diversity, comprising undescribed species, that made it difficult establishing the distribution patterns and their use in bioindication. In the country a descriptive framework was proposed to establish rivers typology, but more biological descriptors are still necessary to sustain it.

The aim of this study is to describe diatom assemblages at a regional scale in relation to the principal environmental factors based on the Colombian river typology mentioned above. As part of a research project about bioindication and water quality held by the Instituto Colombiano del Petróleo- Ecopetrol, studies were performed in a wide region of the country, analysing first macroinvertebrates. Based on these results and considering the hydrogeomorphological heterogeneity of each region, 200 sampling sites were selected. At each station, samples for physicochemical and periphyton analyses were collected. Diatoms were studied with light and scanning electron microscope. In order to establish distributional patterns of the assemblages, a NMDS and an Anosim have been applied to evaluate if there were important differences in richness and composition.

Although up to now only 100 samples were analysed, 1237 subgeneric taxa were identified from which only 22.5% could be identified. Eunotiaceae were frequent and dominant in high plain rivers and Achnanthaceae at the eastern cordillera. At the former sites prevail adnate species while at the latter prevail pioneer species. Higher values of richness and diversity were registered at interandean valleys and at foothills basins. The Anosim shows that assemblages are similar among interandean valleys and foothills, while rivers of the “Catatumbo basin” and high plains were more dissimilar. The PCA showed a group of variables that are clearly asociated to geographical characteristics, independant of those variables related to nutrients and also to those related to industrial activities.

## TAXONOMIC ACCOUNT OF NARROW-CELLED *GOMPHONEMA* SPECIES WITH WIDE AXIAL AREAS FROM EASTERN US AND CANADA RIVERS

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This study presents an account of several *Gomphonema* taxa from rivers in Eastern North America which were found difficult to identify during qualitative counts on the Light Microscope (LM). These taxa are characterized by narrow cells (width  $\leq 7\mu\text{m}$ ) with a wide axial area, short marginal striae composed of a single or double row of areolae, and the presence or absence of a stigma. They were reported by analysts as unidentified *Gomphonema* taxa (*Gomphonema* sp.) or as other similar taxa such as for example *Gomphonema entolejum* Østrup 1903. Samples used in this study were collected as part of four different projects related to water quality assessments. Three of these studies were conducted at the Academy of Natural Sciences of Drexel University (ANSP) in Philadelphia for the US Geological Survey's National Water-Quality Assessment (NAWQA), the New Jersey Department of Environmental Protection (NJ DEP), and the Virginia Department of Environmental Quality (VA DEQ). A fourth sample set was used from a study conducted by the Université du Québec à Trois-Rivières, Québec and the Canadian Museum of Nature, Ottawa, Canada. These *Gomphonema* taxa in the eastern US and Canada (Québec and Ontario) revealed four different taxa: *Gomphonema incognitum* Reichardt, Jüttner & Cox 2004, *Gomphonema stoermeri* Kociolek & Kingston 1999, *Gomphonema apuncto* Wallace 1960 and one taxon potentially new to science. Only by careful examination of ultrastructure on the SEM were we able to differentiate these taxa. This study proves once again the need for more detailed taxonomic work on the North American flora of freshwater diatoms. In particular, it shows the importance of careful taxonomic identification during Water Quality Assessments, since separation of these taxa may help provide more accurate diatom indices.

## EPIPHYTIC DIATOM COMMUNITY RESPONSE TO OCEAN ACIDIFICATION

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Increasing pCO<sub>2</sub> in the atmosphere due to human activity is causing the acidification of the oceans during this century. Perspectives based on mathematical models announced a surface pH lowering of about 0.4 units. For this reason it's fundamental to improve our understanding on marine organism's response to such global change as well as on the long-term effects on marine ecosystems, now still unpredictable. The "Castello" vent site (Ischia Island, Italy) can be considered a natural laboratory to simulate future scenarios of ocean acidification.

In coastal benthic communities, more sensitive to anthropic pressure, *Posidonia oceanica* (L.) Delile assemblages play a great structural and functional role. Diatom community gives a great contribution to the epiphytic biodiversity on *P. oceanica* leaves and changes in their abundance may cause important modifications to the entire food web. Recent researches evidenced different effects of acidification on vegetated communities, suggesting that increasing pCO<sub>2</sub> do not favours all the photosynthetic organisms. Furthermore, acidification combined with other factors such as eutrophication and temperature rising, may cause a significant decrease in abundance and algal diversity. Up to now no studies have been carried out on diatom community response to ocean acidification, inducing an important gap in the knowledge on coastal ecosystem response to this environmental emergency.

We present preliminary results of a two-year study on abundance and diversity changes of diatom community on *P. oceanica* leaves along seasonal and pH gradients at Castello vent site. Adult and intermediate leaves of *P. oceanica* have been bimonthly collected starting from August 2011 and three portions of each leaf blade (i.e. apex, mid and base) have been considered to assess epiphytic diatom community. More than 22 diatom genera have been identified using Scanning Electron Microscopy (SEM) and *Cocconeis* was the dominant genus. Cell density followed the leaves age gradient with higher abundance on the apical portion of the blade especially on the intermediate leaves. Qualitative and quantitative changes in diatom composition have been detected in samples collected along the pH gradient. The encrusting *Cocconeis* genus remained the dominant one but with a lower specie number and a marked reduction of cell density at lower pH environments. Erected (*Lichmophora*, *Gomphonemopsis*) and adnate genera (i.e. *Amphora*, *Mastogloia*) strongly decreased their abundances in correlation to disappearance of calcareous macroalgal turfs. Conversely motile forms seemed to slightly increase their occurrence in the acidified diatom community, especially in terms of species diversity.

## DIATOMS OF NORTH-CENTRAL PENNSYLVANIA

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The initial goal of this study was to inventory the diatom flora associated with the least impaired waters in the State of Pennsylvania, USA. Although the North-Central region has been impacted by logging and mining, the development has been minimal compared to most of the State. Many watersheds and water bodies have been restored to a state similar to their natural condition. This region has the lowest population density and the largest proportion of forested lands in Pennsylvania. 310 diatom samples were collected from 140 sites in streams, lakes, and wetlands in 2009. From these samples we identified 622 diatom taxa including at least five species new for science and several rare, and possibly endemic species. In comparison with other regions of the United States, the native diatom flora of North-Central Pennsylvania is characteristic of low-nutrient, soft waters. The most common diatom species in lake plankton are *Asterionella ralfsii* var. *americana* Körner, *Tabellaria quadrisepitata* Knudson, and *Eunotia romanowii* Siver. Benthic diatom communities are characterized by the high diversity and abundance of *Eunotia*, *Tabellaria*, and *Pinnularia*. Aerophytic diatoms include rear and new species of the genera *Nupela* and *Diadesmis*. The current rapid increase of gas well drilling in the region is expected to stress aquatic ecosystems by decreasing their overall water volume and by increasing amounts of inputs of dissolved solids from wastewater runoff. We are continuing to monitor diatom assemblages in the region using the data obtained in 2009 as baseline for assessing environmental effects of the drilling and other human impacts.

## DIATOM COMMUNITIES FROM A EUTROPHIC RESERVOIR IN SOUTH INDIA

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Krishnagiri reservoir, which is highly utilized for agriculture and domestic purposes, located 7 km (4.3 mi) from Krishnagiri in Tamil Nadu India between Dharmapuri and Krishnagiri. The reservoir is operational from the year 1958 onwards. The hydrobiological resources of this reservoir are studied for a decade, depicting the % occurrence of phyto and zooplankton from various locations and depths. Diatoms serve as a possible bioindicator to detect the past and the present changes that occur in any aquatic ecosystem. Being the dominant primary producers, they play an important role in carbon silica and nutrient budget. In order to create a database on planktonic resources from Krishnagiri reservoir, a detailed study on the diatom population was initiated first. With this background, diatoms were collected from the reservoir sediment cores including upper core fractions (up to 5 cm from the surface layer of sediment) and bottom core fractions (from 6 cm to 15 cm). Intact and frustules/valves from the core sediment samples showed both Pennate and Centric forms. The diatom population and its valve structure present in the sediment cores reported that there were 48 species observed in the upper sediment fraction and 49 species in the bottom sediment fraction. Over all, 24 genera belong to the pennate types and two genera of centric types were found common to both bottom and upper sediment fractions. The SEM studies carried out for a few genera to confirm their taxonomical position, revealed that the genus *Cymbella tumida* (pennate) exhibited cylindrical shape cells with circular valves. Margins were clearly distinct from the central part and have radial striae of 6-10, each. Central area is unarranged. The cells are free floating in water. *Navicula protracta* (Pennate) linear to elliptical valves with broad rostrate and blunt apices. Striae in the center are slightly more spaced than the axial area and finely punctate. In *Nitzschia amphibia* (Pennate) both striae and raphe can be seen under the light microscope. Valve margins had tiny pimple like thick dots formed by raphe endings. The length is 12-50  $\mu\text{m}$  and width would be 4-6  $\mu\text{m}$ . Frustules of the species are bilaterally symmetrical and have a nitzschoid symmetry, valves are linear and have blunt end. *Synedra ulna* (Pennate) cells are linear and valves are narrow with a blunt end. The central area has visible ghost striae, and a roughly square area in outline which is extending to the valve margin. Commonly growing as a free floating species in the water, it also is found on rocks. It has a length of 50 - 250  $\mu\text{m}$  and a width of 2 - 9  $\mu\text{m}$ . *Cyclotella meneghiniana* (Centric) has cylindrical shape cells and circular valves. Margins are clearly distinct from the central part and have 6-10 radial striae each. The central area is unarranged. The cells are free floating in water. The results indicated that diatoms do not depend only on nutrient distribution but depend also on an array of physical parameters. This study is useful further to study the paleolimnological aspect of the reservoir using diatoms as the potential candidate.

## ECOLOGICAL REGIME SHIFTS IN LAKE KÄLK SJÖN, SWEDEN, IN RESPONSE TO ABRUPT CLIMATE CHANGE AROUND THE 8.2 KA COOLING EVENT

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A detailed diatom record from Lake Kälksjön, west-central Sweden, reveals two periods of abrupt ecological change correlative with the 8.2 ka cooling event. Using a combination of abrupt step changes and piece-wise linear regressions, the diatom data were analysed for change points over time, and two sudden and large events that can be described as regime shifts were detected. During the first event at c. 8040 cal. yr BP, a doubling in diatom biomass took place over 5-10 years time. This increase in primary productivity can be connected to a major erosion event in the catchment that resulted in an abrupt increase in nutrient supply to the lake. The second event was characterised by a substantial shift within the planktonic diatom community from taxa indicative of colder conditions to those indicating warm over 5-10 years at c. 7850 cal. yr BP. This event was superimposed on a successive change from periphytic to planktonic diatom dominance over a 250-year period and a gradual diversification of the periphytic community that spanned c. 150 years. Rapid climate warming following the 8.2 ka event likely caused these changes. Both observed regime shifts are examples of abrupt ecological change that is externally driven. In the first case, nutrient input was the primary causative agent, and in the second case climate change is the cause of the more complex shift.

This study demonstrates that it is possible to detect, quantify and test for regime shifts in paleoecological data, and it also highlights the need for high sampling resolution and precise chronological control. High-resolution paleoecological reconstructions of ecological regime shifts in response to climate change can provide useful analogues of future changes in ecosystem structure and functioning with impending climate change.



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**ASTARTIELLA SOCIETATIS SP. NOV. (BACILLARIOPHYTA) ON HOLOTHURIA ATRA  
JAEGER (TAHITI ISLAND, SOUTH PACIFIC) AND OTHER ASTARTIELLA FROM  
INDO-PACIFIC BASIN**

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Several taxa of *Achnanthes* Bory (Bacillariophyta) bearing one stigma to several stigmata on their raphe valve were recently transferred to *Astartiella* Witkowski, Lange-Bertalot & Metzeltin (in Moser et al. 1998). During a survey of the diatom community associated with coral reefs of Tahiti Island (17° 52' S-149° 56' W, Society Archipelago, South Pacific), a few taxa pertaining to *Astartiella* were found on a sample scraped from the teguments of a large specimen of *Holothuria atra* Jaeger, upon which a new and small taxon, *Astartiella societatis* sp. nov. is described. Some other *Astartiella* taxa were also found on nearby marine sediments. We compare the *Astartiella* assemblage from Tahiti Island with those found in similar latitudes of the Indian Ocean (Juan de Nova, Scattered Islands, 17° 03' S-42° 43' E and Mascarene Archipelago, 21° 30' S-55° 30' E). So far, *Astartiella societatis* has been only found in Tahiti. This taxon is close to *Astartiella bremeyeri* (Hustedt) Witkowski et al. but shows several unique features. *Astartiella societatis* is common on the epizoic sample while it is absent on nearby marine and freshwater sediments. We also propose the transfer of *Achnanthes heterostriata* Hustedt to *Astartiella*, since this taxon was shown (Simonsen 1987) to also possess a stigma.

## EXTRACTING DIATOMS FROM INTERTIDAL SEDIMENTS: A COMPARISON OF DIFFERENT METHODS

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Diatom sampling in intertidal coastal and estuarine sediments has always been challenging. Some approaches, like the coverslip and lens-tissue (LT) methods, elegantly use the endogenous migratory rhythms to gather motile diatoms when they surface during low tide. However, these techniques are forcefully selective, as they mainly collect the epipelagic fraction of the diatom assemblages. Contrariwise, direct oxidation of sediment samples is not selective, even to dead and empty frustules, but can be labour-intensive and time-consuming. Finally, the isopycnic separation techniques rely on the differences in density between the sediment and organic matter to separate them, using silica sols (e.g. Ludox®) or others (e.g. nontoxic chemical sodium polytungstate, SPT). They seek a compromise between a satisfactory separation of the diatoms from the different sediment particles (i.e. sands, silts and clays) whilst collecting a representative portion of the diatom assemblages. However, their effectiveness, particularly in sandy sediments, is still subject to debate.

The present study aims to directly compare different extraction methods and determine their effect on the taxonomic structure and diversity of diatom assemblages. Two different sets of samples were studied: 1) Diatom samples extracted using LT and Ludox collected in the Tagus estuary (Portugal), during a two-year survey carried out in 6 stations with different sediment textures; and 2) Diatom samples extracted using three different methods (LT, Ludox and SPT) from freshly collected sandy and muddy sediments from Bourgneuf Bay (France). For both sample sets the correlation between species-abundances matrices was statistically tested in order to determine the level of concordance in taxonomic structure of the assemblages collected by different methods. Their performance in several non-parametric multivariate routines, namely the linkage between biotic and environmental matrices, was further explored in the Tagus LT and Ludox sample set. The Bourgneuf Bay SPT samples also allowed assessing the physiological conditions of diatom cells after extraction, using fluorescence measurements. The SPT method has been rarely used to extract intertidal living diatom material. This preliminary test should allow the development of a simpler way to estimate productivity of natural diatom biofilms in controlled conditions, in particular, in the case of difficult-to-sample epipsammon-dominated communities.

## DIATOM BIODIVERSITY AND DISTRIBUTION IN VOLCANIC LAKES OF NORTHEASTERN CHINA

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The surface-sediments from 36 lakes of Northeastern China have been collected using light coring equipment for sampling the sub-fossil diatom assemblages. Among these 36 lakes, 13 are located in the Long Gang area, 3 in the Changbaishan area (Jilin Province), 5 in the Wudalianchi area (Heilongjiang Province) and 15 in the Aershan and Chaihe areas (Inner Mongolia). Most of these lakes have a volcanic origin (maar, crater or dam formed by a volcanic coulee) although 5 are artificial (reservoirs). These lakes span a wide range of physical characteristics, especially water depth (from 0.3 to 127 m).

In parallel with surface sediments, water samples were collected and analyzed for pH, Electrical Conductivity, total alkalinity, total phosphorus, total nitrogen, DOC, Cl, SO<sub>4</sub>, NO<sub>3</sub>, Ca, Mg, K, Na and dissolved silica. These water samples show wide ranges especially in terms of EC (14 to 425  $\mu$ S/cm), TP (1 to 340  $\mu$ g/l), pH (5.3 to 8.1) and DOC (1.3 to 24.2 mg/L).

Altogether 435 diatom taxa were identified. Light and SEM-photographs have been taken for most of these taxa. The most diverse genera are *Eunotia*, *Fragilaria*, *Gomphonema*, *Navicula*, *Nitzschia*, *Pinnularia*, *Planothidium*, *Sellaphora*, *Stauroneis*, *Staurosira* and *Staurosirella*.

Their relationship between the diatom species and the measured environmental variables was examined using multivariate statistical methods. Canonical correspondence analysis (CCA) with forward selection and Monte Carlo permutation tests identified EC, maximum water depth, DOC and TP as significant environmental controls over the composition of diatom assemblages in this dataset.

## CHANGES IN EPIPHYTIC DIATOM (BACILLARIOPHYCEAE) COMMUNITY COMPOSITION AND STRUCTURE IN AN EUTROPHIC POND (ALALAY POND, COCHABAMBA, BOLIVIA)

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Alalay Pond is a 230-hectare, shallow aquatic ecosystem located within the city of Cochabamba (17°23'43" S, 66°09'35" W), the third largest city in Bolivia. The pond presents extensive growths of emergent macrophytes, and it is highly eutrophic and contaminated due to direct sewage, clothing and plastic factory discharge, as well as surface runoff carrying garbage. With the aim to determine the suitability of epiphytic diatoms as monitoring tools of the ecological status of this ecosystem, their communities developing on *Schoenoplectus californicus* subsp. *tatora* (Kunth) T. Koyama and *Typha domingensis* Pers. were sampled 4 times from three stations in the pelagic zone (away from coastal macrophyte growths) in the period March-September, 2011. Submersed stems of each macrophyte were cut and stored in plastic bags. In the laboratory epiphyte diatoms were brushed off and oxidized for light and scanning electron microscopy observations. Shannon-Wiener and Pielou indexes showed changes in community structure and composition overtime at the three sampling stations. Dendrograms based on species abundance show marked differences among sampling stations. Multivariate analyses suggest that changes in community structure were concurrent with changes in alkalinity, COD, sulfates, ammonium-nitrogen, conductivity, and dissolved oxygen. All these abiotic and biotic changes were related to anthropogenic pollution affecting each station. Regarding composition, 39 taxa were identified, of which *Epithemia adnata* var. *proboscidea* (Kützing) Hendey, *Amphora paraveneta* Lange-Bert. et al., *Gomphonema* sp. 2 ALALAY, *Synedra tabulata* var. *gracillima* Tempère & Peragallo, *Nitzschia* sp. 1 ROCHA, *Lemnicola hungarica* (Grunow) Round & Basson and *Gomphonema* sp. 1 ALALAY were dominant. The remaining 32 taxa had abundances lower than 5%, and, together with the dominant taxa, constantly fluctuated in their relative numbers at each station.

## DIATOMS: AN ALTERNATIVE SOURCE OF OMEGA 3 FATTY ACIDS?

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Numerous epidemiological, animal and clinical studies have shown that the long chain omega 3 polyunsaturated fatty acids (LC-PUFA) EPA and DHA are effective in preventing or treating several diseases, such as cardiovascular disorders and cancers, and that they play a role in brain and nerve development of growing foetuses and infants. However, in many Western countries, the current average intake of these LC-PUFA is below the recommended level. This raises interest in food supplements containing LC-PUFA on the one hand and foodstuffs enriched with LC-PUFA on the other hand. Currently, the main commercial source of LC-PUFA is fish oil. However, several problems are associated with this oil: unpleasant odor, contamination with heavy metals, presence of cholesterol, geographical and seasonal variation in quality, as well as increasingly stringent regulation of fisheries. The aim of this research is to investigate the possibilities of diatoms as an alternative source of these LC-PUFA. The composition of the algal oil (from *Phaeodactylum* and *Thalassiosira*) in comparison with already commercially available sources and oil from other microalgal species (e.g. *Nannochloropsis*) will be discussed. Attention will be paid to the form in which the LC-PUFA are present and the presence of other nutritionally interesting compounds in the oil such as phytosterols and carotenoids.

## DIATOM SUCCESSION IN BOTTOM AND PLATELET ICE IN A COASTAL AREA OF TERRA NOVA BAY, ROSS SEA, ANTARCTICA.

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Pack ice is one of the largest ecosystems on the earth and is considered to be an extreme and highly variable environment. It is typically inhabited by diatoms that grow both within and underneath the sea ice thickness.

During austral spring 1999, we investigated the variations of total phytoplankton biomass and the diatom succession by sampling the bottom and platelet ice every 3 days for three weeks at a station in Terra Nova Bay, Ross Sea (Antarctica).

At the beginning of the sampling, in the bottom-ice chlorophyll *a* (Chl*a*) was 1.30 µg/l, while diatom abundance was  $2.20 \times 10^6$  cells l<sup>-1</sup>. In the platelet ice, biomass was 90.00 µg l<sup>-1</sup> Chl*a* while cell abundance was  $1.20 \times 10^7$  cells l<sup>-1</sup>. Planktonic species (*Fragilariopsis* spp.) dominated in both ice types, while benthic species (e.g. *Berkeleya adeliensis*, *Amphiprora kufferathii*, *Navicula* spp, *Nitzschia* spp.) represented ca. 40 and 35 % of the assemblages in the bottom and platelet-ice layers, respectively.

Over the days biomass values increased up to 432 µg l<sup>-1</sup> Chl*a* and the benthic species *Amphiprora kufferathii* and *Nitzschia* spp. became dominant (< up to 72% ,  $2.6 \times 10^7$  cells l<sup>-1</sup>) in the bottom-ice. In the platelet ice, Chl*a* increased up 382 µg l<sup>-1</sup> and *Fragilariopsis* spp. represented the bulk of the diatom biomass (79%), with *F. cylindrus* as the most abundant species ( $6.6 \times 10^7$  cells l<sup>-1</sup>). The benthic species also increased (<  $2.1 \times 10^7$  cells l<sup>-1</sup>), but their relative contribution decrease down to 21 %.

In conclusion, diatom assemblages showed a different temporal pattern in the bottom and platelet ice, probably in relation with changes in the structure of the two ice types. While the planktonic species could have been trapped in the ice in the previous season and eventually seed microalgal blooms in the water column during the ice melting, the origin and the role of the benthic ice-diatoms still needs to be clarified.

## THE EXPANTION OF THE INVASIVE DIATOM *DIDYMOSPHENIA GEMINATA* IN PATAGONIA, ARGENTINA.

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*Didymosphenia geminata* was registered in Argentina for the first time in August 2010, a few months after it was reported in Chile. The species was observed forming massive proliferations in Futaleufú River, Chubut Province (43°10'44.9"S/71°39'7.7"W). This river belongs to a patagonian Andean basin the same where the species proliferated in Chile. The presence of the species in pristine patagonic waters generated a great concern at the academic field, at the environmental management agencies and also tourism and fisheries institutions. Among other national and regional institutions, the authority encharged of the management of the watersheds of the rivers Limay, Negro and Neuquen–AIC– proposed and coordinated a monitoring program for the early detection of the species in other areas at risk.

In this framework the sampling and the laboratory procedures protocols were elaborated for the analysis of samples considering international recommendations adapted to local necessities. Besides, a sampling program that covered 48 sites from Neuquén and Río Negro Provinces was held. Each station was visited in fall and spring 2011, plankton and periphyton samples were collected and physical, chemical and hydrological data releaved. Samples were concentrated in the field and laboratory and at least ten slides were analysed. Fall sampling gave negative results in all sites but during the spring the species was registered in plankton and perifyton samples at the rivers Chimehuín (40°4'21.3"S/71°3'8.8"W) and Collón Cura (40°3'37.8"S/70°48'54"W) at the northern sector of the study area, far away from the site where the species is installed since 2010. These results evidence the rapid expansion of the species and the vulnerability of patagonian rivers and allowed to intensify the control in the affected area and to perform the actions to minimize the dispersal of the species by fishermen and tourists. Nevertheless, the absence of the species at the intermediate sampling stations was striking and raised the question about the dispersal agents and mechanisms that will be further analysed.

## VALVE MORPHOLOGY OF *DIDYMOSPHENIA GEMINATA* IN PATAGONIA, ARGENTINA.

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The presence of *Didymosphenia geminata* in South America was registered in 1964 in Chile, although this mention was unnoticed when the species was reported as nuisance in other regions of the world. In August 2010 the species was registered in Argentina for the first time a few months after masive proliferations of the species were reported in Chile. The species was found in Futaleufú River, Chubut Province (43°10'44.9"S/71°39'7.7"W), that belongs to a patagonian Andean basin shared by Chile and Argentina.

The early mention of the species in South America made us wonder if it is the same species that was transported through the world by fisherman or if the species was in our rivers but in low densities to be registered. It is important to note that up to now patagonian rivers were not exhaustively studied.

To elucidate this problem we first analysed with light (LM) and scanning electron microscope (SEM) specimens from the population living in the Futaleufú River, compare it with other *Didymosphenia* species and with the materials from Chile.

Periphyton samples were collected in september 2010 and April 2011 and analysed with an Olympus CX31 with a phase contrast LM and a JEOL JSM-6360LV SEM.

This analysis confirms that the specimens collected in Argentina correspond to *Didymosphenia geminata*. The comparison with materials from Chile, show that our specimens are larger but have a lesser length/width relationship and also have diferences in ultrastructural details –e.g., the terminal raphe fissures. Considering that the compared populations live in distant places but in the same basin and are contemporary it is most likely to think that they correspond to two diferent morphotypes adapted to their habitats than recently transported from Chile to Argentina. To reinforce or rejet this idea and to compare Chubut population with others around the world, molecular studies will be held.



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## DIATOMS FROM MARINE COASTAL ENVIRONMENTS OF ARGENTINA. SPECIES OF THE GENUS *PLEUROSIGMA* (PLEUROSIGMATACEAE)

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This study focuses on the analysis of the morphology, taxonomy and distribution of species belonging to the diatom genus *Pleurosigma* W. Smith found in marine coastal waters of Argentina. Net samples were collected from the surface layer of the water column at several coastal stations in Buenos Aires and Río Negro Provinces. Unprocessed and cleaned materials were examined by light and scanning electron microscopy. Species identified were *Pleurosigma decorum* W. Smith, *P. distinguendum* Hustedt, *P. exemptum* Mann, *P. inflatum* Shadbolt, *P. inscriptura* M. A. Harper, *P. normanii* Ralfs and *P. simonsenii* Hasle. Two new taxa are described. *Pleurosigma patagonicum* var. *paucistriatum* nov. var. is compared with type material of *Pleurosigma patagonicum* (Ferrario & Sar) Sterrenburg & Sar var. *patagonicum* and *P. perlucidum* nov. spec. is compared with *P. elongatum* W. Smith, *P. delicatulum* W. Smith, *P. amara* Stidolph and *P. brunii* H. Peragallo based on type materials and/or protologues. New observations on *Pleurosigma decorum*, *P. distinguendum*, *P. inflatum*, *P. inscriptura* and *P. normanii* are included and differences from related species are discussed. *P. inflatum* is recorded for the first time from Argentina and *Pleurosigma distinguendum* and *P. inscriptura* are recorded for first time from the South Atlantic Ocean.

## A REEVALUATION OF GENERA WITHIN THE RHAPHONEIDALES POSSESSING EXTRA-APICAL RIMOPORTULAE.

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The diatom order Raphoneidales Round et al. (1990) is described as: “*Cellulae solitariae vel catenas breves facientes. Valvae circulars vel bipolares vel multipolares. Areolae simplices rotis oclusae. Rimoportulae plerumque as apices, raro circum peripheriam positae. Areae apicales porellorum praesentes sed indistinctae. Copulae plures apertae. Plantae solum maris habitants*”.

This description indicates both broad morphological plasticity and a limited number of unique characters. Despite the extreme morphological variance, it is clear that this does represent a natural grouping of araphid coastal marine diatoms that in several respects defy the centric-pennate dichotomy. Its morphologic diversity in part defines its uniqueness as a group, with high generic diversity and generally low diversity at the species level. *Raphoneis* displays moderate diversity at the genus level, but many genera include but a few known species, despite a long fossil record.

An attempt to categorize morphologic characters of the genera within the Raphoneidales using a cladistic approach, including modern and fossil taxa, resulted in a complex matrix of potential classifications with a distinct lack of parsimony, calling for further character examination. One of the most reliable characters to distinguish genera appears to be the fine structure of the vellum, only visible via SEM.

One of the key morphologic features that appear to make a small but distinct subgroup within the Raphoneidales is the occurrence of marginal rimoportulae outside of an apical area. Known examples include *Diplomenora* (Blaze, 1984) and *Adoneis* (Andrews and Rivera, 1987). An examination of original Janisch (1862) slides identified as *Cocconeis superba* (later transferred to *Raphoneis superba* by Grunow, 1862) from Anagamos guano, northern Chile, is found to include numerous extra-apical rimoportulae. Frenguelli (1949) erected *Detonia superba* based on examination of material from the Mejillones area, though it is unclear whether the material was directly comparable with that examined by Janisch. Frenguelli does not indicate extra-apical processes in his description or illustration. The reexamination of Janisch material calls for a redefinition of *Detonia*, and may call into question the validity of the genus *Diplomenora*.

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## LIGHT-INDUCED GERMINATION OF RESTING SPORES IN A COASTAL DIATOM *LEPTOCYLINDRUS DANICUS*

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Photophysiological and pharmacological approaches were used to examine light-induced germination of resting spores in the red tide diatom *Leptocylindrus danicus*. The equal-quantum action spectrum for photogermination had peaks at about 440 nm (blue light) and 680 nm (red light), which matched the absorption spectrum of the resting spore chloroplast, as well as photosynthetic action spectra reported for other diatoms. DCMU, an inhibitor of photosynthetic electron flow near photosystem II, completely blocked photogermination. These results suggest that the photosynthetic system is involved in the photoreception process of light-induced germination. Results of pharmacological studies of the downstream signal transduction pathway suggested that Ca<sup>2+</sup> influx is the closest downstream neighbor, followed by steps involving calmodulin, nitric oxide synthase, guanylyl cyclase, protein-tyrosine-phosphatase, protein kinase C and actin polymerization and translation.

## HUMAN IMPACT RECORDED IN THE THREE ALPINE LAKES (THE TATRA MOUNTAINS, POLAND): RECENT ENVIRONMENTAL CHANGES BASED ON SUBFOSSIL DIATOMS AND CLADOCERA REMAINS.

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The high mountainous regions of Europe are relatively natural ecosystems; however, there are certain signs of human-induced disturbances that have occurred over the last century. Most lakes in the Tatra Mts. are oligotrophic with water that is cold, well-oxygenated and poor in nutrients. Recently, in certain lakes, an increase in the trophic state was observed. One of the main reasons for the trophic increase in the mountain areas is tourism development; the number of visitors, the location and exploitation of mountain hostels by tourists are all of potential importance. We studied sediments from three lakes: the Morskie Oko (MOK), the Przedni Staw Polski (PSP) and the Czarny Staw Gąsienicowy (CSG), with respect to changes in diatom and Cladocera species composition, because both groups are good indicators of higher productivity in lakes. Year-round shelters exist on the shores of the MOK and the PSP lakes; the CSG Lake was selected as a reference site. Over the last few decades, significant alterations in trophic status, i.e., changes from oligotrophy to mesotrophy, were observed in the lakes located close to the shelters. The lake situated a greater distance from the mountain hostels has been oligotrophic for hundreds of year and is still so today. The recorded trends depict a clear correlation of changes in diatom flora with the increase of tourism in the region over the last few decades, including the following: (1) Diatom-inferred total phosphorus (DI-TP) increased markedly in the lakes with mountain hostels build on their shores; (2) DI-TP decreased in the third lake, and the only symptom of higher productivity in the lake was an increase in the total organic carbon. The cladoceran communities did not present such a clear relationship to the trophic state of the studied lakes. The major factor driving changes in the zooplankton composition of the lakes appears to be fish predation pressure. The chydorid and bosmina resting eggs (ephippia) index did not indicate a correlation with changes in the trophic state; however, the index did indicate a correlation with climate oscillations – the highest production of ephippia is found in sediments deposited during the Little Ice Age.

## DIVERSITY OF PLANKTONIC DIATOM ASSEMBLAGES IN RELATION TO SEWAGE INFLOW IN THE CENTRAL ADRIATIC SEA

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Coastal marine ecosystems are becoming increasingly affected by nutrient loadings from a variety of anthropogenic sources, including domestic wastes and agricultural runoff. The field survey in the central Adriatic Sea that is influenced by sewage inflow was performed through eight year period from 2002 to 2010. Phytoplankton was analyzed from 670 samples taken at six sampling points at different depths. The present study aims to assess the diatom abundances and community structure in relation to season, year and depth with special emphasis on responses of diatoms in two main conditions (before and after activation of water sewage input).

Through the investigation period total of 80 diatom taxa were identified. The majority of diatoms belonged to Centrales group (61) out of which the most common is genus *Chaetoceros*. Among Pennatae diatoms 19 taxa were recorded with *Pseudonitzschia* as most frequent.

Statistical analysis showed differences in diatom communities before and after sewage activation.

## MORPHOLOGICAL VARIATIONS IN *PLIOCAENICUS* AND RELATED THALASSIOSIRACEAE DURING THE LATE PLIOCENE AND PLEISTOCENE IN LAKE EL'GYGYTGYN, CHUKOTKA, NORTHEAST RUSSIA

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In 2009, the 3.6 million-year-old impact crater Lake El'gygytgyn was drilled (ICDP site 5011), and studies of the sediment record are ongoing. Diatom light microscope slides have been prepared and analyzed from systematic down-core sediment samples, and SEM analyses have been completed at selected intervals. Planktonic diatoms are abundant through most of the sediment record. *Pliocaenicus seczkiniae* Stachura-Suchoples, Genkal et Khursevich, has previously only been reported from the modern lake and in previously recovered sediment core records to about 15 ka. This taxon has persisted in the lake since 2.1 Ma with variation in morphology and ranging from the dominant taxon to sporadic occurrence in the diatom assemblage. From 1.2 to 3.5 Ma substantially different *Pliocaenicus* and related Thalassiosiraceae occur and, together with *P. seczkiniae*, dominate the planktonic assemblage. These taxa show extensive morphological variations through time including valve shape and diameter, marginal costae and areolae measures, valve face relief and ornamentation, rimaportula structure and position, valve face fultoportulae position and external openings, and development and structure of the alveolae. Major shifts in these taxa appear to be driven by climatic events inferred from the sediment record.

## THE CENTRIC DIATOMS OF TÜRKMEN MOUNTAIN (TURKEY)

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In Turkey, pennate diatoms are the most frequently reported group, while reports of centrics is fewer. Also, there is little information about centric diatoms for springs. The aim of this study was to reveal centric diatoms of the Türkmen Mountain where belongs to the Sakarya river basin. In large cities administrated by metropolitan municipal authorities, domestic wastewater is treated before being released into nearby streams but water treatment facilities are not installed or are out of use in many smaller towns. However, the mountain is one of the best preserved from anthropogenic changes areas in the basin. The localities are situated at the altitude of 954-1710 m a.s.l., and represent typical rheocrene springs and small streams of the area. Some stations (Türkmenbaba, Güllüdere, İni, Çobanlar stations) represent natural conditions, whereas the others (Darıpınar, Dümbüldek, Güllüdere-Türkmenbaba, Lütfiye-Güllüdere) have been eutrophicated by agricultural and antropogenic activities. In this study, the samples were collected from epilithic, epiphytic and epipelagic habitats in 10 springs and 5 small streams in the mountain between May and December 2007. As a result, seven centric diatoms were totally revealed belonging to species with widespread distribution in Holarctic. Because of new taxonomical concept for invalid genus *Puncticulata* Håkansson, we suggested a new combination for *Cyclotella balatonis* Pantocsek as *Handmannia balatonis* comb. nov. According to the concept, we also suggested a new combination for another species from this group of species *Handmannia praetermissa* (Lund) comb. nov. in this study. Another result of this study, *Handmannia balatonis* comb. nov. is interestingly new record for Turkey according to the last checklist about Turkish freshwater diatoms and among the species, *Cyclotella meneghiniana* and *C. ocellata* was common diatoms while the other species were very rare in Turkish freshwaters. Also, according to the checklist, *Stephanodiscus hantzschii* and *S. minutulus* were present only in Eastern Anatolian freshwater.

MORPHOLOGY AND TAXONOMY OF *PLEUROSIGMA EXEMPTUM* MANN (BACILLARIOPHYCEAE). COMPARISON WITH TYPE MATERIAL OF *P. FORMOSUM* W. SMITH AND *P. DECORUM* W. SMITH.

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This study is devoted to analyze the fine morphology of *Pleurosigma exemptum* Mann, *P. decorum* W. Smith and *P. formosum* W. Smith. The materials examined were samples collected from San Matías Gulf, the published figures of the holotype of *P. exemptum* and the lectotypes and unmounted type materials of *P. decorum* and *P. formosum*. Based on this analysis we conclude that the taxon from San Matías Gulf is conespecific to *P. exemptum*, species that has never been reported after its description. This species shares several ultrastructural features with *P. formosum* and *P. decorum* such as a thick saddle-shaped central raphe nodule, very long overlapping of the central raphe fissures, short hook-shaped terminal raphe fissures, general morphology of the internal hymen-occluded pore crossed by a lower-level bar and presence of some scattered pairs of pores more depressed in the siliceous layer lacking bar. Nevertheless, it differs from both species by size range, some details of the internal hymen-occluded pore of the areolae, the presence of an unperforate area around the helictoglossa expanded toward the concave side, the almost parallel to each other symmetrical bars delimiting the central nodule and the densely arranged row of small areolae bordering the raphe sternum. Taking into account that we expand the species concept by providing the first insight into its ultrastructure, we emend the description of *P. exemptum* and we enlarge its biogeographic distribution.

*Pleurosigma formosum* and *P. decorum* were differentiated up to date by size range, valve shape and striae density. Based on our LM analysis we were not able to determine differences in stria density between these species. In contrast, our SEM analysis of both type materials allow us to reveal a difference in the internal morphology of the areolae pores, circular and rimmed in the former and subcircular to elliptical, not rimmed in the latter.



## NAVICULA BORY AND ITS RELATIONSHIP WITH SALINITY IN SALT MARSH FROM SOUTHERN BRAZIL

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The greatest amount of saltmarshes in southern Brazil is located adjacent to the Patos Lagoon estuary (32°S-52°W) and occupies an area of 70 Km<sup>2</sup>. It is covered with herbaceous vegetation, mainly *Spartina* species, which are adapted to periodic flooding by saline waters. In this region, *Navicula s. str.* is rich in species number. The aim of this study is to understand the composition and distribution of these species in different salinity zones. The samples were collected using a 10 cm diameter core in sites with salinity between 0,1‰ and 35‰, adjacent to the Patos lagoon estuary, in Rio Grande do Sul state (31°57'S-52°06'W). In the laboratory, the living species were isolated from superficial sediment by the "trapping method", using the Whatman® 105 paper. They were later oxidized using nitric acid for analysis of light and scanning electron microscopes. It was found a total of 12 species and one variety from which only *N. erifuga* Lange-Bertalot, *N. gregaria* Donkin and *N. veneta* Kützing are cosmopolitan taxa. Some species were related to specific salinity zone, such as *Navicula cf. recens* (Lange-Bertalot) Lange-Bertalot, *N. gregaria* Donkin, *N. gregaria* Donkin var. *reductissima* Metzeltin, Lange-Bertalot & Garcia-Rodríguez e *N. veneta*, which only occurred in oligohaline zone, on other hand *Navicula amphiceropsis* Lange-Bertalot & Rumrich were restricted to the polyhaline zone. Whereas *Navicula cf. fernandae* Metzeltin, Lange-Bertalot & Garcia-Rodríguez showed a wide tolerance to salinity, once it maintained high frequency since limnetic zone to euhaline zone. It is interesting to observe that *Navicula cf. fernandae* was abundant (corresponding 30% valves observed) in the limnetica zone, while *Navicula cf. cryptotenelloides* (16%) and *Navicula erifuga* (7.79%) were abundant in oligohaline zone. Illustrations of the morphological and structures characteristics of these species are presented, contributing to the taxonomic knowledge of this genus, fairly unknown in southern Brazil.

## NAVICULA VALERIANA, A BRACKISH EPIBENTHIC DIATOM FROM SOUTH AMERICA: DISCUSSION ABOUT ITS TAXONOMIC POSITION

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*Navicula valeriana* Metzeltin, Lange-Bertalot & García-Rodríguez was described to Laguna Rocha (34°15'S-54°17'W), Uruguay in 2005. The species was mentioned as rare and has only been so far registered to this locality. This study is devoted to analyze material of *N. valeriana* isolated by the "trapping method" from superficial sediment of the salt marsh, adjacent to the estuary Patos lagoon (31°57'S-52°06'W), Southern Brazil. The species occurred in oligo and mesohaline zones, showing the highest relative abundance (7.2%) at 15 ‰ of salinity. Specimens of this taxon from several populations were observed with more detail in scanning and transmission electron microscopes. *N. valeriana* presents lanceolate valves, rounded apices and parallel striae, slightly radiate near the apices, and the dimensions are the same as the material from Uruguay. The presence of the external longitudinal strips and striae containing rectangular poroids covered by internal hymens approximate this species to the genus *Haslea* R. Simonsen. Nevertheless, the raphe system and presence de Voight fault approaches this taxon to the genus *Navicula s.str.* This combination of morphological features is shared by *N. valeriana* and other brackish water species (*Navicula duerrenbergiana* Hustedt) and differs from all the others species assigned to *Navicula s. str.* and *Haslea*. Based on these results the generic limits of *Navicula s. str.* and *Haslea* are revised and the taxonomic position of the mentioned species is discussed.

## ARE TOXIC DIATOMS A PROBLEM IN THE ARCTIC? – FOCUSING ON *CALANUS* SPP. AS POTENTIAL VECTORS FOR DOMOIC ACID

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The first record of a domoic acid-producing organism, a diatom *Pseudo-nitzschia seriata*, from the Arctic waters was recently done from the western Greenlandic waters. Knowing that domoic acid can be transferred to higher trophic levels in marine food webs, as it has been found e.g. in whales, sharks and marine birds, we wanted to study if domoic acid-producing *P. seriata* was grazed by zooplankton and whether the grazers could accumulate the toxin also in the Arctic. This is of special interest in Greenland where marine products form the basis for everyday food and are also the most important export products. The experiments were run in Disko Bay, Western Greenland. Three *Calanus* species, *C. glacialis*, *C. finmarchicus* and *C. hyperboreus*, which are the most important zooplankton herbivores in the area, were fed with unialgal cultures of toxic *P. seriata* and non-toxic *P. delicatissima*. All three copepod species fed on toxic *P. seriata* and also retained domoic acid after the grazing and there were no differences in ingestion rates between toxic and non-toxic *Pseudo-nitzschia* species in any of the copepods. *Calanus finmarchicus* and *C. hyperboreus* grazed on toxic *P. seriata* during the first six hours of the experiment but seemed to stop grazing during the last six hours of the experiment suggesting that the copepods may have suffered some kind of physical incapacitation due to ingestion of domoic acid. *Calanus glacialis* grazed on toxic *P. seriata* continuously during the whole experiment probably due to the lower concentration of domoic acid in *P. seriata* cells during the experiment run on *C. glacialis* than the other two copepod species. The results from our experiments show that the three *Calanus* species are potential vectors for domoic acid to higher trophic levels in the Arctic.

## ORGANELLAR DIVISION AND VESICULAR TRANSPORT DURING CELL DIVISION IN THE MARINE PENNATE DIATOM *PHAEODACTYLUM TRICORNUTUM*

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Diatom cells have been used for cytological research for more than a hundred years, especially for studies of mitosis and valve formation. Despite the information accumulated about these two topics, organellar ultrastructural changes with respect to cell division have received little attention. In the marine pennate diatom *P. tricornutum*, it is well known that the single chloroplast changes its form during the cell cycle, however it remains to be elucidated how and when a chloroplast divides into two daughter plastids. To clarify the timing and the machinery of secondary chloroplast division in diatoms, we observed synchronized *P. tricornutum* cells by using transmission electron microscopy (TEM) and confocal microscopy (CM) during the progression of the cell cycle.

By time-lapse CM imaging and TEM, we found that the chloroplast divided following formation of a central constriction prior to karyokinesis and cytokinesis. Interestingly the pyrenoid, located in the center of the chloroplast, was divided at the same position without constriction. After chloroplast division, cytokinesis began before karyokinesis at one side of the longitudinal axis. Additionally, vesicle transport during cytokinesis was tracked using vesicle transport proteins (SEC4 and SYTA) fused with GFP. SYTA-YFP localized to the cleavage furrow, while SEC4-YFP indicated transport of vesicles from the Golgi to the plasma membrane.

These observations elucidate the process of diatom chloroplast division and the plasticity of chloroplast structures during cell division. Moreover the different types of vesicle transport during cytokinesis suggest different roles such as for exocytosis and for addition of new plasma membrane.

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## PALAEOLIMNOLOGICAL RECONSTRUCTION OF HOLOCENE CLIMATE VARIABILITY IN LÜTZOW HOLM BAY, EAST ANTARCTICA

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To date, little is known about past and regional Holocene climate variability in the Southern Hemisphere, despite this information being critical for an improved prediction of the impact of future climate anomalies on the cryosphere and the diversity and functioning of ecosystems. Here, we provide insights in the Holocene climate history of two ice-free regions in Lützow Holm Bay, a largely understudied sector in East Antarctica. Diatoms, fossil pigments and sedimentological proxies were analysed in radiocarbon-dated sediment cores from glacial and isolation lakes from West Ongul Island and Skarvsnes in Lützow Holm Bay. Changes in the moisture balance were quantitatively reconstructed using a diatom-based transfer function for specific conductance. The dominant diatom species in the regional calibration dataset were *Amphora veneta*, *Diadesmis australis*, and *Navicula gregaria*. Deglaciation occurred around c. 11.2 and 7.6 cal. ka BP, in West Ongul Island and Skarvsnes respectively, which is in contrast with previous estimates based on raised beach data, but in agreement with cosmogenic isotope dates. Between c. 2.2 and 1.5 cal. ka BP, a slightly higher specific conductance and increased accumulation of total chlorophylls, carotenoids, carbon, nitrogen and the absolute abundance of diatoms point to an increase in primary production, which is possibly related to the Mid to Late Holocene Hypsithermal detected in ice, terrestrial and marine records elsewhere from Antarctica. Between c. 699 and 476 cal. yr BP, a period of slightly increased primary production and diatom concentration in Skarvsnes can be cautiously linked to warmer conditions. This period clearly postdates the Medieval Climate Anomaly that is detected in records from the Northern Hemisphere. There is no evidence for a Little Ice Age-like event. Proxy data in the upper centimeters of the lake sediment cores point to only very small changes in the diatom communities, the moisture balance and primary production during the past decades. This agrees well with a relatively modest or no recent warming trend recorded in instrumental records from East Antarctica.

## THE USE OF DIATOMS FOR MONITORING RIVER WATER QUALITY IN THE ZAMBEZI AND CONGO SISTER BASINS

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Diatoms are well known and often used indicators of water quality in Europe and other regions of the world. Recently diatom indices have been applied with some success in temperate southern Africa despite regional endemism within some communities. Rural communities in Zambia and the DRC are often almost entirely dependent on the local rivers and streams. These water resources are a source of drinking water, irrigation water for crops, nutrients during flooding and provide fish an essential source of protein for these communities. For this reason it is vital that water quality be maintained and in order to facilitate the necessary good management practices monitoring is needed. Diatoms provide an ideal and cost effective method for monitoring water quality, however, special challenges arise when monitoring the quality of subtropical rivers and streams. The Congo River and in particular the Zambezi River (and drainage basin) is particularly poorly investigated and little is known of the taxa which occur there. In addition there are comparatively few algologists and diatomists in Africa who could establish and maintain such a monitoring program. This poster will present some of the initial findings regarding species diversity from the Zambezi and Congo sister basins as well as discussing possible teaching tools and methods to establish local capacity in using diatom-based water quality monitoring techniques.

## DIATOM VIRUSES ISOLATED FROM JAPANESE COASTAL WATERS

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Since the first reports of large numbers of virus-like particles in natural seawater, the aquatic viruses have been intensively studied. Currently the viruses are regarded as one of the major biological factors that regulate carbon cycling, microbial biomass and the genetic diversity of protists and algae. Most of the virus particles in aquatic environments are considered to be bacteriophages and the viruses infecting eukaryotic phytoplankton may rank second in abundance. The roles of viruses in natural environments are considered to be important from the viewpoint of the ecological dynamics of microalgal host populations. Although the significances of diatoms as key players in the oceanic carbon cycle have been recognized, the existences of diatom viruses are scarcely known until recently. The first diatom virus was reported in 2004, a virus infecting *Rhizosolenia*. After the initial discovery, several *Chaetoceros* viruses have been isolated and characterized. Furthermore, just recently pennate diatom viruses have been successfully isolated. These viruses are grouped into two categories based on genomic features, single-stranded (ss) RNA and ssDNA. The members for ssRNA diatom viruses are RsetRNAV, CtenRNAV, CsfRNAV, Csp03RNAV and AglaRNAV which infect *R. setigera*, *C. tenuissimus*, *C. socialis* f. *radians*, *Chaetoceros* sp. strain SS08-C03 and *Asterionellopsis glacialis*, respectively. They harbor ca. 9kb ssRNA genome with two open reading frames encoding putative replication-related proteins and capsid proteins. Phylogenetic analysis based on the deduced amino acid sequence of the RNA-dependent RNA polymerase domains strongly supported the monophyly of these viruses with a bootstrap value of 99%. The ssDNA diatom virus group includes CsaDNAV, CtenDNAV, Csp05DNAV, ClorDNAV, CsetDNAV and TnitDNAV, infecting *C. salsugineum*, *C. tenuissimus*, *Chaetoceros* sp strain TG07-C28, *C. lorenzianus*, *C. setoensis* and *Thalassionema nitzschioides*, respectively. Their genomes are composed of covalently-closed circular ssDNA (ca. 6kb). The genome of CdebDNAV infecting *C. debilis* is also ssDNA (ca. 7kb) but its structure is unrevealed. These diatom viruses are all lytic to their respective host diatoms; and their infection is strain-specific rather than species-specific. These discoveries are important to understand diatom ecology, the carbon cycles related to diatom production and their evolution.

## PHYLOGENY OF GENUS *SPICATICRIBRA* AND RELATED TAXA

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The genus *Spicaticribra* was described based on the type species *S. kingstonii* by Johansen et al. (2008). This species was found in Fontana Lake, a reservoir in the Tennessee River drainage, North Carolina, U.S.A. The relationship of this genus with other genera was uncertain.

This species was also found in the southern part of Japan including Okinawa Island. A very similar species, *Thalassiosira rudis* Tremarin, Ludwig, Becker et Torgan described from South America, was found at Mae Jork Lung reservoir, Chiang Mai area, Thailand, and transferred to the genus *Spicaticribra* (Tuji et al. 2012). Only two species belong the genus now.

Tuji et al. (2012) reported the phylogenetic position of *S. kingstonii* using a Japanese strain and the 18S rDNA region. The phylogenetic tree using the 18S rDNA shows that the origin of *S. kingstonii* exists in the *Cyclotella meneghiniana* species complex clade.

However, the branch length is extremely long, and phylogenetic research using more genetic regions was essential. In this study, we examined the phylogenetic position of *S. kingstonii* using multi-gene analysis (SSU, ITS, LSU, rbcL, psbA, psbC).



## EVALUATION OF CANDIDATE BARCODE MARKERS ON THE DIATOM GENUS *FRUSTULIA*

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Traditionally, the identification of diatoms has been based on the size, outline, and ultrastructural features of their silica frustules. However lately, the integrative taxonomical approach showed that molecular techniques are often essential for species level identification and that morphological examination usually enables only the identification to the higher taxonomical levels. Even though this crude classification may be sufficient for biomonitoring and paleoecology, it is absolutely unsatisfying once we aim to study diversity or biogeography of diatoms. Possible solution to this problem seems to be DNA-barcoding; however, despite several attempts to find a suitable barcode marker for diatoms a consensus has not yet been made and thus there is a need to examine other “model systems” and DNA regions.

Our data, which were primarily collected for phylogenetic and biogeographical analysis of the genus *Frustulia*, enabled the evaluation of one new (plastidial psbA) and five candidate barcode markers (partial SSU (V4), partial LSU (D1-D2) and 5.8S+ITS2 nuclear rDNA, plastidial rbcL-3P and mitochondrial coi-5P). In order to assess the power, practicality, and universality of each marker both more distantly related *Frustulia* species and sister species from pseudocryptic complex *F. rhomboides* were included in the analysis. In accordance with previous studies all tested markers were suboptimal in at least one of three decisive criteria.

Although the “perfect” diatom barcode marker has not yet been found, it seems advantageous to adopt a unified barcoding methodology including culturing, vouchering and DNA preservation. Thus, once the suitable marker is found its sequences can be still obtained. All in all, we trust that nomenclaturally effective DNA-barcoding will contribute to more stable diatom taxonomy, which has currently the utmost priority in diatomology.

## SPRING PHYTOPLANKTON OF LAKE BAIKAL IN 2007-2009 IN COMPARISON WITH THE 1964-1990 MULTIYEAR

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Lake Baikal situated in the East Siberia is the most ancient (25 My), the deepest (1637 m) and the largest (23,000 km<sup>3</sup>) freshwater body on the Earth (Grachev, 2002). Lake Baikal phytoplankton plays an important role in the creation of primary organic matter and responds sensitively to environmental changes which sometimes cannot be registered by other research methods. Therefore, it is necessary to observe regularly the phytoplankton of this unique lake.

As Lake Baikal is characterized by well-expressed spring maximum of phytoplankton, when the main mass of large-sized phytoplankton is formed (Antipova, 1963; Popovskaya, 1977; Popovskaya, 2000; Popovskaya *et al.*, 2001, Popovskaya, Genkal & Likhoshway, 2008), permanent regime observations were performed immediately after ice break on the lake.

The aim of this work was to study spring phytoplankton of the Lake Baikal pelagic area by data of expeditions around Lake Baikal in 2007-2011 with their compilation with perennial data of 1964-1990.

It is shown that in 2007-2011, diatoms, like during the previous 50-years period, made 80-90 % from total phytoplankton abundance and biomass. Phytoplankton development level and composition of dominant species during the years of studies was not identical. E.g., in 2007, at major part of lake open water, there was abundant development of an endemic species *Aulacoseira baicalensis* (K. Meyer) Simonsen, this year can be considered as a high-productive *Melosira* (*Aulacoseira*) year (Popovskaya *et al.*, 2007). During the next 2008, there dominated in Southern and Central Baikal *Synedra acus* subsp. *radians* (Kützing) Skabitchevsky, its biomass also reached values characteristic for high-productive years. It was possible to observe similar alternation of dominant species as well during previous years. E.g., in 1982, there developed in Lake Baikal abundantly *A. baicalensis*, and 1983 was high-productive for *Synedra*. In 2009-2011, the level of phytoplankton development was slightly lower. These years were characterized in different pelagic area sites as medium-high-productive or medium-low-productive, but the dominant species was as well *S. acus* subsp. *radians* accompanied by *A. baicalensis*, *Stephanodiscus meyeri* Genkal & Popovskaya, *Aulacoseira islandica* (O. Müller) Simonsen and Chrysophyta, mainly *Dinobryon cylindricum* Imhof.

The comparison of phytoplankton abundance and biomass in 2007-2011 and in 1964-1990 showed that they meet the scale of perennial oscillations with a species block characteristic for this period. Consequently, the primary part of Lake Baikal ecosystem exists in the common regime.

This work was carried out as part of the budget topic VII.62.1.3.

## PH INDUCED FLOCCULATION: A COST-EFFICIENT METHOD FOR HARVESTING MICROALGAE

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Diatoms have a relatively high oil content and therefore receive much interest as a potential source of biofuels. A major challenge to make microalgal biofuels cost-effective lies in the harvesting of the microalgae. Harvesting is difficult due to the low biomass concentration in microalgal cultures and the small size of the cells. We studied flocculation induced by high pH to concentrate microalgal cultures, both for a freshwater green algae (*Chlorella vulgaris*) and a marine diatom model species (*Phaeodactylum tricornutum*). In our study, we focus on the underlying mechanism, the influence of the culture growth phase and medium salinity on flocculation. For *Chlorella* was demonstrated that flocculation can be induced by increasing medium pH to 11. Although both calcium and magnesium precipitated when pH was increased, only magnesium (>0.15 mM) proved to be essential to induce flocculation. For *Phaeodactylum*, flocculation was induced at pH 10 but in this case, also calcium played a role in the flocculation mechanism. Using a surface complexation model, the nature of the electrostatic charge at the algae-coagulant-water interfaces are discussed. The combination with precipitate formation models allowed us to predict pH induced coagulation conditions. Practical implications such as cost, medium recycling and contamination risks will be discussed. Finally, this technique is compared to other techniques such as electro-coagulation-flocculation and use of cationic starch as a biopolymer flocculant.

## A NEW CENTRIC DIATOM GENUS FROM THE SUB-ANTARCTIC REGION WITH STRIKING SIMILARITIES TO A CRETACEOUS FOSSIL

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Ile Amsterdam (77°30'E, 37°50'S) is a very young, small volcanic island situated in the southern Indian Ocean, well north of the polar front. Volcanic caves are typical habitats on the island, found at sea level around the scientific station, with a diatom composition dominated by unusual species with unique features.

During a survey of the terrestrial diatom flora in a lava tube cave on the island, an unknown centric diatom was found. The frustules are connected to each other by very large, irregularly branched linking spines. The cells are clearly domed with a narrow to moderately broad mantle. An irregular pattern of slit-like areolar openings are seen between the spines on the valve exterior, usually covered by a continuous silica layer. The areolae are rounded or slit-like on the valve mantle. Internally, the irregularly scattered areolae are occluded by small, disc-like coverings and when these occlusions are lost, complex foramina consisting of several small openings, are visible. Several rimoportulae are found arranged in a ring around the valve mantle. The girdle consists of a large number of perforated, open bands, as is commonly found in species of the Melosiraceae and Orthoseiraceae.

A second population with comparable morphological features was found in a small cave on the nearby sub-Antarctic Ile de la Possession (Crozet Archipelago). Although there are some minor differences in the shape of the spines, reducing the formation of long chains in the Crozet population, the ultrastructure of the areolae seems to be the same.

The poster shows the results of a morphological study of this remarkable genus, with notes on its ecology and its possible systematic relationship to both fossil and extant diatom genera. Based on their unique features, a new genus will be described for these diatoms, showing some similarities with the Cretaceous fossil genus *Bloch* (Witkowski & Harwood 2010) and the extant genus *Clupeoparvus* (Woodbridge et al. 2010).

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## PLANKTONIC DIATOM EVIDENCE FOR HOLOCENE CHANGES IN WIND-DRIVEN MIXING REGIME AND HUMAN IMPACTS IN LAKE VILLARRICA, SOUTH CENTRAL CHILE

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Temperate Southern Hemisphere regions are influenced by climate fluctuations such as ENSO and changes in the strength and position of the Southern Westerlies. These processes can alter physical lake properties like water-column mixing which is an important factor for governing algal abundance and community dynamics. Here we present a low-resolution Holocene diatom stratigraphy of a 14 m long sediment core from Lago Villarrica (39°15'S, 72°02'W), which was combined with sedimentological proxies in order to infer paleolimnological and paleoclimate changes in Northern Patagonia. The reconstruction of past changes in wind-driven mixing regime was aided by a newly developed diatom training set based on standing populations and surface sediment data from 27 Chilean lakes (39°-43°S). Differences in planktonic diatom community structure were mainly related to gradients in euphotic depth and mixing regime.

Between 9369 and 5900 cal yr BP, overall high diatom productivity and high relative abundances of *Discostella stelligera*, *D. cf. mascarenica* and *Urosolenia eriensis* point to the occurrence of thermally stable water conditions likely related to the Early Holocene warm period. During the Mid- and Late-Holocene, enhanced seasonality with longer and more stable lake water stratification during summer and more intense winter mixing could be inferred based upon the notable co-occurrence and high abundances of *Aulacoseira granulata*, *A. granulata* var. *angustissima* and *U. eriensis*. During the last 3000 years enhanced ENSO frequency and intensity could be inferred based on the absolute abundance pattern of *U. eriensis* and the occurrence of lahar laminae in the lake sediment core. These patterns match remarkably well with the ENSO record from Laguna Pallcacocha (Ecuador, 2°S). The most recent period is characterized by a marked shift in the diatom community composition, with a sudden increase of *Asterionella formosa* and *Fragilaria crotonensis*, indicating increased nutrient levels likely related to human activities in the lake and its catchment area.

## A DIATOM AND SEDIMENTOLOGICAL BASED RECONSTRUCTION OF MID AND LATE HOLOCENE PALAEOCLIMATE CHANGES IN SOUTH GEORGIA

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South Georgia's location within the Polar Frontal Zone and the Southern Westerlies wind belt between Antarctica and the mid-latitudes makes it a key location for studying the main drivers of past and present-day climate variability in the South Atlantic. We undertook multi-proxy analyses, including fossil diatom and pigment analysis, of a 5.83 m long, ca. 7800 cal. yr. B.P. old, sediment core from Fan Lake, Annenkov Island, South Georgia (54°29'0''S, 37°5'0''W) to determine whether proxies for Holocene palaeoclimatic variability could be separated from those which reflected local catchment stability and glacier activity. Deglaciation of the Fan Lake site occurred ca. 7800 cal. yr. B.P. A low nutrient/low productivity environment until ca. 3600 cal. yr. B.P., indicating slow development of the lake ecosystem after deglaciation, was followed by a short period of elevated biological productivity 3600-3380 cal. yr. B.P, inferred from the changing carbon content. Anomalous radiocarbon ages of terrestrial mosses between 3380 to 2735 cal. yr. B.P. most likely reflect enhanced inputs of older terrestrial moss from the inflow streams that pass over moss banks in the catchment. The most recent phase of lake development, 2735-0 cal. yr. B.P., was characterized by high and variable biological production. The diatom communities during this entire period were dominated by *Cyclotella stelligera*, but some significant fluctuations in other diatom species occurred in the upper part of the core. For example, a peak of the diatom *Fragilaria capucina* peak can be tentatively linked to increased catchment disturbance ca. 1100 cal. yr. B.P., and was possibly associated with a deglaciation event following a cold spell ca. 1770-1335 cal. yr. B.P. Other brief cold spells possibly took place at 2364 and between 720-495 cal. yr. B.P. Data from Fan Lake complement previous and ongoing reconstructions of past environmental change from peat bogs and lake sediments on South Georgia.

## BCCM/DCG, A NEW AND DEDICATED DIATOM CULTURE COLLECTION INTEGRATED IN THE BELGIAN COORDINATED COLLECTIONS OF MICRO-ORGANISMS

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Diatoms are relatively poorly represented in culture collections, mainly as a result of difficulties for long-term preservation linked to their peculiar size reduction-restitution life cycle. To fill this gap, we are developing a dedicated diatom culture collection integrated in the Belgian Coordinated Collection of Micro-organisms (BCCM) in which long-term preservation of diatoms is achieved via cryopreservation or via the generation of sexual offspring. This diatom collection of diatoms (BCCM/DCG) (<http://bccm.belspo.be/about/dcg.php>) currently contains over 176 well-characterized strains from 23 species of model diatoms belonging to 9 genera for life and cell cycle, speciation, ecophysiological, and trophic interactions research and aquaculture/blue biotechnology. Strains are molecularly and morphologically characterized (including cell size) and, depending on the species, extensive additional ecological, life cycle, physiological and genomics information is available. Current services offered include the public deposition and worldwide distribution of diatom strains. The management system of BCCM/DCG has been assessed and certified as meeting the requirements of ISO 9001:2008 for the accession, control, preservation, storage and supply of diatoms, and related information in the frame of public deposits. Further expansion of the collection will be achieved by (1) improving the collection and (2) offering additional services. For the first, the number of strains and species will be increased, there will be a further integration of genomic and biochemical information, and (cryo)preservation techniques will be improved. Additional services may include the isolation and breeding, and genetic and morphological characterisation of target species upon request, and the distribution of DNA samples for strains which cannot be preserved long-term.

## IDENTIFICATION OF THE MATING TYPE LOCUS IN PENNATE DIATOMS

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Diatoms form one of the most diverse and productive groups of algae. They have a unique life cycle characterized by gradual size reduction which leads to programmed cell death. This can lead to loss of strains and cultures and can only be avoided by sexual reproduction which enables cell size restitution. Sex is cell-size activated, it can only be induced below a species-specific size threshold. Despite these unique features, not much is known about the molecular base of sexual reproduction in diatoms. We are currently identifying the sex determination system of the pennate diatom *Seminavis robusta*. Crossing two strains of opposite mating type (MT+ and MT-) resulted in a F1 mapping population. The segregants were analyzed using AFLP technology. We identified 15 MT- and 13 MT+ linkage groups. The sex phenotype was included as a single marker locus which co-segregated with MT+ specific markers. This strongly suggests heterogamety in the MT+ of *Seminavis robusta*. Two AFLP markers flanking the sex locus were sequenced and mapped to the genome sequence. This resulted in a number of genes possibly involved in sex determination. Currently, we are further identifying the MT locus by complementing the AFLP linkage mapping by a Bulk Segregant Analysis, in combination with high throughput sequencing. In the near future, we will perform a functional characterization of the MT genes by RT-PCR and genetic transformation. The evolution of the MT locus will be studied in related species.



## STRONG COMPOSITIONAL CHANGES IN THE CENTRIC DIATOM COMMUNITIES OF THE SCHELDT-ESTUARY: A RESULT OF IMPROVING WATER QUALITY?

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The river Scheldt has a macrotidal estuarine zone (tidal amplitude up to 6m) that stretches 160 km inland. The estuary covers the complete salinity gradient, including a freshwater tidal area (FTA) of 60 kilometers. During the second half of the twentieth century, water quality was extremely poor due to the discharge of large volumes of wastewater, leading to periods of severe hypoxia and high ammonium concentrations. Since the late 1990's, increasing wastewater treatment caused a gradual recovery of the water quality. As part of a multidisciplinary study on the Belgian part of the Scheldt estuary (OMES/MONEOS projects), the estuarine phytoplankton has been monitored on a monthly basis since 1996. Here we report the main changes in the phytoplankton communities during this period of improving water quality.

The estuarine phytoplankton was always dominated by centric diatoms that mainly bloomed during summer, with bloom intensity depending on the flushing rate. The phytoplankton bloom biomass was considerably higher in the FTA from 2003 onwards and this independent of the flushing rate. This biomass increase was mainly attributable to *Actinocyclus normanii*, which replaced *Cyclotella scaldensis* as the dominant diatom species in the FTA. Field observations and laboratory experiments indicated reduced ammonium concentrations as an important driver for this shift given the low tolerance of *Actinocyclus* for ammonium. In 2011 however, phytoplankton biomass was considerably lower with *C. scaldensis* again dominating the summer phytoplankton. In the brackish part of the estuary, the biomass of *Thalassiosira nodulolineata* increased spectacularly since 2008, coinciding with the start-up of the wastewater purification plant of the city of Brussels, and nowadays forming an extensive recurring spring bloom. This diatom species also extended its range towards the FTA where it now sporadically dominates spring phytoplankton. Simultaneously, the calanoid copepod *Eurytemora affinis*, a predator of both *A. normanii* and *T. nodulolineata*, has colonized the FTA too. Future compositional changes in the estuarine phytoplankton may be anticipated as a result of further increases in water quality, ongoing tidal marsh restoration and downstream deepening of the main water channel.

AN ATTEMPT TO REVISE THE GENUS *PERONIA* BRÉB. ET ARN. EX KITTON WITH TWO SPECIES NEW TO SCIENCE

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*Peronia* Brébisson et Arnott ex Kitton is a small freshwater genus belonging to the Eunotiales Silva, and shares basic characteristics with other genera within this group, e.g. the presence of a rudimentary raphe, the absence of a central nodule, and the ecology. It usually occurs in very low abundances in oligotrophic and dystrophic water, growing as a periphyton attached to the surface by mucilage. Although the genus *Peronia* includes 12 described taxa, only a couple of species are commonly reported. Besides that, they appear to have been confused in the past, with various authors having different concepts for them. After our investigation, it appears that there are only three species of *Peronia*: *Peronia fibula* (Brébisson in Kützing) Ross, *Peronia heribaudi* Hustedt and *Peronia brasiliensis* Hustedt. Despite this resolution, observations on the type material, original drawings in the classical literature and photographs in the modern literature, it still seems that the concept of those few species is still not clear, probably also due a considerable variability in *Peronia* valve shapes. Our goal was to look at available type material and other populations from the North America (mostly from the collection of slides at the Academy of Natural Sciences in Philadelphia), and to demonstrate the morphological variability within the *Peronia* populations. In addition, we present two forms of *Peronia* from our samples morphologically different from all the recognized species: one from the Northeastern U. S., having a drawn - out, acute head pole; the second one from Roraima Tepui (on the border of Venezuela, Brazil and Guayana), which has a bluntly capitate head pole. These *Peronia* forms are presented as new to science.

**FUNCTIONAL GENETIC DIVERSITY WITHIN *FRUSTULIA RHOMBOIDES* SPECIES COMPLEX.**

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A multidisciplinary approach to protist taxonomy and systematics has indicated that molecular genetic diversity is not always associated with morphological and/or ecological differentiation. Consequently, Bland Finlay and Tom Fenchel stated, that molecular markers are neutral with regard to functional diversity; therefore, the genetic variation within microbial morphospecies reflects the accumulation of selectively neutral mutations. In our study, we analyzed genetic diversity of *F. rhomboides* populations from both different geographical regions and various microhabitats within localities in order to assess the importance of environmental factors on the diversity and distribution of lineages. On the basis of sequence frequencies of lineages within samples, it is likely that the distribution of *F. rhomboides* lineages is primarily influenced by environmental requirements and/or dispersal abilities. We suggested that the incongruence between the molecular and ecological data in other studies may reflect undersampling, as the realized niche is represented by different abundances of phylogenetic lineages in natural communities rather than by simple presence or absence.

## A 1000-YR RECORD OF ENVIRONMENTAL CHANGE IN NE CHINA INDICATED BY DIATOM ASSEMBLAGES FROM MAAR LAKE ERLONGWAN

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Past environmental changes based on diatom relative abundances have been inferred from the maar Lake Erlongwan in northeast China. The limnology of Lake Erlongwan is affected by the strongly seasonal regional climate. The composition of diatom assemblages, in turn, responds to changes in the seasonal duration of ice cover in winter, water-column turnover in spring and autumn, and thermal stratification in summer. Statistical analysis of the sedimentary diatom assemblages reveals three significant stratigraphic zones over the past 1000 yr. The highest abundance of the planktonic species *Discostella* species occurs between AD 1050 and 1400 and suggests an annual ice-free period of long duration and well-developed summer stratification of the water column. This planktonic diatom peak between ca. AD 1150 and 1200 suggests that this period was the warmest over the past 1000 yr. The interval between AD 1400 and 1800 is marked by a decline in planktonic diatoms and suggests shorter duration of the ice-free season, weaker water stratification and possibly generally cold conditions. After AD 1800 relative abundances of planktonic diatoms, including *Puncticulata praetermissa* and *Asterionella formosa*, increase again, which indicates lengthening of the duration of the annual ice-free period and a stronger overturn of the water column. All these data imply that the pattern of the seasons is different between the MWP and the 20th century. During the MWP the duration of summer was longer while the spring and autumn were shorter than the 20th century.

## USING BIODIVERSITY OF DIATOMS TO IDENTIFY HYDROLOGICAL CONNECTIVITY IN THE HILLSLOPE-RIPARIAN-STREAM SYSTEM

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In recent years, there have been calls for new eco-hydrological approaches to answer questions on water source and connectivity in the landscape. In this study, hydrologists and ecologists jointly assess the potential for diatoms to infer runoff generation processes. Diatoms from various terrestrial and subaerial substrates (bryophytes, litter and leaves), as well as from aquatic habitats (epilithon, epipelon and drift samples) were collected at seasonal and event scales in two headwater catchments in Luxembourg. Spatial ordination (non-metric multi-dimensional scaling, NMDS) of samples revealed distinct spatial patterns in the organization of diatom communities along three landscape units: hillslope (mosses), riparian zone (mosses, soil, leaves) and streambed (epilithon, epipelon, drift at baseflow, drift during storm events). We also noted a high dissimilarity among diatom communities sampled in the riparian zone and on hillslopes in the Weierbach (schists) and Huewelerbach (sandstone) catchments. Amongst the ca. 400 diatom species identified, very few were common to both geological regions, indicating highly contrasting communities between catchments. This suggests the potential for diatoms to determine the spatial origin of water (i.e. contrasting geological sub-areas) at larger scales. Comparison of diatoms in stream water samples taken during baseflow and storm conditions have shown a systematic flushing of terrestrial diatoms to the stream in response to incident precipitation and increasing discharge. Mixing diagrams (SiO<sub>2</sub> and specific absorbance – 254 nm) suggest a substantial contribution of the soil water component to total runoff. Diatom abundance proportions appeared to be very sensitive to incident precipitation, suggesting rapid onset of connectivity between the soil surface and the stream network. Almost simultaneously to incoming precipitation, the abundance of diatoms from soil habitats summed with those from terrestrial epiphytic habitats increases up to 20-30% of the total number of drift diatoms inside the stream during summer rain events. Species from terrestrial habitats include poorly known taxa such as *Navicula obsoleta* Hustedt, *N. parsura* Hustedt, and *Nitzschia harderi* Hustedt. The diatom community from riparian and hillslope areas was dominated by species belonging to the genera *Eunotia* Ehrenberg, *Diadesmis* Kützing, *Hantzschia* Grunow, *Luticola* D.G. Mann, *Navicula* Bory and *Pinnularia* Ehrenberg.

## TRANSFER OF THE BRYOPHYTIC DIATOM SPECIES *NAVICULA CATARACTARUM* HUSTEDT TO *HYGROPETRA* AND COMPARISON WITH *FRANKOPHILA* SPECIES

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The detailed ultrastructural morphology and identity of *Navicula cataractarum* Hustedt are presented here. This species was originally described by Hustedt from bryophytes collected in a waterfall in 'Los Chorros' village in El Salvador (Central America) as a massive dominant species and is, until now only known from the type locality. The taxon is small sized and characterized by rectangular frustules in girdle view and by elliptic to slightly rhombic valves with bluntly rounded apices. Raphe branches are externally located on the valve face, straight and subpolar in both halves of the valve, with internally slightly deflected terminal fissures. Biseriate striae are short, radiate, and sometimes slightly asymmetric along the apical axis, extending shortly to the valve mantle. The outer openings of the areolae are circular and slightly smaller than the inner openings, which are occluded by a scarcely domed membrane (hymenes). Based on the combination of characters the species belongs to the recently described genus *Hygropetra* Krammer & Lange-Bertalot. This genus is relatively small and comprises only three species restricted to bryophytic habitats, namely: *Hygropetra balfouriana* (Grunow ex Cleve) Krammer & Lange-Bertalot, *H. elongata* Krammer & Lange-Bertalot and the recently described *H. gelasina* Mayama & M. Idei. The genus is characterized by naviculoid small cells, and is similar to *Pinnularia* Ehrenberg, but can be distinguished from it by the position of the areola occlusions and the presence of serrated valvocopula extensions. The genus possesses some similarities to *Frankophila* Lange-Bertalot, which is characterized by even shorter raphe branches, but similar in the overall characteristics. Indeed, in the same sample two closely related populations of *Frankophila* were also discovered and investigated. We present light and scanning electron microscopy micrographs of the internal and external morphology and ultrastructure, along with the morphometry of the distinct populations. Current investigations compared the ultrastructure of the valves and girdle bands of both genera in detail and suggested a close relationship between them with raphe reduction and facultative raphe loss as well as spine formations as genetic control. Similarities with some Neotropical *Planothidium* Round & Bukhtiyarova species are also discussed.

## THE DIATOM GENERA *NITZSCHIA* HASSALL AND *HANTZSCHIA* GRUNOW FROM XINJIANG, CHINA

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The Xinjiang Uygur Autonomous Region (N34°25'-49°10' / E 73°21'-96°21') is located in northwestern China. The province covers one sixth of the territory of China. It borders eight countries including Russia, Kazakhstan, Kirghiziastan, Tajikistan, Pakistan, Mongolia, India and Afghanistan. Its landscape is a mixture of three mountains and two basins alternating with each other. High evaporation, little precipitation and an arid climate result in the upper soils accumulating salt and generating a saline-alkali soil. Despite this, there is a diversity of lentic and lotic habitats, with varying gradients of nutrient levels, pH, temperature and elevation. In addition, the region is in part affected by anthropogenic activity. Thus, the diatom flora in Xinjiang should be diverse at both the generic and specific levels. Up to now, only a few people have studied the diatoms of this region. With respect to the genera *Nitzschia* and *Hantzschia*, only Hustedt (1922) reported four new endemic species from Pamir.

Algae samples were collected from this region in July 2001 and July 2007. Altogether, 311 sites were sampled, spanning a wide variety of habitats at altitudes between 450-3600 m. Nearly 600 diatom species were identified with LM and SEM, among them, about 54 *Nitzschia* and 16 *Hantzschia* taxa. Detailed descriptions have been written for all the *Nitzschia* and *Hantzschia* taxa encountered to date. Most of them are adapted to alkaline-salt and high conductivity waters. Some species are commonly known: e.g. *N. vermicularis*, *N. sigmaidea*, *N. dissipata*, *N. obtusa*, *N. sigma*, *N. linearis*, and *N. palea*. Other species from Xinjiang Province are either very rare or exhibit interesting features as found in SEM. Included among these are *N. cf. eglei*, *N. cf. sublinearis*, *N. cf. elegantula*, and *N. cf. diversa* as well as other possible new, endemic species.

In this poster we present descriptions as well as light and scanning electron micrographs documenting the *Nitzschia* and *Hantzschia* species from Xinjiang Province, and discuss the taxonomic, systematic and biogeographic implications of our findings. We continue to study this interesting region to further assess other diatom species and the floristic diversity of the region.

Supported by the National Natural Science Foundation of China (No. 31100151)

## STUDY OF *CYCLOTELLA* (KÜTZING) BRÉBISSON OF DIATOMS AND THEIR RELATIONSHIP WITH SOME OF PHYSICO- CHEMICAL FACTORS OF WATER IN SIAHROOD RIVER, IRAN

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The Siahrood River is one of the important rivers of the Caspian Sea water basin. This river originates from the Alborz Mountains. It is the most important source of water for agriculture and economic purposes in Ghaemshahr (Mazandaran province). Therefore, a study about the microorganisms in this river is very important for water quality monitoring. Diatoms are the major constituent flora in aquatic ecosystems such as freshwater habitats. Samples were taken from stones and sediments and immediately fixed with formaldehyde 4% in situ. For exact recognition, permanent slides were prepared using the Patrick & Reimer procedure. Identification of diatoms was done by means of light microscope and specialized recognition keys. For the study of diatom communities in aquatic ecosystems, a counting method is used because in this method, identification and determination of diatom communities and morphological variations of species are better known. Simultaneously, physiochemical analysis, temperature, pH, and EC were determined. In this research 4 species were identified belonging to the *Cyclotella* genus (*C. meneghinana*, *C. operculata* and *C. steriata* and *C. glomerata*). *C. meneghinana* was dominant in the whole study period. Likewise, this study revealed that the density of diatom species was higher with increased temperature, high electrical conductivity and a pH of 7.5.



TWO UNKNOWN *LUTICOLA* TAXA FROM THE MARITIME ANTARCTIC REGION

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Species of the genus *Luticola* Mann are often found in a variety of aerial, subaerial and aquatic habitats in Antarctica. Recent studies on Antarctic non-marine diatoms have shown that the species diversity in this genus in the Antarctic region has been underestimated in the past. In the last few years, a large number of new *Luticola* species, most of them previously lumped together as infra-specific taxa of *Luticola muticopsis*, *L. mutica* and/or *L. cohnii*, were recognized and described from the islands in the Antarctic Peninsula area, and the overall number of *Luticola* species, known from this part of the world increased significantly (see Kopalová et al. 2011, Van de Vijver et al. 2011 and references therein for details).

During an ongoing revision of the limnoterrestrial diatom flora of Livingston Island – the second largest of the South Shetland Islands, and James Ross Island, located in the northwestern Weddell Sea, two *Luticola* taxa that cannot be identified using the currently available literature were observed. The first taxon resembles another recently described species from the Antarctic, *L. vermeulenii*. The second species shows some affinities to the European *L. goeppertiana*-complex. Both taxa are studied using light and scanning electron microscopy and compared with other morphologically similar species from the Antarctic and elsewhere to justify their separation as independent species. Based on the results of a preliminary biogeographical analysis, the two taxa have never been observed outside Maritime Antarctica, suggesting once again a separation between this region, the Antarctic Continent and the sub-Antarctic islands in Southern Indian Ocean, as already observed in previous studies.

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## THE GENUS *STAURONEIS* ON LIVINGSTON AND JAMES ROSS ISLAND (MARITIME ANTARCTIC REGION)

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During a survey of the limnoterrestrial diatom flora of two Antarctic islands – Livingston Island (Southern Atlantic Ocean) and James Ross Island (Northwestern Weddell Sea), a relatively high diversity in the genus *Stauroneis* was observed. A total of 11 *Stauroneis* taxa has been recorded. Based on literature data (e.g. Lange-Bertalot et al. 2003, Van de Vijver et al. 2004, Zidarova et al. 2009), three of the taxa have already been known from other islands in the Southern Atlantic Ocean: *S. husvikensis* and *S. pseudoschimanskii* were recently described from South Georgia, and *S. nikolayi* is so far only known from Livingston Island and Signy Island. One species, *S. latistauros*, is shared between the islands in the Southern Atlantic Ocean and the Antarctic continent, whereas *S. pseudomuriella* is common for the islands in the Southern Indian and Southern Atlantic Ocean. Two taxa, *S. obtusa* and *S. subgracilior*, have been found outside the Antarctic region. The remaining four taxa have an unclear taxonomic position. For one species only a few valves have been observed, and therefore it was assigned as *Stauroneis* sp. 1. Three taxa, provisionally named *S. aff. acidoclinata*, *S. aff. jarensis* and *S. aff. reichardtii*, could not be identified using the currently available literature. In order to clarify their correct taxonomic position, the Antarctic populations of these species were compared with the type populations of *S. acidoclinata*, *S. jarensis* and *S. reichardtii* from Europe.

The present poster illustrates the high *Stauroneis* species diversity and discusses their taxonomic position. All taxa are studied in light and, where appropriate, scanning electron microscopy. Notes on their biogeography are added.

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THE GENUS *NEIDIUM* IN THE ANTARCTIC REGION

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A survey of the records for the genus *Neidium* indicates that less than 20 taxa have been reported from the Antarctic and Subantarctic suggesting that *Neidium* species are relatively rare or poorly reported. The recorded biogeographic distribution of this genus is in the Maritime and subantarctic region from James Ross Island to the Crozet Archipelago. *Neidium aubertii* Manguin, and two new potential species are reported here. The three taxa are characterized by linear to linear-lanceolate valves with rostrate to capitate apices, a single prominent longitudinal canal along the valve margins, and lacinia at the distal raphe fissures. The proximal raphe fissures have varying degrees of deflection towards the margins. Two taxa, *Neidium aubertii* and *Neidium* sp.1 have chambered areolae, similar in structure to *Neidium bisulcatum* Lagerstedt, while *Neidium* sp.2. has a simple poroid areola structure covered internally by a fine hymen, similar to *Neidium affine* Ehrenberg. *Neidium aubertii* was the most common of these taxa and is similar to *Neidium bisulcatum* which has also been reported (without specimen verification) from the Subantarctic. *Neidium aubertii* has larger longitudinal canals, and the valve apices are rostrate, not broadly rounded like *N. bisulcatum*. *Neidium* sp.1 has a linear valve with rostrate apices. The longitudinal canal has a row of small round poroid opening on the valve face and another row on the mantle. Areolae have small rounded external foramina, which open into a chamber within the wall. These chambers have an internal hymen cover. In LM, the valve has a similarity outline to *Neidium kozlowi* Mereschkowsky, but the areolae are denser, have smaller more simple external foramina, and the striae are not oblique as observed in *N. kozlowi*. *Neidium* sp. 2 has a linear to weakly concave valve with capitate apices. The areolae are poroid with round external openings and an internal opening covered by a hymen. The longitudinal canal has a row of small pores opening onto the valve face close to the striae. Although similar in outline to *Neidium affine* var. *longiceps* (Gregory) Cleve, *Neidium* sp. 2 has only one longitudinal canal (i.e. not related to *Neidium affine*) and the apices are capitate. The presentation here of three distinct *Neidium* taxa from the Maritime Antarctic and Subantarctica suggests that a more thorough investigation of this genus from the Antarctic region is required.



# Author Index



Abe, K.	56,121	Bones, A.M.	31,124
Abarca, N.	65,115	Bonet, B.	191
Ács, É.	95	Bosak, S.	29,135
Adam, F.	43	Bouchez, A.	30,59
Adamska, I.	106	Bourgougnon, N.	47
Admiraal, W.	49	Bowler, C.	52,228
Agogué, H.	128	Brady, M.	107
Ahn, J.H.	122	Brembu, T.	31,124
Ai, X.	188	Brito, A.	25
Alakananda, B.	77	Brotas, V.	25,210
Allinger, L.	123	Brown, M.H.	70
Almandoz, G.	158,159	Bruneel, C.	213
Almeida, S.F.P.	191	Bryan, A.	222
Alverson, A.J.	21	Buczkó, K.	113
Amato, A.	227	Buia, M.C.	205
Andersen, M.V.	124	Bužančić, M.	221
Anderson, N.J.	79	Caccamo, M.	44
Andersson, J.	114	Čalić, M.	136
Andrejić, J.Z.	125	Campeau, S.	11,204
Annenkov, V.V.	22,126,149	Cangelosi, A.	123
Anokhina, L.	162	Car, A.	169
Antonucci, A.	151	Carbonelle, S.	41
Armbrust, E.V.	23,85	Card, V.	137
Artigas, J.	84	Carić, M.	136
Ashworth, J.	23,85	Carrère, V.	165
Ashworth, M.P.	24,107	Carstensen, J.	210
Ast, M.	143	Cartaxana, P.	40,68
Aston, B.	36	Caruso, A.	47
Aumeier, C.	127	Casco, M.A.	215
Baart, G.J.E.	41,42,76	Cefarelli, A.	158,159
Baez, J.	43	Chamnansinp, A.	72,138
Balasubramanian, K.	77	Chang, T.-P.	139,140
Baliga, N.S.	23,85	Chanthirath, I.	141
Bardeau, J.-F.	47	Chapligin, B.	172
Barillé, L.	25,82,131,164,165	Charles, D.F.	32,39
Barker, P.	98	Chaumeil, P.	59
Barlaan, E.A.	77	Cheminant, S.	37
Barnett, A.	26,82,128,134,210	Chen, C.-P.	33,142,163,187,188
Bartelme, B.A.	55	Chen, D.D.	187
Basharina, T.N.	22,126,149	Cheong, C.	77
Basu, S.	44	Chepurnova, O.	239
Batistić, M.	175	Cherepanova, M.	222
Battegazzore, M.	27,129	Chierici, M.	114
Beals, J.	130,206	Choquet, P.	170,190
Beauregard, L.	128	Chu, L.	143
Belton, T.	39	Chu, G.	244
Benyoucef, I.	25,131,165	Chudaev, D.	144
Bes, D.	132	Cocquyt, C.	160,230
Beszteri, NB.	88,133	Coesel, S.	23
Bhaskar, M.V.	63	Collier Valle, K.	31
Bhatt, J.P.	185	Collins, L.	67
Bixby, R.J.	28	Comeau, A.M.	145
Blommaert, L.	26,134	Congestri, R.	151
Bojović, S.	125	Conley, D.J.	208

Coppola, G.	151	Engelken, J.	106
Coquery, M.	84	Epp, L.S.	105
Corcoll, N.	191	Escandell, A.	155
Cornet, C.	146	Espinosa, M.A.	155
Coste, M.	160	Evans, K.M.	112
Cox, E.J.	34,47,111,179,184,236	Ewe, D.	156
Crawford, R.M.	35	Ezat, M.	51
Cremer, H.	147	Ezequiel, J.	40,68,161
Crosta, X.	93	Fabris, M.	41,42,76
Cruces L., F.	148	Fabro, E.	159
Cvijan, M.	125	Falciatore, A.	37,52,69
da Motta Marquez, D.	157	Fenner, J.	43
Dagnelie, J.	146	Ferrante, M.	44
Danilovtseva, E.N.	22,126,149	Ferrario, M.E.	158,159,216
Davidovich, N.	47,150	Ferreira da Silva, E.	191
Davidovich, O.	47	Ferrington Jr., L.	38
Davies, S.	36	Fevola, C.	44
de Baar, H.	108	Finn, D.P.	67
De Batist, M.	229,237	Fleurence, J.	47
Debeer, A.-E.	112	Fofana, C.A.K.	160
De Cooman, L.	166	Fortin, C.	11
de Haan, M.	251	Fortunato, A.E.	37,52
De Martino, A.	228	Foubert, I.	166,213,235
de Souza Cardoso, L.	157	Fraeye, I.	235
De Stefano, M.	87,151,205	Franc, A.	59
De Stefano, L.	151	Frankenbach, S.	40,68,161
De Tommasi, E.	151	Frenkel, J.	45
De Veylder, L.	52	Frentress, J.	245
del Monte, F.	67	Fritz, S.C.	208
Depauw, F.	37	Fuertet-Mazel, A.	61
Detongre, O.	239	Gabriels, W.	162
D'Hondt S.	112	Gale, D.	46
Di Caprio, G.	151	Gallo, L.	27
Diekmann, B.	172	Gao, Y.-H.	33,77,142,163,187,188
Ditmarsen, Z.	152	Gąsiorowski, M.	220
Dogra, S.	70	Gastineau, R.	47
Dohet, A.	200	Gaudin, P.	26,47,82,131,164
Domingues, N.	68	Gemeinholzer, B.	117
Dominy, J.N.	193	Gernez, P.	25,165
Donders, T.	147	Gillard, J.	45
Doseth, B.	124	Giraud, L.	129
Drou, N.	44	Giuliano, D.	159
Dupuy, C.	26,128	Gladenkov, A.	48
Durbinova, U.	153	Glöckner, G.	117
Economou-Amilli, A.	169	Godhe, A.	167
Ector, L.	77,83,132,160,170, 171,179,190,199, 200,212,245,246	Goiris, K.	166
Edlund, M.B.	38	Goldenberg Vilar, A.	49
Egan, A.	38	Gonzalez, P.	61
El'gygytgyn Sci. Party	222	Goossens, A.	41,42,76
Ellis, A.V.	70	Gosselin, M.	145
Elster, J.	183	Grinham, A.	46
Enache, M.	39,154	Gross, S.	167
		Gruber, A.	69,96,106,143,156
		Gu, Z.	244



Guasch, H.	191	Jendyk, J.	70
Guerrero, J.M.	159,168	Jessen, C.	208
Gunn, H.	79	Jesus, B.	25
Guruprasad, S.	77	Jewson, D.H.	28,54
Gusev, E.	64,101,115	Johansen, J.R.	55,242
Haferkamp, I.	143	John, J.	176,177
Hafner, D.	169	Johnsen, G.	31
Haimovich, M.	156	Johnston, M.	70
Hájek, M.	90	Jones, M.	36
Hamilton, P.B.	77,204,212,251	Jones, V.J.	79
Han, J.	244	Jordan, R.W.	56,121,178
Hancke, K.	31	Juggins, S.	92
Hansen, G.	47	Julius, M.L.	57,77,102,141,152,180
Hara, S.	75	Jüttner, I.	179
Hardivillier, Y.	47	Kaczmarska, I.	47
Harwood, D.M.	78	Kahlert, M.	58
Hayles, J.	70	Kamei, Y.	219
Hayworth, E.Y.	50	Kamiya, M.	66
Hedblom, M.	114	Kaplan, A.	156
Heirman, K.	229,237	Katoh, K.	77
Hellio, C.	47	Kaufhold, S.	43
Hem, C.D.	124	Kawachi, M.	66
Higashi, S.-I.	219	Kelly, M.	9
Hinz, F.	133	Kermarrec, L.	59
Hisabori, T.	75	Khim, J.S.	201
Hlúbiková, D.	170,171,190,200	Khursevich, G.K.	60,64,110
Hobbs, W.	38	Kikutani, S.	75
Hodgson, D.A.	229,238	Kildea, T.	70
Hoff, U.	51,172	Kim Tiam, S.	61
Hoffmann, L.	83,170,171,190,199, 200,245,246	Kimura, K.	62,109,181,231
Hofmeester, C.	177	Kiran, T.M.	63
Hogan, E.J.	79	Kobos, J.	116
Hoogstraten, A.	108	Koç, N.	51
Houben, G.	43	Kociolek, J.P.	60,71,110,180,182,247
Hojić, A.	135	Koh, C.-H.	201
Humbert, J.-F.	59	Kooistra, W.H.C.F.	29,197
Husain, M.	173	Koolmees, H.	147
Huysman, M.J.J.	52,228	Kopalová, K.	183,184,249,250,251
Ichinomiya, M.	66	Kozlov, A.S.	126
Idei, M.	174	Kreike, A.	49
Imura, S.	229	Kristiansen, S.M.	86
Inzé, D.	52	Krizmanić, J.	125
Iseki, M.	219	Krock, B.	227
Isla, F.I.	155	Krokowski, J.	179
Iwataki, M.	178	Kromkamp, J.C.	73
Jacques, A.	229	Kroth, P.G.	10,52,69,75,96,106, 143,156
Jacquette, I.	47	Kršinić, F.	136
Jahan, D.	164	Kudoh, S.	229
Jahn, R.	53,64,65,77,94,101, 115,117	Kuijpers, A.	86
Jarlman, A.	179	Kulikovskiy, M.	64,77,101,115,223
Jasprica, N.	104,136,169,175	Kumar, A.	185
Jelačić, A.	221	Kuriyama, Y.	178
		Kusber, W.H.	65

Kuwata, A.	66	Mathieu, B.	228
Labollita, H.	215	Matias de Faria, D.	157
Lafrançois, T.	38	Matsuda, Y.	75
Lamaro, A.	159	Matsunaga, S.	219
Lambert, A.-S.	84	Matsuoka, T.	194,196
Lang, P.	230	Matthijs, M.	41,42,52,76
Lang, Y.	67	Mayama, S.	77
Lange-Bertalot, H.	64	Mazalová, P.	90
Large, A.	98	Mazella, N.	61
Larras, F.	30	McCartney, K.	78
Launeau, P.	25,82,131,165	McDonagh, M.E.	215
Lavaud, J.	26,68,69,82,106,128 134,210	McDonnell, J.J.	245
Laviale, M.	40,68,161	McGowan, S.	79
Lavigne, A.	217	Medlin, L.K.	80,81
Lavoie, I.	11,204	Meire, P.	241
Lechnes-Wiens, H.	43	Méléder, V.	25,26,82,128,131, 164,165,210
Lee, A.	23,85	Menzel, D.	127
Lee, J.H.	77	Metcalfe, S.	36
Leignel, V.	47	Mettbach, U.	127
Leng, M.	36	Meyer, H.	172
Lepetit, B.	69	Mialet, B.	241
Leterme, S.C.	70	Michel, C.	91
Levkov, Z.	249	Miele, M.	44
Li, X.-S.	163	Mikame, Y.	195
Li, Q.-Y.	33	Moestrup, Ø.	72,138
Li, Ya.	72	Mohri, Y.	232
Li, Yu.	186	Monnier, O.	171
Liang, J.-R.	33,142,163,187,188	Montresor, M.	12,44,197
Lidberg, R.	141	Montuelle, B.	30
Lin, S.-L.	187	Morais, M.M.	199
Liu, J.	244	Morales, E.A.	83,154,168,212
Lobo, E.A.	77,132	Morançais, M.	47
Louvrou, I.	189	Morin, S.	61,84
Lovejoy, C.	145	Mouget, J.-L.	47,164
Lowe, R.	71	Muangphra, P.	77
Lu, H.	244	Muylaert, K.	166,213,235
Lucadamo, L.	27	Nagumo, T.	122,174,186,194, 195,196
Luddington, I.	47	Nakov, T.	24,107
Luís, A.T.	170,190,191	Nanjappa, D.	29,197
Lundholm, N.	72,138,227	Nat, E.	49
Ly, J.	73	Nedbalová, L.	183
MacKay, A.W.	244	Nelson, H.	28
Main, S.P.	192	Nesterovich, A.	198
Mangoni, O.	214	Nielsen, T.G.	227
Mann, D.G.	74,97,112,174	Ninčević Gladan, Z.	221
Manoylov, K.M.	193	Novais, M.H.	83,199,120
Marasović, I.	221	Nymark, M.	31
Maris, T.	241	Ohki, K.	66
Martínez Chávez, E.	168	Ohtsuka, T.	201
Martinez-Carreras, N.	245	Olney, M.	218
Masoudian, N.	248	Omori, H.	77
Massé, G.	86	Orellana, M.V.	23,85
Mather, L.	47		

Ovalle, H.	203	Rodi, B.	248
Palmer, P.	32	Rodriguez, B.	67
Pandit, M.K.	185	Rogato, A.	37,69
Pandit, A.	67	Romagnoli, T.	87
Pang, W.	247	Romero, O.E.	53,93,94,209
Panizzo, V.N.	244	Rostaing, P.	228
Park, J.	201	Rottberger, J.	96
Patil, S.	44	Ruck, E.C.	107
Pearce, C.	86	Rusanov, A.G.	95
Pedraza, E.	203	Ryckebosch, E.	213
Penant, P.	146	Sabbe, K.	26,99,112,134,229, 238,240,241
Pennesi, C.	87	Sachse, M.	69,96
Pesce, S.	61,84	Saggiomo, M.	214
Petrov, A.K.	126	Saitoh, K.	66
Pfister, L.	245	Sala, S.E.	168,203,215,216
Philippart, C.J.M.	73	Sanges, R.	44
Philippe, B.	145	Santinelli, N.	158,216
Philippe, R.	131	Sar, E.	217,224,225
Pilny, J.	55	Sarno, D.	29,135
Pimienta, A.	203	Saros, J.	100
Pinkernell, S.	88,133	Sastre, V.	158,216
Pither, J.	89,202	Sato, S.	97,174
Plata-Diaz, Y.	203	Sato, N.	66
Pletikapić, G.	135	Sawada, K.	66
Pohnert, G.	45	Scalco, E.	44
Ponader, K.C.	204	Schellenberg-Costa, B.	96
Popovskaya, G.I.	234	Scherer, R.	218
Poretti, V.	39	Seidenkrantz, M.-S.	86
Porzio, L.	205	Seino, S.	77
Potapova, M.	130,154,204,206	Serôdio, J.	40,68,161
Poulíčková, A.	90	Shikate, T.	219
Poulin, M.	87,91,131,145	Sienkiewicz, E.	220
Poulsen, N.	13	Sims, P.A.	113,236
Pouvreau, J.-B.	47	Skejić, S.	221
Prasetiya, F.S.	47	Skibbe, O.	65
Quesnel, F.	146	Skogen Chauton., M.	31
Raes, J.	14	Slupphaug, G.	125
Ramanibai, R.	207	Snell, M.A.	98
Randsalu-Wendrup, L.	208	Snowball, I.	208
Rasmussen, T.L.	51	Snyder, J.	222
Ravichandran, S.	207	Soeprbowati, T.R.	77
Reavie, E.	92,123	Solak, C.N.	77,223
Reichardt, E.	179	Souffreau, C.	99
Reyes, F.	203	Sow, E.H.	160
Reynisson, N.F.	86	Spaulding, S.A.	28,100
Riaux-Gobin, C.	209	Srivibool, R.	77
Ribeiro, L.	210	Stachura-Suchoples, K.	101
Rimet, F.	30,59	Stapleton, C.	102
Rines, J.	47	Štěpánková, J.	90
Rioual, P.	211,244	Sterken, M.	238
Riss, W.	203	Sterrenburg, F.A.S.	217,224
Rivera, S.F.	212	Stevenson, R.J.	103
Rivera R., P.	148	Stickley, C.E.	56
Roberts, S.J.	238		

Stijepo, L.	104	Van der Putten, N.	238
Stoof-Leichsenring, K.R.	105	van Ee, G.	49
Sturm, S.	69,96,106	van Leuken, R.	49
Sun, L.	33	Van Nieuwenhuyze, W.	238
Sunesen, I.	217,224	van Rensburg, L.	230
Šupraha, L.	29	Van Wichelen, J.	241
Surridge, B.	98	Vandamme, D.	235
Suto, I.	56,121	Vanormelingen, P.	99,112,239,240,241
Suzuki, H.	122,186,195	Vanstechelman, I.	240
Svetličić, V.	135	Verhaegen, G.	162
Sylwestrzak, Z.	116	Verkhovina, O.N.	149
Tackx, M.	241	Verleyen, E.	17,99,229,237,238
Taffs, K.	46	Verstraete, T.	112
Takaichi, S.	66	Veselá (C. Budejovice), J.	55,242
Takao, Y.	109,231	Veselá (Prague), J.	233,243
Talgatti, D.	225,226	Viličić, D.	29,134
Tammilehto, A.	227	Vouilloud, A.	215
Tanaka, J.	122,186,195,196	Vugrinec, S.	106,143
Tanaka, A.	228	Vuylsteke, M.	240
Tavernier, I.	229	Vyverman, W.	26,41,42,45,52,76, 99,112,134,229,237, 238,239,240,241
Taylor, J.	160,230	Wagstrom, R.	137
Thaler, M.	145	Wang, P.	187
Theriot, E.C.	24,107	Wang, Q.	247
Tiedemann, R.	105	Wang, L.	244
Timmermans, K.	108	Watanabe, M.	219
Tirichine, L.	228	Wetzel, C.E.	83,211,245,246
Tomaru, Y.	62,109,181,231	Williams, D.M.	113,182
Torgan, L.C.	132,225,226	Winge, P.	31
Torstensson, A.	114	Witkowski, A.	64,77,201,209
Totti, C.	87	Witkowski, J.	78,236
Toyoda, K.	109,174,231	Wolin, J.	103
Trauth, M.H.	105	Wu, W.-Z.	163
Tremblay, R.	47	Wulff, A.	47,114
Trobajo, R.	15	Wyseur, J.	166
Tuji, A.	232	Xu, H.-L.	142
Turcotte, F.	47	Xu, B.	187
Turpin, V.	164	Yang, D.	244
Udy, J.	46	Yokoyama, J.	178
Umadevi, K.	63	Yoshiakwa, S.	66
Underwood, G.J.C.	16,91	You, Q.	247
Unoura, K.	178	Yu, A.	107
Urbánková, P.	233,243	Zadeh, A.A.N.	248
Usoltseva, M.	110,153,234	Zalack, J.	103
Uyua, N.	216	Zelinskiy, S.N.	149
Vaché, V.	170,190	Zetsche, H.	64,101,115
Vadstein, O.	31	Zgrundo, A.	116
Van Burm, E.	241	Zhao, D.-H.	163
van Dam, H.	49	Zheng, M.-H.	33
Van de Vijver, B.	71,83,99,111,160, 171,179,183,184, 199,236,249,250,251	Zhou, Q.-Q.	33
Van de Vyver, E.	237	Zidarova, R.	183,184,249,250,251
Van den Daele, H.	52	Zimmerman, J.	53,65,94,117
van der Geest, H.G.	49	Zingone, A.	197,214







