

**15<sup>th</sup>**  
**EUROPEAN**  
**DIATOM MEETING**  
Ohrid, North Macedonia  
**7<sup>th</sup> – 9<sup>th</sup>**  
**May, 2024**

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## PREFACE

After three years planning and postponing, European Diatom Meeting is organized in Ohrid, North Macedonia. Initially planned for 2021, then postponed for 2022 due to the Covid 19 pandemic, it was finally scheduled for 2024. Having in mind the exceptional biological features of the Lake Ohrid, which were recognized by UNESCO in 1979, as well the cultural tradition and importance of town of Ohrid, it was not preferred to organize an online meeting during pandemics and restrictions. The conference is a great opportunity for participants to experience our famous lake that was already visited by several famous algologists such as Bruno Schröder, Bohuslav Fott, Friedrich Hustedt, Anto Jurilj, Horst Lange-Bertalot, Ditmar Metzeltin etc. One of the most famous excursions was organized by Dr. Siniša Stanković in 1934 during the 7<sup>th</sup> Congress of Limnologists in Belgrade, Serbia, when the most prominent limnologists of the time visited for the first time Lake Ohrid and subsequently published numerous studies highlighting the lake as an important biological and geological site.

This year EDM is organized on the shore of Lake Ohrid, one of the biodiversity hotspots in Europe. The lake is considered to have the highest index of endemic diversity of animal species, with 80% of endemism in some animal groups such as Porifera, Tricladida, Amphipoda, Gastropoda etc. Such high diversity and endemism, is most likely the result of multiply colonization and intralacustrine speciation in combination with longevity of the lake without catastrophic events during its geological history. The lake existed continuously at least 2 million years, although some geological studies and biological data suggest much older lake spanning from the late Miocene.

The commission of UNESCO also recognized the importance of the town of Ohrid as cultural, architectural and religious centre. The town of Ohrid is one of the oldest human settlements in Europe. As one of the best preserved complete ensembles encompassing archaeological remains from the Neolithic Era up to the Middle Ages. Ohrid is a testimony of Byzantine arts, displayed by more than 2,500 square metres of frescoes and more than 800 icons of worldwide fame. The churches of St. Sophia (11<sup>th</sup> century), Holy Mother of God Perivleptos and St.

John Kaneo notably display a high level of artistic achievements in their frescoes and theological representations. The Lake Ohrid region boasts the most ancient Slavic monastery and the first Slavic University in the Balkans – the Ohrid literary school that spread writing, education and culture throughout the old Slavonic world.

**T**he EDM conference is a continuation of the Central European Diatom Meeting (CEDM) and the Treffen Deutschsprachiger Diatomologen (DDT), which has been held regularly for almost 50 years. Almost 140 participants from 29 countries attend the 15<sup>th</sup> EDM conference, not only from Europe, but also from Africa, Asia and North America. A total of 116 scientific studies will be presented at the conference, including three keynote speeches, 41 oral presentations and 72 posters, covering various topics of diatom research: taxonomy, phylogeny, evolution, genetic, ecology, paleoecology, physiology, forensic, 3D printing and materials that makes the 15<sup>th</sup> EDM one of the largest EDM conferences. The EDM will be followed by a one-day Workshop on diatom taxonomy “European species of the araphid genera *Staurosira* and *Staurosirella*” organised by Bart Van de Vijver.

**O**n behalf of the conference committee, we would like to welcome all participants to our EDM conference in Ohrid and wish them a successful conference with constructive discussions accompanied by traditional Macedonian food, beer and wine. We would like to take this opportunity to thank the keynote speakers, presenters and authors for contribution, and wish you all a fulfilling experience and very pleasant stay in Macedonia.

Danijela Mitic Kopanja, Dušica Zaova & Zlatko Levkov

**KEYNOTE  
PRESENTATIONS**



**EXPLOSIVE DIVERSIFICATION IN DARWIN'S  
DREAMPOND: THE CICHLID FISHES OF LAKE  
TANGANYIKA**

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Adaptive radiation is the likely source of much of the ecological and morphological diversity of life on Earth. Owing to their spectacular taxonomic, phenotypic, ecological and behavioral diversity and propensity for explosive speciation, the assemblages of cichlid fishes in the African Great Lakes Victoria, Malawi and Tanganyika are prime role models for this evolutionary process. The cichlid fishes of Lake Tanganyika constitute the morphologically, ecologically and behaviorally most diverse cichlid species flocks. Our in-depth examination of nearly all its approximately 250 cichlid species revealed that this spectacular adaptive radiation proceeded in a non-gradual manner, in the form of three consecutive and trait-specific pulses of accelerated phenotypic evolution. In addition, there is evidence that diversification with respect to behavior as well as pigment patterns contributed to the cichlid adaptive radiation in Lake Tanganyika.

## **UNDERSTANDING THE DARK MATTER OF THE DIATOM CHLOROPLAST**

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Diatoms, as photosynthetic eukaryotes, depend on the metabolic activities of their chloroplasts to support their primary production and cellular biology. But what sustains this organelle? The diatom chloroplast is distantly related to that of plants, derived from a secondary or higher endosymbiosis of a eukaryotic red alga, and surrounded by an intricate membrane system that necessarily complicates the import of nucleus-encoded proteome. Identifying what proteins function in the diatom chloroplast, and why, is crucial to understanding their biology, and present-day environmental distributions.

Here, I will discuss a decade of work, using combined phylogeny, meta-genomics, physiology and biochemistry to characterise the unknown components of the diatom chloroplast proteome. First, I will show that the diatom chloroplast is an evolutionary mosaic, supported by nucleus-encoded and endogenous components derived not only from the red endosymbiont, but also the host, and ancestral and progressive horizontal gene transfers from both eukaryotic algal and bacterial sources. I will also show the importance of diatoms for understanding the evolutionary dynamics of other major algal orders, particularly pelagophyte, haptophyte and dinoflagellate algae.

Next, I will outline attempts from my group to place individual proteins that support the diatom chloroplast into a functional and eco-physiological context, particularly using the experimental model *Phaeodactylum* and global environmental data from the Tara Oceans Expedition. These include: a complete plastidial glycolytic-gluconeogenic pathway of host origin, which may facilitate diatom acclimation to variable day lengths and

environmental temperatures ; an unusual homologue of molybdate transporters that localises to the diatom periplastidial membrane and may enable photoacclimation under Fe limitation; and a distant subunit of ATP synthase subunit A that is broadly shared, but unique to marine eukaryotic algae. Finally, I will consider how characterising the diatom chloroplast proteome may provide clues into algal resilience and fragility to anthropogenic climate change.

**THE PALAEO LIMNOLOGICAL SIGNIFICANCE  
OF ANCIENT DIATOMS IN ANCIENT LAKES**

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In this talk, I will discuss how diatoms, often endemic and often extinct, have furthered our understanding of both ancient lake ecosystems in general, and of the interplay between climate and basin morphology in influencing long-term patterns of diatom diversity through time. Recent work has also highlighted the importance of environmental drivers in influencing diatom morphology and ecosystem function through time. Case studies will be drawn on from ancient lakes around the world.

**ORAL  
PRESENTATIONS**



## INTERMITTENT RIVERS AROUND EUROPE AND THE MEDITERRANEAN – DIATOM COMMUNITY VARIABILITY CAUGHT THROUGH AMPLICON SEQUENCING

**Andrea M. Burfeid Castellanos**<sup>1\*</sup>, Maria H. Novais<sup>2</sup>, Manuela Morais<sup>2</sup>, Zoltán Csabai<sup>3</sup>, Bálint Pernecker<sup>3</sup>, Eman Calleja<sup>4</sup>, Armin Lorenz<sup>1</sup>, Frédéric Rimet<sup>5</sup> & Agnès Bouchez<sup>5</sup>

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Intermittent rivers and streams are a common feature of the Mediterranean region due to the climactic tendency to have dry – hot summers. However, the trend of more and more rivers and streams becoming intermittent is becoming evident in other climates. Only a few organisms survive the drying, and benthic, biofilm-dwelling diatoms can be able to do it if the humidity suffices. In different climates, the proportion of survivability is variable, as increased drought can dry out the biofilm, shifting the community to terrestrial. Proper monitoring of these types of rivers and conserving their intrinsic diversity has not been adequately studied yet. As all rivers and streams are in danger of being at least temporarily intermittent due to climate change, it is important to confirm the universality of amplicon sequencing, which is the main aim of this study. To characterize the community, the use of amplicon sequencing – in this case *rbcL* ASVs – was undertaken. A selection of samples from France, Germany, Hungary, Israel, Malta, Portugal and Tunisia was used to compare the usability of the *rbcL* marker gene to display the genetic variability found in the samples. Species assignment varied according to distance from France. Genetic variability per site was highest in Portugal. The proportion of living, non-terrestrial diatoms was reduced in the dry locations. Pools represented a higher diversity of diatom species than flowing rivers. Temporal recolonization after

rewetting in rivers showed that diatom diversity was reduced directly following the drought, and that the resilience of the organisms in the rivers was affected by it. Effects of the anthropogenic effort in perennializing intermittent rivers also resulted in changes in the diatom community, as salinization was often associated with them.

**INTEGRATIVE TAXONOMIC AND ECOLOGICAL CHARACTERIZATION OF *EUNOTIA TENELLA* (GRUNOW) HUST., A DIATOM RED-LIST SPECIES TYPICAL OF OLIGO-DYSTROPHIC HEADWATER HABITATS**

**Marco Cantonati**<sup>1\*</sup>, María Cid-Rodríguez<sup>2</sup>, Nicola Angeli<sup>3</sup>, Priscillia Gourvil<sup>4</sup>, Michele Grego<sup>4</sup>, Daniel Spitale<sup>5</sup>, Francesco Trenti<sup>6</sup> & Graziano Guella<sup>6</sup>

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*Eunotia tenella* (Grunow) Hustedt in A. Schmidt et al. 1913 is a characteristic diatom species of undisturbed, electrolyte-poor, oligo- to dystrophic habitats (e.g., low-alkalinity seepage and pool springs on siliceous substrata). The study of this species was performed using a polyphasic approach. Besides marker gene sequences, such as 18S and *rbcL*, the species was characterized by morphology and ultrastructure (LM and SEM), by the content of pigments and lipids (carotenoids and lipid “fingerprint”), and its ecology. After some time in culture, the strain exhibited atypical (aberrant) morphology which was as well documented. Several blasts confirmed that there is no known sequence of *Eunotia tenella* in GeneBank or other databases (e.g., AlgaTerra) but revealed a match with a sequence of “*Eunotia* sp.” that might therefore be attributed to this species. Pigment analysis by LC-MS techniques indicates the presence, besides chlorophyll *a*, chlorophyll *c* and fucoxanthin, of a group of photoprotective carotenoids which includes  $\beta$ -carotene and the xanthophylls, diatoxanthin and diadinoxanthin. Lipid analysis, on the other hand, shows the presence of several classes of phospholipids (PE and PG as the most abundant), glycerides (very abundant TAG and DAG) and galactolipids (MGDG, DGDG and SQDG). The latter bear highly unsaturated  $\omega$ -3 fatty acyl chains, in particular eicosapentaenoic

acid (EPA) and docosahexaenoic acid (DHA). For the ecological characterization we used our databases on springs, high-mountain lakes, mires of siliceous mountains of the Alps and central Europe. Efficient desiccation-resistance strategies, apparently widespread in the genus *Eunotia*, are important for life in high-mountain springs, mires and lakes. These adaptations might be supposed also for *Eunotia tenella* on the basis of the characteristics of the habitats colonized. Low-alkalinity, naturally slightly-acidic, strictly-oligotrophic mountain freshwater habitats host diatom assemblages which possess a high species richness. They include many species considered rare and belonging to the Red List making the conservation diatom biodiversity of these habitats essential. These relatively-pristine aquatic habitats on siliceous bedrocks are however highly sensitive, and endangered because of diffuse pollution. Climate- and environmental-change driven increased release of organic acids, and contamination by airborne inorganic acids and nutrients are all potential threats to these still high integrity but fragile systems. The detailed characterization of their diatom assemblages, that are excellent monitors of acid-base and nutrient status is thus of the foremost importance. In particular the distinction between naturally-acidic and acidified systems is a relevant topic that might benefit from detailed knowledge of taxonomy and ecology of both communities and indicative taxa of the diatom assemblages. *Eunotia tenella* appears to belong to the category of species with thrive in naturally-acidic (= non-acidified) aquatic ecosystems.

**SEXUAL REPRODUCTION LIMITS THE USE OF CLONAL LINES TO CONTROL PHENOTYPIC VARIATION OF *COSCINODISCUS GRANII* FRUSTULE MORPHOLOGIES FOR PHOTONIC APPLICATIONS**

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The porous nanostructures presented by the silicon dioxide frustules of diatoms exhibit light-modulating properties, reminiscent of photonic crystals commonly produced in cleanrooms. As the current production of these materials relies widely on non-sustainable techniques, diatom frustules are promising nanomaterials as an eco-friendly alternative to artificial nanofabrication. Recent research has confirmed that the girdle bands of *Coscinodiscus granii* exhibit highly ordered nanopores arranged in square or hexagonal lattices forming slab photonic properties which can present photonic bandgaps. Such properties are highly demanded in applications spanning sensing application, telecommunication, solar energy technologies, or quantum computing. The precise use of this properties, however, can be limited by phenotypic variation of some structural parameters, such as pore diameter, which controls refractive index contrast inside the slab. Here, we tested if structural parameters can be controlled by cultivating clonal lines. These clonal lines are accomplished by single cell isolation and subsequent propagation by mitotic cell division, during the asexual phase of the diatom life cycle. Regular cell monitoring ensured assessment of clonal culture progression. Scanning Electron Microscopy, coupled with microscopic techniques for assessing photonic properties were subsequently used

to investigate girdle structure and optical characteristics, respectively. We found that cells within a clonal line showed significant reduction in phenotypic variation of pore diameters, while pore-to-pore distances remained highly conserved within and in between different clonal lines. This led to confined parameters for the use of girdle bands of that species. However, sexual reproduction - occurring within an inbreeding clonal line - reversed the experimental observation of pore size confinement. Sexual reproduction seemingly occurred randomly, within few days, or weeks, and without an obvious environmental trigger. Control of sexual reproduction might therefore be a key in producing larger-scale amounts of photonic materials from diatoms with defined lattice symmetries.

The potential technological application of diatom frustules is tightly linked to better understanding the molecular regulation of biosilicification, including control of lattice symmetries and pore size. The occurrence of sexual reproduction currently poses a limitation to producing large quantities of genetically identical clones. However, this methodology also holds promise in contributing for the future of diatom nanotechnology applications. Ultimately, it might enable on demand tailored natural photonic crystal nanomaterials, based on genetic manipulation methods.

## **EVIDENCE OF LAKE-LEVEL FLUCTUATIONS BASED ON DIATOMS IN LAKE VRANSKO, CRES ISLAND (ADRIATIC COAST) DURING THE LATE HOLOCENE**

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An investigation of Lake Vransko, located on the island of Cres in the Adriatic Sea, was performed in order to reconstruct the palaeoenvironmental changes that occurred during the late Holocene, based on diatoms. Ten diatom zones (DZs) and the water/sediment interface (WSI) were recorded from the deeper core (CS-51) and two from the shallower (CS-52) core, along with the WSI, based on which an abrupt fluctuation in the level of the lake was interpreted. A dominance of *Epithemia adnata* (DZ 2, DZ 4, DZ 7) and *Gomphonema pumilum* (DZ 5) in the CS-51, and the last mentioned (DZ 1, WSI) in the CS-52 indicated a shallow phase recorded in the lake. A dominance of *Amphora inariensis*, *Pseudostaurosira* spp., *Cyclotella* spp. and *Pantocsekiella ocellata* in the rest of the zones indicated deeper, more alkaline lake settings. Fluctuations in the lake level were mostly driven by the intensification of climatic oscillations (wet periods during North Atlantic Oscillation intervals and summer drought during Azores High intervals) and anthropogenic influences (e.g. the extraction of drinking water). The DZs accord with depth variations in Adriatic coastal lakes that are key sites of more extreme recent changes in the local climate, as recorded from the shallow core (CS-52).

### 3D PRINTING OF DIATOMS

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The group of Ille C. Gebeshuber, physics professor at TU Wien in Austria, has more than a quarter of a century experience in diatom research and outreach. In the last millennium, they investigated live diatoms with atomic force microscopy in Santa Barbara, California, USA. Then they concentrated on diatom tribology and especially hinges and interlocking devices in diatoms, as biomimetic inspiration for 3D MEMS (minute components in electronics). This work was mainly done in South East Asia. Subsequently, listening to diatoms with hydrophones and the establishment of structure-function relationships (especially in *Corethron criophilum*) was the focus of their research at TU Wien. Nowadays the group concentrates on the development of scalable designs for 3D printing of diatoms, for use in science, outreach and art. Investigating mechanical properties of diatoms can be tedious due to their minute size. 3D printed models on a larger length scale increase the accessibility and promote understanding of complex functionalities. For biologists, digital repositories such as Thingiverse are of specific interest because of the possibility to provide and download 3D models of complex biological structures and custom laboratory equipment. Such platforms democratize access to customizable designs, fostering innovation in experimental methodologies and the development of specialized tools tailored to specific research needs in the biological sciences. By leveraging this repository, biologists can significantly accelerate the iterative process of design and testing, thereby enhancing the efficiency and precision of scientific inquiry and experimentation. In the course of the last years, our group developed designs of the diatoms *Ellerbeckia arenaria*, *Kittonia elaborata* and *Hyalodiscopsis plana*. These designs can be downloaded from <https://www.thingiverse.com/calopteryx92/collections/41458463/things>.

The presentation will support scientists in creating 3D models of their specific diatoms of interest on their own, by providing established protocols, and widens the pool of available designs for diatoms that can be utilized in science, art and outreach.

## **PHOTONIC CRYSTAL SLABS IN DIATOMS: FUNCTIONALITY, DISTRIBUTION, AND POTENTIAL PHYSIOLOGICAL IMPLICATIONS**

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Researchers who study photonics - the study of the interaction of light with nanoscale features - find diatoms to be an intriguing platform due to their highly perforated frustules. Recent investigations have revealed advanced photonic properties within the girdle bands of selected species belonging to the Coscinodiscophyceae. Termed photonic crystal slabs, they exhibit photonic properties with remarkable reproducibility among individuals of the same species, specifically under natural conditions when immersed in water. Photonic crystal slabs, among the most sophisticated optical materials known, facilitate waveguiding and establish stopbands within defined spectral ranges of light. Diatoms currently stand as the exclusive group of organisms capable of naturally constructing such intricate photonic structures. Despite this revelation, the functional significance of these photonic structures remains enigmatic, as does their prevalence across the diverse landscape of diatoms. To address these uncertainties, we conducted a comprehensive analysis of the girdle band topologies of approximately 400 diatom species. Our findings revealed that only two genetic clades within the Coscinodiscophyceae (*Coscinodiscus* spp. and *Proboscia* spp.) and one within the Mediophyceae (*Trieres* spp.) harbour these distinctive structures, all of them with a planktonic lifestyle. The stopbands associated with square lattice types primarily occupy the visible light spectrum, yet certain species exhibit hexagonal

slab photonic crystals with anticipated stopbands extending into the near to mid-infrared spectral range. Interestingly, the Bacillariophyceae lack these slab photonic crystals within their girdle bands; however, preliminary evidence suggests that some raphid pennates may exhibit them within their valves. This observation hints at the possibility of slab photonic crystal topologies adapting for niche differentiation during the diversification of diatoms. The close association observed between slab photonic crystals, phylogeny, and lifestyle strongly implies a functional role for these structures within the organism, but one without a universal benefit, as these properties appear and disappear across the diversity of diatoms. Nonetheless, the exact nature of this role remains speculative, whether involved in light harvesting, photosynthesis facilitation, sensory perception, energy dissipation, or other mechanisms.

While humans conceptualized slab photonic crystals in the 1980s and have since utilized them in cutting-edge technologies such as lasers, quantum computers, sensing devices, and thermal management systems, their functional mechanisms within diatoms remain elusive. It appears that diatoms have employed these advanced structures for millions of years in ways that surpass our current understanding.

**INSIGHTS INTO TAXONOMY, MOLECULAR PHYLOGENY AND SEXUAL REPRODUCTION OF MONORAPHID DIATOMS CLOSELY RELATED TO STAURONEIDACEAE**

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The taxonomic position of diatoms selected for this study was uncertain or inappropriately designated. Species belonging to *Schizostauron* and *Astartiella*, are often misidentified due to their confusing taxonomic history in case of *Schizostauron* or lightly silicified frustule in case of *Astartiella*, impeding proper identification. Morphological features of *Schizostauron* and *Astartiella*, such as the stauros, fistula, and coaxial internal proximal raphe endings, are found in other genera belonging to Stauroneidaceae, whereas the only common feature with monoraphid diatoms as whole group is the heterovalvy of frustules. Although no unambiguous morphological synapomorphies between the two genera are currently recognized, they are closely related by DNA sequence data. Heterovalvate frustules of *Schizostauron* are characterized by a bifid stauros on the raphe-bearing valve and intricate areolate occlusions on the sternum valve. In *Astartiella*, the raphe-bearing valve is characterized by a process resembling a fistula by morphology, while the sternum valve presents a particular striation pattern. Observations by light and electron microscopy were made, along with a molecular phylogenetic analysis using a three-gene (SSU, *rbcL*, and *psbC*) concatenated dataset. Additionally, the sexual reproduction of *Schizostauron trachyderma* was induced and each stage of reproduction was observed and documented, that allowed to determine its mating type.

**EFFECT OF MOBILE BOTTOM TRAWLING  
ON MICROPHYTOBENTHIC COMMUNITIES  
IN THE GERMAN BALTIC SEA**

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Mobile bottom fishing has a direct and indirect effect on benthic communities, sediment characteristics and nutrient fluxes at the sediment-water interface due to heavy fishing gear. Marine protected areas (MPA) are currently not exempt from bottom trawling and thus there are hardly any undisturbed areas in the Baltic Sea and almost no research to estimate the impact of bottom trawling on benthic marine habitats. The present study is part of a comprehensive joint project, which deals with the effect of bottom trawling on benthic and demersal ecosystem components, with our focus on microphytobenthic communities. Microphytobenthos, mostly dominated by diatoms, are primary producers and thus key players in the benthic community of marine soft bottoms. In the past years, we collected sediment cores from several areas in the Baltic Sea (MPA and Reference) and examined microphytobenthic primary production and diversity (top 1–2 cm). For a quantitative and qualitative data analysis, we measured primary production, chlorophyll a content as well as sediment characteristics. Furthermore, in this study we present for the first time an insight into benthic communities from deeper areas of the Baltic Sea. The diatom communities were investigated morphologically via light microscopy and molecular markers using high-throughput sequencing. We found benthic diatom species in all samples even as deep as ~36 m. The presented data show no significant differences for primary production, chl *a*, carbon and nitrogen content between reference and MPA indicating that bottom trawling should be excluded from all protected areas.

## **EUNOTIA ISOLATED FROM SWEDISH STREAMS – A STORY OF BEAUTIFUL TAXA WITH SOME LOOSE ENDS**

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During the national barcoding project FRESHBAR (Barcoding of freshwater taxa for improved assessment of biodiversity, 2019-2023), we isolated and cultured freshwater benthic diatoms from Swedish streams and analyzed the strains with molecular and morphological methods. The main purpose was to contribute to the work of populating diatom reference databases with new sequences to improve the use of metabarcoding for environmental monitoring and research. FRESHBAR also had the aim to contribute to the development of the necessary metabarcoding infrastructure in Sweden.

More than 80% of the Swedish freshwater benthic diatom species have been found to be lacking in the most important diatom barcode databases - and one genus important for the Fennoscandian region but represented by only few sequences is *Eunotia*. Thus, we chose to focus the isolation of diatom cells from humic and acid sites with a known high abundance of *Eunotia*. Indeed, then, many (51) of the in total 301 cultured diatom strains being successfully sequenced for the barcodes *rbcL* and 18SV4, belonged to this genus. However, the work of identifying the strains turned out to be quite challenging. The sequences clustered into approximately 11 taxa. The morphological analyses showed that some of those taxa matched already described species well, so we are quite confident in the species name. In other cases, the morphology only matched “almost”. Very minor characters like the presence or absence of small thorns at the valve edges could rule out otherwise well-fitting descriptions. Some taxa fell in between two species having overlapping characters, and three of the clusters could not be given a name at all yet. Thus, we are populating reference databases with sequences, but further

research is needed to fit both molecular and morphological characters of the taxa into a lineage-based species concept for species delimitation.

FRESHBAR is one of eight research projects focusing on the development of DNA methods for national environmental monitoring. The project was financed by the Environmental Protection Agency's environmental research grant in collaboration with the Swedish Agency for Marine and Water Management.

## **DARK SURVIVAL MECHANISMS IN POLAR BENTHIC DIATOMS**

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The polar regions represent an extreme habitat for phototrophic algae due to long periods of darkness caused by the polar night. Benthic diatoms, which dominate microphytobenthic communities in coastal soft bottom regions, can survive this dark period, but the underlying physiological, biochemical and cell biological mechanisms are not well understood. Over the last decade numerous benthic diatom strains from Arctic Svalbard and Antarctic Peninsula were isolated and established as clonal cultures, and used for controlled dark incubation experiments mimicking the polar night along with other polar conditions. The data indicate an array of response patterns, such as, for example, a reduced basic metabolism, utilisation of stored energy products, chloroplast shrinkage/degradation etc. Dark exposure for 10 months dramatically reduced the chloroplast ultrastructure, thylakoid stacking, and led to a higher proportion of cells with compromised membranes compared to light-adapted cells. However, photosynthetic oxygen production was readily measurable after such a long dark period indicating that the main adaptive strategy in polar benthic diatoms is the maintenance of a functional photosynthetic apparatus that guarantees rapid recovery after re-irradiation.

**SEGMENTATION OF DIATOM IMAGES: FROM IDEALIZED EXPERIMENTS TOWARDS REAL WORD SETTINGS**

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Over the recent years, a growing number of deep learning-based approaches for automated and computer-assisted identification of diatoms from light microscopy samples have been proposed. However, these mostly used very “clean” diatom material, consisting of either carefully preselected images depicting single valves, or originating from planktonic samples which usually come with only small amounts of disturbing background. Nevertheless, in benthic material, as it is investigated e.g. in routine biomonitoring, usually “dirty” samples prevail, which contain not only diatom valves, but also loads of irrelevant objects such as mineral particles or diatom aggregates, girdle bands and small valve fragments. These can hamper the process of automatic diatom segmentation and identification by presenting ambiguous structures similar to relevant diatom valves, or by partially occluding them. Here we present the first study investigating the influence of such disturbing objects on the performance of diatom segmentation models, advancing the segmentation problem from idealized conditions towards real word settings.

## **USING DIATOMS TO TRACK SOURCES OF PARTICULATE ORGANIC MATTER IN AN ANTARCTIC GLACIAL-FED STREAM**

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Stream particulate organic matter (POM) is derived from the surrounding landscape and streambed, and is a source of energy and nutrients to downstream communities, as well as a vector for organism dispersal. Yet, quantifying different sources of POM is notoriously difficult, though vital for understanding the ecology of stream systems and their connectivity with other habitats. In this context, the glacier-fed streams of Antarctica's McMurdo Dry Valleys represent an excellent model system for investigating POM transport due their relative physical and biological simplicity. Here, adjacent riparian zones are completely devoid of vegetation, and in-stream microbial growth is the dominant source of POM. These streams experience daily flow pulses that mobilize POM, and diatoms recovered from underlying stream sediments suggest that microbial mat material is retained there through hyporheic exchange. Yet, 'how much' different in-stream habitat types and growth forms contribute to POM, and 'when' along daily hydrographs, is unknown. To quantify the contribution of different in-stream habitat types to the POM, we collected time-integrated POM samples over four diel experiments, which spanned a gradient of flow conditions over three Antarctic summers. We then identified and quantified diatoms from POM samples, using them as environmental 'tracers' for the different in-stream habitat types (i.e. benthic 'orange' mats, marginal 'black' mats, and bare sediments). We found that both the bulk POM and diatom cell concentrations followed a clockwise hysteresis pattern with stream discharge over the daily flow pulses, indicating their supply limitation. We then compared POM diatom communities with the three major habitat types, finding that different habitat types harboured significantly different diatom communities, and that POM-associated diatom assemblages were furthermore distinct from these. Given observed

community differences by habitat type, we then created Bayesian mixing models to quantify the contribution of different habitats to POM diatom assemblages over time. The resulting models revealed that a substantial proportion of POM diatoms originated from bare sediments, especially under baseflow conditions. At the same time, orange and black mats contributed diatoms to POM primarily during daily flow pulses. Since flow pulse periods occurred when both cell concentrations and discharge were at their greatest, microbial mats are therefore the most important contributor to the POM. These observations indicate a time-dependence of different in-stream habitat types to POM generation and export on daily to seasonal timescales, with implications for biogeochemical cycling and the local diatom metacommunity. Importantly, they also highlight the utility of diatoms as environmental tracers, and we suggest that this method may have further applications in other habitats, and biomes, outside of Antarctica.

**TRYBLIONELLA COMPRESSA SHOULDN'T BE PUT IN TRYBLIONELLA (WITKOWSKI WAS RIGHT!) AND IT MAY ALSO NOT BE COMPRESSA!**

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*Tryblionella compressa* or *Nitzschia compressa* are the names currently used for a diatom known for more than 100 years as *Nitzschia punctata*, from Grunow's transfer of *Tryblionella punctata* into *Nitzschia* in 1878 to Poulin's decision [in Poulin et al. 1991, *Naturaliste Canadien* 117: 73-101] to synonymize *T. punctata* with J.W. Bailey's *Pyxidicula compressa*. Just before this, in 1990, Round et al. had resurrected the genus *Tryblionella*, which Grunow had treated as a group within *Nitzschia*, and restored *punctata* to its original position within it, among many other changes. These changes were not accepted by some, including Witkowski et al. in their key work on coastal marine diatoms [2000, *Diatom Flora of marine coasts*] nor in a later paper describing four new tryblionellid species [Witkowski et al. 2004, *Phycologia* 43: 579-595]. In the case of *T. compressa*, which we have studied by SEM and via DNA sequence data, Witkowski et al.'s caution was justified. Although *T. compressa* has wide valves, an almost marginal raphe system, a strongly undulate valve face and a marginal ridge, like most species assigned to *Tryblionella*, molecular evidence [Mann et al. 2021, *Mol. Phyl. Evol.* 158: 106985] indicates that it is not related to the group containing the type of *Tryblionella* (*T. acuminata*) but belongs in a clade (Bacillariaceae clade 8B) containing lanceolate *Nitzschia* species, such as *N. amphibia*, *N. inconspicua* and *N. reskoi*, as well as *Denticula kuetzingii*. Nor is it related to *T. debilis*, and presumably, therefore, not to *T. gracilis*, *T. victoriae*, *T. plana*, *T. circumscuta* and similar species. *Tryblionella* in its present form has to be reformed.

Despite the fact that the relationship between *T. compressa* and other clade 8B species has never, as far as I know, been suggested on the basis of morphology, some morphological characters do seem to support its position in clade 8B, notably the structure of the girdle, in which

there are alternating wide and narrow bands (the so-called ‘winawina’ configuration). The same girdle structure occurs also in *T. lanceola*, which shares similar areola and marginal ridge structure, external ornamentation, and the presence of a ‘circumvallation’ on the valve margin, indicating that it is probably closely related to *T. compressa* and also belongs to clade 8B.

The taxonomic (and hence nomenclatural) consequences of these findings are unclear. Complications include that *compressa* is probably the wrong name for the species of Bacillariaceae currently bearing this name and that, as currently circumscribed, it is almost certainly a complex of several species. For example, authentic material of Smith’s *T. punctata* contains two distinct demes. Furthermore, clade 8B contains members of at least three currently accepted genera, does not contain the type species of two of them (*Nitzschia* and *Tryblionella*) and, at first sight, seems an unlikely home for most accepted species of the third (*Denticula*). Two further genera that may belong here, *Giffenia* and *Zothea*, need further examination. I predict this mess will not be cleared up quickly!

## **NANOPLANKTONIC DIATOM FRUSTULES AS MICROENCAPSULATION DEVICES FOR DRUG DELIVERY APPLICATIONS IN AQUACULTURE**

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The development of novel microencapsulation technologies for application in aquaculture settings is posited to bring a new era of sustainable development to the sector. Microencapsulation devices i.e. immobilized nutrients, chemicals, or formulations inside a digestible capsule; offer technological solutions for increased delivery efficiencies of specific formulations to aquatic livestock. Dosing bioactive substances in aquatic environments require highly efficient delivery vehicles to promote chemical stability and reduced losses from leaching.

In the quest for innovation in drug delivery system development designed for aquatic livestock, we proposed exploring diatom derived biomaterials as the primary architectural scaffold of a new microencapsulation platform for aquaculture applications. Diatoms, naturally produce 3-Dimensional cell walls made of silicon dioxide with unique structural, mechanical, and chemical features (high micro- and nanoscale porosities, large surface areas, biocompatibility, and tuneability) potentially superior to available microencapsulation technologies for application in aquaculture. Frustule biomaterials can be easily fabricated by cultivating specific microalgae strains and harvested after mild refining processes, tailored to specific sizes and shapes with resource to green technologies.

In a proof-of-concept approach, we conducted the first empirical studies exploring the potential use of these biomaterials as novel microencapsulate systems for application in aquaculture. One nanoplanktonic diatom strain – *Minidiscus* sp. – was cultivated and used as model for frustule-microencapsulate design due to its minute size, making it suitable for

passive uptake by aquatic fauna. Protocols were optimized for organic matter removal from cultivated biomass, while keeping *Minidiscus* sp. frustules' ultrastructure intact. In addition, frustules were fluorescently tagged with Rhodamine B for tracking upon *in vivo* administration trials. Loading of the 3D hollow frustules was undertaken using a fluorescent dye (Dichlorofluorescein, DCF) as a surrogate of an organic compound for encapsulation and delivery trials, easily studied via fluorescent microscopy. A model aquatic crustacean – *Artemia* sp. – was used for administration bioassays and toxicological screenings of the newly designed system. The fate and behaviour of fluorescently tagged frustules loaded with DCF was assessed using Confocal Fluorescence Microscopy.

We demonstrated for the first time the successful i) extraction 3D hollow microshells from cultivated *Minidiscus* sp.; ii) loading of an exogenous organic compound into the hollow nanoplanktonic frustules; iii) administration of the functionalized frustules to *Artemia* sp. via passive oral uptake; and iv) the ability to monitor the trajectory and behaviour of these functionalized materials post-ingestion. Detailed analysis further revealed that *Minidiscus* sp. frustules accumulated inside the organisms' digestive tract and endured mechanical stress upon ingestion/digestion i.e. compaction and decreased frustule height. Encapsulated DCF was successfully delivered/liberated within the organisms possibly due to mechanical stress imposed on frustules during ingestion/digestion. Additionally, the studied microencapsulate system exerted no toxicological effects on the target aquatic organism.

Our findings validate the potential of using diatom frustules as microencapsulation devices in aquaculture settings and provide the foundational basis of a new class of drug delivery system especially adequate for aquatic livestock.

## DIATOM BIOSTRATIGRAPHY OF THE SARMATIAN SHALLOW-WATER SUCCESSION IN THE ZELENKA SECTION, NE BULGARIA

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The large epicontinental Paratethys Sea occupied the vast territory of West Eurasia's interior during the Neogene. At 12.6 Ma (the late Middle Miocene) the Paratethys became hydrologically isolated from the global ocean and the largest eastern branch became an endorheic basin – the Eastern Paratethys. The hydrological balance of this basin was primarily controlled by the climate (ratio of evaporation/precipitation). Variations in the hydrological balance affected water chemistry, leading to the high level of aquatic ecosystem endemism/radiation and later to their extinction. Despite relatively well-documented trends of biotic evolution in the Sarmatian s.l. (12.65-7.6 Ma), aspects such as comprehensive age constraints, (substage) biozonation, and depositional characteristics of strong water-level fluctuations are still missing.

This study presents our new biostratigraphic data from the Sarmatian s.l. deposits of the key section “Zelenka”, NE Bulgaria. Detailed description of the studied section is published in previous publications elsewhere. The fossil diatom microflora originated from the sediments in the area of NE Bulgaria, belonged to a shallow inland Varna-Balchik Bay of the vast Euxinian Basin. The aim is to trace the response of the fossil diatom record to the environmental basin evolution on the boundary Upper Bessarabian – Lower Khersonian substages, Sarmatian s.l.

Fossil diatoms are determined in the whitish clayey sediments (package 1 from bottom to top) whereas the aragonite crystals are very rare. Species characteristic for the association of the *Achnanthes baldjikii* var. *podolica* Subzone have indicated: *Achnanthes baldjikii* (Bright) Grun., *Achnanthes baldjikii* var. *podolica* Miss., *Campylodiscus fastuosa* var. *baldjikiana* (Grun.) Van Landingham, *Navicula palpebralis* var. *sempilena* Greg., *Grammatophora hungarica* Pant., *Cocconeis scutellum* var. *inaequalipunctata* Miss., *Navicula cancellata* Donk. But, in this benthic association *Caloneis liber* var. *zagrebiensis* Jurilj and

*Licmophora* representatives are dominated. Its stratigraphic range is within the Sarmatian Stage (Bessarabian Substage).

A new transgressive cycle begins with a layer of dark clays with carbonate layers (package 2 from bottom to top). These layers dominated by benthic forms – mainly “*Achnanthes brevipes* Ag. – complex”. The index species of the Middle Sarmatian are no longer present here, or they are only with abundance “rare” (1-3 specimens per slide). During the Late Sarmatian Stage (Khersonian Substage) the changes in the number of species and genera are gradual. Subzone *Navicula zichyi* has indicated. It is characterized by a great species diversity of genera *Navicula* Bory sensu lato, *Amphora* Ehr. Ex Kütz., *Achnanthes* Bory sensu lato, *Rhopalodia* O. Müll., and *Cocconeis* Ehr.

This work has been carried out in the framework of the National Science Program “Environmental Protection and Reduction of Risks of Adverse Events and Natural Disasters”, approved by the Resolution of the Council of Ministers № 577/17.08.2018 and supported by the Ministry of Education and Science (MES) of Bulgaria (Agreement No. DO1-27/06.02.2024).

## MORPHOLOGICAL AND MOLECULAR REVISION OF APICULATAE *TRYBLIONELLA* SPECIES

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*Tryblionella* (Bacillariaceae) was described by Smith in 1853. Several years later Grunow stated that the morphological traits of *Tryblionella* species are not adequate for distinguishing a genus, and he placed them among *Nitzschia* in four different sections: *Tryblionella*, *Pseudotryblionella*, *Apiculatae*, and *Circumsutae*. At the beginning of the '90s, morphological analysis of former *Tryblionella* species demonstrated a necessity to restore this genus and led to a description of a new genus: *Psammodictyon* D.G Mann. Recent molecular analysis based on the *rbcL* marker suggests that *Tryblionella* is a polyphyletic genus with *T. apiculata*, *T. hungarica* and *T. gaoana* forming a group distinct from *Tryblionella debilis*.

The current study aimed to document morphological traits describing *Tryblionella* species from the *Apiculatae* section, for the purpose of further taxonomic investigation. Several strains of *Tryblionella* spp. were analyzed for morphological and DNA sequence data. A three-gene dataset (*rbcL*, *psbC*, and SSU) from 150 diatom taxa was used in a phylogenetic analysis, which resolved one major monophyletic clade of Bacillariales with several subclades. A subclade of *Tryblionella* taxa from the *Apiculatae* section consists of clades corresponding respectively to *T. apiculata*, *T. hungarica*, *T. gaoana* and *T. marginulata* taxa. Morphological observation using SEM showed traits shared by taxa

belonging to these four clades, i.e. one sternum centrally located along an apical axis, each fibula corresponding to one or two ribs, striae interrupted by the fibulae and continuous on the junction between the valve face and mantle and presence of the warts externally on the pars exterior of valvocopula.

## **DIVERSITY OF MICROEUKARYOTES IN DIFFERENT LAKES IN CROATIA**

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Microeukaryotes represent a remarkably diverse clade of eukaryotic microorganisms, showcasing the utmost morphological complexity and differentiation among single-celled organisms. The taxonomic identifications of microeukaryotes traditionally relies on microscopic techniques involving observation of live or fixed cells. DNA barcoding is a method of identifying species on the based on short DNA sequences linked to morphologically identified species. Our research covered four natural freshwater temperate lakes in Croatia, two in the Croatian continental region and two in the Mediterranean region. Water samples were collected from different depths, depending on the presence of the thermocline or oxycline of all four lakes from May 2021 to February 2023. Water temperature, conductivity, salinity, pH, total dissolved solids, dissolved oxygen and oxidation-reduction potential was measured in situ Multi Meter for Water. Total genomic DNA was extracted with the DNeasy PowerWater kit. The samples were sequenced using 18S V4 amplicon sequencing using specific primers (18S V4: 528–706R as the barcode). Microeukaryotes were strongly divided by lake location especially in the Mediterranean region. DOM, nitrate and chloride were the major factor for the community diversity. A similar pattern was detected in the free living and in the attach community. In the Mediterranean lakes the majority of the community belonged to Chlorophyta, Cycliophora and Cryptophyta. In the continental part, Cryptophyta were the dominant population. In the context of the carbon source utilizations in the Mediterranean region phagotrophs are the dominant and in the continental mixotrophs (Lake Kozjak) or phagotrophs (Lake Prošće) dominated. Our research represents the first attempt to describe the microeukaryotes diversity in Croatian lakes at seasonal and vertical level.

**ALONG THE COAST: UNVEILING THE IMPACT OF PEATLAND WATER ON BALTIC SEA AND PEATLAND BENTHIC DIATOM GROWTH**

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Shallow coastal waters are unique ecosystems intricately involved in the dynamic exchange processes across marine-terrestrial gradients. Within the coastal regions of the southern Baltic Sea, adjacent terrestrial habitats, notably peatlands, are frequently delimited by dunes and dikes, preventing natural biogeochemical fluxes between these ecosystems. With the ongoing climate change, driving sea level rise and increasing storm surge events as well as increasing renaturation of coastal peatlands, the naturally or artificially removal of these barriers is increasing. Consequently, the heightened exchange between both ecosystems exposes inhabiting organisms, such as benthic diatoms, to different environmental conditions, characterized by fluctuations in salinity, light availability, and nutrient dynamics, potentially exerting profound effects on their physiological processes, such as photosynthesis and growth. Through in-vivo fluorimetry, the growth response of five Baltic Sea and peatland benthic diatoms to ten different growth media, comprising variations of Baltic Sea and peatland water with assorted additives were investigated. Diatoms exhibited an enhanced growth in media based on peatland water, indicating a stimulating effect. In addition, growth of two Baltic Sea species was shown to be inhibited in the freshwater peatland media, evincing salinity as a critical co-factor influencing species-specific growth during merging ecosystems. While measurements of species-specific photosynthetic rates under increasing photon fluence rates (0-1400  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ , recorded at 20°C) in two selected media illustrated a considerable photo-physiological plasticity across in the five diatom strains, only one species displayed a difference in photosynthetic performance depending on the growth medium.

## **DIATOM COMMUNITY RESPONSE TO THE SUDDEN REWETTING OF A COASTAL PEATLAND**

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The coastline of the shallow southern Baltic Sea is a highly dynamic system. Irregular water level changes, erosion and coastal currents lead to the dynamic transport, resuspension and deposit of sediments, shaping a coastline of spits, lagoonal systems and subtidal flats. On the land side, coastal peatlands are common. Intact peatlands are important carbon storages and sinks, representing approximately 30% of the global carbon. However, many coastal peatlands have been severely degraded due to peat cutting and drainage for agricultural use or urban expansion. At present, many coastal peatlands are separated from the southern Baltic Sea by artificial dunes or dykes in order to facilitate drainage and coastal protection measures. Due to their potential to act as carbon sinks and as buffer zones against sea level rise and flooding events, there is an increasing interest in the restoration and rewetting of coastal peatlands. However, rewetted peatlands may also become a source of nutrients for the adjacent coastal waters. Interactions between shallow coastal waters and adjacent peatlands are among the important exchange processes along the southern Baltic Sea coastline. A variety of different processes can facilitate the exchange of organic material, minerals and nutrients, which will modify the biogeochemical processes in both systems, the extent of which is widely unknown. All these possible processes will interact with the biota of these systems, however, nature and scale of these effects are equally poorly understood. Within the framework of Baltic TRANSCOAST the key objective is to deepen the understanding of the interactions of land- and sea-borne processes at the coast, including the structure, diversity and function of microphytobenthos. Diatoms are often the dominant microphytobenthic group in temperate regions. They are highly diverse and important primary producers which may contribute up to 30% of the carbon fixation in coastal systems. In our project, we

study the diatom communities at three different coastal peatland sites with different flooding regimes. One rewetted and frequently flooded, one rarely flooded and another one that was intentionally flooded with sea water. The aims are to investigate the poorly known diatom community composition of coastal peatlands, to determine the effects of flooding events on diatom composition and biomass in coastal peatlands as well as the effects of nutrient and substance release from the peatlands on the diatom communities of adjacent coastal waters. In November 2019, the removal of the dyke that separated the Polder Drammendorf (a drained peatland), from the adjacent Baltic Sea lagoon Kubitzer Bodden represented a unique chance to study the sudden rewetting of an oligohaline, drained peatland with brackish water from the Baltic Sea and its effect on the microphytobenthos. We investigated and documented the impact of this sudden rewetting on the benthic diatom communities of the peatland and the adjacent lagoon over the course of one year, applying the latest taxonomy and measuring key environmental factors. The results deepen the understanding of diatom community composition, taxonomy and ecology and explore the influence of land-sea exchange processes on the benthic diatoms of the Baltic Sea coast.

## LONG-TERM PHYTOPLANKTON DYNAMICS AND DIVERSITY IN THE COASTAL ADRIATIC SEA: A COMPREHENSIVE ANALYSIS OF 14 YEAR DATASET

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The phytoplankton communities of the Adriatic Sea have been the subject of extensive studies, particularly in the northern region, which is known for its higher trophic levels compared to the central and southern areas. In the late 1980s, attention was drawn to the central Adriatic and particularly its coastal areas due to increased eutrophication, leading to studies on phytoplankton blooms and their harmful effect. In recent years, however, there have been few studies focusing on the plankton community, so our understanding of community dynamics in the central Adriatic is patchy. The present study addresses this gap by covering 14 years of recent study in coastal area of central Adriatic, particularly in Kaštela Bay. A total of 1207 samples were collected and analyzed at two stations across the vertical depth profile. This revealed a remarkable species diversity with 375 taxa observed, an average of 19 species per sample.

The most dominant groups, in terms of abundance are diatoms and phytoflagellates. Over the years, interannual trends show an increase in species diversity, which is reflected not only in species richness but also in various diversity indices such as Shannon, Margalef and Menhinick. This increase in diversity over the study period was observed for all phytoplankton groups.

Among the individual phytoplankton genera, certain diatom genera such as *Pseudo-nitzschia*, *Chaetoceros* and *Leptocylindrus* dominated the community, with *Pseudo-nitzschia* showing a decreasing trend in abundance over time. Interestingly, the occurrence of *Pseudo-nitzschia* was not characterized by specific seasons. *Chaetoceros* was abundant in spring, especially in March. Among the dinoflagellates, *Gymnodinium* and small, unidentified dinoflagellate species (<20 µm) were abundant

throughout the year. Although dinoflagellates are generally low in abundance, they exhibit a distinct seasonal pattern, peaking in the summer months. We calculated IndVal for each species and showed the most represented species in certain seasons and years of the studied area.

**CLARIFICATION OF THE ECOLOGICALLY IMPORTANT SPECIES, *COSCINODISCUS OCULUS-IRIDIS* EHRENB.**

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In high arctic marine waters, *Coscinodiscus* species are often low in relative abundance and in many planktonic collections, members of the genus are not even reported. Yet, recent studies show that periods of time with high concentrations of biogenic silica in marine sediments may be attributed to arctic surface water with enhanced concentrations of Si and P (Jones et al. 2003, Kelleher et al. 2020). In fact, such high periods of primary production result in significant concentrations of *C. oculus-iridis* being deposited in marine sediments. However, the taxon has been characterized as being present in a variety of ecological conditions (cold water, high April sea ice, or no sea ice; Oksman et al. 2019, Luostarinen et al. 2020). Such disparate conditions lead us to question the identity of this taxon and if more than one species of *Coscinodiscus* is present. To further the modern and paleo-reconstruction of arctic conditions, we examine the original Ehrenberg material of *C. oculus-iridis* along with the *Coscinodiscus* from high arctic marine sediments and clarify the identity and morphological features of the specimens.

**MARINAS AS ARTIFICIAL HABITATS ALONG THE SHORELINE OF LAKE BALATON FAVOUR MORE HOMOGENEOUS PERIPHYTIC DIATOM ASSEMBLAGES**

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Construction of marinas along Lake Balaton's shoreline has led to the alteration of the natural habitat, resulting in an artificial environment. We studied this impact on the periphytic diatom communities, hypothesising an effect on the composition, abundance, and diversity. We selected twelve municipalities with four habitats at each site (marinas and reeds exposed to, and protected from the waves), and installed semi-artificial substrates in the water for a six-week period. We collected and analysed the biofilm from the substrates, while also analysing physical and chemical parameters from the water.

Our results showed that environmental variables varied spatially, with nutrient concentrations being significantly higher in the marinas. Periphyton at these habitats had higher biomass, with a higher proportion of diatoms and a lower autotrophic index compared to other habitats. The diatom composition within marinas was significantly different from that of the other three habitats, and although alpha diversity was higher at these sites, beta diversity was lower, and the communities were more homogeneous.

We showed that the intense alteration of the natural shoreline of Lake Balaton by marina construction has a noticeable impact on the primary producers and potentially on the entire trophic network, making it imperative for stakeholders to consider these factors when constructing new marinas.

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## **SUBFOSSIL DIATOMS OF PERIALPINE LAKES REVEAL EARLY LAKE RESPONSES TO CLIMATE WARMING AND HUMAN IMPACT IN THE 20<sup>TH</sup> CENTURY**

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The majority of Perialpine lakes suffered from nutrient enrichment since the early 1960s because of the demographic growth and the economic boom that followed World War II. However, limnological surveys have remained scattered and irregular for both large and small Perialpine lakes during the early stages of nutrient enrichment, while regular monitoring programs started mainly during the most acute anthropogenic eutrophication or in conjunction with the launching of lake restoration measures. As a result, the early-stage responses of Perialpine lakes to major human-related perturbations occurred during Anthropocene can only be reconstructed based on information preserved in deep lake sediment records. Subfossil diatoms are among the most reliable biological remains in lake sediments and have been extensively used for the reconstruction of the trophic evolution of temperate lakes at secular scale. In this contribution we provide evidence of the capacity of subfossil diatoms to provide information on indirect effects of early global warming occurred in the first half of the 20<sup>th</sup> century in both large and small Perialpine lakes.

We present results of subfossil diatom studies conducted during the last ca. 10 years on sediment cores from a set of large Perialpine, and two additional smaller mid altitude lakes, located on both the northern and southern Alpine slopes. Diatom based environmental reconstructions

show that lake biological responses to major changes in lake nutrient availability during the post-war economic development was particularly rapid and coherent in different lake types north and south of the Alps. In addition, these studies reveal that first changes in the diatom species composition occurred already during the first half of the 20<sup>th</sup> century in several lakes. Although the investigated lakes differ in location, morphology (e.g. altitude, size, depth), and exposition to direct and indirect human impacts, subfossil diatoms first relevant changes mainly occurred between the 1930s and the 1940s. In most of the lakes early diatom changes consist in the rapid substitution of small centric taxa by pennate taxa mainly belonging to the genera *Asterionella* and *Fragilaria*. These changes could be interpreted as indirect community response to the first documented climate warming after the end of the Little Ice Age, that mimicked nutrient enrichment effects although being mediated by lake-specific hydrological and thermal dynamics.

The interpretation of the observed changes is difficult for some lakes due to the combination of climate related effects with superimposed and simultaneous anthropogenic perturbations that ranged from early eutrophication to hydroelectric exploitation. These results underscore: a) the importance of the paleolimnological approach for complementing and expanding limnological surveys and for predicting future lake ecological trends based on the understanding of past lake responses; b) the potential of subfossil sediment diatoms as a proxy to understand indirect effects of climate change on the planktonic lake biota.

## REVISION OF THE ARAPHID GENUS *STAUROFORMA* (STAUROSIRACEAE, BACILLARIOPHYCEAE) BASED ON GRUNOW'S ORIGINAL MATERIAL

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The genus *Stauroforma* was first described by Flower et al. (1996 in Diatom Res. 11) to accommodate a former variety of *Fragilaria virescens* Ralfs (now in the genus *Fragilariforma*), because it lacked a rimoportula. However, the taxonomic history of *Stauroforma exiguiformis* is far from being clear, mainly because of confusion about the valid publication of the basionym *F. virescens* var. *exigua*. A second species, *S. inermis*, was placed in *Stauroforma* based on samples from the Maritime Antarctic Region. These species differ with regard to linking spines (present in *S. exiguiformis*, but absent in *S. inermis*) and the formation of long chain-like colonies in *S. exiguiformis* while *S. inermis* is only present as solitary cells. In the present contribution, we unravel the taxonomic history of *F. virescens* var. *exigua* by examining Grunow's original drawings and several historic Grunow samples containing the taxon. Different Antarctic populations of *S. inermis* were added to the analysis and compared with its presumed European records, showing that, in our opinion, these European populations should be split from the Antarctic taxon. Additionally, two other varieties of *F. virescens* described by Grunow, such as var. *oblongella* and var. *subsalina*, have been analysed. Based on this, *F. virescens* var. *subsalina* belongs to the genus *Stauroforma* and can be considered identical to the European records of *S. inermis*, but is different from the Antarctic populations. The analysis of the different populations and taxa indicated that the current concept of the genus *Stauroforma* should likely be modified by its circumscription, as currently only *S. exiguiformis* fits this description,

whereas all other *Stauroforma* taxa differ markedly in having mantle striae and apical pore fields, but lacking linking spines almost completely.

## TRACKING DIATOM SEXUAL EVENTS USING MARKER GENE EXPRESSION

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Although most diatoms are obligate sexual organisms and need to pass a sexual phase to complete their life cycle, observations of reproductive events in the field remain scarce. Consequently, our understanding of the role of meiotic recombination in shaping genetic diversity and for the adaptive potential of diatom populations is limited. Similarly, it is unknown how life cycle variation has contributed to the extraordinary species diversification observed in diatoms. Using a comparative genomics approach based on the combination of experimental gene expression studies and computational analyses of genomic data, we identified a set of three pennate and four centric specific sex marker genes, respectively, as well as one diatom-wide marker gene. We validated the applicability of these markers using a metatranscriptome experiment in the Schelde freshwater tidal estuary. In this well-studied system, intense seasonal diatom blooms have occurred during the past 20 years, and are dominated by thalassiosiroid diatoms. According to V4 metabarcoding, the 2023 bloom was dominated by a single *Cyclotella scaldensis* ASV, coexisting with other, rare Thalassiosirales taxa. Mimicking the effect of salt water incursions upstream in the estuary, we applied a salinity increase to field samples in a microcosm experiment to trigger sexual reproduction. Spermatogenesis, fertilization and auxospore formation were microscopically observed within two days following salinity increase. In parallel, we observed significant upregulation of all five centric sex markers among a total of 27,947 differentially expressed genes. Based on comparative analysis using genome data from a large number of thalassiosiroid diatoms, we were able to phylogenetically assign the expression of sex marker genes to four different taxa. Interestingly, sexual reproduction was not only detected in the very abundant *Cyclotella scaldensis*, but also in exceedingly rare species, such as *Thalassiosira allenii/angulata* and *Conticribra* sp., both of which took up only about 0.01% of the library. Together, our results demonstrate the feasibility of the use of sex marker genes to track sexual reproduction events in

natural diatom populations with a high taxonomic resolution and high sensitivity.

## **MORPHOLOGICAL ANALYSIS OF TYPE MATERIAL OF *MAYAMAEA ATOMUS* AND ITS TERRESTRIAL ALLIES, WITH INSIGHTS INTO THE GENETIC DIVERSITY OF THE GENUS**

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As in most historic diatom studies, the description and figures of *Amphora atomus* Kützing are not particularly informative, and the understanding of the circumscription of *Navicula atomus* (Kützing) Grunow differs among diatomists. Most authors seem to have built their concept of the species primarily based on descriptions from Grunow (1860) or from the Types de Synopsis des Diatomées de Belgique No. 149 by Van Heurck, or more recently by Mayama & Kobayasi (1988), without examination of the original material using scanning electron microscopy (SEM). The species is currently placed in the genus *Mayamaea* Lange-Bertalot (1997), which develops in ephemeral habitats as well as nutrient-rich waters; *Mayamaea* populations can reach high abundance in certain polluted habitats. To date, the genus comprises 30 taxa, most of which are aerophiles and found in humid subaerial environments, temporary water bodies, and soils. *Mayamaea* frustules are small (<10 µm). The axial area is thickened and contains a straight raphe. Sometimes, the axial area is all that remains after acid preparation. This study presents, for the first time, original materials of *Amphora atomus* Kützing (1844) found

in the collection of Van Heurck in Meise (B), *Synedra atomus* (Kützing) Nägeli in Kutzing (1849) sensu Grunow in the collection in Vienna (W), from which the material was used for the transfer of *Synedra atomus* to *Navicula atomus* (Kützing) Grunow and the historical information found the Natural History Museum in London (BM). This study also presents illustrations of *Mayamaea* in SEM from several materials, and this species is compared to other unpublished materials, such as *Mayamaea asellus* (Weinhold) Lange-Bertalot, *Mayamaea spirans* (Hustedt) Lange-Bertalot, and *Navicula peratomus* Hustedt. Moreover, several taxa are discussed and illustrated, such as *Mayamaea agrestis* (Hustedt) Lange-Bertalot, *Mayamaea alcimonica* (E.Reichardt) C.E.Wetzel, Barragán & Ector, *Mayamaea arida* (W. Bock) Lange-Bertalot, *Mayamaea excelsa* (Krasske) Lange-Bertalot, *Mayamaea lacunolaciniata* (Lange-Bertalot & Bonik) Lange-Bertalot, *Mayamaea nolenoides* (W.Bock) Lange-Bertalot, *Mayamaea pseudopermitis* Lange-Bertalot, Cavacini, Tagliaventi & Alfinito, and *Mayamaea recondita* (Hustedt) Lange-Bertalot. The high genetic diversity of *Mayamaea* found in soils in Luxembourg is also illustrated and discussed. It is reasonable to think that the study of soil diatom communities could increase the diversity of the genus in the near future. Numerous specimens identified as « unclassified » *Mayamaea* and found in terrestrial samples collected from different types of cultivated and natural environments (forests and meadows) in Luxembourg allow us to say that the genus is as important as the genus *Hantzschia* Grunow in terrestrial habitats.

## **INSIGHT INTO BIOLOGICAL STRUCTURES USING VARIOUS IMAGING AND IMAGE ANALYSIS TECHNIQUES**

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High-resolution imaging techniques are essential for understanding the hierarchical structures formed in the self-assembly processes, such as the one governing the architecture of diatom frustules. The most extensively studied diatom characteristic is the intricate cell wall structure, called the frustule, which is mainly made of biosilica. To obtain detailed and multi-length scale descriptions of frustules one may use characterization techniques and procedures of modern materials science, such as SEM, TEM, FIB, and nano-CT. These powerful tools enable imaging fine details of the structure of diatom shells. However, an intrinsic limitation of this technique is the post-mortem character. Recently, it has been demonstrated that a new insight into the structure of diatoms can be provided by an in-situ fluid SEM system that allows one to investigate liquid samples in the SEM system. The obtained images are subjected to qualitative as well as quantitative evaluation using image analysis procedures. The results obtained with this tool are discussed in terms of the new knowledge generated in diatom research and diatom engineering applications.

## **USE OF JEOL SCANNING ELECTRON MICROSCOPE IN BIOLOGY AND DIATOM RESEARCH - EXPERIENCES FROM SCAN DOO**

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SCAN, d.o.o. is specialized in providing high quality laboratory instruments in the field of electron microscopy, vacuum technique and cryogenics as distributor of the world largest most important companies in these fields such as JEOL, Pfeiffer Vacuum and Cryopal. During this presentation the performances of the newest JEOL SEMs will be presented.

JEOL introduced its first Scanning Electron Microscope (SEM) in the early 1960s. Since then, JEOL innovations in resolution and SEM functionality have enabled microscopists to image and characterize a new generation of nanomaterials, reveal intricate biological details, analyze forensic evidence, and perform failure analysis and quality control. JEOL scanning electron microscopes are very well known worldwide as one of the best and the highest quality instruments. SEMs are used to observe morphology of the samples from small magnifications (5x or less) to highest magnifications with resolutions below 1 nm. SEMs provide very high depth of field and are therefore very suitable in imaging diatoms. It is possible with optional EDS detector to do also qualitative and quantitative elemental analysis of the sample under observation with SEM. The smallest easy-to-use entry level Benchtop SEM- scanning electron microscope model JCM 7000 is shown in the image.

Brochures for all models of the JEOL SEMs and other analytical instruments can be downloaded from the JEOL website: [www.jeol.com](http://www.jeol.com)



**ORAL PRESENTATIONS  
BY THE STUDENTS**



## FOR RICHER OR POORER: DIATOMS AS INDICATORS OF BIOLOGICAL CONDITION IN NORTHERN PEATLANDS

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Owing to their sensitivity to changes in water quality, diatoms have been established as bioindicators in a wide range of aquatic ecosystems. Compared with other aquatic environments, we know relatively less about the reliability of diatoms as bioindicators in peatlands. The goals of this study were to 1) document spatial and temporal variation in diatom species composition across a gradient of northern peatlands and 2) determine how underlying environmental conditions influence diatom species distribution. We sampled diatoms in relation to environmental conditions across a gradient of peatlands in interior Alaska, U.S.A., over the course of a summer growing season (after snowmelt in May until August 2017). A total of 100 species were observed across all peatland types (i.e., rich, moderate-rich, poor fens), 17 of which were present at >1% relative abundance, and 15 were selected as indicator species using Indicator Species Analysis. General linear model results showed that fen type affected the richness, diversity, and composition of diatom assemblages and differences among fens were driven by changes in the most common, but not in the least common species. Time was not a significant factor among fens for measures of diversity (Shannon-Weiner diversity, Simpson's diversity, and Pielou's evenness), and some species (e.g., *Eunotia subcapitata*, *Eunotia julma*, *Eunotia ambivalens* and *Eunotia cf. glacialispinosa*) were affected by time among fens, while the rest of the species encountered were only affected by time within fens. Diatom species richness declined along a gradient of rich to poor fens. Redundancy analysis showed conductivity, dissolved organic carbon concentration, water temperature, pH, and water depth explained 63% of the total variation in diatom assemblage structure among fens. The diatom assemblages within the rich and moderate fens shared two indicator species (*Tabellaria flocculosa* and *Eunotia subcapitata*) and were dominated by *Tabellaria flocculosa*, and the poor fen was dominated by *Eunotia mucophila* and shared no indicator species with the rich and moderate fens. Our study demonstrates that diatoms are

useful indicator species across a range of peatlands that commonly occur across the boreal landscape and provides resource managers another tool to monitor ecological conditions in the face of environmental change occurring across the boreal landscape.

## DEVELOPMENTS IN THE FOSSIL RECORD AND EARLY EVOLUTION OF MARINE DIATOMS

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It is generally accepted that diatoms have inhabited the world's oceans for at least the past 120 Ma since their first appearance in the Lower Cretaceous. There are also records of older diatoms, from the Jurassic dating to ca. 172 Ma and ca. 165 Ma, these are poorly documented, however. The predicted origin time of diatoms using molecular phylogenetics yields an earlier date of origin of 200 Ma. These dates point towards some expected gaps in the fossil record, which may bias our understanding of early diatom evolution. To advance current knowledge on the origin and early evolution of diatoms, we revisited the Cretaceous fossil record of diatoms and explored a set of Mesozoic sediment samples expected to contain fossilized diatoms. An extensive search for Lower Cretaceous and Jurassic diatoms revealed no new fossils. Instead, scarce sponge spicules and radiolarians were observed at several study sites, exhibiting a high degree of secondary dissolution and alteration. This finding suggests that diagenetic processes potentially led to a loss of diatom fossils from these areas. Critical re-evaluation of the previously described oldest diatoms from the Lower and Middle Jurassic age revealed these fossils were not diatoms but most likely calcareous nannofossils and testate amoebae, respectively. This discovery extended the gap between the oldest fossils and estimated origin time to 80 million years. The lack of diatoms in ancient sediments inspired further research on published Cretaceous diatoms. To identify trends in distribution, diversity, and emergence of genera in time and space, we compiled the Cretaceous Diatom Database. We identified extant diatom genera as far back as 100 million years and compiled a list of well documented fossils for future use as calibration points in molecular clock studies. We also identified areas for future taxonomical work. Overall, based on the biogeographical dispersal of the

lower Cretaceous diatoms and their morphological diversity, together with diagenetic evidence from radiolarians and sponge spicules, we suggest that undocumented diversity in diatoms likely existed before the lower Cretaceous. Our research highlights the importance of the fossil record and the significance of rigorous verification of diatom remnants in ancient sediments to support the current knowledge on the early diatom evolution and to progress both paleontological and molecular studies.

## **MAJOR CHANGE IN THE DISTRIBUTION OF DIATOMITE DEPOSITS IN THE MIDDLE EOCENE (~44 MA)**

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Diatoms are currently the main primary producers and exporters of organic carbon and silica in the oceans. However, their importance in the carbon and silicon cycles during the Paleogene (~66 to ~23 million years ago [Ma]) is unknown, primarily due to their vulnerability to diagenetic processes, which complicates deep-time paleoproductivity reconstructions. Another complication is that global-scale syntheses are usually based on deep-sea drilling data, and thus do not consider data from onshore sites, which represent an archive of geological and environmental processes taking place in marginal marine settings.

In order to gain new insight into changes in diatom production through the Paleogene - a period of initial extreme warmth followed by a gradual climate cooling, punctuated by short-term climatic events of extreme magnitude termed hyperthermal events – we compiled stratigraphic and geographic occurrences of diatomite, with age control based mainly on diatom biostratigraphy. Diatomite is a sedimentary rock formed by the accumulation of diatom frustules in freshwater and shallow marine environments, and thus represents an indirect proxy for high rates of biosiliceous production.

Our global-scale compilation, comprising data from over 100 sites, reveals a high number of diatomite occurrences in the Paleocene, followed by a slow decrease from the Paleocene-Eocene Thermal Maximum (PETM, ~56 Ma). A clear minimum occurs around ~44 Ma, before increasing again until the end of the Eocene (~56 to ~34 Ma). The high number of diatomite occurrences recorded are consistent with the high rates of silicate weathering that occur during these periods, but the drop at ~44 Ma is not associated with any known paleoceanographic or climatic

event.

A statistical comparison with reconstructed sea-level and proxies of carbon and oxygen reveals an absence of correlation with our data. Paleogeographic reconstructions of the Paleogene period, however, appear to be the key to fluctuations in diatomite deposition.

Our data show that diatoms play a major role in the study of changes in shallow marine environments and contribute to the reconstruction of paleoceanographic and climatic fluctuations.

**DESCRIPTION OF TWO NEW *LUTICOLA*  
(BACILLARIOPHYTA) SPECIES FROM  
A SUPRATIDAL ZONE OF KOREA**

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Two new *Luticola* species have been found from the gut contents of a terrestrial gastropod *Ellobium chinense*, which inhabits supratidal flats located on the western shores of Korea. Detailed morphological characterizations of the two newly discovered species have been presented based on light microscopy (LM) and scanning electron microscopy (SEM). Comparisons with similar taxa have also been made. *Luticola jinii* sp. nov. is most similar to *L. celebesica*, yet the former has coarser striae with 12–15 in 10 µm while the latter has striae with 17–21 in 10 µm. *Luticola witkowskii* sp. nov. is most similar *L. halongiana*, however, the *L. witkowskii* (12–17 in 10 µm) can be distinguished by striae density, which are coarser than in *L. halongiana* (20–24 in 10 µm). The present study has expanded our understanding of the diversity and ecology of the genus *Luticola* within the study area and also discussed the biogeography of marine and brackish *Luticola* species from East Asia.

## **HISTORICAL WATER QUALITY RECONSTRUCTION OF TINKERS CREEK, OH (USA) THROUGH EXAMINATION OF DIATOMS FROM FISH GUT**

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Historical water quality in Tinkers Creek, Ohio was examined through study of diatom species composition in the intestines of preserved fish from curated collections (1922-1998) and newly acquired fish and periphyton samples (2012, 2023). Therefore, study period spanning 1922 to 2023 was possible. Within that period, a total of 330 diatom species were found. Water quality in the monitored years during the past century was inferred using several types of diatom-based indices. The indices evidenced poorest water quality in the 1976 samples and best in 1996 samples. However, the ways of pollution seem to be changing throughout the studied period. Moreover, this study provides evidence that water quality may once again be deteriorating in Tinker's Creek. This technique allows for accurate historical water quality reconstructions for rivers where archived fish voucher specimens are available and is comparable to standard methods based on periphytic diatom assemblages.

## **ASSESSING THE FUNCTIONAL AND COMPOSITIONAL RESPONSES OF STREAM MICROALGAL COMMUNITIES TO STRESSORS INCREASE AND RELEASE IN A MESOCOSM EXPERIMENT**

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Most studies on microalgal communities in freshwater ecosystems are based on field observations. The number of mesocosm experiments conducted is limited, but they provide important insights into how various stressors affect photosynthetic biomass and the community composition of microalgae. In addition, mesocosm studies have paid less attention to the recovery process after stressor alleviation. Here, we fill this gap by presenting the results of a riparian mesocosm experiment that investigated how increases in temperature and salinity and decreases in flow velocity affect microalgal communities. A comprehensive analysis of individual and combined stressor influences was possible thanks to the full factorial design and semi-randomised distribution of treatments, which included two levels of each stressor and eight (2 x 2 x 2) replicates, yielding a total of 64 circular mesocosms. We aimed to clarify how photosynthetic biomass and community composition of microalgae responded to the applied environmental stressors. Our data showed that temperature was the most important predictor of photosynthetic biomass, although this effect was weak. We also found notable effects of temperature and flow velocity on diatom community composition during the stressor phase, with temperature exerting a persistent influence during the recovery phase. Conversely, amplicon analysis highlighted

the significant influence of flow velocity during the stressor phase, while temperature and salinity emerged as key factors during the recovery phase, with a notable interaction between temperature and flow velocity observed in this phase. Although our results partially contradicted our predictions, they illustrate how sensitive photosynthetic biomass and community composition of microalgae are to even minor changes in environmental variables, particularly flow velocity and temperature. These results highlight the complexity of ecological responses in benthic systems and call for more research into the subtle interactions between microalgal communities and their ecosystems.

## SHIFTING SHELLS: ENVIRONMENTAL DRIVERS OF DIATOM COMMUNITY COMPOSITION ON LOGGERHEAD SEA TURTLES

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Apart from some truly epizoic diatom species that show a high degree of host specificity, loggerhead sea turtles often carry a diverse range of opportunistic benthic diatom species on their carapaces, likely acquired during feeding activities that disturb the sediment. Although turtle-associated diatoms show a strong regionalisation pattern, the nature and strength of this association remains unclear. Furthermore, there is limited understanding of how the environment might influence the composition of these epizoic diatom communities. To explore this, samples were collected from turtle carapaces at two different time points: upon arrival at rescue centres (Aquarium Pula, Croatia, and Blue World Institute, Lošinj, Croatia) following injury, and again after a month of recovery at the centres. Additionally, the recovery basins and the environment of the Verudela Peninsula (Pula, Croatia) were sampled to make comparison to the environmental diatom communities. A Detrended Correspondence Analysis revealed that the diatom communities at the specific time points differ substantially. Interestingly, a very high level of community similarity was discovered between turtles housed for a prolonged time period and the recovery basins themselves, indicating a swift turnover of diatom species on loggerheads when introduced in a new environment. During this process, existing species are shed or disappear (e.g. *Berkeleya fennica*, *Hyalosira* cf. *delicatula* and *Licmophora debilis*) while newcomers are incorporated from the surrounding environment (e.g. *Craspedostauros alatus*, *Nitzschia* cf. *amabilis*, *Fallacia* cf. *subpygmaea*). This underscores the remarkable ability of

diatom communities to promptly acclimate to changing environments by changing the species composition, highlighting their capacity for swift environmental modification.

## MARINE BENTHIC DIATOMS FROM ULLEUNGDO, KOREA

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Ulleung Island is an isolated, remote island off the Korean mainland. The island is influenced by warm and cold currents, resulting in particularly high productivity and diversity in the surrounding waters. In the present study, we used a light microscope and a scanning electron microscope to observe and identify marine benthic diatoms in and around Ulleung Island. Five sediment samples were collected, four from the subtidal zone and one from the intertidal zone. A total of 240 species belonging to 70 genera have been identified, with the most dominant genera being *Amphora*, *Diploneis* and *Cocconeis*. In addition, 12 prospective new species were found, five of which are considered to belong to rare genera reported in recent years (e.g. *Majewskaea*, *Disymmetria*) or very small and neglectable genera (e.g. *Alveus*, *Austariella*, *Lunella*). As the first report of marine benthic diatoms from the intertidal and subtidal habitats of Ulleung Island, the present study would shed light on the diversity of Korean diatoms.

**DISTINCT OR SIMILAR? MARINE BENTHIC DIATOM DIVERSITY IN ANTARCTIC AND ARCTIC COASTAL ZONES INVESTIGATED BY DNA METABARCODING**

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Polar regions play a critical role in the Earth's climate system and global nutrient circulation and comprise many different habitats with unique organisms. The seasonal change from the polar night to the midnight sun distinguishes the polar regions from all others of the world. The marine organisms living in these regions must cope with extreme seasonality of light, temperature, salinity and sea ice. However, diatom biodiversity is much more extensive and ecologically diverse than previously thought. The Antarctic and Arctic coastal zones offer a unique opportunity to test hypotheses regarding biodiversity and evolution of marine benthic diatoms. While many environmental features are similar in the two polar regions, they are separated from one another by the temperate and tropical belts, and their origins as well as their cold-water history differ considerably. The Diatom Research group of the Botanical Garden Berlin initiated a first study to expand the knowledge about the biodiversity of benthic diatoms in Antarctic and Arctic shallow water coastal zone environments by using a combined approach of morphology, culturing and DNA metabarcoding. This research project aims to investigate how this ecologically important group of protists is distributed. This includes the question, if dispersal limitation is a mechanism for diversification of diatoms or if unlimited dispersal and similar environments have led to similar benthic diatom communities in the polar regions. Benthic samples (biofilm from stones and epipsammic biofilm) from marine habitats were taken in the Austral summer 2020 at the Potter Cove (Antarctic Peninsula). In summer 2022 biofilm samples were taken in Kongsfjorden (Svalbard, Arctic). A total of 160 clonal cultures were established from Antarctica resulting in the identification of 60 taxa. Until March 2024 over 120 clonal cultures were isolated from the Arctic samples. Based on those cultures, a taxonomically validated reference library for polar benthic diatoms is established at the BGBM Berlin, with

comprehensive information on habitat, morphology, and DNA barcodes. Here, first insights will be presented comparing marine benthic diatom species composition from both polar habitats by a DNA metabarcoding approach. Biodiversity and relative abundance will be assessed, and the number of endemic species will be estimated. Due to the 10-fold longer cold-water history and geographic isolation of Antarctica a higher rate of endemism is expected in this region. Further, the rate of species assignment through the metabarcoding pipeline will be assessed to draw conclusions about the completeness of the taxonomic reference library.

## **BRYOPHYTES: A NEUTRAL OR TARGETED MICROHABITAT FOR DIATOMS IN KARSTIC SPRINGS**

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Karstic springs are known for their stable hydrological and ecological characteristics, providing a suitable habitat for bioindicator flora and fauna. This study focuses on epibryon diatoms, previously identified as key indicators in such environments. We assessed the relationship between diatom communities and bryophytes in 30 karstic springs located in Apuseni Mountains (Carpathians, Romania). Our methodology involved collecting samples of all available species of bryophytes from five distinct submerged points within the eucrenal area of these springs. We identified nine bryophyte taxa, with *Rhynchostegium riparioides* as the most prevalent, found in 20 of the karstic springs. The other taxa, including *Cinclidotus aquaticus* and *Chiloscyphus polyanthos*, were less common. The diatom samples were obtained after digesting the entire tufts of mosses. The qualitative list summarized 145 diatom taxa, with species such as *Meridion circulare*, *Cocconeis lineata* and *C. placentula* present in more than 85% of the samples. In quantitative terms, 89 taxa were identified, with *Achnantheidium minutissimum*, *A. biasoletianum*, *Amphora pediculus*, *Planothidium lanceolatum* and *M. circulare* being the most abundant species in all samples. The IndVal analysis showed few significant ( $p < 0.05$ ) indicators for specific bryophyte species, for instance *M. circulare* for *C. polyanthos*; *Encyonema minutum* and *Planothidium frequentissimum* for *Cratoneuron ilicinum*; *Gomphonema micropus* and *G. minusculum* for *Palustriella commutata*; *Achnanthes pyrenaicum* for *Dichelyma falcatum*. Bryophytes from the genus *Cratoneuron* were commonly found in springs with high conductivity and  $\text{HCO}_3$  levels, while *D. falcatum* and *C. aquaticus* preferred springs with lower values. Karstic springs distant from anthropogenic influences showed greater  $\alpha$  diversity and equitability in diatom samples. However, the  $\alpha$  diversity

of the diatoms did not seem to be influenced by the presence of specific bryophytes in the eucrenal area.

**BENTHIC DIATOM COMMUNITIES IN TWO PROGLACIAL LAKES WITH DIFFERENT GLACIAL INFLUENCE (CEVEDALE GLACIER, ITALY)**

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Climatic changes induce cascade effects on high mountain headwaters. Due to the current deglaciation, proglacial lakes increased worldwide in number and volume in the last decades, thus becoming more represented ecosystems in high mountain landscapes. With the progression of glacier retreat, proglacial lakes rapidly evolve from ice contact conditions towards ice distal conditions. The final evolution stage is a clear water mountain lake, with no glacial influence. In the European Alps, ice distal proglacial lakes represent the largest proportion of high mountain lakes, in comparison with ice contact ones and clear lakes. Glacial runoff is typically cold and contains large amounts of inorganic suspended solids (so called “glacial flour”). Due to low water temperature and high turbidity, proglacial lakes are highly selective habitats, where planktonic communities are taxonomically simplified and quantitatively scarce. Nonetheless, littoral habitat conditions may sustain algal growth. However, benthic assemblages have been scarcely addressed by studies on Alpine proglacial lakes regarding qualitative and especially quantitative aspects.

Here, we provide a first characterisation of the littoral diatom assemblages of two Alpine proglacial lakes with different glacial influence and discuss differences in community composition between the ice contact and the ice distal lake habitat. The two lakes are located between 2700 and 2900 m a.s.l. in the Martello Valley (Stelvio National Park, Province of Bolzano, Italy). They formed in the past decades in the depressions of the land surface shaped by the retreat of the Cevedale glacier and are both dammed by moraines. The ice contact lake (CL) receives glacial runoff flowing on debris-covered ice. The ice distal lake (DL) is about 500 m downstream and hydrologically connected to CL. In the ice-free

seasons 2022 and 2023, we collected quantitative diatom samples from colonised substrata (stones or cohesive sediment) during the different stages of the Alpine glacial summer (snow melt, glacier ablation and base flow). In parallel, we collected water samples to analyse chemical habitat conditions and installed temperature dataloggers in the water column to calibrate a 2D laterally-averaged hydrodynamic model that allowed to reconstruct lake thermal dynamics.

The two lacustrine habitats differed mainly in water turbidity, littoral water temperature and thermal dynamics, i.e., stratification patterns. In addition, we found differences in the benthic diatom communities of the two proglacial lakes. Alpha diversity was higher in the ice distal lake (DL), where we found species that were not observed in the ice contact lake (CL). In CL, diatom density peaks corresponded to the increase of *Achnantheidium minutissimum* s.l. (Kützing) Czarnecki, which was the numerically dominant species in all the samples in this lake. Density peaks in DL were characterised by different dominant species during the Alpine summer and the community composition showed a species succession. Furthermore, in DL, we observed a stable population of *Pinnularia bullacostae* Krammer & Lange-Bertalot. At the best of our knowledge, this is the first record of the species in the European Alps.

## **LIFE BENEATH THE GLACIER: UNRAVELING THE DIATOM DIVERSITY IN CHANGING STREAMS OF GLACIER FLOODPLAINS**

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Climate change is shrinking glaciers worldwide, fundamentally altering the streams that drain them, and exposing new terrestrial habitat for microbial colonization. By the end of the century, most small mountain glaciers will likely disappear. As a result, the glacier-fed streams currently draining alpine regions will be replaced by streams primarily fed by precipitation and/or groundwater. However, the biological response to this transition is not well known, though relevant for understanding effects for downstream ecosystems. Diatoms are one of the few primary producers able to survive the harsh conditions present in glacier-fed streams, giving them great significance in terms of elemental cycling and providing the basis for the food chain. Yet, the diatom species diversity and composition of this habitat type is poorly explored, and little is known how these communities might look in the future despite the rapid changes occurring in these ecosystems. Here, we compare the diversity and structure of diatom communities between glacier-fed streams and corresponding non-glacial tributaries from three different proglacial floodplains in the Swiss Alps. Since glacial-fed streams have glacial meltwater as their main water source, whereas the non-glacier-fed tributary streams are fed by precipitation and groundwater, the characteristics of the glacier-fed streams are expected to more closely resemble those of the tributaries as glaciers recede and eventually disappear. Thus, comparisons between the glacier-fed streams and their tributaries within these floodplains creates a space-for-time substitution.

Benthic sediments were collected from 131 patches across these floodplains and stream types in the summer of 2019. In the laboratory, we characterized a subset of these samples for their morphological diversity through light microscopy and extracted DNA from all sediment patches. DNA was then sequenced for both 18S and *rbcL* genes. Overall, we observed a significant difference between glacier-fed and non-glacier-fed tributary streams regarding diversity and species composition for both genes. Tributaries hosted more than two times the amount of diatom amplicon sequence variants (ASVs) found in the glacier-fed streams. In contrast to the stream types, diatom richness was not significantly different between the different glacial floodplains. Employing indicator species analysis, we have confirmed the presence of specific diatom ASVs associated with each stream type. Our examination identified 32 ASVs affiliated with glacier-fed streams and 61 ASVs affiliated with non-glacier-fed tributary streams, employing the *rbcL* method. When entire communities were compared through ordination (i.e. glacier-fed vs. tributary for each floodplain individually), patches clustered together within stream types, and stream types were significantly different when the results from both genes were compared with PERMANOVA. These insights reveal that glacier shrinkage will indeed lead to changes in Alpine stream diatom communities, and collectively contribute to our understanding of ecosystem dynamics in rapidly changing glacial environments, as well as the analytical methods providing them. Lastly, these findings can have implications for future research and conservation efforts in alpine ecosystems.

## **DIATOMS OF THE LARGEST SODA LAKE IN THE WORLD: INSIGHTS FROM MOLECULAR AND MORPHOLOGICAL STUDIES**

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Lake Van is located in the eastern part of Turkey, in the Eastern Anatolia Region, and is a volcanic crater lake, formed by the eruption of Mount Nemrut. It is recognized as the largest soda lake worldwide and has unique ecological attributes, including its saline water composition. The perimeter of the lake is characterized by volcanic rocks to the north and west, metamorphic rocks to the south, and predominantly sedimentary rocks to the east. The geological diversity of this region has drawn the attention of researchers, particularly concerning the chemical parameters of Van Lake's water across different periods. We are making a comprehensive study of the diatom diversity within Van Lake, currently with a particular focus on four species of the genera *Craticula*, *Halamphora* and *Navicula*. By combining morphological analyses conducted with light microscopy (LM) and scanning electron microscopy (SEM), we provide detailed insights into the morphological characteristics of diatom species inhabiting the lake. Furthermore, molecular investigations employing ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene (*rbcL*), 18S ribosomal RNA gene (18S), and photosystem II protein D1 gene (*psbC*) data enhance our understanding of the taxonomic composition, phylogenetic relationships, and genetic diversity of diatoms in this unique ecosystem.

**POSTER  
PRESENTATIONS**



**IN LOVING MEMORY OF PROFESSOR ANDRZEJ  
WITKOWSKI (1955 - 2023) A LIFE DEDICATED TO  
UNDERSTANDING MARINE DIATOMS**

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A poster in memory of Professor Andrzej Witkowski is a tribute to an outstanding scientist, mentor and visionary in the field of diatomology and paleoceanology.

The Professor's scientific achievements are enormous. He was the author or co-author of 250 scientific articles, 14 monographs and 26 chapters in monographs, and the editor of 27 monographs, including 20 from the Diatom monographs series. Through meticulous research and innovative observations, Professor Witkowski made a significant contribution to the development of diatomology and paleoceanology, inspiring subsequent generations of researchers in striving for excellence and pushing the boundaries of knowledge. In his research, he searched for the practical use of marine and freshwater algae to obtain substances and products of utility importance (e.g. in the food, chemical, medical industries, nanotechnology and other fields).

In 2021, Professor Witkowski became a Full Member of the Polish Academy of Sciences. He was also a member of two of its committees: the Sea Research Committee and the Geological Sciences Committee at the Faculty III of Exact and Earth Sciences, and also sat on the Scientific Council of the Institute of Oceanology of the Polish Academy of Sciences. Apart from his scientific activities, Professor Witkowski was valued as a mentor. He nurtured scientific talents with enthusiasm and generosity, imparting not only knowledge but also invaluable wisdom and guidance. His mentorship went beyond mere academic teaching, fostering a culture of intellectual curiosity, creativity and integrity. He was extremely appreciated and liked by his PhD students, students and colleagues for his extensive knowledge, modesty and cordial nature. He inspired subsequent generations of scientists and instilled in them a sense of

wonder and curiosity about the world. He supervised 18 doctoral theses. Many of his protégés became leaders in their fields, a testament to his lasting influence and legacy. He left an indelible mark on academia and the lives of countless people around the world.

The poster in memoriam is a visual tribute to the diverse legacy of Professor Andrzej Witkowski and is a moving reminder of the profound impact one person can have on the world through unwavering dedication to the pursuit of knowledge.

In summary, the poster in memory of Professor Andrzej Witkowski is a testimony to an extraordinary person whose brilliance, mentorship and support left an indelible mark on the academic fabric and society as a whole. We remember and honor his legacy, drawing inspiration to push the boundaries of knowledge and work for a more just and enlightened world.

## **B BERLIN – MORE THAN ONE DIATOM COLLECTION**

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The Herbarium Berolinense is part of the Botanical Collections of the Botanischer Garten und Botanisches Museum, since 1996 ‘Zentrale Einrichtung der Freien Universität Berlin’. With 3.8 million specimens, the herbarium is the largest in Germany and one of the largest worldwide. The standard abbreviation according to Index Nominum Algarum is “B”. As of 2023 the Algae part of the collection comprises more than 45,000 herbarium specimens and approximately 30,000 wet samples.

In the early 20th century, the Herbarium Berolinense had a rich stock of macro algae, such as rhodophytes, phaeophytes and charophytes, dried on paper. The algal collection suffered a dramatic loss during WW II when the library and the herbarium wing of the building were destroyed. Because the exhibition of the German colonies and the wet collection were not affected, all material that had been housed there is still available. These include samples and collections from East Africa, from which Otto Müller (1837-1917) described many new species, as well as the archived material of Willi Krieger (1886-1954) who had sampled all over Germany and Central Europe.

Beginning with the curatorial work of Regine Jahn 1991, orphaned diatom collections were obtained, such as the collection of Ursula Geissler’s (1931-2018) group at the Freie Universität, of Friedrich Fricke (1863-1926), of Linda Medlin, of Hannelore Håkansson (1957-2014), of Erwin Reichardt as well as collections from limnological research and from WRRL biomonitoring (Plankton collections of the Max-Planck-Institute, Plön; of the Willi Ripl-Group, Berlin; of the Staatliche Betriebsgesellschaft für Umwelt und Landwirtschaft (BfUL), Saxony).

In 2004, the diatom collection expanded its scope and started gathering molecular data of diatoms. These were obtained from unialgal strains whose DNA and morphology provide the basis for research on integrated

taxonomy and phylogeny as well as a reference library for eDNA-metabarcoding. With the growing internationality and important research projects of the Diatom Research Group diatom strains from different parts of the world were established: besides Germany and Central Europe, also Korea, Mexico, North America and recently both polar regions. As of 2023, the Diatom Reference Strain Collection comprises about 4000 unialgal strains with deposited DNA (GGBN), molecular data (GenBank) and morphology (LM & SEM photos).

In 2024, the diatom collection of Friedrich Hustedt (1886-1968) which includes a number of further collections such as of Kurt Krammer (1925-2020) will be transferred to B. We hope to have the Hustedt Collection accessible by early 2025. Colleagues are welcome to explore all our diatoms collections with us!

## **PHYCOBANK REGISTRATION DATABASE – WHY REGISTRATION MATTERS**

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Taxonomy, as theory and practice of classification, defines taxa on any rank as well as their circumscription (description of the characters that identify a taxon), their diagnoses (characters that establish the borders to similar taxa) and the appropriate methods of documentation. Integrative taxonomy refers to taxonomy that integrates all available data sources to frame taxon limits, in this case LM, SEM, and molecular data and deposits type specimens and research data for further studies.

Botanical Nomenclature is a set of rules and provisions to name taxa, developed more than 100 years ago, and updated regularly by the scientific community, e.g. 2024 at the Nomenclatural Section before the XX. International Botanical Congress in Madrid. Nomenclatural rules need to cover routine procedures in naming as well as all particular cases. Thus, the rules are now much more complex than in the past. Novelties are nomenclatural acts, which name taxa (new genera, new species, replacement names, new combinations etc.) and typify them in line with the appropriate sets of nomenclatural rules.

At the turn of the millennium within the AlgaTerra Project, members of the Berlin Diatom Research Group started in the evaluation of names, protologues, types and in databasing novelties. They had been disillusioned because publications with incorrect nomenclatural acts were relatively frequent at that time, resulting in invalid or illegitimate names, or incorrect typifications that had no standing. Since not even the reviewers or scientific editors were aware of these nomenclatural mistakes, the question arose how to make this knowledge available to the phycological and especially the diatom community. As a result, ‘PhycoBank’, an online registration system for nomenclatural novelties, was developed and launched in 2017. Since that time the system is

working as a nomenclatural repository in-line with the provisions of the International Code of Nomenclature. PhycoBank does not provide taxonomic views but a curated view on nomenclatural acts.

The PhycoBank registration workflow needs to be started by the author of the nomenclatural act when submitting the paper. During the registration process authors get feedback from the curator, thus incorrect names and types can still be corrected before publication. Using http-identifiers a direct link between the PhycoBank record(s) and an electronic publication is established. Because taxonomy is a lively science, novelties may also be linked to related actions, such as recombinations or comments on relocated specimens etc. Also, novelties published in conventional printed books are not overlooked, if registered. As an additional service for users, PhycoBank can be searched for names, references or types not cited in the original material, such as lectotypes, neotypes, and epitypes. To face the challenges of a modern integrated taxonomy the community needs a reliable tool that works in real-time. PhycoBank is a curated repository that keeps registered data stable and transparent. This is why PhycoBank matters. PhycoBank was funded by a DFG Grant (JA 874/8-1) and subsequently institutionalized at the Botanic Garden Berlin and is available at <http://phycobank.org>.

**TYPIFICATION OF *COLLETONEMA VIRIDULUM* WITH THE CORRECT NAME FOR *FRUSTULIA ERIFUGA* AND THE DESCRIPTION OF A NEW *FRUSTULIA* SPECIES (AMPHIPLEURACEAE, BACILLARIOPHYTA)**

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The name *Frustulia erifuga* Lange-Bertalot & Krammer was introduced in 1996 in the book on Julma Olkky lake flora, replacing the name *Frustulia rhomboides* var. *viridula* (Brébisson) Cleve (Lange-Bertalot & Metzeltin 1996). The species was initially described in 1849 under the name *Colletonema viridulum* Brébisson ex Kützing, the *typus generis* of the genus *Colletonema* Brébisson. *Colletonema viridulum* was transferred to the genus *Frustulia* under the name *F. viridula* (Brébisson) Schaarschmidt, the latter unfortunately being a younger homonym of *F. viridula* Kützing (now transferred to the genus *Navicula*). Cleve (1894) recombined the species into a variety of *Frustulia rhomboides* [*F. rhomboides* var. *viridula* (Brébisson) Cleve] but Lange-Bertalot & Jahn (2000) analyzed *N. rhomboides* and concluded that the transfer of the latter to the genus *Frustulia* by De Toni was not correct because the species did not present the typical characteristics of the genus *Frustulia*. As a result, they changed the taxonomic status of all other old varieties of *F. rhomboides*, necessitating a new name for *F. viridula*. Two morphotypes were separated based on observations in the famous Julma Olkky blade with clearly different morphologies.

Analysis of the Brébisson type material of *Colletonema viridulum* from Falaise showed that the largest morphotype of *Frustulia erifuga* at Julma Olkky is conspecific with the type material of

de Brébisson and therefore represents *F. erifuga*. The second, smaller morphotype II of *F. erifuga* shows sufficient morphological differences to be separated as a separated species, described as a new species, *Frustulia subtorfacea* Van de Vijver, Jüttner & Kusber. During the analysis of de Brébisson's sample, another species of *Frustulia* was studied. *Frustulia torfacea* Braun ex Rabenhorst was initially described from Titisee (Germany) (Rabenhorst 1853), and later added as a synonym of *F. viridula* but subsequently forgotten in history. Analysis of the original sample from Rabenhorst 761 showed that the species is definitely conspecific with *F. erifuga* and according to the rules of the International Code of Botanical Nomenclature, thus the correct name.

The results of this work have recently been published in the journal *Botany Letters*.

**TWO NEW *ENCYONEMA* SPECIES FROM EUROPE  
COMPARED TO THE TYPE MATERIAL OF *E. MINUTUM*  
AND *E. SILESIACUM* (GOMPHONEMATACEAE,  
BACILLARIOPHYCEAE)**

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*Cymbella minuta* Hilse and *C. silesiaca* Bleisch, transferred in 1990 by D.G. Mann into the genus *Encyonema*, are two fresh water species that are frequently observed in our European rivers. The original material of these two species was included in Rabenhorst's historical exsiccata series (numbers 1261 & 1802 respectively) and further illustrated by Kurt Krammer in 1997. Despite this publication, the morphological variability of these two species is poorly known and often too widely or incorrectly interpreted.

Recently, several populations of small-celled taxa of the genus *Encyonema* have been observed in France and Belgium showing an intermediate morphology between *E. minutum* and *E. silesiacum*, making a reliable identification of these taxa impossible. Based on the currently available literature, two taxa could not be identified and are described as new: *E. morvanensis* and *E. vandammeanus*.

The poster illustrates and discusses the morphology of the type material of *E. minutum* and *E. silesiacum* and compares it with the new species. *Encyonema morvanensis* is characterised in having usually protracted, rostrate apices, a low length/width ratio, the presence of an isolated pore, and areolae that are discernible in LM. On the other hand, *E. vandammeanus* also possesses protracted apices, but a higher length/width ratio, only very weakly discernible areolae, and lacks an isolated

pore. Comparisons with other *Encyonema* species, described in 1997 by Krammer (such as *E. brevicapitatum*, *E. minutiforme* and *E. simile*), are added in order to better understand the differences and similarities between these species.

The results of this work have recently been published in the journal *Botany Letters*.

## THE GENUS *GOMPHONEMA* IN SUBALPINE FRENCH LAKES, AN UNEXPECTED AND UNKNOWN HIGH BIODIVERSITY

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French subalpine lakes harbor a very diverse and abundant diatom flora. These lakes, often calcareous and oligo-mesotrophic, are usually dominated by the genera *Achnantheidium*, *Brachysira*, *Denticula* and several cymbelloid taxa belonging to the genera *Cymbella*, *Cymbopleura* and *Encyonopsis*. During a survey of several larger lakes in the French region Rhône-Alpes-Corse, an unexpected high diversity of larger-celled *Gomphonema* taxa has been observed. Most of the taxa belong to the *Gomphonema vibrio*-group but could not be identified using the currently available literature.

The present poster illustrates several type specimens of *Gomphonema vibrio* Ehrenberg from the Seychelles and compares these with the observed *G. vibrio* populations observed in the French lakes. Additionally, the type material of *G. dichotomum* Kützing was investigated as some of the observed taxa showed some resemblance to the latter. Despite these type material analyses, it proved to be impossible to identify the observed *Gomphonema* taxa in this group recorded from the French lakes.

By illustrating their morphology, we hope to open the discussion on the identity of these taxa. Together with the *G. vibrio*-group, several other interesting, though at present unknown species were recorded. A large

*Gomphonema*, resembling a Chinese spoon, was identified as a new species following comparison with taxa such as *G. apicatum* Ehrenberg, *G. apiculatum* Ehrenberg and *G. neoapiculatum* Lange-Bertalot et al. Another *Gomphonema* taxon presented cymbelloidly bent valves and reminded of *G. hebridense* W.Gregory, although the latter was described from acid environments.

This survey of the *Gomphonema* flora in the rather limited number of French lakes, shows once more our lack of good knowledge of these environments and highlights the importance of more taxonomic work based on morphology. A better understanding of this biodiversity and its ecological preferences will improve the use of diatoms in monitoring the ecological quality of these environments.

## Student Poster

P7

**UNMASKING INVISIBLE VARIABILITY: THREE NEW *HALAMPHORA* TAXA ASSOCIATED WITH LOGGERHEAD SEA TURTLES****Margaux Pottiez**<sup>1\*</sup>, Ralitsa Zidarova<sup>2</sup>, Sunčica Bosak<sup>3</sup>, Myriam de Haan<sup>1</sup> & Bart Van de Vijver<sup>1,4</sup><sup>1</sup> *Meise Botanic Garden, Research Department, Nieuwelaan 38, B-1860 Meise, Belgium*<sup>2</sup> *Institute of Oceanology – Bulgarian Academy of Sciences, Department of Marine Biology and Ecology, Parvi May Str. 40, BG-9000 Varna, Bulgaria*<sup>3</sup> *University of Zagreb, Faculty of Science, Department of Biology, Horvatovac 102a, HR-10 000 Zagreb, Croatia*<sup>4</sup> *University of Antwerp, Department of Biology – ECOSPHERE, Universiteitsplein 1, B-2610 Wilrijk, Belgium*\* [margaux.pottiez@plantentuinmeise.be](mailto:margaux.pottiez@plantentuinmeise.be)

During light microscopy (LM) observations of diatom samples obtained from the carapaces of loggerhead sea turtles near Pula, Croatia (in the Adriatic Sea), as well as from the recovery tanks where these turtles were housed after arrival, we identified multiple populations that could be assigned to a single *Halamphora* species. In LM, this taxon had a more or less narrowly semi-lanceolate to semi-elliptic valve outline with moderately convex dorsal margins, and rostrate, subrostrate to subcapitate apices, slightly bent to the ventral side of the valves. Valve length of the measured valves in LM varied between 7.0 and 17.0  $\mu\text{m}$ , with a valve width of 2.0 to 3.5  $\mu\text{m}$ . Teratological valves with distorted valve outlines can also be observed. In all populations, only dorsal striae were visible, almost parallel in the valve middle, becoming weakly radiate towards the apices, usually slightly punctate, 22–27 in 10  $\mu\text{m}$ , although some valves had finer, less discernible striae in LM, suggesting a denser striation. Following scanning electron microscopy (SEM), it became obvious that this provisionally single taxon contains at least three different species with overlapping dimensions and stria number. Separation of these taxa was only achievable in SEM based on the raphe ledge structure, central raphe endings, dorsal areola arrangement, and ventral striation patterns. Two of the taxa seemed to be exclusively present on turtle carapaces, whereas the third taxon was detected with

a few valves both in the recovery tank and on a turtle carapace after the same turtle had spent several months in this tank. One of the taxa, present on the turtle carapaces upon arrival, showed a broad morphological variability, in terms of dorsal valve margin, areola shape and width of the raphe ledge. However, after examining numerous valves in SEM, we could not detect sufficient evidences for the separation of these valves as different taxa, and we concluded that they all belong to a single taxon. A search across the literature did not help us with the identification of all three observed *Halamphora* taxa, suggesting that they most likely represent unknown and yet undescribed species.

## Student Poster

P8

**OBSERVATIONS OF ORIGINAL WILLIAM SMITH MATERIAL OF *CYMATOPLEURA APICULATA* AND ITS TRANSFER TO THE GENUS *SURIRELLA* (SURIRELLACEAE, BACILLARIOPHYTA)**

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William Smith (1808–1857) separated in 1851 the genus *Cymatopleura* W.Smith from the genus *Surirella* Turpin based on “the undulated valves with margins not produced into alae” subsequently placing three species into the new genus [*C. solea* (Brébisson) W.Smith, *C. elliptica* (Kützing) W.Smith, and *Cymatopleura hibernica* W.Smith] and in 1853 adding another two: *Cymatopleura apiculata* W.Smith and *C. parallela* W.Smith. Lectotypes for the different *Cymatopleura* species, including *C. apiculata*, were designated by Kurt Krammer in Lange-Bertalot & Krammer (1987) and lectotype slides, made from original Smith material, were deposited in the Van Heurck collection, (Meise Botanic Garden, Belgium).

In 2016, Ruck & al. published a detailed phylogenetic analysis of the orders Surirellales and Rhopalodiales concluding that the genus *Cymatopleura* should be included within the genus *Surirella*. As a result, several *Cymatopleura* species were transferred to the genus *Surirella*, taking into account an analysis of several Ehrenberg species that had been described earlier than some of the Smith species. *Cymatopleura solea* for instance, thus became *Surirella librile* (Ehrenberg) Ehrenberg and *C. elliptica* is now generally accepted as *Surirella undulata* (Ehrenberg) Ehrenberg.

*Cymatopleura apiculata* was left in the genus *Cymatopleura*, most likely because it was seen as a simple outline variation of *C. solea*. However,

both species can be separated based on the more apiculate valve apices and the distinctly smaller valve width of *C. apiculata*, never exceeding 18  $\mu\text{m}$ .

The present poster illustrates and discusses the morphological analysis of the type material of *C. apiculata*, providing an overview of its taxonomic history (including the correction of the lectotypification). The taxon will be transferred to *Surirella* as *S. microlibrile*. The differences with *S. librile* are discussed using historic *S. librile* and *S. microlibrile* populations to highlight their morphological features.

**DIVERSITY AND DISTRIBUTION OF *SELLAPHORA* AND *PLACONEIS* SPECIES FROM LAKE TANGANYIKA****Neda Raposka**<sup>1\*</sup>, Zlatko Levkov<sup>1</sup> & Elena Jovanovska<sup>2</sup><sup>1</sup> *Institute of Biology, Faculty of Natural Sciences, Ss. Cyril and Methodius University in Skopje, Arhimedova 2, 1000 Skopje, North Macedonia*<sup>2</sup> *Department of Paleoanthropology, Senckenberg Research Institute and Natural History Museum, Senckenberganlage 25, 60325 Frankfurt am Main, Germany*

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Ancient lakes are characterized by high diversity and a great endemism of benthic diatom species. Recent studies on the diversity of pennate diatoms in Lake Tanganyika have shown that there are many unknown and endemic species. However, the genera *Sellaphora* and *Placoneis*, which were previously known to be diverse in the lake, have not yet been sufficiently studied in Lake Tanganyika. Therefore, several expeditions to the lake along the Zambian and Tanzanian shoreline were carried out between 2018 and 2022 and more than 500 samples were collected and analysed with light and electron microscope. Over 40 taxa from the genus *Sellaphora* and 10 taxa from the genus *Placoneis* were recorded, most of which are unknown to science and are likely endemic species of the lake. Most of the *Sellaphora* taxa belong to the species complex around *Sellaphora nyassensis* and *S. pupula* sensu lato, opposite to Euro-Asian continental ancient lakes where *Sellaphora bacillum* show a greater diversity. The genus *Placoneis* is characterized by a smaller number of species and by a lower morphological diversity. Most of the taxa belong to the species complex around *P. gastrum*. The highest number of species were observed at Crocodile Island and Isanga Bay on sandy and clay substrate at a water depth of 10–20 m. Such a high and unique species richness, could indicate an evolutionary radiation as recently demonstrated in the genus *Diploneis* from the lake. Further molecular and microscopic analyses are needed to determine the number of species within the two genera, their origin and their phylogenetic position. This will shed light on the nature of the potential evolutionary radiation and help to explain the origin of the great diversity of diatoms in the lake and in general.

***DISCOSTELLA ASTEROCOSTATA* (B.Q.LIN, S.Q.XIE & S.X.CAI) HOUK & KLEE ARRIVED IN EUROPE: DISTRIBUTION, MORPHOLOGICAL AND ECOLOGICAL FEATURES OF EUROPEAN SPECIMENS**

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Water quality monitoring is a necessary tool for water management, but it is also an important tool for collecting biodiversity data. As such, it is one of the first tools for discovering new species. This study includes data from the monitoring of phytoplankton in Croatian Pannonian very large rivers, which was started in 2010. Phytoplankton was collected together with water for physical and chemical parameters on a monthly basis from April to September. The phytoplankton was analysed with an inverted microscope according to the Utermöhl method, whereby the diatoms were identified on permanent slides. During routine analysis, *Discostella asterocostata* was identified for the first time in 2016 in the samples from the Mura River and in the following years in Drava and Danube rivers. The species was originally described in China and later found in the USA, but there was a gap in Europe until now. The morphometric characteristics were counted and measured under the light microscope and additionally analysed in the SEM. PCA analysis of eight European characteristics of *D. asterocostata* with populations from China and the USA showed significant overlap. The species was never the dominant centric diatom in phytoplankton samples, but it is common with a relative biomass of up to 3.2 %. The negative correlation to nutrients, TOC, BOD and COD indicates that it favours water with lower organic load, while the positive correlation to water temperature confirms it as mid-summer species. Studies from USA suggests that the spread of *D. asterocostata* was due to the introduction of invasive cyprinid fish, but further research is needed to confirm the same pattern

in Europe. *Discostella asterocostata* was originally described as a lake species, so this European discovery as part of the river phytoplankton provides scope for future studies in nearby lakes and a more detailed distribution.

**A NEW DIATOM (BACILLARIOPHYTA) SPECIES - FALLACIA SP. NOV. - FROM KAKLIK CAVE IN THE WESTERN ANATOLIAN KARST REGION, REPUBLIC OF TÜRKIYE**

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Kaklık Cave is in Western Anatolian Karst Region, Türkiye. In this region, characteristic formations of hydrothermal karsts caused by tectonic deformation are common. The Pamukkale travertines on the surface (25 km northwest of the cave) and travertines of the Kaklık Cave are typical examples of surface collapses due to thermal corrosion. The stepped travertine system in the Kaklık Cave is likened to the unique Pamukkale travertines and is called “Little Pamukkale” or “Cave Pamukkale” by tourists. The cave is quite unique with highly calcareous water chemistry (pH = 8.2). The samples were collected in September 2015 from travertine surfaces from thin biofilm composed primarily of musilage. *Fallacia angarae* Metzeltin, Kulikovskiy & Lange-Bertalot, *F. helenensis* (Schulz) D.G.Mann, *F. lange-bertalotii* Reichardt, *F. lenzii* and *F. pseudohelensis* Kulikovskiy, Metzeltin & Lange-Bertalot are similar in morphology and general outline. Among them, *Fallacia lange-bertalotii* co-occurs and is the most similar taxon, but *Fallacia* sp. has distinct apical pore occlusions. Detailed description of the holotype of the new species was documented by using scanning electron microscopy

(SEM) and the morphology of the newly described species was compared with its relatives.

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**INTERESTING *ACHNANTHIDIUM* (BACILLARIOPHYTA) SPECIES INCLUDING TWO NEW SPECIES FROM SAKARBAŞI SPRING, REPUBLIC OF TÜRKIYE**

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Three species of the genus *Achnantheidium* Kützing have been found in a limnocene spring (Sakarbaşı) in Eskişehir, Turkey; they are described based on light and scanning electron microscopy observations and associated with specific water conditions. Among them, *A. jiuzhaiensis* and *A. sp. 1* sp. nov. belong to the general *A. pyrenaicum* complex, while *A. sp. 2* sp. nov. falls within the *A. minutissimum* complex. *A. jiuzhaiensis* and *A. sp. 1* can be distinguished from *A. pyrenaicum* by valve apices, round and slightly drawn-out in *A. pyrenaicum* vs. subcapitate to capitate in *A. jiuzhaiensis* and rostrate in *A. sp. 1*. *Achnantheidium jiuzhaiensis* has structured areolae that interconnect internally along the striae, a feature only known from one other species. *Achnantheidium sp. 2* is distinguished from *A. minutissimum* by long transapically areolae on the external raphe valve and the evenly distributed striae on the valve. Valve outline, broadly linear-lanceolate with rounded valve apices in *A. sp. 2* are different from the linear-lanceolate with protracted, rostrate to subcapitate apices in *A. minutissimum*. The three species are compared to other related species:

*A. sp. 1* to *A. deflexum*, *A. druartii*, *A. linannulum*, *A. sublaceolatum* and *A. chitrakootense*; *A. jiuzhaiensis* to *A. gracillimum*, *A. acerosum* and *A. ertzii*; and *A. sp. 2* to *A. straubianum*, *A. dolomiticum*, *A. caravelense* and *A. saprophilum*. The species are found in a unique spring environment with, alkaline waters of reasonable oxygen levels, pH 7.7–7.9 and higher conductivity (545–575  $\mu\text{S}\cdot\text{cm}^{-1}$ ).

**TWO NEW *HALAMPHORA* (AMPHIPLEURACEAE: BACILLARIOPHYTA) SPECIES FROM GÖKOVA BAY IN REPUBLIC OF TÜRKIYE**

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Gökova Bay (Turkey), which is situated in the South Aegean Sea, has been declared as a Special Environmental Protection Area (SEPA) since 1988. The study area, which is fed by freshwater inputs, is located within one of the sub-zones of this protected area. The region is in the 'Mediterranean Basin' global biodiversity hotspot, and WWF Global 200 ecoregion as well. The samples were collected in November-2023 by using toothbrush from stones. In this study, two new *Halamphora* taxa were found in the bay. *Halamphora* sp. 1 has linear-lanceolate with truncate ends, convex dorsal and straight ventral margin. The other taxon is *Halamphora* sp. 2. *Halamphora* sp. 2 has semi-elliptic with strongly arched dorsal and straight ventral margin. It is similar to *H.* sp. 1 however, *H.* sp. 2 has dorsally more arched outline and marginal areolae structure is two-line. Additionally, *Halamphora* sp. 1 has larger areola than *H.* sp. 2 in ventral striae.

This work is supported by the Scientific Research Projects Coordination Unit of Istanbul University (Project Number: FBA-2023-39521 and FBA-2023-39425). SEM work was performed with the facilities of Istanbul University Nano & Optoelectronics Research Laboratories.

**TWO NEW *HALAMPHORA* (AMPHIPLEURACEAE: BACILLARIOPHYTA) SPECIES FROM NEGEV DESERT IN ISRAEL**

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Geographically, the Negev Desert is critical as it is the only land bridge between Africa and Asia is located. The desert is arid and semi-arid, ranging from 50 mm annual precipitation in the south to up to 300 mm annual precipitation further north. The samples were collected in January-2012 by using toothbrush. In this study, two new *Halamphora* taxa were found in a saline pond. *Halamphora* sp. 1 has strongly dorsiventral with typically arched dorsal margin and slightly convex ventral margin. The taxon is similar to *Halamphora minima*, *H. veneta* var. *somalica*, and *H. pratensis* are similar taxa. Among them, *Halamphora minima* is the most similar one however, *H. sp. 1* has longer capitate endings. The other taxon is *Halamphora* sp. 2. Valves are semi-lanceolate with smoothly arched dorsal margin and slightly convex ventral margin. *H. poianensis* is similar taxon. In *Halamphora poianensis* has larger valves. Additionally, areolation in dorsal and ventral striae is quite different.

The SEM work was supported by TUBITAK (Grant no: 123Y267) and Dumlupınar University Foundation (Grant no: 2023-38).

**A NEW DIATOM SPECIES—*TETRAMPHORA* SP. NOV.  
(MASTOGLOIACEAE: BACILLARIOPHYTA)—IN ARADE  
ESTUARINE, PORTUGAL**

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The Arade is the second largest river that flows into the southern coast of Portugal. It originates in the Serra do Caldeirão and flows for 75 km in an ENE-WSW direction until it reaches its mouth near Portimão city. The Arade River Estuary extends to the town of Silves and is approximately 15 km long. Along its banks, there are urban areas, artificial marshes, salt marshes, salt pans and agricultural areas. In May-2010 sample were collected on the salt marches of Arade River estuary and a new diatom species present in those samples is here described. *Tetramphora* sp. nov. has semi-elliptic and strongly dorsiventral outline with smoothly arched dorsal margin, slightly tumid in central area and slightly convex ventral margin. Valve endings narrowly rounded and slightly ventrally deflected. Typically, valves are surrounded by visible silica thickness in both externally and internally. *Tetramphora croatica* Gligora, Udovič, Caput Mihalić, Stanković & Levkov, *T. fontinalis* Stepanek & Kociolek, *T. lineolata* (Ehrenberg) Mereschkowsky, *T. lineolatoidea* Stepanek & Kociolek, *T. ostrearia* (Brébisson) Mereschkowsky, *T. robusta* Stepanek & Kociolek, *T. securicula* (Peragallo & Peragallo) Stepanek & Kociolek and *T. sulcata* (Brébisson) Stepanek & Kociolek are similar in morphology and general outline.

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**TWO NEW *HALAMPHORA* (AMPHIPLEURACEAE: BACILLARIOPHYTA) SPECIES FROM AYDAR-ARNASAY SYSTEM OF LAKE IN UZBEKISTAN**

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The Aydar-Aynasar Lake System (AALS) are located in Central Asia which connected with Kyzylkum Desert. Ecologically, salt dust raises from the sediment so, the lake is brackish characteristics. AASL has an area of 3791 km<sup>2</sup>, a water volume of 44,19 km<sup>3</sup>, a length of 350 km from West to east and a width of 2 km to 40 km from North to South. Aydar Lake is called “Turquoise River between the sands”. The lake system is a unique antipode of the Aral Sea and it is granted the status of Internationally Protected Lakes by the Ramsar Convention. The samples were collected in May 2023. In this study, two new *Halamphora* taxa were found in the lake system. *Halamphora* sp. 1 has strongly dorsiventral with arched dorsal margin and slightly tumid ventral margin. Typically, it has quite long ventral striae. The taxon is similar to *Halamphora moncheviana* however, *H. moncheviana* has more or less straight ventral side. The other taxon is *Halamphora* sp. 2. Valves are semi-elliptic with smoothly arched dorsal and almost straight ventral sides. The taxon is similar to *Halamphora banzuensis*, *H.guhanensis*, *H. margalefii* var. *lacustris*, and *H. moelleri*. Among them, *Halamphora banzuensis* is the most similar outline however, it has larger conopeum in the detailed morphological investigation (SEM).

The SEM work was supported by TUBITAK (Grant no: 123Y267) and Dumlupınar University Foundation (Grant no: 2023-38).

## PRELIMINARY RESULTS OF DIATOM BIODIVERSITY ON INDONESIAN ISLAND JAVA

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Diatoms are diverse, with an estimated 20,000 to 2 million species existing worldwide. This diversity is reflected in their morphology, habitat preferences, and ecological roles. They have a wide range of sizes, shapes, and frustule structures, from simple, unornamented forms to complex, intricately patterned designs, and are found inhabiting coral reefs as microphytobenthic communities attaching corals, macroalgae, sand and also coral rubbles. Light microscopy analysis revealed an extremely high diversity of the diatom assemblages studied with a species richness amounting to several hundred. We found at least two genera and five species of diatoms which are potentially new to science. In terms of abundance, dominants were a few: *Halamphora*, *Nitzschia*, *Amphora*, *Cocconeis*, *Psammodictyon*, *Paralia*, *Mastogloia*, and *Navicula* species that occurred almost at all stations studied. In our study, we identified more than 30 species of *Mastogloia* and *Cocconeis* – diatom that are known as common species in tropical areas. Several taxa from Java Island are not clearly identified and will require taxonomical identification in the future.

## PRELIMINARY RESULTS OF DIATOM BIODIVERSITY ON INDONESIAN ISLAND SULAWESI

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Indonesia is the world's largest archipelago. It consists of more than 17,000 islands and a coastline spanning approximately 81,000 kilometres. Among these islands, the five largest are Papua, Kalimantan (former known as Borneo), Sumatra, Sulawesi (formerly known as Celebes), and Java. For this reason, Indonesia boasts mega-biodiversity in marine resources, including diatoms. It is indicated that two of the world's 23 biodiversity hotspots are located in Indonesia: Sundaland, situated in the western part, and Wallacea, found in the eastern part. In terms of surface area, the island of Sulawesi ranks at the 4<sup>th</sup> place. However, it is characterized by the overly complex shape of its coastline. The island of Sulawesi has been poorly investigated for what concerns the global biodiversity of marine diatoms. This poster presents the first results of a PhD thesis dedicated to this topic. Samplings were carried out on Transpatoa and Molas beaches, North of Sulawesi in September 2022, with water quality parameters measured concomitantly. A total of 113 species and 35 genera were observed (the results are not final yet and will be continued). *Mastogloia*, *Amphora*, *Halamphora* and *Diploneis* genera display the highest species richness for now. These early results suggest that the biodiversity of marine diatoms in Sulawesi is high and deserves further exploration. Since the first samplings were conducted in the northern part of the Island, a more exhaustive study would include samplings in the central and southern parts.

## Poster presentation

P19

**MORPHOLOGY OF *EUNOTIA FLEXUOSA* (BRÉBISSEON EX KÜTZING) KÜTZING, *E. FLEXUOSA* VAR. *BICAPITATA* GRUNOW (IN VAN HEURCK 1881) AND *SYNEDRA BICEPS* W.SMITH**

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The type materials of *Synedra biceps* W.Smith and *Eunotia flexuosa* (Brébisson ex Kützing) Kützing (1849) found at the Van Heurck collection, Meise, and material of *Eunotia flexuosa* var. *bicapitata* Grunow (in Van Heurck 1881) from the Walker Arnott collection, Meise, were studied in light and scanning electron microscopy. Kützing originally described *Eunotia flexuosa* as *Synedra flexuosa* Brébisson ex Kützing (Kützing 1846, p. 248, “Falaise: Bréb.!”) later transferring it to *Eunotia* (Kützing 1849, p. 6, “Falaise. Specimina communicavit amiciss. De Brébisson, sub. no. 51”). Smith (1853, p.69) described *Synedra biceps* based on material from “Boggy pools, Dorsetshire, Dec. 1849, W. Sm.”, and “Dolgelly, Mr. Ralfs”, a taxon differing from *Synedra biceps* Kützing (1844, p. 66). Grunow (1881, pl. XXXV, fig. 11) established *Eunotia flexuosa* var. *bicapitata* referring to “*Synedra biceps* W.Smith (Kütz. partim.) and *Eunotia biceps* Ehr. partim ??”. The three taxa present are identical in their morphology making *S. biceps* and *E. flexuosa* var. *bicapitata* later synonyms of *Eunotia flexuosa* (Brébisson ex Kützing) Kützing (1849).

The species could be described as follows: Frustules rectangular, valves slightly arcuate to almost linear with more or less parallel valve margins, not or slightly tapering near inflated ends, 131–313 µm long, 5.5–7.0 µm wide. Striae parallel, slightly radiate near valve ends, parallel or slightly

convergent at apices, 10–12 in 10  $\mu\text{m}$ . Raphe fissures long, curved on mantle, curving onto valve close to apices, recurving towards valve centre for some distance. One large rimoportula present in centre or slightly above centre of apex. Small spines of varying size located at valve margin, larger spines present at apex.

## NEW AND RARE DIATOMS SPECIES FROM A KARSTIC REGION IN CROATIA

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Karstic aquatic ecosystems play a crucial role as global water resources, particularly in the Mediterranean region of Croatia, where the karst constitutes a large part of the southern Dinaric belt within the Adriatic Sea basin. Throughout history, Croatian karstic areas, especially the freshwater environments, have gained recognition as biodiversity hotspots, in particular for diversity of diatom taxa. Notable among these are several species described by Hustedt in 1945 from the Croatian Plitvice Lakes National Park. More recently, several new and interesting species from this region have been recorded, described and deposited in the Croatian National Diatom Collection (HRDNC). Despite the challenges, karstic freshwater ecosystems persist in harbouring a rich diversity of unexplored diatom species. In the current study, we present diatom species discovered in Croatian karstic freshwaters, including two newly described species from the Krka River (*Cymbopleura amricula* Gligora Udovič & Levkov, *Aneumastus visovacensis* Gligora Udovič & Levkov), as well as one new species from the genus *Berkeleya*. Species from the genus *Berkeleya* are typically associated with marine environments, making this discovery the first record of a freshwater species within this genus. Furthermore, our investigation has yielded two new species observed in Lake Kutina (*Brachisira* sp. nov. 1 and *Brachisira* sp. nov. 2), a new small species of *Encyonema* sp. nov. found in the Lešće hydroelectric reservoir, and a new species from the Cetina River identified as *Geissleria* sp. nov. The karstic region of Croatia undeniably constitutes a part of the unique Mediterranean biodiversity hotspot, yet it remains perpetually threatened by various anthropogenic impacts, including excessive surface and groundwater abstraction, water pollution, and ultimately,

climate change. This region boasts geographical and hydrological uniqueness, characterized by high habitat heterogeneity and biodiversity. Therefore, prioritizing sustainable management practices for protection of this vulnerable area is of critical importance. Nonetheless, scientific research has the potential to raise awareness of its sensitivity and unique biodiversity, thus paving the way for enhanced protection measures in the future.

## TERRESTRIAL DIATOMS FROM ULU PENINSULA (JAMES ROSS ISLAND, MARITIME ANTARCTIC REGION)

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Diatoms constitute an important and diverse component of terrestrial protist communities, but remain poorly studied, especially in the Antarctic realm. We investigated the diversity and community structure of the terrestrial diatom flora from the Ulu Peninsula, James Ross Island (Maritime Antarctic Region) using a morphology-based dataset and physico-chemical measurements. A total of 97 taxa belonging to 27 genera was identified in 59 samples from terrestrial environments, including soils and rock walls. The flora was dominated by the genera *Hantzschia*, *Luticola*, and *Humidophila*. Eight distinct diatom assemblages could be distinguished and were mainly structured by differences in environmental characteristics such as vegetation coverage, moisture, conductivity, pH, and nutrient concentrations. In general, James Ross Island harboured

a unique diatom flora as evidenced by very low similarity values with other (sub)Antarctic localities. Only 16% of the taxa have a typical cosmopolitan distribution, whereas 70% showed a restricted Antarctic distribution, supporting previous indications of high species level endemism in environments characterized by harsh abiotic conditions. In addition, several of the cosmopolitan species uncovered in this study might harbor substantial levels of hidden diversity, including endemic taxa, as previously revealed for the *Pinnularia borealis* species complex on James Ross Island. Taken together, the present study improves our knowledge and understanding of the diversity, ecology, and community structure of the terrestrial diatom flora of Ulu Peninsula, and highlights that soils and wet rock walls represent important terrestrial habitats in this transitional zone between Maritime and Continental Antarctica.

**THE GENUS *NAVICULA* IN SPRING ECOSYSTEMS, WITH DISCUSSION OF A PUTATIVE NEW SPECIES**

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Spring ecosystems are unique habitats, which are increasingly menaced because of the exploitation of the strategic water resource, the availability of which is reduced in many geographic areas due to climate change. Spring biota and ecology are receiving more attention in recent decades but are still grossly understudied in many geographic areas. Diatoms in springs have been more investigated than other components of the biota, but new species (sometimes even new genera) are still being described from springs in the frame of in-depth research on diatom communities. Using LM and SEM micrographs and comparison with the morphologically most similar species, here we present a potentially new

species. This *Navicula* species has medium-small size (width = 5.5-6.5  $\mu\text{m}$ , length = 15-21  $\mu\text{m}$ ; 13-15 striae in 10  $\mu\text{m}$ ), lanceolate outline, subrostrate ends, and a variable, moderately large central area. It was collected in a mountain spring located above the tree line at 1613 m a.s.l. in the Northern Apennines. The Fontana del Vescovo (Bishop's spring), which is the locus classicus of the new species, has a low conductivity (60-70  $\mu\text{S cm}^{-1}$ ), temperature of ca. 5  $^{\circ}\text{C}$ , circumneutral pH (7.3-7.5), and relatively low nitrate (ca. 1  $\text{mg L}^{-1}$ ), and has suffered a discharge reduction from 1 to 0.1  $\text{L s}^{-1}$  from 2011 to 2023. The putative new species was confirmed by a second finding in Northern Macedonia, and we here thoroughly document this second population as well. We seized the occasion of the discussion of this putative new *Navicula* to review the global literature on the diatom genus *Navicula* in spring ecosystems. Using the results of this literature review and own databases on springs and their artificial counterpart (drilled wells) in central Europe and North Africa (Egypt), we discuss main *Navicula* species and their environmental preferences in spring habitats.

## NEW DIATOM SPECIES (BACILLARIOPHYTA) SPECIES FROM SOUTHERN BRAZILIAN SUBTROPICAL COASTAL LAGOONS

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New diatom species from the genera *Catenula*, *Chamaepinnularia*, *Planothidium* and *Pseudostaurosiropsis* discovered in epiphytic samples from southern Brazil's coastal lagoons are here illustrated. *Catenula* sp.1 shows similarities to other two species. *Catenula javanica* Witkowski, Kryk, Risjani & Yuniarta has a distinct valve shape with elongated, protracted apices. In *Catenula adhaerens* (Mereschkowsky) Mereschkowsky valve shape changes from very narrow with slightly protracted apices in the longest specimens to strictly lanceolate with somewhat broader and obtusely rounded apices in the shortest specimens. In *Catenula* sp.1 raphe slit lies almost along the ventral valve face margin, whereas in *C. adhaerens* it is on the valve face at a greater distance from the margin. The raphe is also straight (i.e. not undulate as *C. javanica*) showing proximal and distal raphe endings slightly deflected towards the dorsal valve face margin with a broad external central nodule, whereas in *C. adhaerens* it is straight with a much narrower external central nodule. We also observed significant differences in valve width. The second new species, *Chamaepinnularia* sp.1 is similar in outline and size to the other two species found in either thermal or saline environments. *Chamaepinnularia thermophila* (Manguin) C.E. Wetzel & Ector and *Chamaepinnularia tongatensis* (Hustedt) Lange-Bertalot, but the main difference is the central area. *Chamaepinnularia* sp.1 possess wider central inflation on the median portion of the valve, being wider in relation to the apices. Furthermore, we illustrated the *Planothidium* sp.1, which is distinguished from the most similar species by its elliptical, slightly asymmetrical valves with pronounced short rostrate ends and

coarse striae consisting of one to two rows of areolae. This taxon bears resemblance to the cavum-bearing species in the “*Planothidium rostratum* (Östrup) Lange-Bertalot sensu lato” group and the recently described *Planothidium xinguense* K.S.Morais, C.E.Wetzel & C.E.M.Bicudo from the Brazilian Amazon. Finally, one commonly found species related to the short-striated diatoms of the genus *Pseudostaurosira* E.A.Morales is also presented. The first species was found in a lake plankton collection made during the Wallacea-Expedition in the early 1930s; it was originally described as *Fragilaria construens* var. *javanica* Hustedt but based on its ultrastructure (i.e. closing plates circular, flattened, plate-like structures almost entirely occluding the areolar openings) we propose its transfer and new status. The second species was often observed in the epiphytic diatom communities of southern Brazilian coastal lagoons, *Pseudostaurosira* sp.1. Each taxon is described morphologically and compared with closely related published taxa, using characters such as axial area, virgae, vimines, areolar shape, volae, internal striae depositions, spines, flaps and apical pore fields. Moreover, a detailed account on associated flora and ecology is discussed. This study compares the morphology and ecological preferences of the new species with those of related taxa, providing insights into its occurrence in southern Brazilian subtropical coastal lagoons.

## ADDING MORE TAXA TO THE *COCCONEIS PLACENTULA* GROUP (BACILLARIOPHYTA): TWO NEW SPECIES FROM STREAMS IN BIODIVERSITY HOTSPOTS

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Two new *Cocconeis* species are described as new based on unialgal strains, cultivated from samples collected in streams of Mexico, California, and Germany. For *Cocconeis* sp. nov. 1, strains from Mayotte Island and Spain were re-analysed. Although these two new species belong morphologically within the *C. placentula* group, genetic distances for the nuclear-encoded 18S-V4 and the plastid gene *rbcL* show that these species are distinct from other species with available sequencing data. Morphologically, these two taxa can be distinguished from similar species by a combination of characters such as length, width, striae and areolae count, size of hyaline rim, number of submarginal areolae, by closed or open valvocopulae of both valves and their additional features fimbriation and smoothness. These characters are difficult to assess in

environmental samples containing several taxa from this group especially when only small specimens are identified with light microscopy. Despite the disjunct origin of the 13 analysed *Cocconeis* sp. nov. 1 strains, encompassing different climatic zones, their diagnostic morphological features are broad but stable, and share identical sequences for the two markers, indicative of a cosmopolitan distribution. Lastly, we provide occurrence notes for freshwater species of the placentuloid group based on eDNA metabarcoding data from subpolar, temperate, and tropical zones, further supporting the global distribution of *Cocconeis* sp. nov. 1 and the probable endemic occurrence of *Cocconeis* sp. nov. 2 in North America.

## **METABARCODING INVESTIGATIONS ON THALASSIOSIRALES SPECIES OF PHYTOPLANKTON FROM DANUBE RIVER**

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Metabarcoding is an increasingly widely used method for studying different communities of organisms. The method is fast and getting cheaper, so it is becoming more and more common. Spatial and temporal changes of the species of Thalassiosirales order were studied during a vegetation period in the middle section of Danube River with metabarcoding method to identify key factors shaping the community. Phytoplankton samples were collected from 13 sampling points in the Hungarian section of the Danube River from the main arm and two side arms (Mosoni Danube and Ráckevei-Soroksári Danube) with monthly sampling from May 2021 to April 2022. The results obtained by amplicon sequencing were also compared with the results of the SEM investigations once per season (except in winter), in each sampled location. Our studies were carried out in a period with a more or less balanced water discharge, so the relationship between species and environmental variables was barely affected by changes in water flow. Among the measured environmental variables, temperature and total nitrogen had the most significant effect on the composition of the order Thalassiosirales, but the effect of pH, turbidity and soluble reactive phosphorus were also significant. The highest algal biomass was in March in the Danube, then highly decreased by the end of summer and remained low till the end of vegetation period. This decrease was related to the changes in the total nitrogen: total phosphorus ratio, which was very low in the warm water period (mostly below 10), which could potentially limited

algal growth. Temperature is one of the most fundamental drivers of microbial nitrogen dynamics in rivers. As a result of global warming, the average temperature of the Danube is increasing. Since the speed of microbial processes is higher in warm water, the conditions are more favourable for denitrification.

In total, 35 taxa of the order Thalassiosirales were detected by DNA and morphological analyses. Of these, 23 were detected only by both methods, 7 only by morphological and 5 only by DNA analyses. The main reason for the discrepancies was the shortcomings of the reference database. The research was carried out within the framework of the Széchenyi Plan Plus programme with the support of the RRF 2.3.1 21 2022 00008 project.

**MORPHOLOGICAL AND PHYLOGENETIC DIVERSITY OF THE DIATOM GENUS *ENTOMONEIS* ISOLATED FROM THE TIDAL FLATS OF KOREA**

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The diatom genus *Entomoneis*, first named by Ehrenberg in 1845, was redefined by Patrick & Reimer (1975) and Round et al. (1990) as a species with panduriform cells, sigmoid raphe located along the keel, numerous bands, biseriate or multiseriate striae, and basal fibulae (junction line). Currently, 34 species are listed in Algaebase, but only three species are recorded in Korea: *E. alata*, *E. costata*, *E. paludosa* var. *subsalina*. This study reports the morphological and phylogenetic characteristics of six species newly discovered in Korea. From November 2022 to April 2023, mud, sand, and stones were collected from the west and south coasts of Korea, including Jeju Island. Single strains were isolated and cultured. The ultrastructure of the cells was observed using an electron microscope, and the gene sequence of *rbcL* was analyzed.

Six species of *Entomoneis* were newly discovered. *Entomoneis* sp. 1 has grooves on both ends of the valve and the cells are long. *Entomoneis* sp. 2 has two Y-shaped horns at central ends of raphe. *Entomoneis* sp. 3 is panduriform in both girdle and valve view. *Entomoneis* sp. 4 is very similar to *E. paludosa*, but shows differences in the ultrastructure. The two other species were *E. punctulata* with short curved basal fibulae at both ends of the valve, and *E. annagodheae* with fascia in the center of the valve. Among the six species, *Entomoneis* sp. 1 to sp. 4 are likely new species, and two species are unrecorded species in Korea.

## **A POLYPHASIC TAXONOMY OF THE PSEUDOCRYPTIC SPECIES COMPLEX *PINNULARIA ACIDICOLA*, WITH DESCRIPTION OF NEW SPECIES**

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Diatoms are among the most species-rich and environmentally significant protists on Earth, playing important roles in biogeochemical cycles and food webs of many aquatic and semiaquatic ecosystems. However, a large portion of their diversity appears to lie beyond the resolution of the traditional microscopy-based methods routinely and sometimes exclusively utilized in the investigations of their populations, species, and communities. One such cryptic species complex is *Pinnularia acidicola* which was recently shown to consist of at least three different species-level lineages with distinct geographic ranges on the islands of the Southern Hemisphere and (when looked beyond morphological characters traditionally utilized in the identification of diatom species) promising species-specific morphological differences. In this study, we are (1) adding one more *P. acidicola* complex species represented by seven monoclonal strains from the Northern Hemisphere, (2) analyzing all available evidence including morphology, genetics, phylogenetics, biogeography and ecology, and (3) suggesting taxonomic changes to formally resolve the *P. acidicola* complex. The name shall be reserved for the type population found on Ile de la Possession and Marion Island, while the populations found on Amsterdam Island, Réunion, and in Sweden should be described as three new species. For now, the

species appear not only unambiguously distinguishable using standard diatom barcode markers 18S rDNA and *rbcL*, but also semicryptic (i.e., distinguishable by distinct geographic ranges), and to some extent even pseudocryptic (i.e., three out of four species appear distinguishable even under the LM due to discontinuities in relative fascia coverage). On the other hand, the species does not appear to be differentiated ecologically, with all of them preferring slightly acidic soils, lakes, or streams.

## MOLECULAR AND ECOPHYSIOLOGICAL VARIABILITY AMONG *ACHNANTHIDIUM* STRAINS

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Diatoms are commonly used as bioindicators for water quality assessments due to their sensitivity to environmental changes. Effective biomonitoring requires identification down to the species level, as different species within a genus may have varying ecological preferences. One of the more problematic genera is *Achnantheidium*, a benthic diatom genus that is common in all types of freshwater habitats comprising several morphologically similar groups of species, such as the *Achnantheidium minutissimum* complex, that may differ in their ecological profiles. It will be important to combine morphological, molecular, and ecophysiological methods to better understand boundaries between species alongside their autecological differences. Stressors like salinity and temperature are considered important environmental factors affecting aquatic biota resulting in direct and indirect effects on photosynthetic organisms, such as diatoms. We conducted ecophysiological experiments on five *Achnantheidium* strains collected in Germany and Spitsbergen to determine the influence of temperature and salinity on their growth and survival. Using light microscopy and scanning electron microscopy, two strains were identified to belong to *Achnantheidium minutissimum* s.l., one as *Achnantheidium jackii* and two as belonging to the *Achnantheidium minutissimum* complex.

All strains were also characterized by sequencing the plastid-encoded *rbcL* gene to ascertain their phylogenetic relationships. The strains from Spitsbergen did not differ morphologically, molecularly, or

ecophysiologically from each other, but they differed in all these aspects from the strains from Germany by having different morphological characteristics, an *rbcL* difference of 37 base pairs, and a higher tolerance regarding salinity and temperature changes. We found two different *rbcL* sequences in the strains from Germany. One strain of *A. minutissimum* had an identical *rbcL* sequence but showed a broader tolerance than the strain identified as *Achnantheidium jackii*. The other strain of *A. minutissimum* differed from the two previously mentioned strains by only two base pairs and showed similar ecological preferences with the strain of *A. jackii*. It can be concluded that the strains examined in Germany possibly consist of three distinct species rather than just two. These results demonstrate the need to combine morphological, molecular, and ecophysiological characterization of widely distributed species complexes such as *A. minutissimum* s.l. More strains are necessary to investigate the different *Achnantheidium* species, their tolerance ranges, and their role as indicator species.

**MOLECULAR PHYLOGENETIC STUDY OF THE GENUS  
*NITZSCHIA* WITH SPECIAL FOCUS ON THE *NITZSCHIA*  
*PALEA* COMPLEX**

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Previous studies have shown that the water quality of many bodies of water is heavily disturbed, especially in urban areas. As a result of these influences, precise monitoring is necessary, and indicator organisms are required for this purpose. *Nitzschia palea* (Kützing) W. Smith is a widespread diatom that occurs in various lotic and lentic freshwater habitats and is known as a cosmopolitan for these. Due to their wide distribution, their different variants are used as indicators of water quality. However, it is also taxonomically problematic, as their different morphologies are not clearly distinguishable. In order to dissect the *Nitzschia palea* complex, individuals were sampled and isolated from different regions, mainly from the Ruhr catchment. The genetic diversity of the strains was analyzed by sequencing the *rbcL* gene. The results are in line with previous results showing that *N. palea* is not a single homogeneous taxon and may need to be subdivided into three or more species. Through utilization of *rbcL* analysis and the resulting distance matrices and phylogenetic trees, it can be concluded that there is no clear barcode gap between the potential species at this stage and further studies are needed to distinguish the different cryptic and pseudocryptic species.

## Student Poster

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**14 UNIQUE *HALAMPHORA* SPECIES, POTENTIALLY NEW, DISCOVERED IN THE TIDAL FLATS OF KOREA****Jeong Yung Jin\***, Jong-Gyu Park, So-Yeon Kim & Byoung-Seok Kim*Department of Oceanography, College of Ocean Science & Technology, Kunsan National University, 558, Daehak-ro, 54150 Gunsan-si, Republic of Korea*

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*Halamphora* is difficult to distinguish from *Amphora* (Kutzing, 1844; Stepanek & Kociolek, 2013, 2014). Research on *Halamphora* has been conducted in Europe and the United States, and important results have been published (Levkov 2009; Stepanek & Kociolek 2013, 2014). However, in Korea, there has been little research on the *Halamphora*. In this study, we studied the genus *Halamphora* living in tidal flats of Korea. At low tide, the surface layer of the mud flats, sand, and stones was collected. The collected samples were isolated and cultured, and their morphology was observed using LM and SEM. The base sequence of *rbcL*, a chloroplast DNA, was analyzed. Data for these base sequences were collected from NCBI, and a phylogenetic tree was constructed using the ML analysis with the GTR+G model and 1000 bootstrap replicates. Fourteen species of *Halamphora* from single cultures were obtained, and both morphological observations and genetic analyses were conducted on them. The observed species showed differences in morphology and genetic characteristics from previously reported species. *Halamphora* sp. 1 differs from *H. arcus* and *H. angustiformis* in areola shape, fascia, and the end of the raphe ledge. *Halamphora* sp. 2–5 differs from *H. nipponensis* and *H. maritima* in stria density, proximal raphe end and raphe ledge. *Halamphora* sp. 6–11, while similar in shape to *H. isumiensis* and *H. nagumoi*, show differences capitata, raphe ledge, and stria density. *Halamphora* sp. 12 show differs from *H. aponina*, *H. scatebra* and *H. tenuis* in the shape of the raphe ledge. *Halamphora* sp. 13 is very similar to *H. holsatica*, but differs in the shape of the raphe ledge and stria density. *Halamphora* sp. 14 shows differences in the shape of stria compared to *H. formina*. This study suggests that the species diversity of the genus *Halamphora* previously reported in Korea is richer than expected. Additionally, if we conduct research on various types of diatoms that have recently been studied overseas, it is expected that more new or unique species will be discovered.

**A POLYPHASIC METHOD FOR THE CHARACTERIZATION OF EPIPHYTIC DIATOMS GROWING ON *GELIDIUM CORNEUM***

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Benthic marine algae play a fundamental role in shaping ecosystem structure and function, with diatoms standing out as the most abundant and diverse component within this group. *Gelidium* spp. frequently hosts epiphytes, especially during the summer months, resulting in epiphytic loads that are 30-40% higher on shallow bottoms. The ecological significance of epiphytes in seaweed-microbial interactions is well-recognized. Nevertheless, diatoms are considered the primary structural elements of epiphyton on seaweeds and seagrasses. Given the absence of standardized methods for obtaining comparable results, this study proposes a methodological protocol for the quantitative and qualitative analysis of epiphytic diatom assemblages growing on *Gelidium corneum* (Hudson) J. V. Lamouroux mats. This protocol can be easily adapted to other macrophyte-microbial host scenarios. Three areas along the Cantabrian Coast (Pais Vasco, Asturias, and Cantabria) were sampled at two different depths (5 and 12 m) and during two seasonal periods (Winter-Spring) in 2022. Additionally, this material was utilized for characterizing and identifying the diatom community found in *G. corneum*. For this purpose, samples were divided and treated according to two types of analysis: morphological and molecular. Morphological analysis involved standard procedures following the UNE-EN 13946 guide, including sample concentration, removal of organic matter, and mounting of samples for microscopic examination. Molecular analysis utilized DNA extraction, PCR amplification of the *rbcL* marker, and subsequent sequencing. Bioinformatic analysis involved processing sequences with the DADA2 package in R, taxonomic assignment using the Diat.barcode v7 reference database, and subsequent discussion

of findings. Overall, this study addresses the lack of standardized methods for studying epiphyte communities on marine macroalgae, particularly diatoms on *Gelidium corneum* (Hudson) J.V.Lamouroux. The proposed protocol allows for comprehensive analysis of epiphytic diatom communities, offering insights into their ecological roles and interactions with host macroalgae. The integration of morphological and molecular techniques enhances our understanding of these communities and facilitates future research on their dynamics and responses to environmental changes.

**BENTHIC DIATOM COMMUNITY IN LAKE BATUR (BALI, INDONESIA) INVESTIGATED BY MORPHOLOGY AND MOLECULAR APPROACHES**

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Lake Batur is the largest lake in Bali, and it was formed as a result of the eruption of Mount Batur. The eruption obliterated the summit of the mountain, creating a caldera within which the lake developed. Lake Batur is situated within an active caldera and is located at an altitude of 1,050 meters above sea level. The lake covers an area of 16.05 km<sup>2</sup> and has a maximum depth of approximately 60–70 meters. Being a closed water system with no outward flow, Lake Batur exhibits a distinctive ecological profile. The lake typically exhibits a pH value ranging from 8.81 to 9.50 and is significantly influenced by fish farming, which now plays a crucial role in the lake's ecosystem. The convergence of these factors highlights the environmental and ecological importance of Lake Batur. The primary objective of the study is to characterize the benthic diatom community of the lake and investigate its morphological and molecular features. Morphological approaches involve microscopic examination and identification of diatom species, while molecular approaches include genetic analyses and DNA sequencing techniques. These two approaches are combined in a preliminary investigation whose ultimate aim is to comprehensively understand the diversity and interspecies relationships within the diatom community of Lake Batur.

## THE CROATIAN NATIONAL DIATOM CULTURE COLLECTION: STRAINS FROM BIOTA AND TURTLEBIOME PROJECTS

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The Croatian National Diatom Collection (HRNDC) is primarily a collection of permanent microscopic slides of diatom specimens collected on the territory of Croatia, and as such is the first institutional collection of diatoms in Croatia. However, our algology group has also extensively cultured diatoms from marine plankton and benthic/epizoic habitats from the Adriatic Sea during the last decade. We have succeeded in culturing diatom taxa included in the genera such as *Achnanthes*, *Diploneis*, *Entomoneis*, *Navicula*, *Nitzschia*, *Psammodyction*, or *Tabularia*. The information obtained on these strains has also been made available to the scientific community and interested fellow researchers through the HRNDC. We have successfully cultured 181 diatom strains during the Turtle BIOME and 61 (of which 39 are sequenced) diatom strains during the BIOTA projects. These strains have been taxonomically curated and assembled in a library of reference DNA sequence data and morphological information. SEM and LM images are also available upon request for 161 and 145 of these strains respectively. As part of these two projects 416 original verified sequences were assembled and published in the GenBank database: 173 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*), 147 photosystem II CP43 chlorophyll

apoprotein (*psbC*); and 96 small subunit ribosomal RNA (SSU gene sequences). To place these strains in a wider context, Maximum Likelihood (ML) and Bayesian Inference (BI) phylogenetic analyses made on *rbcL* gene were constructed with other available diatom sequences obtained from University of Texas, Austin (UTEX) culture collection and reference literature sequences available from GenBank using IQTree2 v2.2.6 and MrBayes v3.2.7a. The alignment was created using Muscle v3.8.425, and included a total of 806 sequences, 1553 sites of which 686 parsimony informative, 115 singleton sites and 752 constant sites. The resulting consensus tree verified the sequence identities and placements within correct nodes, therefore clarifying morphological curation and providing perspective for future investigations. The next step will be integration of *rbcL* sequences in Diat.barcode database with our vouchered specimens. This will provide the diatom scientific community with correct taxonomic annotations in future systematic, phylogenetic and metabarcoding surveys of marine diatoms.

## Student Poster

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**MOLECULAR AND MORPHOLOGICAL CHARACTERISTICS OF *PSEUDO-NITZSCHIA* SPECIES IN THE EAST ADRIATIC SEA**

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*Pseudo-nitzschia* species are diatoms known to produce domoic acid, a neurotoxin responsible for Amnesic Shellfish Poisoning (ASP). It is a diatom genus known for its cryptic and pseudo-cryptic diversity. Some species within a genus show differences in morphological characteristics, while others require molecular identification. In our study, diversity was observed by both morphological and molecular analysis of established cell cultures isolated from the studied area. Sampling was carried out monthly at four shellfish farming sites on the eastern Adriatic coast (Kaštela Bay, Mali Ston Bay, Šibenik and Velebit Channel). The samples were collected using a plankton net with a mesh size of 20 µm. *Pseudo-nitzschia* cell cultures were established by isolating single cells or cell chains from live plankton field samples net. The isolated species were analysed morphologically by Field Emission Scanning Electron Microscopy and molecularly by analysing sequences based on the internal transcribed spacer (ITS), large subunit region (LSU), and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (*rbcL*) of ribosomal DNA. By molecular analyses and morphological features, 10 species have been identified: *P. allochroa*, *P. calliantha*, *P. delicatissima*, *P. fraudulenta*, *P. galaxiae*, *P. hasleana*, *P. mannii*, *P. multistriata*, *P. pseudodelicatissima* and *P. subfraudulenta*. Approximately 350 cultures were established during the study period, of which 173 were sequenced in both the ITS and LSU regions, while 97 were additionally sequenced on the *rbcL* region. The ITS region contained greater intra-specific variation among *Pseudo-nitzschia* species, while the coding LSU region was beneficial for understanding the phylogenetic relationships among different *Pseudo-nitzschia* species. As the *rbcL* gene is less variable than the ITS region, it provides valuable insights into species

identification and phylogenetics. The generated Sanger sequences will be used in further research to prepare libraries for metabarcoding of the phytoplankton community. Phylogenetic analyses were performed on all markers to study evolutionary relationships. The study has led to the discovery of species that were previously not recorded in the Central and South Eastern Adriatic Sea, such as *P. hasleana*, which is genetically closest to *P. calliantha* and *P. manni*, but whose genetic distances differ significantly due to interspecific diversity. In addition, *P. allochroa* has been confirmed as a species within the *P. delicatissima* complex. In general, the majority of analysed species were found in all studied areas. Species isolated from specific areas were *P. hasleana* and *P. fraudulenta* established from Kaštela Bay, and *P. multistriata* from Šibenik Bay. By combining molecular techniques targeting ITS, LSU, and *rbcL* gene regions, along with electron microscopy, this study allows more detailed understanding of genetic diversity and morphological features of *Pseudonitzschia* species in the Central and South Eastern Adriatic Sea.

**PSEUDO-NITZSCHIA GENUS DIVERSITY IN THE CENTRAL AND SOUTHERN ADRIATIC SEA**

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The marine diatom genus *Pseudo-nitzschia* was studied for two years (2022-2023) in coastal areas of the central and southern Adriatic Sea. Since certain species of the genus *Pseudo-nitzschia* are known to produce the neurotoxin domoic acid, which causes amnesic shellfish poisoning (ASP), our study was conducted in four major aquaculture areas (Veľebit Channel, Šibenik Bay, Kaštela and Mali Ston Bay). Samples were collected monthly and analysed by light microscopy (LM) and scanning electron microscopy (FE-SEM).

Light microscopy analyses revealed that *Pseudo-nitzschia* species were present in the phytoplankton community in all areas throughout the study period. *Pseudo-nitzschia* abundances mainly were below  $1 \times 10^5$  cells L<sup>-1</sup>, except for two bloom events in February and March 2022, when the abundance of *Pseudo-nitzschia* in Mali-Ston and Šibenik Bay was  $3.6$  and  $2.5 \times 10^6$  cells L<sup>-1</sup>, respectively. On average, *Pseudo-nitzschia* accounted for 20.1% to 32.4% of the total diatom abundance, increasing its contribution to the southern areas.

The seasonal distribution showed that *Pseudo-nitzschia* was more abundant in autumn and winter and least abundant in spring in all areas except Kaštela Bay, where higher abundances were recorded in winter/spring.

Twelve species of *Pseudo-nitzschia* were identified by morphological analysis using FE-SEM. The most frequent in all areas were *P. delicatissima* complex and *P. cf. pseudodelicatissima*, followed by *P. calliantha* and *P. mannii*. Although most of the identified species were found in all studied areas, *P. brasiliiana* was found only in Šibenik Bay and Kaštela Bay, as well as *P. multistriata* in Šibenik Bay and Mali Ston Bay.

Different spatial and seasonal patterns were observed, as the species *P. linea* and *P. calliantha* were more common in the Velebit Channel and *P. galaxiae* in Kaštela and Mali Ston Bay. Regarding seasonal occurrence, *P. linea*, *P. subfraudulenta* and *P. multistriata* were more pronounced in autumn/winter, while *P. cf. pseudodelicatissima* prevailed in spring/summer. The observed blooms were not monospecific. In Mali Ston Bay, species *P. calliantha*, *P. subfraudulenta* and *P. delicatissima* complex were equally represented ( $\approx 30\%$ ), while in Šibenik Bay, along with the mentioned species *P. fraudulenta*, *P. linea*, *P. manni* and *P. cf. pseudodelicatissima* were found in addition to the aforementioned species.

**TOWARDS A MONOGRAPH OF THE GENUS *ODONTELLA*  
C.AGARDH: PRELIMINARY RESULTS**

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The genus *Odontella* C.Agardh was created to include just one species of *Diatoma* Bory: *Diatoma auritum* Lyngbye (1819), as *Odontella aurita* (Lyngbye) C.Agardh (1832), a species commonly found in European coastal waters. De Brébisson considered Lyngbye's *Diatoma auritum* to be a species of *Biddulphia* S.F.Gray (1821), *Biddulphia aurita* (Lyngbye) Brébisson (1838), perhaps initiating the protracted confusion between *Biddulphia* and *Odontella* – and of *Isthmia* C.Agardh (1832) as well. *Odontella*, its relationships within *Odontellaceae* P.A.Sims, D.M.Williams & Ashworth, and some details of the type specimens of *Odontella aurita* (= *Diatoma auritum*), were discussed by Sims et al. (2018) – but they did not address the diversity within the genus. This study is intended as a preliminary account of that diversity, and will describe some new species, seven new combinations and re-describe eight of the named species. A (geologically) young genus (Miocene), it is difficult to convincingly confirm if any of the currently included fossil species are actually extinct. Nevertheless, our account will be divided into two sections each with two parts: I (a) those species previously described as Recent, (b) along with the newly described Recent species and new combinations; and II (c) previously described fossil species, (d) along with a newly described fossil species and the new combinations.

## ARCTIC AND CARIBBEAN PHYTOPLANKTON DISTRIBUTION

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Oceanographic cruises have been conducted in tropical and polar regions to study the impact of Sea-Ice melting and Marine Heat Waves (MHW) on the production and the distribution of phytoplankton. Biannual cruise Chinare 2018 (China Arctic Expedition) and seasonal cruises in the Caribbean Sea (Caracalhis 2015 & Caraibe 2019), provided a large number of phytoplankton samples. The Chinare campaign started in 2008 and document sea-surface conditions and sea-ice cover from the Bering Strait to the North Pole (Coupel et al. 2015). In the Caribbean Area, sampling was made in June 2019 (C1) and November 2019 (C2) to study seasonal phytoplankton distribution.

Samples were collected at surface (0–5m) and at SCM (surface chlorophyll maximum) using polycarbonate and/or nitrocellulose filters. Physical and chemicals proxy were measured. A portion of filter was cut, mounted on a stub and coated for SEM study. Taxonomy is based on morphology approach using a Hitachi FlexSEM1000 II electronic microscope. We present here preliminary results from twenty phytoplankton samples from the Arctic and the Caribbean, categorized into Coccolithophores, Diatoms, Dictyochales and Dinoflagellates.

In the Chinare 2018 expedition, only Coccolithophores *Coccolithus pelagicus* and *Emiliana huxleyi* along with rare *Michaelsarsia* sp. were observed. Centric Diatoms are dominants with *Chaetoceros* (two

species), *Thalassiosira* (diverse with in particular, *T. nordenskiöldii*, *T. eccentrica*, *T. tenera*, *T. angulata* and *Thalassiosira* cf. *tealata*), *Fragilariopsis*, *Rhizosolenia*, *Asteromphalus*, *Actinocyclus*. Rare pennates as *Luticola*, *Navicula* and *Nitzschia* are observed in the Bering Strait with the Dinoflagellate *Gonyaulax catenella*. The occurrence of Dictyochales are represented only by *Dictyoca* cf. *speculum*.

In Caraibes 2019, Coccoliths are abundant and diverse including genera such as *Umbellosphaera*, *Rhabdosphaera*, *Syracosphaera*, *Helicosphaera*, *Calciosolenia*, *Discosphaera*, *Michaelsarsia*, *Emiliana*.

Pelagic diatoms are mainly represented by genus *Chaetoceros*: *C. muelleri*, *C. atlanticus*, *C. decipiens*, *C. didymus*, occurring with *Asteromphalus*, *Bacteriastrum*, *Thalassionema*, *Skeletonema*, *Ardissonia*, *Cyclotella* and *Actinocyclus*.

Notably, *Chaetoceros muelleri*, *Cyclotella* cf. *striata* and *Thalassionema nitzschioides* were the dominant assemblage in Atlantic areas, particularly in the Angola Basin.

In Caribbean coastal sites, pennate diatoms prevail as *Luticola*, *Halamphora*, *Cocconeis*, *Diploneis* and *Nitzschia*. *Pseudo-nitzschia* is observed in the warmest coastal part of the Caribbean Sea together with dinoflagellate.

What is striking at this stage of observations is that the genus *Chaetoceros* is abundant in both regions even under the ice in the Pole, although further details in species taxonomy are pending. These preliminary observations are invaluable for detecting the impact of climate change in two of the most affected regions of the ocean on these primary producers.

## DIATOMS: TINY BUT NUMEROUS INHABITANTS OF CAVES

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Caves are considered to be unique and extreme ecosystems in which specific ecological parameters allow the survival of some characteristic and/or extremophilic microorganisms. Aquatic surface habitats have been intensively studied, in contrast to freshwater cave habitats, which are much less studied with respect to diatoms. Data on diatoms from lampenflora and cave entrance zone biofilms have increased considerably in recent years compared to those from cave waters and aphotic cave zones. The diatoms from cave biofilms in Serbia have been studied several times, but knowledge about this group is still limited. As for the diatoms of water bodies in caves, these studies are deficient in Europe, and in Serbia this is the second insight into the diatom community in a cave stream and the first in relation to this stream (Ponorac stream in Ravništarka Cave). The aim of this study is to investigate diatom community composition of the cave stream and lampenflora in Ravništarka Cave (Eastern Serbia). The sampling was carried out in May 2023. The diatoms were collected by scraping stones along the Ponorac stream (6 sampling sites) and biofilms from the inner walls of the cave near artificial lights (two sampling sites). Some of the physical and chemical parameters were measured directly on the field and some in the laboratory. Regarding lampenflora samples *Humidophila contenta* was observed as a dominant species. In addition, two more species of this genus (*H. paracontenta* and *H. perpusilla*) as well as *Orthoseira roeseana* were noticed, but with low abundance. A total of 95 diatom taxa from 39 genera were identified analyzing diatom community diversity of Ponorac stream. The highest number of taxa (60) was found at the second site, which is characterized by the highest flow velocity of the stream water and a pronounced artificial

light, while the lowest number (19) was found at the third site, where the water is almost standing and there is no any type of light. Genera with the highest number of present taxa were *Navicula* (12), *Nitzschia* (12) and *Stauroneis* (8), while 30 counted one or two species. The same *Humidophila* species as in the lampenflora were also found in the stream. *H. contenta* was detected at four sampling sites along the stream, with the highest relative abundance of 60.88% at the first site, where it was the dominant taxon. Its relative abundance at the other sites ranged from 0.48 to 3.18%. Individuals of *H. paracontenta* and *H. perpusilla* were recorded at two sites. This study is the starting point for future research on microphototrophs from different habitats in the aphotic cave zones which are poorly investigated.

## UNVEILING THE DIATOM FLORA IN THE STARA PLANINA MOUNTAIN WATERFALLS

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Waterfalls create a range of aquatic and wetland microhabitats supporting diverse species and enhancing overall biodiversity. Diatoms are one of the dominant groups within these ecosystems. However, their presence and ecological preferences are unexplored in Serbian waterfalls. This study aims to fill this gap by exploring the floristic composition and ecology of epilithic and epiphytic diatoms in selected waterfalls of Stara Planina Mountain Nature Park. Stara Planina Mountain is located in the eastern part of the Balkan Peninsula and spans from the Black Sea in the east to the Vrška Čuka peak in the west. During the summer (2019 and 2021) and autumn (2019), a total of 17 epilithic and epiphytic samples from five waterfalls were collected (Bigar, Tupavica, Bukovački Do, Donji Piljski and Gornji Piljski waterfalls). Epilithic samples were collected by scraping the stone surface with a toothbrush, while epiphytic samples were collected by squeezing different mosses. Microscopic analysis was done using a light microscope Carl Zeiss AxioImager.M1 with DIC optics, AxioCam MRc5, and AxioVision4.9 software. Selected slides were observed with Apreo S LoVac (Thermo Fisher Scientific) SEM at the University of Duisburg-Essen. Shannon's diversity index, Pielou's equity index, and ecological groups were calculated using the OMNIDIA software. A total of 189 diatom taxa distributed among 57 genera were identified. The genera with the highest number of taxa were *Gomphonema* (15), *Eunotia* (15), *Navicula* (12) and *Nitzschia* (11). Through this study, 23 new species for the flora of Serbia, belonging to 16 genera were observed. *Hannaea yalaensis*, a species that has not been recorded in Europe so far was noted in Donji Piljski and Gornji Piljski waterfalls. The Bigar waterfall displayed the highest values of Shannon's

diversity index (H), whereas the lowest values were observed at the Tupavica waterfall. The equity index (E) values correlate with diversity index values. In the investigated waterfalls different diatom taxa prevail. *Achnantheidium minutissimum* and *A. jackii* were dominant in the Bigar waterfall, *Fragilaria tridentina* and *F. nevadensis* in Tupavica waterfall, while *Amphora pediculus* dominated in Bukovački Do waterfall. *Cocconeis* species were dominated in Donji Piljski and Gornji Piljski waterfalls, along with *Humidophila perpusilla* and *Odontidium hyemale*, respectively. According to recorded diatom taxa, the water of the investigated waterfalls in Stara Planina Mountain is oxygen-rich, neutral to alkaline, and oligo to  $\beta$ -mesosaprobic.

## DIATOM DIVERSITY OF FIVE THERMAL SPRINGS IN SERBIA

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Due to their high water temperature and special water chemistry, thermal springs offer unique ecological conditions for microbial communities. They are considered to be very interesting and important habitats for the study of algal flora, including diatom biodiversity. However, their occurrence in Serbian thermal springs is scarcely investigated. The aim of this study was to assess the diversity of the diatom community in five thermal springs in central Serbia (Vrujci spa, Bukovička spa, Omoljica, Ovčanska spa and Poljane). Samples were collected from natural thermal springs except from Bukovička spa, where they were taken from the tap. In Vrujci spa, samples were collected both from the tap and from the natural spring. During the autumn and winter in 2023, a total of 11 biofilm samples were collected with a sterile scalpel. The samples were treated with HCl and KMnO<sub>4</sub> in the laboratory, afterwards permanent diatom slides were made. The Carl Zeiss AxioImager.M1 light microscope with DIC optics, AxioCam MRc5 and AxioVision4.9 software were used for microscopic analysis. During survey, in total 82 diatom taxa belonging to 33 genera were identified. The most taxon-rich genera were *Nitzschia* and *Navicula* with 13 and 10 taxa, respectively. Our preliminary results show that the samples from the Vrujci spa have the highest diatom diversity (62 taxa) and from Bukovička the lowest (5 taxa). In the Vrujci spa, 40 taxa were identified in the natural thermal spring, while 22 were recorded at the tap. The highest temperature (25.1°C) was measured at this site compared to the other thermal springs. The dominant taxa were *Staurosira venter* and *Achnantheidium straubianum*, which distinguishes this natural thermal spring from the others. At the tap in Vrujci, the dominance of *Navicula veneta*, *Nitzschia*

*palea* and *Achnanthydium microcephalum* showed that the communities at natural and artificial sites differ greatly. In Bukovička spa, the most abundant taxa included *N. palea* and *N. veneta*. In the Omoljica thermal spring, 19 taxa were identified with dominance of *N. palea*, followed by *Gomphonema parvulum* and *N. veneta* with slightly lower abundances. In Poljane, *N. palea* and *A. microcephalum* were dominant from 11 recorded taxa. In Ovčanska spa, *Nitzschia thermaloides* was found to be predominant. In addition, the other 18 taxa found at this thermal spring occur with very low abundances. Additional taxonomic insights are required to more precisely define the diatom communities and the ecology of the taxa inhabiting thermal springs. Besides, occurrence of rare species can be expected in this types of extreme habitats.

## FIRST INSIGHTS INTO THE DIATOM FLORA OF THE WORLD'S GLACIAL-FED STREAMS

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Climate change is having a significant impact on global cryospheric ecosystems. This is particularly true for mountain glaciers, and up to 90% could disappear by the end of the century. Despite this urgency, the ecology of the streams draining these glaciers (glacier-fed streams; GFSs) remains understudied, although they serve as crucial connectors between mountain glaciers and downstream ecosystems. In general, GFSs are considered harsh environments, characterized by low temperatures, high turbidity, scant organic matter concentrations, and distinct temporal fluctuations which include drying/freezing in winter and turbulent discharge in summer. Due to their simplified food web structure and similar environmental conditions worldwide, GFSs are ideal model systems for studying controls on community assembly and biogeography within aquatic ecosystems. Interestingly, diatoms are often abundant primary producers within GFSs, and contribute energy to these otherwise energy-deficient ecosystems. However, to date, their diversity and ecological roles remain largely unexplored. Herein, and as part of the Vanishing Glaciers Project, we present our initial findings on the diversity of diatoms found in GFSs across major mountain regions worldwide. Our study examines GFS samples taken from the European Alps (Switzerland, Austria, and Italy), Himalayas (Nepal), Andes (Ecuador and Chile), Rwenzori Mountains (Uganda), North America (Greenland and Alaska), and the Southern Alps (New

Zealand). From each GFS, sediments were sampled from the benthos using sterilized graded sieves. In the laboratory, sediment samples were cleaned of organic matter, and morphological observations made using light and scanning electron microscopy. Across these mountain ranges, we found that the most prevalent and abundant diatom genera belonged to *Achnantheidium*, *Psammothidium*, *Planothidium* and *Gomphonema*. These were followed by genera such as *Neidium*, *Reimeria*, *Odontidium*, *Nitzschia*, *Cymbopleura*, *Encyonema*, *Eunotia*, *Pinnularia* and *Hannaea*. Our observations suggest common patterns among diatom communities in glacial regions worldwide, which we argue are conducive to their survival under these challenging conditions. Specifically, species from the aforementioned genera are exclusively pennate, typically exhibit smaller cell dimensions, and are often monoraphid. Furthermore, a substantial portion of the genera are aerophilic (having the ability to endure desiccation), and many form stalked colonies. The high elevation and geographical isolation of GFSs almost hinders dispersal, which may also explain their typically small cell sizes. Geographical isolation may also foster speciation and endemic taxa, which aligns with our difficulty in identifying many of the observed valves to species, and suggests they may be new to science. Our future research will further explore patterns and controls on diatom diversity in the world's major mountain ranges by the sequencing of 18S and *rbcL* amplicons from the same material. Considering the significant alteration and potential disappearance of numerous GFSs worldwide within this century, this work may represent one of the final opportunities to investigate the diversity of these unique habitats before they vanish permanently. In this context, we hope our observations will shed some light on the previously understudied diversity of GFS diatom communities before they are gone forever.

## **THE EPIPHYTIC DIATOM BIODIVERSITY IN THE SOUTHERN PARTS OF THE EUPHRATES-TIGRIS RIVERS, IRAQ**

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The Euphrates-Tigris catchment area is one of the major drainage basins in southwestern Asia. The excessive use of water in Iraq and the construction of dams at the source have pushed this region to the brink of environmental degradation. The significant decrease in water level coupled with climate changes has led to salinization and affected the natural distribution of diatoms in the region.

The aim of the presented work is to study the composition of epiphytic diatom species and the impact of specific environmental variables on their occurrence in the Tigris and Euphrates Rivers during August 2019 (summer or dry season) and February 2020 (winter or rainy season). 47 samples were taken at six epiphytic sampling sites from the rivers Tigris and Euphrates in Northern Basra, Southern Iraq. A total of 283 taxa belonging to 59 genera were identified, with 46 species newly recorded for the region. The taxonomic composition observed was dominated by taxa of the genera *Nitzschia*, *Navicula*, *Gomphonema* and *Tryblionella*. Non-metric multidimensional scaling analyses of occurring taxa (based on morphology) showed a clear separation of diatom communities according to the seasons. A species new to science was identified in the epiphytic communities of the Euphrates River whose valve morphology corresponds to the *Prestauroneis* genus concept.

The findings of this study provide insights into the diversity, distribution, and ecological preferences of diatom taxa and communities in the Euphrates-Tigris River system. They contribute to the baseline knowledge of diatom biodiversity and ecological dynamics. The findings also emphasize the need for further monitoring efforts for the studied waterbodies, since the good ecological condition of both river ecosystems is of crucial importance for all living organisms in the region.

## DIATOM ALGAE ON THE PLASTIC SURFACES NEAR ZMIINYI ISLAND (THE BLACK SEA, UKRAINE)

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In recent years there has been increasing interest in «plastisphere» studies and microalgae communities on plastic surfaces. Diatoms and cyanobacteria are the primary colonisers of the marine litter and main components of biofilms on plastic.

The diatom that directly attach to the plastic surfaces can affect its decomposition and structural changes influencing the degradation of marine litter to smaller fragments ending up in microplastics. In addition, lightweight plastics are capable to float and migrate throughout aquatic environments, becoming one more path of potential harmful and invasive species penetration. The studies of biofouling on plastic substrates in the Black Sea started in 2018 (Sapozhnikov et al., 2020; Snigirova et al., 2019; 2022). The present study is focused on the species composition of diatoms on the polymer surface (polyethylene-terephthalate) near the Zmiinyi Island (N 45.266894; E 30.203224) the unique rocky island located in the marine protected area in Ukrainian marine waters. The plastic bottles, attached by the rope to the bottom, were collected in November 2018 on the depth of 9 m in the water column. The bottles were fully covered with marine biofouling (1.2-4.5 kg per item) representing the assemblage of *Mitylus galloprovincialis*. The state of biofouling and age of molluscs indicated that the bottles spent over 2 years in the marine environment. The biofilm diversity was analysed using temporary ethanol-glycerol slides and the permanent slides with Naphrax medium under the light microscopy; air-dried samples were proceeded under SEM.

In total over 46 species of diatoms were identified. Most of the species were common for the region. The genus spectrum includes 24 genera,

among which the most species-rich representatives are: *Amphora* Ehrenb. (5 species), *Navicula* Ehrenb. (5), *Nitzschia* Hassall (5), *Cocconeis* Ehrenb. (4), *Licmophora* C.Agardh (3), *Diploneis* Ehrenb. ex Cleve (3), *Halamphora* (Cleve) Mereschk. (2), *Tabularia* (Kützing) D.M.Williams ex Round (2). Other 16 genera were represented by a single species. The habitat preferences included plankto-benthic, benthic, and epilithic-epiphytic-benthic microalgae. The species diversity observed in the biofilm formed on plastic surfaces consisted of cosmopolitan species (65%) and boreal species (35%). Under the macrofauna fouling we observed the cover of the surface by colonies of *Navicula* sp., *Berkeleya* sp., separate fragments contained scattering of *Cyclotella baltica*, *Cocconeis neothumensis* var. *marina* were revealed. Diatoms were distributed within the matrix of cyanobacteria and other diatom colonies. The main differences in the algae biofilms on the seafloor litter compared to other locations from the Northwestern Black Sea lied in absence of the dense layer of the *Cocconeis* species on the plastic from Zmiinyi area. We can explain that with the differences of the eutrophic conditions between these areas, which affect the biofilm composition (Minicheva et al., 2021). The signs of the decomposition of the plastic surface were noticed.

**BIODIVERSITY AND ENVIRONMENTAL FACTORS  
STRUCTURING DIATOM COMMUNITIES IN TWO RICE  
PADDY FIELDS IN NW-ITALY****Elisa Falasco\***, Gianluca Vacca, Tiziano Bo & Francesca Bona*Department of Life Sciences and Systems Biology, Univeristy of Turin, via  
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Rice paddy fields are very dynamic and unique man-made freshwater ecosystems where anthropogenic activities (such as agrochemical use and water level manipulations) combined with the extreme water temperatures reached during the summer, create a harsh environment for biota living in it. Here we present the results of a research conducted in two rice paddy fields and their associate supply canal, in the North-Western Italy. Samplings were performed every two weeks during the rice growth cycle (June-September 2023), with the aim to 1) follow the colonization on artificial substrates of the three main autotrophic groups composing the periphyton (namely diatoms, cyanobacteria and green algae) by using a portable fluorometric probe 2) analyse the taxonomic and functional composition of benthic diatom communities inhabiting supply canal and paddy rice fields at different stages of the plant growth. Results showed that diatom chlorophyll *a* was the dominant component in the periphyton of the supply canal, whereas cyanobacteria and diatoms co-dominated biofilms in rice fields, especially during the early stages of colonization. Diatom communities of the supply canal were taxonomically and functionally significantly different from those colonizing the two rice fields. In the canal, we mainly observed low profile and motile species, adapted to medium conductivity and classified as meso- or  $\beta$ -meso-saprobous. Communities in rice fields were dominated by motile taxa mainly classified as  $\beta$ -meso or  $\alpha$ -meso-saprobous. Periphyton has been recently recognized as a promising bio-based solution for reducing heavy metals concentration in water and soil in rice paddy fields, being also able to lower the transfer of Cd to rice grains, an issue of great importance for food safety and human health. At the same time, diatoms are known to be able to reduce nutrients in wastewater, thereby mitigating the negative effects of eutrophication. Our results shed light on colonization dynamics

of periphyton communities in rice paddy fields, with a special focus on diatom taxa able to withstand the harsh conditions that characterize this environment.

## RESULTS FROM THE 3<sup>RD</sup> ITALIAN INTERCALIBRATION EXERCISE ON BENTHIC DIATOMS

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Starting from 2007, intercalibration exercises on benthic diatom taxonomy and related indices has been spreading throughout Europe. Indeed, in the framework of the WFD application and the use of diatoms as indicators for the ecological status assessment of rivers, interlaboratory harmonization has been fully recognized as mandatory to assure the reliability and comparability of results. In the present work we show the results of the third Italian intercalibration exercise, carried out in 2023-2024, involving 39 diatomists from different Italian laboratories and 2 referees. Two sampling sites were chosen, both classified as belonging to the Central (lowland) river type and having their origin in lakes. The two sampling localities were chosen to represent different ecological status, respectively good and moderate. The Ticino sampling site is located just downstream the Lago Maggiore (Lombardy, Northern Italy), the second largest lake in Italy; in the chosen stretch, riverbed is 50-80m large

and the main human pressure is represented by wastewater treatment plants. The Chiese sampling site is located downstream the Idro lake (Lombardy, Northern Italy) and is affected by several human pressures, due to the presence of residential and industrial areas, as well as water captations for agricultural and hydroelectric purposes. The results of the participants (respectively taxonomical composition and diatom index calculated using the Italian ICMi) were evaluated based on the Bray-Curtis similarity index (60% threshold) and Z-scores.

Important differences could be observed between the different participants. Most of these differences arose from taxonomic misidentifications, mainly concerning the genera *Achnantheidium* (in particular the *A. minutissimum* complex and *A. delmontii*) and *Fragilaria* (in particular the *F. rinoi* and *F. vaucheriae* complex). The overlooking of small taxa such as *Fistulifera saprophila* and *Mayamaea permitis* also led to a wrong status classification, resulting in an overestimation of the water quality in many inventories. Even though long experience in diatom identification or the number of samples counted per year are important, these do not guarantee the similarity of results between participants and auditors. In this context, the main objectives of intercalibration exercises, training courses, or workshops—problem sharing and knowledge transfer—are the best tools for reducing differences in results.

## DIATOM DNA METABARCODING AND DIGITAL MICROSCOPY METHODS FOR BIOASSESSMENT OF LARGE LOWLAND RIVERS IN SERBIA

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Anthropogenic influences, such as industry, thermal power plants, untreated communal water, and agriculture have led to the disappearance of natural reference conditions for large lowland rivers which makes it difficult to standardize tools for routine biomonitoring.

The present study provides detailed information about benthic diatoms from the Sava and Tisa rivers analyzed using digital microscopy and two gene markers (*rbcL* and 18S). Samples were collected in June 2022 along the Serbian part of the Sava and Tisa rivers. Using the BIIGLE 2.0 image annotation system, a total of 204 and 193 diatom taxa were identified in the Sava and Tisa rivers, belonging to 66 and 61 genera, respectively. Molecular analyses showed a higher species richness using the *rbcL* marker (265 Sava River, 87 Tisa River) than 18S (87 Sava River, 68 Tisa River). *Navicula* and *Nitzschia* were the most dominant genera in the Sava River, while mostly centric diatoms (*Discostella pseudostelligera* and *Stephanodiscus hantzschii*) dominated in Tisa. After summarizing the diatom community in both rivers, both markers were tested as tools to assess water quality by calculating diatom indices in two ways: using the relative abundance of taxa and the presence/absence of taxa. In both rivers, the values of diatom indices based on molecular data showed one or two classes of lower ecological status compared to the data obtained by morphological analysis. However, the mean values of diatom indices based on molecular presence/absence data were higher than the indices that considered the relative abundance of taxa. The data obtained with *rbcL* were more reliable than with 18S. The dominant taxa were

mostly identified at the species level with *rbcL*, while with 18S the most dominant taxa were from the genus *Surirella*, whose presence was only detected by microscopic analyses. This comprehensive study involves, for the first time, the use of molecular analysis for the bioassessment of large lowland rivers in Serbia and proposes the use of the *rbcL* marker using the presence/absence of taxa for routine monitoring.

**ASSESSMENT OF THE ECOSYSTEM RECOVERY ON KAMENICKA RIVER (R. NORTH MACEDONIA): COMBINED APPROACH USING MACROINVERTEBRATE AND ALGAE COMMUNITY STRUCTURES**

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Algae and macroinvertebrates are quite sensitive to the changes in the aquatic environment, and therefore they are often utilized in the determination of the ecological response of different waterbodies under pressure. Here we present a case study conducted on Kamenicka River during the period from September 2020 to May 2022. After the discharge of waste mine material from a pulp leakage of “SASA” mine into Kamenicka River, a detailed study of the algae and macroinvertebrate structure was conducted. The surveys included analyses of the spring-autumn dynamics in their structure for two years after the incident, aiming to assess the remediation measures for cleaning up the pulp tailings from the watercourse. Algae and macroinvertebrate samples were collected from 12 sampling sites on Kamenicka River. During the first sampling (after the pulp leakage), we have detected extremely low algae and macroinvertebrate diversity in the river, and at some sampling points the algae and macroinvertebrate communities were completely “washed out” implying that the force of the leakage itself was lethal. The follow-up analyses after the remediation measures have disclosed clear and unequivocal gradual, step-by-step successive recolonization, remediation and improvement of the communities composition and abundance, reflecting a gradual return of the natural communities and conditions in Kamenicka River, which had probably existed before the dangerous tailings leakage event. Only one year after the hazardous event, Kamenicka River was already recolonized by many cyanobacteria,

algae and macroinvertebrate taxa. This study demonstrates the stages of recovery of the ecosystem followed through the algae and macroinvertebrate diversity, highlighting their importance as an “early warning system” for the ecosystem condition.

## **DIATOM FLORA OF ACID MINE DRAINAGE POLLUTED WATERS**

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Acid Mine Drainage (AMD) affects lotic ecosystems all over the world, being also a prevalent issue concerning water quality in the Roşia Montană mining area (Romania). Diatoms are good indicators of the features of the water due to its ubiquity and sensitivity to environmental variables. For this reason many diatom-based indices are being used. In this context, the main objective of this work has been the study of the diatom communities spatial and temporal pattern variation in the polluted catchment basin of the Abrud River located near the Roşia Montană mine area. Additionally, this study investigates the occurrence of teratological diatoms in AMD affected waters that could shedding light on environmental stressors and potential ecological implications.

The results showed the identification of 274 diatom taxa in the Abrud River catchment area, 35 of them recorded for the first time in Romanian waters. The spatial and temporal pattern variations of species richness in the study area highlight the effects of water pollution on diatom communities and the differences between the main stream of the Abrud River, with species richness ranging between 38 and 102 taxa, and the tributaries with some very species-poor sampling points where almost no taxa were identified. Only in the clean waters upstream of Roşia Valley tributary, a very rich diatom community was found.

On the other hand, the results from the analysis of the teratologic diatoms growing on AMD-polluted waters have shown that the presence of abnormal individuals can be attributed to the fact that the diatom

communities were affected by this kind of pollution released from mining works and waste rock deposits. Intermediate perturbations are responsible of the appearance of large proportions of abnormal individuals, with a variable typology of malformations, reaching almost 58% in the case *Fragilaria rumpens* (Kützing) Carlson. Also, a new type of teratology regarding the species *Achnanidium minutissimum* (Kützing) Czarnecki and *Achnantheidium macrocephalum* (Hustedt) Round & Bukhtiyarova, affecting the shape of the frustule cingulum, has been reported.

## **FLOODPLAINS AS KEY SITES FOR SPECIES-RICH DIATOM COMMUNITIES**

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Floodplains are areas of aquatic-terrestrial interfaces adjacent to rivers and streams, which occupy wide surfaces and constitute valley bottoms. As these plains develop, the river channel's sideward migration produces dynamic, highly connected systems and oxbows, oxbow lakes, sloughs, natural levees, and backswamp deposits disconnected from the main channel. These ecosystems are disturbed naturally through periodic hydrological connections that respond via complex and variable trajectories, creating dynamic environments with pronounced spatial heterogeneity. Floodplains are usually considered 'hotspots' for biodiversity, which is possible due to the shifting habitat mosaic with ideal conditions supporting species-rich ecosystems.

The worldwide distribution of diatoms suggests that they occupy diverse aquatic habitats with a wide range of ecological niches. Their local diversification can explain this to varying environmental conditions that have made diatoms a highly successful group. The work aims to investigate the diversity of the diatom algae of the Danube floodplains near Vienna. Understanding species distribution patterns and species assembly processes, related to environmental niches, dispersal abilities and the drivers, could explain diatom ecological preferences. Also, evaluating diatom diversity across space and time is essential for understanding species speciation and distribution.

Qualitative samples were collected from different habitats of the floodplains along the Danube River on two sampling occasions in the years 2022 and 2023. For this, 19 sites with different hydrological connectivity with the Danube River, which varied from 0 to 365 days per year were chosen. Algae were collected from different substratum types: macrophytes, stones, and sediments. Further preceding in the lab allowed us to receive clean diatom frustules for the identification following the European standards CEN 13946 2003 and CEN 14407 2004.

More than 300 species were determined, and many are still under taxonomic identification. According to Bray-Curtis similarity that was calculated based on presence-absence data, we found that the studied sites on the Danube River are mostly (grouped into one cluster. However, as for the other stations, we see differences in grouping, and it is clear that the degree of connectivity and substratum types are essential.

Our results reveal floodplains as habitats having rich species diversity. Thus, considering the importance of various habitats in floodplains, it's crucial to foster their sustainable management.

**SEASONAL “WINDOWS OF OPPORTUNITY” IN  
ALPINE HEADWATERS: IMPLICATIONS FOR DIATOM  
ASSEMBLAGES**

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In the European Alps, around 80% of glacier volume is predicted to vanish within the end of this century because of global warming. The progressing glacier retreat is affecting the Alpine hydrological dynamics as well as the distribution and biodiversity of glacier-fed streams. Within this scenario of vanishing glaciers, mountain permafrost is becoming increasingly significant since the thawing of its subsurface ice occurs at a slower rate in comparison to surface glacier ice. The most common evidence of mountain permafrost are the rock glaciers, i.e., creeping rocky landforms made of rock fragments that host subsurface ice. Springs and streams emerging from intact (containing ice) and relict (not containing ice) rock glaciers are increasingly considered as a climate-resistant source of cold water, as well as ecological refugia for cold-stenothermic aquatic organisms. Nonetheless, the knowledge of benthic biodiversity and dynamics in relation to the seasonal changes of habitat and water chemical setting is still patchy and incomplete.

Ecological Windows of Opportunity (WOs) are defined as seasonal periods of mild environmental conditions supporting the development of benthic primary producers, especially diatoms, in Alpine glacial streams. The primary WO occurs in autumn when glacier ablation is reduced, and glacier-fed streams have stable channels and less turbid waters. Differently, the spring WO more strongly depends on stochastic meteorological factors and is more irregularly paralleled by benthic growth. Although WO has been conceptually modelled based on field surveys of primary producers in glacial streams of the European Alps,

much scarcer field evidence is available for headwaters of different origin. In particular, the seasonal development of diatom biomass and diversity in Alpine headwaters fed by rock-glaciers and in non-glacial reference streams (i.e., not influenced by permafrost and glaciers) in the present context of Alpine deglaciation is poorly known.

Here we present the first results of a two-year investigation on seasonal development of diatom biomass and diversity in headwater streams of different origin in two deglaciating catchments of the Italian Central-Eastern Alps. The study has been conducted within the Euregio project “Rock-me” (2022-2025, <https://rock-glaciers-euregio.fmach.it/>) by investigating physical (temperature, turbidity) and chemical (nutrients, major ions, and trace elements) parameters of water, organic and chlorophyll-a content of epilithic biofilm, as well as density and taxonomic composition of epilithic diatoms in streams fed by glaciers and rock glaciers, and in non-glacial reference streams in both catchments.

We found different patterns of seasonal WOs, outlined by higher diatom density and biofilm chlorophyll content, either in early or late summer, in headwaters of different origin in relation to differing hydrological dynamics and habitat settings. The early summer WO appears to be more pronounced in headwaters fed by glaciers or rock glaciers, as a possible effect of the climate-related hydrological dynamics in deglaciating Alpine catchments. On the other hand, non-glacial springs and streams with more stable water discharge are characterised by a more evident late summer WO. Seasonal changes of diatom biodiversity seem to be related to differing hydrological dynamics at catchment scale.

## UNEXPECTED RECORD OF DIATOMS ON GAMMARIDS FROM SPRINGS OF OHRID LAKE

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Lake Ohrid and its surroundings have been designated as a UNESCO World Heritage Site for being a refuge for numerous endemic species of freshwater fauna and flora. Its isolation and unique geological history have contributed to the development of a highly specialized ecosystem. The lake is considered the oldest in Europe, ca. 1.3 My old. Lake Ohrid is fed by karstic springs located mostly on the eastern shoreline and also beneath the water surface, with a total in-flow of 50% while the main sources are the St. Naum and Biljana's spring complexes. The spring complexes, providing a stable, clean, cold, shallow water ecosystem, host various endemic animals. Among them, are endemic species from the genus *Gammarus* (Amphipoda), benthic crustaceans, key elements in freshwater ecosystems. During our study on the *Gammarus* species found in the Biljana, we discovered that many of the specimens are covered with diatoms. These diatoms were found attached to the pereopods and mostly on the dorsal part of the pleonites and telson of three species, *G. parechiniformis*, *G. roeselii* and *G. sketi*. This phenomenon has, so far, not been discovered on species found in the lake itself. What's more, epizoic diatoms have not been recorded on *Gammarus* species so far, although their presence on living and dead animals has been confirmed. The association of diatoms with marine vertebrates such as turtles, porpoises, dolphins, and whales is well known. Also invertebrates are known to host diatoms as epibionts. Diatoms on invertebrates have been recorded, among others, on cladocerans, copepods, polyps of marine hydroids, and

krill. However, these quotes are mainly related to marine ecosystems. Diatoms that were identified as attached to *Gammarus* belong mainly to genera *Cocconeis*, *Achnantheidium* and *Planothidium* as well as *Caloneis*, *Navicula* and *Meridion*. *Cocconeis* species were most frequent, which are considered typical epizoic taxa, most often found in other animals. We suspect that the phenomenon is an effect of the fact that the water springs from Lake Ohrid basin are particularly clean and shallow, allowing good light penetration, while gammarids are dominant animals here, favouring such interaction.

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## **THE INFLUENCE OF THE SUBSTRATE ON THE DIATOM SPECIES COMMUNITY IN ARTIFICIAL AND HEAVILY MODIFIED WATER BODIES**

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In Pannonian part of Croatia, a total of six types of artificial water bodies (AWB) and heavily modified water bodies (HMWB) on streams are defined. The calculation of the ecological potential is based on taking over the assessment system of ecological status of natural water bodies of the Pannonian ecoregion. Methods for assessing the ecological status include the taxonomic composition of diatoms at the species level and the relative abundance of species.

According to the national methodology for sampling biological elements of surface water quality, when sampling phytoplankton, in this case diatoms, it is necessary to collect a sample from a hard movable substrate of mesolitoral size. Other types of moving substrate such as mosses or macrophytes and soft sediment such as psammal or argyllal are used as alternative substrates for diatom sampling.

Given that in AWB and HMWB we often do not find a hard moving substrate of mesolitoral size, it is necessary to sample diatoms from alternative substrates. In order to see the potential impact of sampling diatoms from alternative substrates on the composition of the community, we selected 74 samples collected on AWB and HMWB from the area of Pannonian Croatia, which were collected in the period from 2018 to 2023. Of the total number of samples, 40 samples were collected from the hard moving substrate and 34 from alternative substrates. From the Bray-Curtis analysis of the similarity of the established communities, it is evident that the communities are grouped depending on the type of substrate from which they were sampled. In the RDA analysis, in addition to the two types of substrate, we also included the mean annual values for the specified physico-chemical indicators TOC, BOD<sub>5</sub>, dissolved oxygen, total nitrogen, nitrates, orthophosphates and total phosphorus. The results indicated that of all the physico-chemical

indicators, orthophosphates have the greatest effect on the composition of diatom communities. But somewhat unexpectedly, the results indicated that the greatest influence on the composition of the diatom communities of all the included abiotic indicators is the type of substrate, i.e. whether the samples were collected from hard movable substrates of mesolithal size or from alternative types of substrate such as psammal or argyllal. Thus, the following species were attached to the hard movable substrate of mesolithal size: *Gyrosigma sciotoense*, *Nitzschia inconspicua*, *Navicula antonii*, *Navicula trivialis* and *Craticula subminuscula*. The species *Ulnaria ulna*, *Aulacoseira granulata*, *Halamphora veneta*, *Nitzschia amphyoaxis* were attached to alternative substrate types.

**GREENHAB MONITORING POTENTIAL HARMFUL ALGAL BLOOMS (HABS) IN THE WEST COAST OF GREENLAND: PAST, PRESENT AND FUTURE****Andrea M. Burfeid Castellanos\*** & Bánk Beszteri*University of Duisburg-Essen, Universitätsstr. 2, 45141 Essen, Germany*

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Marine harmful algal blooms (HABs) are represented by various types of algae, including diatoms, dinoflagellates, haptophytes, and raphidophytes. They usually appear with the combined presence of a higher water temperature, increased salinity, and increased nutrient availability. As the Arctic Sea is suffering from Atlantification, entailing all the previously mentioned effects, it might be subject to harmful algal blooms in the future. Since 2012, the identification of species through amplicon sequencing has been used in Greenland's West Coast with special focus on the presence of HAB forming algal strains. The proportion of these strains seems to be slightly increasing with time, and a monitoring strategy would be necessary to protect the fish-based economy of the Greenlandic indigenous population. The hypotheses posited are based on a local aspect – around Disko Bay - and an extended monitoring of the western Coast to observe whether the distribution of HAB-forming algae keeps migrating North in a meso- and macroscale. Using historical records to keep track of changes in the algal community, we take a special look at *Pseudo-nitzschia delicatissima* (Cleve) Heiden s. l. and other non-diatom species like *Alexandrium catenella* (Whedon & Kofoid) Balech and *Alexandrium ostenfeldii* (Paulsen) Balech & Tangen from the region. Within the GreenHAB project we will continue this time series, with a summer and a spring sampling of Disko Bay, as well as a ship Expedition through Greenland's West Coast to ascertain the current upper northern limit of HAB-forming algae presence.

**DIATOM COMMUNITY DURING VERTICAL MIXING IN THE OPEN SOUTHERN ADRIATIC SEA, NORTHEASTERN MEDITERRANEAN****Nenad Jasprica\***, Iris Dupčić Radić & Nika Pasković*University of Dubrovnik, Institute for Marine and Coastal Research, Kneza Damjana Jude 12, 20000 Dubrovnik, Croatia*

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Deep water convection in winter is one of the major processes driving primary productivity in open waters, such as in the open Southern Adriatic Sea (eastern Mediterranean Sea). This process is highly variable in time, depending on the specific oceanographic and meteorological conditions (circulation, stratification, sea-atmosphere interactions). Phytoplankton community can be a good indicator of occurrence of such convective events. The high abundances and biomass (chlorophyll *a*, Chl-*a*) of phytoplankton in the open Southern Adriatic Sea observed in March 2017 are the result of convection-driven nutrient enrichment in the upper layer, followed by a period of water column stability that promotes phytoplankton development. The diatom bloom commenced in early March 2017, reaching its peak approximately two weeks later, as also evident from satellite-derived Chl-*a* data. It occurred during the greatest SiO<sub>4</sub>, PO<sub>4</sub> and NH<sub>4</sub> availability. Diatoms had the highest contribution to the phytoplankton abundance (21.1-41.0%) from 3<sup>rd</sup> to 20<sup>th</sup> March 2017, with values from 6.1×10<sup>2</sup> to 5×10<sup>4</sup> cells L<sup>-1</sup> and 2×10<sup>2</sup> to 1.9×10<sup>5</sup> cells L<sup>-1</sup> on 3<sup>rd</sup> and 20<sup>th</sup> March, respectively. During the peak (20<sup>th</sup> March) diatoms were mostly composed of *Pseudo-nitzschia* spp. (19.8%), *Chaetoceros* spp. (13.4%), *Ch. lorenzianus* (12.0%), *Ch. affinis* (11.1%) and *Asterionellopsis glacialis* (9.6%). The majority of the diatom population occurred in the surface layer (0–50 m). Subsequently, four days later (24<sup>th</sup> March), the contribution of diatoms markedly decreased (to 1%), while that of coccolithophorids slightly increased (to 2–3%). In general, the bloom-type diatom community (*Chaetoceros*, *Pseudo-nitzschia*, *Nitzschia*, etc.) suggests that a high nutrient environment supported the growth of species typical for the summer bloom of the southern Adriatic coastal waters, i.e. in areas of higher trophic levels and influenced by anthropogenic nutrient loads. These species are

generally the main contributors to high Chl-*a* in cases of intense physical dynamics, i.e., during winter/early spring convection. Some species (e.g., *Skeletonema*) may originate from the northern Adriatic source area, and reach the deep Southern Adriatic Sea either by southward currents along the western Adriatic coast and/or by crosswise flow within the cyclonic gyre and mesoscale eddies from the eastern or western Adriatic coast.

**SUMMER DIATOM COMMUNITY COMPOSITION IN THE SOUTH ADRIATIC SEA (LASTOVO ISLAND)****Maja Mucko\***, Antonija Matek, Sunčica Bosak & Zrinka Ljubešić*University of Zagreb, Faculty of Science, Biology Department, Trg Marka Marulića 20, 10000 Zagreb, Croatia*

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Oligotrophic South Adriatic Sea is characterized by low average productivity influenced by a strong summer water stratification with omitted vertical mixing and nutrient supply to the euphotic layer. During two-week July investigation at Lastovo Island, we investigated phytoplankton diversity with light microscopy approach, Chl *a* measurements and amplicon sequencing of 18S rRNA gene of three fractions: pico- from 0.2-3 µm, nano- from 3-20 µm and micro- from 20-200 µm. LM counts and amplicon sequencing was done on samples from two layers: surface and deep-chlorophyll maximum (DCM) in fine-scale daily manner, resulting in total of 24 biological samples for eDNA and 180 for LM/Chl *a* analysis. Diatoms (Bacillariophyceae+Mediophyceae+Coccolithophyceae, part of the Gyrista phylum) represented total of 1% of identified ASVs in pico-, 2% in nano- and 2.3% in the micro- fraction, while most of the phytoplankton community was represented with Dinoflagellata (56.7%), Gyrista (13%), Ciliophora (6%), Chlorophyta (6%) and Haptophyta (6%). Dominant diatoms with highest detected number of ASVs across all samples were *Pseudo-nitzschia* spp., *Cylindrotheca closterium*, *Entomoneis* spp., *Thalassiosira* spp., *Rhizosolenia imbricata*, *Ceratulina pelagica* and *Chaetoceros diversus*. There was no statistical significance between surface and DCM diatom community composition based on robust Aitchison's index (PERMANOVA *p-value* = 0.15). Diatoms were more abundant in DCM and micro- fraction, which was corroborated with frequency of LM counts, where diatoms represented 58.57% of the whole microphytoplankton community. Dominant species revealed with LM were *P. delicatissima*, *C. closterium*, *Nitzschia longissima*, *Proboscia alata*, *Guinardia flaccida* and *R. imbricata*, while average dissimilarity between surface and DCM analyzed with SIMPER (73.56%) distinguished *P. alata*, *P. delicatissima*, *R. imbricata* and *C. closterium* as most contributing species to this difference. This study was a part of

broad interdisciplinary experiment from project “ISLAND” and gave an interesting perspective in comparisons of amplicon sequencing vs. LM microscopy in fine-scale sampling survey.

**PLANKTONIC DIATOMS IN THE OPEN SOUTHERN ADRIATIC SEA IN 2022**

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The Adriatic Sea, situated as a semi-enclosed basin within the eastern Mediterranean, with its deepest part in the southern Adriatic, reaching depths of approximately 1250 meters in the Southern Adriatic Pit (SAP). Characterized as oligotrophic, the southern Adriatic is primarily dominated by nanophytoplankton and picophytoplankton. Nevertheless, higher microphytoplankton (cells  $>20\ \mu\text{m}$ ) abundances ( $>10^5\ \text{cells L}^{-1}$ ) are a recurrent phenomenon, especially in early spring. In 2022, comprehensive seasonal studies were conducted on the hydrography, nutrient concentrations, biomass, structure and dynamics of the phytoplankton community in the SAP. Four sampling cruises were carried out, during which 41 phytoplankton samples were collected. Phytoplankton abundances were estimated using the Utermöhl method. Nanophytoplankton abundances prevail the microphytoplankton by an order of magnitude in all seasons. The highest nanophytoplankton ( $1.37 \times 10^6\ \text{cells L}^{-1}$ ) and microphytoplankton ( $3.3 \times 10^5\ \text{cells L}^{-1}$ ) abundances were found in the spring, coinciding with the highest observed chlorophyll *a* concentrations. A total of 142 taxa (species and intraspecific taxa) were recorded: 77 dinoflagellates, 55 diatoms, 9 coccolithophores, and one silicoflagellate. The diatom genera with the greatest number of taxa were: *Chaetoceros*, *Thalassionema*, *Navicula*, *Leptocylindrus*, and *Pseudo-nitzschia*. The nutrient concentrations were characteristic of the oligotrophic conditions in southern Adriatic. Maximum concentrations of phosphate and silicate were noted in autumn, and total inorganic nitrogen reached its maximum in summer. Vertical water column mixing occurred during winter, creating conditions suitable for phytoplankton development. In 2022, the phytoplankton abundances were dominated by nanophytoplankton (2–10  $\mu\text{m}$ ) in all seasons, while diatoms dominated the microphytoplankton in winter-spring period (80.7–97.1%). Dinoflagellates prevail during summer (76.9%) and autumn

(54.4%). The primary production and diversity of phytoplankton in the southern Adriatic are influenced by hydro-meteorological conditions and the inflow of different water masses. The circulation of the Bi-modal Oscillating Mechanism (BiOS) between the Adriatic Sea and Ionian Sea determines the water mass entering the southern Adriatic.

**PHYLOGENY THROUGH A STRUCTURAL LENS:  
CHARACTERISING PYRENOID PROTEINS IN THE DIATOM  
*THALASSIOSIRA PSEUDONANA***

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Diatoms are eukaryotic algae important for global biogeochemical cycles and responsible for up to 20% of global carbon fixation. To maximise photosynthetic efficiency, algae have evolved biophysical carbon concentrating mechanisms (CCMs), concentrating inorganic carbon to saturate Ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) with CO<sub>2</sub>. Central to the algal CCM is the pyrenoid, a dynamic, Rubisco-containing organelle located in the chloroplast. Current understanding of the diatom CCM is incomplete with key pyrenoid components unknown or uncharacterized. Recently, we have used Rubisco co-immunoprecipitation in the model diatom *Thalassiosira pseudonana* to identify putative pyrenoid proteins, including 7 'Shell' proteins. Fluorescent tagging has shown the 7 *T. pseudonana* Shell homologues (Shell1-7) localise to the surface of the pyrenoid, however differ in suborganelle distribution. This study has combined phylogenetic and structural analysis to characterise Shell1-7, and gain insight into diatom CCM component evolution. BLAST search identified 137 Shell protein homologues across diverse algal lineages. Phylogenetic analysis revealed homologues from diverse species are widespread, pointing to Shell proteins being present in the diatom common ancestor. Within *T. pseudonana*, the distinct clade positions revealed a sequence of evolutionary similarity between Shell1-7. Interestingly, this trend was reflected in Shell protein structural analysis. The Shell1-7 AlphaFold structures (which possess a characteristic beta fold) were aligned, giving root-mean-squared-deviation (RMSD) values as a measure of structural similarity. When comparing RMSD values to Shell protein clade positioning, increased structural difference is reflected by more distant evolutionary relationships. Further in silico structural analysis revealed subtle differences in beta fold curvature between Shell1-5. As the Shell proteins with increased curvature localise to similar regions,

it is possible that Shell protein secondary structure is associated with distinct sub-pyrenoid localisation.

**RHOPALODIOID DIATOMS AS HOSTS FOR  
CYANOBACTERIAL ENDOSYMBIONTS – A PHYLOGENOMIC  
AND TAXONOMIC APPROACH**

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The convergence of taxa from different lineages through endosymbiosis is widely recognized as the accepted explanation for the origin and diversity of numerous life lineages encompassing groups referred to as “Algae”. Some of these symbioses are more than 1 billion years old, however, some are surprisingly recent in their origin. These more recent symbioses can offer insights into the early process of endosymbiosis, which might be hidden in those associations of much longer duration. One such instance involves a lineage of canal raphe diatoms known as the Rhopalodiales, which host obligate endosymbiotic blue-green algae termed spheroid bodies (SBs). SBs fix gaseous nitrogen for their hosts, allowing the Rhopalodiales to live in nitrogen-limited habitats, whereas the diatom host provides fixed carbon and supplies energy to SBs. Our research aims to understand the evolutionary dynamics of this symbiosis through taxonomic studies, phylogenomics, biogeography, and ecological investigations. Preliminary findings on the phylogeny and evolution of diatoms suggest intriguing patterns, which will be further elucidated through the ongoing analysis of entire plastomes and mitogenomes of Rhopalodiales.

**TEMPORAL EVOLUTIONARY PATTERNS IN ENDEMIC DIATOM SPECIES FROM LAKE OHRID****Dušica Zaova**<sup>1\*</sup>, Elena Jovanovska<sup>2</sup>, Bánk Beszteri<sup>3</sup> & Zlatko Levkov<sup>1</sup>

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Evolution is fundamental to the survival of species, as this process allows populations to adapt to environmental changes by altering genes and creating new traits. This usually leads to speciation and emergence of new species, which in turn increases biodiversity. However, due to the general lack of high-resolution fossil records, very little is known about how this process operates within species. Using high-resolution fossil record spanning 1.36 million years, we investigate the evolutionary processes in diatoms from Lake Ohrid. We observed the degree of morphological change, persistence, and abundance of endemic planktonic species and compared them with the non-endemic planktonic species in the lake. While all planktonic diatoms showed morphological changes, the endemic *Cyclotella cavitata* exhibited the strongest and especially unidirectional morphological changes and persisted the longest in the lake. Typical for this species were periods of slow morphological changes, followed by the coexistence of different morphotypes with relatively high abundance, interrupted by rapid morphological changes and disappearance. This suggests diversification processes and subsequent extinction events. Similar changes were also observed in the endemic *C. hustedtii*, which appeared in the lake in the more recent past. Such pronounced and unidirectional morphological changes were not detected in the non-endemic and short-lived species, which occasionally appeared in relatively small numbers in the lake probably through colonisation, supporting the possible *in situ* diversification of the long-lived and morphologically variable species in the lake. Incorporating paleoenvironmental data into further analyses will reveal more about

the adaptive potential of the species and provide more details about the nature of the evolutionary processes taking place in these and the other diatoms in the lake and similar isolated ecosystems.

Student Poster

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**ENDEMIC DIATOM DIVERSITY IN LAKE OHRID THROUGH THE TIME: COMPARATIVE STUDY BETWEEN FOSSIL AND CONTEMPORARY FLORA**

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The survival of the species is a complex mechanism and depends on the ability for adaptation to changing environmental conditions including shifts in climate, habitat loss, and/or introduction of new predators or competitors. Those processes were particularly challenging during the Pleistocene when intensive fluctuations in the climate were noted. As a result, several mass extinction events were recorded from different groups of organisms including both, terrestrial and marine species. Although in small numbers, some ecosystems, such as ancient lakes, served as refugia providing opportunities for many organisms to survive. One extraordinary example is Lake Ohrid, a lake with tectonic origin from the early Pleistocene that still exists. Numerous taxonomic publications have demonstrated the extraordinary diatom contemporary diversity, with high levels of endemism. Several studies for origin and biostratigraphy of diatoms have been conducted by using fossil sediment samples from the lake covering the last 1.3 million years. However, to what extent the diatom species composition has been changed from the period of early lake formation until the present has not been studied in detail. To gain insight into this aspect, we use fossil diatom data covering the period between 1.3–0.7 million years. Two different drilling sites i.e. DEEP from the central part of the lake basin and Peštani from the eastern part have been used not only to cover the earliest stage of lake formation, but also to include possibly different past habitats from the more littoral zone of the lake. Moreover, we compared them with data from the modern diatom flora of Lake Ohrid. In total, 124 endemic and 12 relict benthic diatom taxa were recorded from the fossil record of Lake Ohrid. As a difference, the contemporary flora of the lake shows a higher diversity of endemic taxa or 213 in total. Of the recorded endemic

taxa, 52 have been found only in the fossil record and therefore could be considered extinct. From the relict taxa, only nine have been present in the modern flora of the lake and three are considered extinct. However, in total 84 taxa are documented as shared species between the fossil and recent diatom flora of the lake. This significantly points to the ability of Lake Ohrid as an important refugee ecosystem for diatoms. In addition, the increased number of endemic taxa in the recent flora indicates that Lake Ohrid was an evolutionary active ecosystem during the time. This is also supported by the presence of species complexes within the genera *Gomphonella*, *Navicula*, *Sellaphora* and *Placoneis*. Further molecular analyses on them and other diatom genera can provide more insights into the processes of species evolution and speciation. Moreover, additional analyses are necessary to understand the causes of species extinction in the lake.

Student Poster

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**MAJOR PATTERNS OF DIATOM BIODIVERSITY IN LITHUANIAN LITTORAL SEDIMENTS****Irina Sosnina**\* & Vaida Šeiriene

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In the course of its development, the Baltic Sea passed through several phases. The sea level and salinity changed multiple times, reflecting the relief of the Lithuanian coastline and the nature of the deposited material. The most significant changes occurred at the beginning of the Middle Holocene when the Curonian Spit began to form actively. To determine how alterations in hydrological and sedimentological patterns have affected diatom biodiversity, a 5 m sediment core was studied. The core was obtained from a nearshore depth of 57 m, and the diatom assemblages were analysed and supplemented with organic matter content data.

The findings allow us to distinguish changes in the diatom biodiversity along the section. The complex of the lowermost part mostly consists of benthic taxa *Amphora* sp. and epipsammic *Staurosirella martyji*, the diatom taxa predominantly freshwater. In the middle of the section, a complex with dominant *Staurosira* and *Staurosirella* genera was found. This zone is characterised by a high abundance of valves, most of which are benthic. In samples from the uppermost part of the section, the number of valves is low and the composition of the complex varies from interval to interval. Prevail planktonic *Cyclotella choctawhatcheeana* with benthic *Ellerbeckia arenaria* and tychoplanktonic *Staurosira inflata*.

Whereas radiocarbon dating results were estimated as unreliable, diatom flora revealed that the stage of a low-water steady complex with freshwater taxa was substituted by the littoral zone complex under unstable hydrological conditions and global climate changes. Diatom development may have been limited by the lack of nutrient-rich water input from the Nemunas River Delta, and the changes in biodiversity may have been influenced by general warming in the Holocene.

**THE PRELIMINARY RESULTS ON THE DIATOM DIVERSITY FROM THE HOLOCENE LACUSTRINE SEDIMENTS IN THE GREAT WESTERN ERG OF THE SAHARA DESERT IN ALGERIA**

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The Great Western Erg is located in the northwestern part of the Algerian Sahara. It is part of the large Saharan dune sets with a length of 500 km and width from 150 to 200 km. There is limited knowledge on the diatoms of the region. In this study, the samples collected from holocene lacustrine sediments from Hassi Mouina, Daïet Sidi El Ala and Abou II depressions that extend for within the Great Western Erg of Sahara Desert in Algeria.

A total of 77 diatom species were observed, belonging to 22 genera. The most commonly occurring diatoms in sediments belonged to the genera *Nitzschia*, *Navicula*, *Pinnularia*, *Fragilaria*, *Cymbella*, *Diploneis*, *Epithemia*, *Gomphonema* and *Pantocsekiella*. On the other hand, *Epithemia* cf. *argus* Kützing was the most common species recorded at all sites.

In two sites (Daïet Sidi El Ala, Hassi Mouina), the assemblage is characterized by the dominance of periphitic diatoms with scarce coastal planktonic forms, suggesting a shallow sedimentary environment; pond or swamp type. Whereas the Abou II site, marked by the abundance of planktonic forms (*Pantocsekiella* spp.) suggests a deeper environment.

**RESPONSES OF EPILITHIC DIATOM TO CHEMICAL RECOVERY FROM ACIDIFICATION: INSIGHTS FROM LONG-TERM STUDIES IN THE WESTERN ALPS**

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Air pollution has increased dramatically during the Anthropocene, causing detrimental effects on ecosystems and human health. To face this issue, in 2019, the National Emission Ceilings Directive (NECD) was launched by the European Union to set national emission reduction commitments of the most relevant air-borne pollutants for each Member State. For the first time, EU countries were also asked to monitor the effects of the reduced emissions on both freshwater and terrestrial ecosystems, as specifically addressed in Art. 10 of the NECD. For freshwaters, the monitoring is mainly targeted to assess the effects of sulphur and nitrogen deposition and the acidification status. High mountain lakes (HMLs) are among the most threatened ecosystem by global change, including atmospheric deposition of pollutants and climate warming. In Italy, HMLs, due to their location far from direct anthropogenic disturbance, have been used since the 1980s to monitor the response of freshwaters to atmospheric pollution. To further evaluate the suitability of these sites for the monitoring under the NECD, a two-year chemical and biological survey of 8 HMLs was performed in the Central Alps, Italy. These sites, which have been subject to previous chemical and biological monitoring, lie on a gradient of sensitivity to acidification (as the main pressure) and are affected by medium-high nitrogen deposition.

Besides sampling for chemical analysis, samples have been collected for epilithic diatoms, included among the biological indicators suggested by Annex V of the NEC Directive, following a European wide standardized sampling protocol. Despite the documented effectiveness of diatoms as indicators of atmospheric pollution and climate change impacts on freshwater ecosystems, their monitoring at sensitive sites in Italy have

been performed on a few occasions, mainly in the in the framework of EU-funded projects.

The main aims of this study were to expand knowledge on HMLs as under-studied ecosystems and evaluate the applicability and reliability of some metrics commonly used to assess the impacts of atmospheric pollutants on these sensitive water bodies, with a focus on acidification. Previous and newly acquired chemical and biological data of HML were analysed to assess the chemical and biological responses to reduced surface water acidification, detect temporal trends, identify the major environmental gradients that structure biological assemblages and evaluate the possible use of diatoms as indicators of other pressures than acidification. All the lakes showed evident signs of chemical recovery, with increasing pH and alkalinity, but in the most sensitive sites the changes were less evident. Results indicated that diatoms are highly responsive to acid-related parameters and confirmed their suitability as indicators of acidification and recovery. Beside acidification, other drivers, including nutrients and climate change may affect HMLs diatoms assemblages. Hence, collecting new data will represent a benchmark to evaluate the combined effects of multiple stressors (e.g., acidification, nitrogen enrichment, climate change, microplastics pollution) on sensitive habitats.

**PLANKTONIC DIATOM SUCCESSION TO RECONSTRUCT TROPHIC LEVEL AND CLIMATIC CHANGES IN LAKE SCHLIERSEE, GERMANY**

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In this study, we present a continuous Late Holocene sediment record spanning the last ca. 4000 cal. yr BP from Lake Schliersee, a perialpine lake in the Bavarian Alps. Overall, we aim to identify the palaeohydrological dynamics based on a multidisciplinary approach, to show changes in past productivity and their relation to times of human activities. Here we focus on how the diatom community has been affected by climatic and anthropogenic induced environmental changes. Planktonic diatoms were exclusively analysed for the palaeolimnological reconstruction because they are dominant in this 40m-deep lake, and we wanted to know whether the exclusive analysis of planktonic diatoms is suitable.

Our results show a strong dominance of planktonic diatoms during the past 4000 years. These are *Pantocsekiella comensis*, *P. hinziae* and *P. costei*, mainly present in oligo- to mesotrophic waters. The first shifts in the dominance ratios were detected around 1000 cal. yr BP and corresponds with strong deforestation and increased lake productivity due to higher human activity during the Medieval. While the abundance of the most common *P. comensis* decreases, the proportions of *P. costei*, *Cyclotella distinguenda* and *Lindavia glomerata* increase.

The most dramatic change in the planktonic diatom community occurred around 1970 with the abrupt appearance of species that favour higher trophic levels, such as *Stephanodiscus parvus*. At the same time, the concentration of diatom valves also increased visibly. The gradual decline of *St. parvus* at the beginning of the 1990s, accompanied by a simultaneous increase in the abundance of *P. hinziae* and *L. glomerata*

documents a decrease in trophic conditions, which still prevail in Lake Schliersee today.

Our results show a strong sensitivity of planktonic diatom species to climate- and human-driven lake system shifts.

**THE CLIMATE AND PALEO-ENVIRONMENTAL  
CONDITIONS OF THE ZAWADOWSKIE POND IN GORCE  
MTS. (SOUTH POLAND)**

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The history of Zawadowski Pond begins ~8,000 to 7,300 years ago, with a warm and humid climate. The lack of pollen from aquatic plants at this time may indicate low water levels in Zawadowski Pond. Later, until ~4300, there is a gradual cooling and humidification of the climate, as well as an increase in the water level in the Zawadowskie pond, as indicated by the appearance of pollen from aquatic plants - yellow water lily). The formation of the Zawadowskie pond at ~3.3–2.5 ka cal BP was related to the largest concentration of landslide dates in the Polish Carpathians. The landslide zone was successively formed by several mass movement generations induced by headward erosion. Increased mass movements activity in the Gorce Mts (Polish Outer Carpathians) relate to cold and humid periods of the Holocene. This cooling period has been linked with glacier advances in the Alps.

In order to follow diatom flora changes in landslide ponds, a paleolimnological study was applied as one of the best recognized and applied methods used to track long-term environmental changes worldwide. Diatoms are an underexploited tool for tracking ecosystem responses to climate change in the Polish mountain regions. The aim of this study was to investigate the diatom species composition of the Zawadowskie pond (landslide lake) and the potential application of diatoms in paleoecological studies.

Little is known about the direction and nature of diatom changes in landslide environments, therefore the study focused on qualitative changes in diatom assemblages preserved in sediments collected from Zawadowskie pond. The landslide Zawadowskie pond sediment layers contain perfectly preserved diatom shells and pollen grains. Moreover, in the Zawadowskie pond diatoms are present in large amount in all layers, for 7,500 years. The survey shows high species variability - from

very small, definitely dominant *Staurosirella* taxa (pioneering diatom assemblages) to the taxonomic group of huge *Pinnularia* (climax species), which presence is most likely related to the water availability and more calm surroundings.

A crucial question in palaeoclimatology in the perspective of a possible global warming scenario is the variations in the hydrological cycle associated with the Holocene natural climate variability. Lake levels are influenced by climatic parameters affecting both evaporation and precipitation, but they can also be induced by a variety of local, non-climatic factors. However, synchronous changes in water-levels within a region can be assumed to be climatically driven. Given the scarcity of data to document climatic variations, our results offer precious data to refine palaeoclimatic reconstructions.

Student Poster

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**DIATOMS FROM THE HOLOCENE LACUSTRINE  
SEDIMENTS OF ERG ER RAOUI IN SAHARA DESERT,  
ALGERIA**

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The Erg Er Raoui is located in the northwestern part of the Algerian Sahara. In the region, numerous inter-dune depressions are marked by Holocene lacustrine sedimentation (detrital layers overlain by white biogenic carbonates containing diatoms). The samples were collected from the depressions by scraping the surface of the hills. The samples were digested with HCl to remove minerals. After repeated washing with distilled water, the materials was air-dried on cover glasses and mounted in Naphrax. Light microscope (LM) observations of the diatoms were performed using Nikon Ni compound microscope equipped with full immersion differential interference contrast optics capable of 1000X and NA of 1.40 at Dumlupınar University. Scanning electron microscope (SEM) observations were made with a FEI NovaNanoSEM 650 at Dumlupınar University and Eskişehir Technical University by using ZEISS Ultra, Türkiye. As a results, the members of *Halammphora*, *Caloneis*, *Pinnularia* and *Mastogloia* were dominant taxa.

## **ADVANCEMENTS IN FORENSIC DIATOM TESTING: A HIGH-EFFICIENCY EXTRACTION METHOD**

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This study introduces a novel forensic diatom testing technique involving nitric acid digestion and vacuum filtration using polycarbonate membrane filters, achieving recovery rate of 93% for diatom standards added to liver samples. In contrast, conventional methods employing strong acid and hydrogen peroxide digestion, along with centrifugation for sample purification, yield a maximum of 1% of added valves. The resulting permanent slides enable precise diatom identification at the species level using light microscopy at a magnification of 1000x, facilitating detailed comparisons, including multivariate analysis, between inhaled diatoms and those present at the drowning site.

The first experiment, involving three non-drowned pig cadavers (50-60 cm in length) submerged in a natural water body for one month, reveals minimal diatom presence (maximum = 5, mean = 1 per gram of wet weight) in heart, liver, lung, and spleen tissues. However, brain, pancreas, kidney, femur, and rib tissues exhibit diatom concentrations up to two orders of magnitude higher (maximum = 650, mean = 140 for kidney).

Subsequent experimental results from the analysis of human drowning cases (eight subjects) demonstrate the high efficiency of the new method, with an average yield of 55,000 valves per gram of lung tissue (minimum

= 183, maximum = 160,000). Conversely, diatom content in drowned human liver, heart, kidney, spleen, pancreas, and brain does not exceed 3 diatom valves per gram on average (minimum = 0, maximum = 12), with the exception of the rib, exhibiting an average value of 750 valves per gram (minimum = 0, maximum = 2500).

The low contamination rate observed in non-drowned cases (the first “pig“ experiment) and a very high diatom content in drowned cases (second experiment) advocate for the application of the new method on lung tissue, offering a reliable means to unequivocally resolve controversies between drowning and non-drowning cases.

## DIATOM BIOSILICA AS A SOURCE FOR NOVEL BIOCOMPOSITES IN THE INDUSTRY OF TOMORROW

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An innovative approach to the synthesis and design of new inorganic composite nanomaterials involves the use of microorganisms, such as single-celled microalgae like diatoms, which can synthesize unique mineral composites with complex, hierarchical structures at micro- and nanoscale. Diatoms exhibit an astonishing variety of intricately decorated silica exoskeletons with unique three-dimensional structures, found in more than 100,000 known species. This diversity arises from the precise silica structures with systems of pores, spikes, ribs, and processes, rendering them desirable materials for modern technologies.

The focus of our research is the synthesis of various metal nanoparticles (i.e., Ag, Au, Fe), which are particles ranging in size from 1 to 100 nanometers, using diatom-based biosilica. In production of silver-silica (Ag-SiO<sub>2</sub>) hybrid nanoparticles we utilized two species of *Gedaniella* (*G. flavovirens* and *G. mutabilis*, both isolated from the Baltic Sea) as a source of nanosilica. Characterization techniques including UV-Vis spectroscopy, energy dispersive X-ray spectroscopy (EDS), and scanning (SEM) and transmission (TEM) electron microscopy confirmed the production of flower-shaped multiple layered Ag-SiO<sub>2</sub> hybrid nanoparticles with autofluorescent properties under blue light excitation. Additionally, the nanostructures displayed catalytic activity in methylene blue decolorization, suggesting potential applications in eco-friendly wastewater and dyes removal.

The biosilica derived from the strain of *Pseudostaurosira trainorii* was used in the subsequent series of studies on the production of iron-oxide nanoparticles (IONPs). *Pseudostaurosira trainorii* efficiently produced

IONPs from a 0.01 M Fe<sup>+3</sup> aqueous solution, resulting in dark orange coloration of both biomass and media. SEM images revealed complete coverage of *P. trainorii* frustules by synthesized nanostructures. The loaded frustules exhibited catalytic properties by decolorizing nitrophenol, while TEM confirmed the spindle-shaped morphology of the particles.

Other study explored standard fucoxanthin and extracted from strain of *Nanofrustulum shiloi* for its ability to reduce gold ions and synthesize gold nanoparticles (GNPs). Results indicate both isolated and standard fucoxanthin effectively reduce gold ions within 12 hours, while the entire diatom biomass turns pink after 72 hours. Characterization via UV-Vis spectroscopy and SEM reveals successful GNP synthesis, while TEM showed that GNPs synthesized by the whole biomass had triangular, spherical, and hexagonal shapes, whereas those produced by extracted and standard fucoxanthin were spherical.

Research on the synthesis of metal nanoparticles and the utilization of diatom-based biosilica is of great importance for both science and modern economy. This enables the development of nanotechnology and the creation of new materials with unique properties that can be applied in various fields such as electronics, medicine, and environmental protection. The use of natural materials for nanoparticle production can lead to more efficient and environmentally friendly manufacturing processes, which is significant for the nanotechnology industry. Additionally, these discoveries may have applications in environmental remediation by removing pollutants from water.

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## **DIATOMS FROM THE BALTIC SEA AS A SOURCE OF BIOMATERIALS**

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The most extensively studied diatoms character is their intricate cell wall structure, a bipartite exoskeleton, called frustule and made of silicon dioxide. The size of the single shell ranges from micrometres to millimetres and the size of the cell of each species decreases from generation to generation during a vegetative reproduction, while the size of open volumes does not scale with the cell size and remains almost constant.

The diatom frustules are considered as materials with specific architecture in micro- and nano-scale. The in-situ fluid SEM system for the scanning electron microscopy observations will be used to establish a conditions-structure-properties relationship.

The long-term impact of such study is to set the stages for the design and synthesis of new materials based on the hierarchically organized bio-grown modifiers in the form of frustule biomineralized by the diatoms. From the materials science perspective, this research will generate new knowledge on the nano- to macro-properties of the particulate systems with intricate architecture of the particles. Successful isolation of the Baltic Sea diatom strains allows us to test their growth and frustule properties using electron microscopy in variable environments. Considered and investigable conditions are related to the gas/fluid environment within a wide temperature range.

The scientific problem, and thus short-term outcome, is to understand and develop the ability to conduct imaging of the biological samples

(materials) in-situ, in-vivo in various environmental conditions using electron microscopy and the impact of these on the structure of diatoms' frustules will be defined.

**OBTAINING AND FUNCTIONALIZING THREE-DIMENSIONAL HOLLOW DIATOM FRUSTULES FROM CULTIVATED BIOMASS: A NEW TECHNOLOGICAL PLATFORM FOR DRUG DELIVERY APPLICATIONS**

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The unique combination of chemical, mechanical and structural properties featured by diatom frustules make them promising biomaterials for drug delivery applications. Conceptually, the cultivation of specific diatom species allows the production of frustules with properties on demand, such as defined size and nanoarchitecture, exploitable as biosilica microcapsules. In such a system, diatom cultures can operate as living factories to produce tailor-made three-dimensional (3D) drug delivery devices. However, before functionalization, it is necessary to remove the organic matter from cultivated biomass. This step typically leads to the frustule subunits disassembling into valves and girdle bands, thus dismantling the complete 3D frustule structure.

The present study aimed to develop a method that enables organic material removal from cultivated diatom biomass while preserving the natural 3D structure of the frustules - this achievement would allow maximizing the drug loading capacity, should be fast and inexpensive, while using non-hazardous reagents and thus suitable for upscaled production. Three different purification methods were tested on different centric diatom strains – “strong acid”, “mild oxidizing”, and “surfactant/chelating”. Subsequently, the frustules integrity was evaluated using Scanning Electron Microscopy and Optical Microscopy techniques.

Once accomplished, different functionalization processes were optimized on 3D purified frustules of *Coscinodiscus granii*, namely encapsulation and coating trials, using Confocal and Widefield Fluorescence Microscopy.

Our results showed that the surfactant/chelating-based purification method maintained the biosilica layers and the 3D shape of the frustules, whereas strong acid and oxidizing methods led to the dismantling of the frustules 3D structure. The encapsulation of both substances was successful, but in different ways: the organic dye filled the internal hollow space of the microshells, while the phytochemical was loaded onto the biosilica surface. The chitosan coating layer deposited onto the surface of the microshells.

This study presents an optimized purification method that preserves the original topology of cultured centric diatom frustules, allowing the extraction of natural 3D biosilica microcapsules with a hollow space available to encapsulate substances, with obvious advantages towards increased loading capacity. We also demonstrated how this biomaterial can be functionalized, including i) encapsulation of an organic dye inside the hollow 3D frustules, ii) encapsulation of a phytochemical on the surface of the frustules, and iii) coating the microcapsule with a natural polysaccharide.

We have verified the feasibility of using the internal diatom frustule's biovolume for encapsulation, providing a new approach to drug delivery research with potential biomedical applications while establishing a basis for future developments in other diatom nanotechnology sectors.

**THE ANALYSIS OF THE CHITIN NANOFIBER PRODUCTION CHARACTERISTICS OF THE MARINE DIATOM *CYCLOTELLA CRYPTICA* (CCMP 333) DURING FED-BATCH GROWTH IN A BUBBLE COLUMN PHOTOBIOREACTOR**

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Chitin is a natural polymer used in the biomedical, pharmaceutical, and food industries. Currently, chitin extracted from harvested shellfish is almost the only feedstock used for production. This reliance is the reason behind several chronic issues experienced both on the production and consumption sides. Chitin in shellfish is ingrained in a matrix of organics and inorganics, which requires the use of environmentally damaging chemicals at elevated temperatures. Furthermore, even after extensive purification, an important amount of these molecules usually persists. These impurities can limit the applicability, particularly for uses requiring high purity. In contrast to the embedded nature in crustaceans, diatoms, those that belong to the *Cyclotella* and *Thalassiosira* genera, are known for their ability to biosynthesize chitin in nanofiber form and extrude them from dedicated cell openings. Owing to this unique production method, diatoms have the potential to serve as a reliable source and solve the issues of the current market. To realize this potential, cultivation methods must be developed to enhance the production metrics of interest, particularly the production rate and final concentration. In this regard, fed-batch cultivation can supersede other methods because of its ability to establish nutrient-replete growth conditions, which is particularly challenging for the cultivation of diatoms due to the limited solubility of silicon in seawater. The current study aims to understand the effects of temperature and salinity on fed-batch growth-based chitin production and utilize the outcomes to develop methods for maximized productivity. For this, *Cyclotella cryptica* CCMP 333 is cultivated under fully controlled process conditions of a bubble column photobioreactor

under constant availability of all the major nutrients, including silicon, nitrogen, and inorganic carbon, and the resulting chitin productivity metrics were assessed and compared at two different temperature and salinity levels. The results reveal the critical roles these two process conditions play in chitin biosynthesis, both in terms of productivity and physicochemical biopolymer properties. Future studies should cultivate the strain under various levels of these critical process parameters and assess trends to devise protocols maximizing productivity.

**THE NEW UNIVERSITY OF DUISBURG-ESSEN  
FRESHWATER DIATOM IMAGE DATA SET (UDE DIATOMS  
IN THE WILD 2024)**

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Here we present the dataset “UDE DIATOMS in the Wild 2024” (University of Duisburg-Essen – Digital annotated open-source microscope slide scans from real-world samples, version of 2024), a collection of more than 83,000 light microscopy images of individual diatom valves or frustules, covering a broad range of taxa and morphology by about 320 samples from 15 different river and lake ecotypes. The diatoms were imaged at high resolution ( $< 0.1 \mu\text{m}/\text{pixel}$ ) by transmitted light bright-field microscopy, with focus stacking to artificially increase focal depth up to  $25 \mu\text{m}$ , allowing simultaneous observation of valve ornamentation and shape. Taken from real-world settings, the images partly also include debris, mineral particles or other diatoms. Four experts identified over 500 diatom species; more than 100 species are represented by at least 100 specimens, and nearly 150 by at least 50 specimens each. This data set is about one order of magnitude larger than previously published diatom data sets, and its high interspecies similarity makes it a valuable resource e.g. for benchmarking fine-grained out-of-distribution (OOD) detection, on which we present preliminary results.

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Biodiversity of coral reef diatoms from Java and Bawean Island, Indonesia based on morphology approach

**Deevena Elias**, Forest Research Institute, Dehradun, India  
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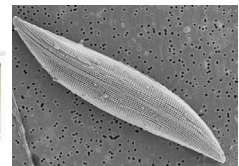
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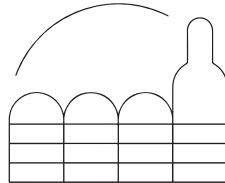
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