

# “Microbes in the Baltic: Small things, small sea, big questions”

18-21 November 2014  
Gdynia, Poland

**BOOK OF ABSTRACTS**

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### Tuesday 18<sup>th</sup> November 2014

#### Plenary lecture:

### **N<sub>2</sub> fixation by heterotrophic diazotrophs in temperate estuarine waters**

**Lasse Riemann**

Department of Biology, University of Copenhagen, Helsingor, Denmark

**Abstract:** Nitrogen (N) fixation is fueling planktonic production in a multitude of aquatic environments. In meso- and polyhaline estuaries, however, the contribution of N by pelagic N<sub>2</sub> fixation is believed to be insignificant due to the high input of N from land and the presumed absence of active N<sub>2</sub>-fixing organisms. In this overview, I will talk about our work in the Baltic Sea and in Danish estuaries with emphasis on N<sub>2</sub> fixation by heterotrophic diazotrophs. In general, we have measured N<sub>2</sub> fixation, examined nitrogenase (nifH) gene composition by next-generation sequencing, and used real-time PCR to quantify distribution of key nifH genes or transcripts in situ.

In the Baltic Sea, we found N<sub>2</sub> fixation by the surface and in the anoxic waters at 200 m depth (Farnelid et al. 2013). The 454-pyrosequencing nifH analysis revealed a diverse assemblage of nifH genes related to Alpha-, Beta-, and Gammaproteobacteria (nifH Cluster I) and anaerobic bacteria (nifH Cluster III) at and below the chemocline. Abundances of genes and transcripts of seven diazotrophic phylotypes were investigated revealing abundances of heterotrophic nifH phylotypes of up to  $2.1 \times 10^7$  nifH copies L<sup>-1</sup>. Abundant nifH transcripts (up to  $3.2 \times 10^4$  transcripts L<sup>-1</sup>) within nifH Cluster III and co-occurring N<sub>2</sub> fixation ( $0.44 \pm 0.26$  nmol L<sup>-1</sup> day<sup>-1</sup>) in deep water suggested that heterotrophic diazotrophs are fixing N<sub>2</sub> in these anoxic ammonium-rich waters. Hence, N<sub>2</sub> fixation in the Baltic Sea is not limited to illuminated N-deplete surface waters.

We examined diazotrophy over one year in two contrasting, temperate, estuarine systems (Roskilde Fjord and the Great Belt strait; Bentzon-Tilia et al.). In Roskilde Fjord, N<sub>2</sub> fixation was mainly accompanied by transcripts related to heterotrophic (e.g. *Pseudomonas* sp.) and photoheterotrophic bacteria (e.g. unicellular diazotrophic cyanobacteria group A). In the Great Belt, the first of two N<sub>2</sub> fixation peaks coincided with a similar nifH expressing community as in Roskilde Fjord, whereas the second peak was synchronous with increased nifH expression by an array of diazotrophs, including heterotrophic organisms as well as the heterocystous cyanobacterium *Anabaena*. The study documents for the first time that significant planktonic N<sub>2</sub> fixation takes place in mesohaline, temperate estuaries and that the importance of heterotrophic, photoheterotrophic, and photosynthetic diazotrophs is clearly variable in space and time.

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**Session 1- “Diversity of microbes in the Baltic Sea”:**

**Salinity structures bacterial communities at contrasting seasons in the Baltic Sea**

**Daniel PR Herlemann<sup>1</sup>, Daniel Lundin<sup>2</sup>, Anders F. Andersson<sup>3</sup>, Matthias Labrenz<sup>1</sup>,  
Klaus Jürgens<sup>1</sup>**

<sup>1</sup>Leibniz-Institut für Ostseeforschung Warnemünde, Warnemünde, Germany; <sup>2</sup>Linnaeus University  
Kalmar, Sweden; <sup>3</sup>Science for Life Laboratory, Solna, Sweden

**Abstract:** Understanding basic processes that control bacterial community composition improves our ability to predict bacterial distributions and functions in ecosystems. Bacterial communities in the Baltic Sea are structured in accordance to the salinity gradient but exhibit also significant seasonal changes. We used the Baltic Sea as a model system to examine the importance of salinity and season as driving factors for bacterial community composition. Abundances of 16S rRNA gene ampliconsequencing reads were assessed from samples taken on similar geographic locations in summer (June/July) and winter (February/March) at brackish (salinity 3-9) and marine (10-32) conditions. Significant changes in species richness were only found between summer and winter conditions but not at different salinities. In contrast to species richness, we found major differences in the bacterial community composition at the different salinity regimes and seasons. Principle coordinate analysis revealed that salinity explains most of this variability, whereas temperature, as proxy for seasonality, was the second most important factor. By changing the dissimilarity radius that defines an operational taxonomic unit (OTU) gradually from 0.01 to 0.25, the impact of temperature on bacterial community composition decreases drastically at an OTU dissimilarity radius of 16% (resembles differences at the taxonomic order). The influence of salinity remained high until a dissimilarity radius of 24% (resembles differences at the taxonomic phyla). The analysis indicates that salinity determines the broad phylogenetic composition of bacterioplankton whereas seasonality determines the succession within the phylogenetic groups. Since the dissimilarity radius of an OTU reflects the evolutionary distance between species on 16S rRNA level, our study underlines the insurmountable character of salinity for most bacterial taxa.



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### The bacterioplankton of the Gulf of Finland - 'gold mine' of biodiversity?

Peeter Laas<sup>1</sup>, Lips Inga<sup>1</sup>, Simm Jaak<sup>2</sup>, Kisand Veljo<sup>3</sup>, Lips Urmas<sup>1</sup>, Metsis Madis<sup>4</sup>

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**Abstract:** The easternmost sub-basin of the Baltic Sea, Gulf of Finland, is a temperate estuary, which undergoes many seasonal changes in environmental parameters. The strong stratification in the central part of the gulf due to the permanent halocline and seasonal thermocline often hinders mixing in the water column. This has led to annually re-occurring hypoxia or even anoxia in the near-bottom layer of water column. The presence of redoxcline results in redox-driven niche partitioning of bacterioplankton community composition. In addition, the surface microbial communities in the gulf influenced by relatively large input of freshwater compared to the Baltic Proper and southern basins, therefore influx of bacterial groups usually associated with fresh water communities. We studied bacterioplankton communities using flow cytometry and massively parallel sequencing technology for enumeration and identification, respectively. Our results indicate that the cumulative bacterial species richness present in the gulf is staggeringly high and the community composition is always accompanied with 'long tail' rare species. Hence, increase in the depth of investigation (by sequencing more indexed reads per sample) also raises the observed bacterial diversity. We used up to thirty thousand sequences per sample, but the plateau was still not reached by the rarefaction curve. This raises the question how large should be the minimum sequencing depth to adequately determine the status of microbial diversity. We also observed that communities with higher species richness tended to occur in low temperature conditions, which in relation to recent discoveries may explain the dipolar dynamics of bacterioplankton community on global scale.

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### NGS of the Baltic Sea microbiome - from diversity patterns to genome-ecology links

Luisa Hugerth<sup>1</sup>, Johannes Alneberg<sup>1</sup>, John Larsson<sup>2</sup>, Jarone Pinhassi<sup>3</sup>, Anders Andersson<sup>1</sup>

<sup>1</sup>KTH / SciLifeLab, Stockholm, Sweden; <sup>2</sup>Linnaeus Universit, Stockholm, Sweden; <sup>3</sup>Linnaeus Universit, Kalmar, Sweden

**Abstract:** Recent years progress in high-throughput sequencing has enabled us to investigate spatiotemporal patterns of Baltic Sea microbial communities at a taxonomic scale. These studies have provided important insights on the taxonomic composition and e.g. revealed that the Baltic appears to be composed of a mixture of bacterioplankton with closest relatives in freshwater and marine habitats, respectively. Since cultured bacteria rarely represent the dominant members of a natural community we have very little genomic information about the microbial plankton of the Baltic Sea. Here, we have conducted Illumina shotgun metagenomic sequencing on a large number of samples from the Linnaeus Microbial Observatory (LMO) Time-series. By using our novel program CONCOCT for clustering contigs into genomes we were able to reconstruct 30 nearly complete microbial genomes that together constitute ca. 25% of the DNA content in the surface water of the Baltic Proper. The genomes represent members of Actinobacteria, Bacteroidetes, Proteobacteria, Verrucomicrobia, Cyanobacteria and Thaumarchaeota. Similar to what earlier taxonomy-based studies have shown, these are a mixture of marine and freshwater relatives. However, by comparing with metagenomic data from different aquatic habitats, the high phylogenetic resolution provided by the genomic data shows that the brackish genomes are distinct from marine and freshwater populations, and that a global brackish microbiome exists. Linking the reconstructed genomes to the spatiotemporal distribution of their respective 16S genotypes allows us to infer links between the ecology and the functional potential of these populations.



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### Genetic and metabolic diversity of the Baltic cyanobacterium *Nodularia spumigena*

Anna Toruńska-Sitarz<sup>1</sup>, Mireia Bartros-Fortis<sup>2</sup>, Agata Błaszczuk<sup>1</sup>, Hanna Mazur-Marzec<sup>1</sup>

<sup>1</sup>University of Gdańsk, Gdynia, Poland; <sup>2</sup>Linnaeus University, Kalmar, Sweden

**Abstract:** *Nodularia spumigena* is one of the dominant cyanobacterial species in the Baltic phytoplankton. Despite high morphological diversity of the cyanobacterium, the 16S rRNA gene sequencing showed that in the Baltic there is only one planktic *Nodularia* species.

In the present work, we aimed to study the diversity of the Baltic *Nodularia spumigena* isolates in terms of their genetic and metabolic features. In addition, the potential ecological implications of this diversity was considered.

Over 20 strains of the Baltic *N. spumigena*, isolated in years 1986-2012, were analyzed. Molecular studies comprised amplification and sequencing of the phycocyanin operon intergenic spacer and its flanking regions (PC-IGS), as well as phylogenetic analysis. To define the metabolic diversity of the isolates, peptidomic approach was used. The profiles of nonribosomal peptides (NRPs) were determined by LC-MS/MS. The effects of the genetically and metabolically distinct *N. spumigena* strains on heterotrophic bacteria and crustaceans were tested.

On the basis of our research we can conclude that Baltic population of *N. spumigena* is composed of several genetically and metabolically different sub-populations. The diversity of the cyanobacterium determined on the basis of peptide profiles correspond well with the diversity determined using the PC-IGS sequences.

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**Plenary lecture:**

**The ‘structure’ or ‘composition’ of bacterioplankton communities**

**Jakob Pernthaler**

Limnological Station, University of Zurich, Zurich, Switzerland

**Abstract:** Environmental microbiology -like all science- employs a number of conceptual frameworks to plan and interpret observational or experimental studies. These concepts may be organized in a hierarchic manner, the top-level one defining the larger context (e.g., the biogeochemical cycling of carbon), and one or several more focussed sub-concepts that guide the actual data collection. The introduction of molecular biological methods has rendered it possible to identify and eventually quantify different pro- and eukaryotic microbes in environmental samples. This advance necessitated the adoption of an additional broad concept that has formed the base of community ecology for more than a century. Since then, research on the ‘structure’ or ‘composition’ of microbial assemblages has rapidly proliferated. On the one hand fundamental insight has been gained which microbial genotypes are common in particular biomes and habitat types, including the freshwaters. On the other hand, a more ‘holistic’ approach has focussed on understanding changes at the level of (meta)communities and their relationship to ecosystem processes. While the concept of community composition and structure has become omnipresent in most if not all areas of environmental microbiology, it is probably most familiar to microbial ecologists working in planktonic systems. In my presentation I will first point out some limits of a quantitative use of Next Generation Sequencing of rRNA gene tags for this purpose. Next, I will present different angles on how to interpret the terms ‘structure’ and/or ‘composition’ of bacterioplankton assemblages, distinguishing in particular between ataxonomic approaches and others that include phylogenetic information.

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### Session 1 (cont) - “Diversity of microbes in the Baltic Sea”:

#### **Genetic diversity and adaptive potential of an expanding toxic dinoflagellate in the Baltic Sea**

**Anke Kremp<sup>1</sup>, Pia Tahvanainen<sup>2</sup>, Johanna Oja<sup>1</sup>, Anniina Le Tortorec<sup>1</sup>, Päivi Hakanen<sup>1</sup>, Sanna Suikkanen<sup>1</sup>**

<sup>1</sup>Marine Research Centre, Finnish Environment Institute, Helsinki, Finland; <sup>2</sup>University of Helsinki, Helsinki, Finland

**Abstract:** Recent studies suggest that phytoplankton populations may harbor high levels of genetic diversity. When reflected by phenotypic variability such standing genetic diversity might help populations to cope with environmental changes. In this study we examine the relationship between genetic diversity of toxic bloom forming *Alexandrium ostenfeldii* from the Baltic Sea and phenotypic variability in the species' response to climate related salinity and temperature changes. Analyses of Amplified Fragment Length Polymorphism (AFLP) of multiple isolates established from resting cysts revealed a complex and hierarchic genetic structure of *A. ostenfeldii* in the Baltic. While gene diversity was generally low, clonal diversity was high with all genotypes exhibiting unique AFLP profiles. When the response of a subset of genotypes to salinity and temperature changes was investigated in culture experiments, effects on growth rates, toxicity and bioluminescence emission were found to be genotype specific. Strain variability buffered the net- or population specific responses to the changes, though some population specific effects were observed on specific traits. Different temperature and salinity conditions selected for specific sets of competitive isolates with different toxicity and bioluminescence capacities. A general relationship between particularly competitive strains and high toxicities or strong bioluminescence could not be established for any of the tested conditions. Our results suggest that the gene pool of *A. ostenfeldii* harboured in Baltic propagule banks provides the diversity that is necessary to ensure adaptation to short term fluctuations and predicted temperature and salinity conditions resulting from climate change.

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**What electrophysiology can do for aquatic ecology?**

**Ilya Pozdnyakov, Sergei Skarlato**

Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russian Federation

**Abstract:** In the present time researchers use interdisciplinary approach more and more often, because it allows considering scientific problems from an unexpected perspective. Despite the long-term cooperation between ecologists and cell physiologists, new areas of ecophysiology continue to appear. One of them is the investigation of ion channels of marine microorganisms for answering important ecological questions. Ion channels play a crucial role in a wide range of essential cellular processes including cell proliferation, differentiation, death, and sensitivity. An important property of ion channel functioning is the ability to initiate both fast (e.g. motility) and slow (e.g. gene expression) responses to various environmental factors. Therefore, ion channels are the crucial part of cellular machinery for adaptation. In the present work we discuss the progress and problems in this field with the emphasis on the ecophysiological role of ion channels in marine dinoflagellates. Despite the importance of ion channels and their potential role in toxin production and release, as well as harmful algal bloom proliferation, the information about them still remains poor because of obstacles in applying electrophysiological techniques to the cell wall containing organisms. We developed a new approach that allows using the patch-clamp technique to study ion channels of a dinoflagellate *Prorocentrum minimum*. *P. minimum* is a potentially toxic and very successful invasive species in the Baltic Sea which is able to form large blooms. In order to understand molecular mechanisms of its great ability for adaptation to a broad range of environmental conditions and its success in the Baltic Sea, we started to study the role of ion channels of *P. minimum* in these processes. By means of our approach we made the first single-channel recordings in dinoflagellates. The first data revealed the presence of putative sodium channels with rather high conductance in this dinoflagellate. Therefore, an important question arises: what is the role of the channels permeable for toxic sodium ions in the cells living in a concentrated sodium solution (sea water)? On the one hand, these channels can be involved in maintenance of internal sodium concentration in euryhaline *P. minimum*, allowing its success in the salinity gradient of the Baltic Sea. On the other hand, similar to neurons, dinoflagellates could evolve a mechanism to split the sodium and calcium signaling systems in order to avoid undesirable activation of a number of calcium-dependent processes.

This work was supported by the RFBR grant № 13-04-00703-a.

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**Oceanographic and ecological drivers of genetic diversity and differentiation of a marine diatom species along the Baltic sea salinity gradient**

**Conny Sjöqvist<sup>1</sup>, Anna Godhe<sup>2</sup>, Lisa Sundqvist<sup>2</sup>, Per Jonsson<sup>2</sup>, Anke Kremp<sup>1</sup>**

<sup>1</sup>Finnish Environment Institute/Marine Research Centre, Helsinki, Finland; <sup>2</sup>Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden

**Abstract:** The Baltic Sea is an estuary composed of several sub-basins connected to the North Sea via the Danish Belts. A northeast-southwest salinity gradient ranging from 3-30‰ creates a spatially heterogeneous environment. Genetic structure of a marine diatom, *Skeletonema marinoi*, was studied from ten locations along the gradient. In total 354 resting stages were germinated and individual clones established, which were subsequently genotyped by eight microsatellite loci. Oceanographic connectivity was compared to gene flow patterns using an asymmetric dispersal index. Local salinity adaptation of individuals from three different sites was experimentally tested by growing ten strains per site in 0-35‰. F-statistics indicated significant differentiation between sampling sites and slightly increasing levels of genetic diversity towards southwest. STRUCTURE identified two populations living in low respectively high saline habitats. Patterns of asymmetric gene flow correlated with oceanographic connectivity, potentially contributing to spatial genetic divergence. Reaction norms of tested strains coincided with local salinity conditions. Our results suggest that there is a reduced probability of dispersing cells to thrive in neighboring areas, leading to genetic population structure and supporting local adaptation.

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**Are diatom populations locally adapted?**

**Sirje Sildever<sup>1</sup>, Josefin Sefbom<sup>2</sup>, Inga Lips<sup>1</sup>, Anna Godhe<sup>2</sup>**

<sup>1</sup>Marine Systems Institute, Tallinn University of Technology, Tallinn, Estonia; <sup>2</sup>Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden

**Abstract:** Previous studies have shown that phytoplankton from neighbouring marine areas can be genetically differentiated without apparent dispersal barriers. We designed an experiment to investigate if local adaptation, and thus native competitive advantage exists between differentiated diatom populations. Environmental influence to the growth of *Skeletonema marinoi* was investigated by using strains from Mariager Fjord, Denmark and from Kattegat, Baltic Sea. It is previously known that those populations are genetically different, although mixing between them is enabled. Thus, we hypothesized that the strains are adapted to their place of origin, therefore exhibiting higher fitness in the water collected from their native habitat. In order to test this, 10 strains from both locations were grown in native and foreign water to investigate potential differences in fitness, evaluated based on maximum biomass yield. The results supported the hypothesis as all the strains from Mariager Fjord showed significantly decreased yield in foreign water ( $p < 0.05$ ,  $n = 10$ ) and similar pattern was also shown by the strains from Kattegat ( $p < 0.05$ ,  $n = 5$ ). In addition, yield was significantly higher for the strains originating from Mariager Fjord both in native and foreign water compared with strains from Kattegat.



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### Discovering grounds for the protistan species maximum in the horohalini- cum of the Baltic Sea

Irena Telesh<sup>1</sup>, Hendrik Schubert<sup>2</sup>, Sergei Skarlato<sup>3</sup>

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**Abstract:** The recently discovered paradoxical protistan species maximum in the horohalini- cum of the Baltic Sea (Telesh et al., 2011) revealed an inverse trend of microplankton species number/salinity relation, if compared with the previously accepted species-minimum model for macrozoobenthos (Remane, 1934). We reviewed the long-term data and the present knowledge on biodiversity of organisms of different size classes, taxonomic and ecological groups in the Baltic Sea salinity gradient. Our findings disclosed the facts that eukaryotic microorganisms and some prokaryotic microbes (e.g. Cyanobacteria) in the Baltic plankton demonstrated the maximum species richness in the challenging zone of critical salinity 5-8 psu, whereas the bacteria exposed neither minimum nor maximum number of the operational taxonomic units (OTUs) in this environment. These results mismatch the data on the large-bodied bottom dwellers (macrozoobenthos, macroalgae and aquatic higher plants) that experience the pronounced salinity stress, especially at 5-8 psu, which leads to the impoverished diversity of the latter groups in brackish waters. We analyzed possible reasons of this peculiar microbial diversity pattern and proposed a new conceptual model to explain why the diversity of small, fast-developing, rapidly evolving unicellular planktonic organisms benefits from the relative vacancy of brackish-water ecological niches and impaired competitiveness therein (Telesh et al., 2013). The ecotone theory, Hutchinson’s Ecological Niche Concept, species-area relationships and the Intermediate Disturbance Hypothesis are considered as a theoretical framework for understanding extinctions, speciation and variations in the evolution rates of eukaryotic microbial species in brackish-water ecosystems with the pronounced salinity gradient. Recent molecular data, e.g. the experimental studies of cell cycle characteristics, DNA and RNA synthesis, and HSP expression under salinity stress allow revealing the specific cellular and molecular mechanisms which underpin fast and effective adaptations of eukaryotic microorganisms in the changeable brackish-water environment. The work was funded by RFBR grant 13-04-00703, grant 5142.2014.4 for the Leading Scientific School on Production Hydrobiology, the RAS Programs “Biodiversity” and SPbSC 2013-2-10, and the IB/BMBF grants RUS 09/038, 01DJ12107. References: Telesh I.V., Schubert H., Skarlato S.O., 2011. Revisiting Remane’s concept: evidence for high plankton diversity and a protistan species maximum in the horohalini- cum of the Baltic Sea. *Marine Ecology Progress Series*, 421: 1-11. Telesh I.V., Schubert H., Skarlato S.O., 2013. Life in the salinity gradient: Discovering mechanisms behind a new biodiversity pattern. *Estuarine, Coastal and Shelf Science*, 135: 317–327.

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### Wednesday 19<sup>th</sup> November 2014

#### Plenary lecture:

### **Microbial food webs and carbon cycle in the Baltic, any significant changes in a discourse during last decades?**

**Veljo Kisand**

Institute of Technology at University of Tartu, Tartu, Estonia

**Abstract:** As an introduction I will give a short overview about development of the concepts and terminology from more simple to more complex, sometimes back to simplifications to stress important phenomena in interactions between plankton organisms and aquatic carbon cycle. How the linear food chain was implemented with microbial loop, how microbial loop complicated into microbial food webs, back again - viral shunt? I will discuss whether still simple “black box” type approach is useful on high wave of richness and diversity studies? Is that reflected in better understanding of degree of respiration i.e. efficacy of trophic levels?

The Baltic Sea is a huge transient system between marine and freshwater systems different from most of much more open coastal regions. It has considerable latitudinal (different climate, ice cover etc), generic spatial (salinity gradients) and temporal (seasonal) variability. There are several basins, each basin harbors two or more compartments with distinctive properties. The microbial loop concept for oxygenated surface layer was introduced to the Baltic Sea studies in late 80ies early 90ies, and with ~10 years it developed into quite well established framework of the microbial food web processes. Only viruses, studied in the Baltic Sea as early as in beginning of 90s, was not fully recognized link in this concept, sometimes brought in light, sometimes not. It included following knowledge:

- Seasons: spring, inorganic nutrients, growth of phytoplankton, larger organisms, increased rates of sedimentation, low importance of microbial food web. Summer, development of higher trophic levels, regeneration of DOM and microbial food web becomes more important. Autumn, temperature declines, wind driven mixing events and larger algae may have slight advantage. Winter, low autochthonous primary production; ice cover in several regions, plankton abundance and biomass declines.

- Basins: the Bothnian Bay is a net heterotrophic, strongly influenced by humic DOM, low phosphate. Primary production increases from north to south, along that nitrogen limitation becomes more important towards south and in anthropogenically polluted bays in north-east (GoF, GoR).

One common prediction is that climate change brings the warmer and more humid conditions to the Baltic Sea region. Temperature is quite closely related to respiration rates of the whole plankton.

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More precipitations might change the salinity gradient together with different substrate supply which may modulate changes in growth and growth efficiency of bacteria. This could lead, with amplifying effect by increased respiration, to higher heterotrophy of the system and potential increase threat of hypoxia.

Changes in land use i.e. increase of intensity in forestry and agriculture of so far pristine regions in boreal regions should be also not neglected.



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### Session 2 - “Microbial food webs and carbon cycling”:

#### Spring bloom community change modifies carbon pathways and C : N : P : Chl-a stoichiometry of Baltic Sea material fluxes

**Kristian Spilling<sup>1</sup>, Anke Kremp<sup>1</sup>, Riina Klais<sup>2</sup>, Kalle Olli<sup>2</sup>, Teresa Camarena<sup>1</sup>,  
Tobias Lipsewers<sup>1</sup>, Timo Tamminen<sup>1</sup>**

<sup>1</sup>Finnish Environment Institute, Helsinki, Finland; <sup>2</sup>University of Tartu, Tartu, Estonia

**Abstract:** Diatoms and dinoflagellates are major bloom-forming phytoplankton groups competing for resources in the oceans and coastal seas. Recent evidence suggests that their competition is significantly affected by climatic factors under ongoing change, modifying especially the conditions for cold-water, spring bloom communities in the Baltic Sea. We investigated the effects of phytoplankton community composition on spring bloom carbon flows and nutrient stoichiometry in multi-year mesocosm experiments. Comparison of differing communities showed that community structure significantly affected C accumulation parameters, with highest particulate organic carbon (POC) build-up and dissolved organic carbon (DOC) release in diatom-dominated communities. In terms of inorganic nutrient drawdown and bloom accumulation phase, the dominating groups behaved as functional surrogates. Dominance patterns, however, significantly affected C : N : P : Chl a ratios over the whole bloom event: when diatoms were dominant, these ratios increased compared to dinoflagellate dominance or mixed communities. Diatom-dominated communities sequestered carbon up to 3.6-fold higher than the expectation based on the Redfield ratio, and 2-fold higher compared to dinoflagellate dominance. To our knowledge, this is the first experimental report of consequences of climatically driven shifts in phytoplankton dominance patterns for carbon sequestration and related biogeochemical cycles in coastal seas. Our results also highlight the need for remote sensing technologies with taxonomical resolution, as the C : Chl a ratio was strongly dependent on community composition and bloom stage. Climate-driven changes in phytoplankton dominance patterns will have far-reaching consequences for major biogeochemical cycles and need to be considered in climate change scenarios for marine systems.

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### Role of urea in nutrition of dinoflagellates: is it merely a supplementary N source?

**Olga Matantseva<sup>1</sup>, Sergei Skarlato<sup>1</sup>, Maren Voss<sup>2</sup>**

<sup>1</sup>Institute of Cytology, Russian Academy of Sciences, St. Petersburg, Russian Federation; <sup>2</sup>Leibniz Institute for Baltic Sea Research, Warnemuende, Rostock, Germany

**Abstract:** Urea is a well-known nutrition source for many marine microbes including planktonic eukaryotes. Even some photosynthetic species, such as dinoflagellates, possess molecular machinery to utilize this organic substance. It is commonly assumed that urea can be a supplementary nitrogen source for such organisms. However, it is still not clear how dinoflagellates respond to the presence of urea when one of the other, ‘canonical’ inorganic N sources, e.g. nitrate, is also available. This question appears particularly important in the light of extensive eutrophication of coastal waters which results in elevated concentrations of dissolved organic matter. We performed laboratory experiments with the culture of a widely spread dinoflagellate *Prorocentrum minimum*, a successful invasive species of Baltic Sea. Using stable isotope tracers (<sup>15</sup>N and <sup>13</sup>C), we showed that urea may inhibit nitrate uptake when both substrates are present in the medium. In its turn, urea uptake is almost completely inhibited in the night (dark) time along with the uptake of bicarbonate. This fact supports a suggestion that in dinoflagellates urea mainly serves as a nitrogen source for photosynthesis. However, we also received some indications for urea carbon assimilation by *P. minimum*. Although at the present time the ability to use urea as a carbon source does not seem environmentally important due to unlimited bicarbonate availability, it is an interesting physiological finding.

*This work was supported by the RFBR grants 14-04-32146-mol\_a and 13-04-00703-a.*

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### Action of procaryotes on a large-scale precipitation anomaly in the northern Baltic Sea

**Johan Wikner**

Dep. of Ecology and Environmental Science, Umeå, Sweden

**Abstract:** An all-time high precipitation event occurred in the northern Baltic Sea at the millennium shift. The bacterioplankton community was shown to sustain biomass productivity despite markedly lower phytoplankton productivity. The trophic balance consequently shifted to be dominated by carbon flow through the microbial community, leading to a less efficient supply of biomass to higher trophic levels. This was preceded by a 2-year depression in bacterial productivity, indicating a lingering adaptation of the bacterial species composition, transformation of riverine dissolved organic matter (DOM) to bioavailable substrates, or a combination of both. Increase of riverine DOM promoted development of *Flavobacteria* sp. that appeared to be immigrants from the riverine environments establishing in the estuarine environment (0.25 % of total abundance). They contributed to import allochthonous carbon and energy to the marine food web. This in a marine community of Actinobacteria,  $\alpha$ -Proteobacteria (summer) and Bacteroidetes (spring) as major phylogenetic groups in the Gulf of Bothnia. Increased ionic strength in the marine environment and photolysis seem to promote increased bioavailability of riverine DOM when entering the estuarine environment. Bacterial respiration was promoted by riverine DOM with high C:P ratio and led to high temperature sensitivity ( $Q_{10}$ - value) for this process, especially during winter. Consequently, climate projections with higher precipitation and temperature may cause a net heterotrophic food web, also enhancing respiration and the risk of hypoxia.



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### Food web interactions in the northern Baltic Sea during autumnal phytoplankton bloom manipulated with top predators

**Harri Kuosa**

Finnish Environment Institute SYKE, Helsinki, Finland

**Abstract:** The dynamics of plankton communities in the northern Baltic Sea have been extensively studied from the early 1980's onwards, but fewer have focused on autumnal food web with dominance of higher heterotrophic food web, e.g. zooplankton. The introduction of American comb jelly, *Mnemiopsis leidyi*, in the southern Baltic Sea has questioned whether it can expand further north from its current distribution and whether it competes with *Aurelia aurita* in controlling zooplankton abundances. To evaluate the impact of gelatinous top-predators on the brackish water plankton food web during autumnal phytoplankton bloom we carried out a 6-day mesocosm experiment and manipulated our experimental units with *M. leidyi* and *A. aurita*. During an intensive growth of the euglenophyte *Eutreptiella gymnastica* we recorded strong grazing by jellies and strong control on the lower food web by protozoans, but very little of direct cascading impact caused by gelatinous top-predators on the mesozooplankton food web. The cascading effects were nonexistent at the bacteria-microbial loop level. The results indicate some top predator effects on nutrient dynamics, which appears to be the main mechanism throughout the food web in our experiment.

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### The phytoplankton community composition during the spring bloom affects bacterial productivity in the Baltic Sea

**Maria Teresa Camarena Gomez**

Finnish Environment Institute (SYKE), Helsinki, Finland

**Abstract:** In the last decades, a shift from diatom to dinoflagellate dominance in the phytoplankton spring bloom community has been observed in parts of the Baltic Sea. This shift in the community may affect the vertical flux of organic nutrients and alter the trophic interactions of the microbial food web. There is a clear connection between phytoplankton community composition and secondary bacterial production by the quality and quantity of the release dissolved organic matter. In this study we tested if the phytoplankton species composition affects bacterial production, which potentially could have important consequences in the biochemical cycling and the fate of the new production.

We carried out a microcosm experiment, with natural water collected from SW Finland in spring 2013. The water was collected at the ice outside Tvärminne Zoological Station. The experiment was set up using 4 different treatments, each with 3 replicates. The treatments were: natural community (control) and three treatments with addition of a small inoculum of phytoplankton monocultures: *Achnanthes taeniata* (diatom), *Thalassiosira baltica* (diatom), and *Biecheleria baltica* (dinoflagellates). Inorganic nutrients were at a wintertime high ( $\text{NO}_3 \sim 100 \mu\text{g L}^{-1}$ ,  $\text{PO}_4 \sim 20 \mu\text{g L}^{-1}$ ), and no further additions were made. Primary production and bacterial production (bacterial DNA production and bacterial protein production) were determined in addition to inorganic nutrients and particulate organic nutrients

A phytoplankton bloom developed to a chlorophyll a max of  $43\text{-}56 \mu\text{g L}^{-1}$  (day 15). After the inorganic nutrients were completely depleted (day 18), the temperature was increased from  $4^\circ\text{C}$  to  $9^\circ\text{C}$ , and the quickly sedimented material was removed on day 22. There were two peaks of primary production. The first peak appeared in the day 13 of the experiment and reached  $70 \mu\text{g C L}^{-1} \text{ h}^{-1}$  in the control treatment. The second peak occurred after the temperature increased and was between  $20\text{-}45 \mu\text{gC L}^{-1} \text{ h}^{-1}$ , being the highest value in the *B. baltica* treatment. The bacterial production was highest in the treatments with *A. taeniata* dominance ( $\text{BPT} = 1.88 \pm 0.113 \mu\text{gC L}^{-1} \text{ h}^{-1}$ ,  $\text{BPL} = 2.71 \pm 0.67 \mu\text{gC L}^{-1} \text{ h}^{-1}$ ) than in the treatments with *B. baltica* dominance ( $\text{BPT} = 0.95 \pm 0.089 \mu\text{gC L}^{-1} \text{ h}^{-1}$ ,  $\text{BPL} = 0.99 \pm 0.040 \mu\text{gC L}^{-1} \text{ h}^{-1}$ ). The control (diatom dominance) and *T. baltica* presented intermediated values. The dissolve organic matter results were relativity constants during the experiment.

Even the peak of chlorophyll a was the highest in *B. baltica* treatment, the bacterial production achieved in the *A. taeniata* treatment were the greatest values. This suggests a better quality and quantity of the dissolve organic matter excreted by diatoms species. The high constant values of dissolve organic matter suggest a refractory quality of the organic matter inaccessible for the

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bacterial community, typical for the Baltic Sea. Once the phytoplankton community die, the labile organic matter is rapidly remineralized by bacteria. This experiment point out that the phytoplankton species composition affects bacterial production, which in turn modifies the cycle of organic carbon.



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### Dynamics of main nanophytoplankton groups in brackish, coastal waters of the Gulf of Gdansk (Baltic Sea)

Kasia Piwosz<sup>1</sup>, Jörg Villiger<sup>2</sup>, Jakob Pernthaler<sup>2</sup>

<sup>1</sup>National Marine Fisheries Research Institute, Gdynia, Poland; <sup>2</sup>Limnological Station, University of Zurich, Zurich, Switzerland

**Abstract:** Nanophytoplankton (3-20 µm) is important contributor to total phytoplankton biomass, especially after the phytoplankton bloom. However, the composition of phytoplankton communities, and dynamics of specific groups remains poorly investigated. We investigated diversity, community composition and dynamics of nanophytoplankton groups by Catalysed Reported Deposition-Fluorescence in situ Hybridization and high throughput sequencing. Samples were collected weekly from mid-April till November 2012 at a coastal station in the Gulf of Gdańsk (southern Baltic Sea, salinity 6-7). Our aim was to identify groups of mixotrophic algae present in the Gulf of Gdańsk, assess their importance in nanophytoplankton assemblages, and to elucidate environmental factors that best explain the variability in the community composition of mixotrophs.

Chlorophytes, haptophytes, cryptophytes, pedinellids and chrysophytes contributed up to 100% to the total abundances of nanophytoplankton. Chlorophytes were the most numerous of the investigated groups, but due to their small size their contribution to total nanophytoplankton biomass was similar or lower than of the other groups. 86 OTUs were affiliated with chlorophytes, and these included members of Chlorophyceae (e.g. *Chlamydomonas* sp., *Chlorococcum* sp.) Mamiellophyceae (e.g. *Micromonas pusilla*, *Ostreococcus tauri*), Trebouxiophyceae (e.g. *Chlorella* sp., *Nannochloris* sp., *Oocystis* sp.), and others. Haptophytes and cryptophytes were the second most numerous groups, exhibiting a clear seasonal succession. Haptophytes were abundant from end of May till end of July, when they were key contributors to algal biomass at that time. Sequences well represented in the libraries affiliated with *Prymnesium* sp., *Chrysochromulina* sp., *Haptolina* sp. and a coccolithophorid *Ochrosphaera* sp. Cryptophytes were abundant from beginning of July till mid-October. They substantially contributed to algal biomass from August until mid-October. Only 10 OTUs affiliated with cryptophytes, mainly with *Teleaulax* sp. and unclutred group P1-31. Pedinellids formed two peaks of abundance and biomass: in May and September. Only 7 OTUs fell into pedinellids, including heterotrophic *Petridomonas* sp., and phototrophic *Pseudopedinella elastica* and *Apedinella radians*. Surprisingly, phototrophic chrysophytes were at the detection limit through the whole sampling period.

Our results showed high temporal dynamics of different groups and species within the nanophytoplankton. This likely have an impact on food web dynamics, biogeochemical cycles, and community-level responses to environmental changes, because distinct phylogenetic groups have different physiologies and growth efficiencies. Project no. PSRP-036/2010 supported supported by a grant from Switzerland through the Swiss contribution to the enlarged European Union (project DEMONA: Diversity and Ecology of Mixotrophic Nanoflagellates in the Gulf of Gdańsk).

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Plenary lecture:

### Microbiology and biogeochemistry of pelagic redoxclines of the central Baltic Sea

**Klaus Jürgens**

Leibniz-Institut für Ostseeforschung Warnemünde, Warnemünde, Germany

**Abstract:** The central Baltic Sea constitutes the largest, anthropogenically induced hypoxic area in the world but oxygen-deficient waters are currently expanding also on a global scale due to climate change and eutrophication. Microbial community metabolism in oxygen deficient systems has received considerable interest recently because of the impacts on marine nutrient and energy flow patterns, resulting in biological nitrogen loss, climate active trace gas production, H<sub>2</sub>S detoxification and significant chemoautotrophic production. Recent research, combining “metaomics” data with process rate measurements and physiological and genomic studies on representative isolates has provided mechanistic insights into coupled biogeochemical cycling in different types of oxygen-deficient marine waters and revealed general patterns in global distributions of microbial biodiversity. Our studies on the anoxic basins of the central Baltic Sea, combining different molecular and metaomics approaches with experiments, cultivation and process measurements, revealed that the pelagic oxic-anoxic interface is dominated by chemolithoautotrophic prokaryotes. Several groups can be considered as key players for distinct biogeochemical processes. These include ammonia-oxidizing Thaumarchaeota, chemoautotrophic denitrifying Epsilonproteobacteria and potentially sulfur-oxidizing Gammaproteobacteria. Accounting for up to one fourth of total cell counts in their respective redox zones, these organisms link the carbon, nitrogen and sulfur cycles, and their chemoautotrophic production is the basis of a microbial food web. Close relatives of these groups have been found in marine oxygen minimum zones worldwide. As such, the Baltic Sea represents an ideal model system for an in-depth understanding of the structure and regulating mechanisms of the biogeochemistry and microbiology of marine pelagic anoxia.

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### Session 3 - “Human borne pathogens in the Baltic Sea”:

#### Diversity of integrons in *Escherichia coli* from wastewater and marine water origin

Ewa Kotlarska<sup>1</sup>, Aneta Łuczkiwicz<sup>2</sup>, Artur Burzyński<sup>1</sup>

<sup>1</sup>Institute of Oceanology Polish Academy of Sciences, Sopot, Poland; <sup>2</sup>Gdansk University of Technology, Gdańsk, Poland

**Abstract:** Nowadays, wastewater treatment focuses mainly on parameters that may cause oxygen depletion and eutrophication of the receiving waters. Health aspects are considered only in terms of fecal contamination and evaluated only in bathing areas by monitoring fecal indicators (*Escherichia coli* and *Enterococcus* species). However, other important aspects of wastewater discharge are currently under debate. It is suspected that clinically relevant bacteria and mobile genetic elements can survive the wastewater treatment plant processes and be disseminated in the receiving waters. Thus, domestic and municipal wastewater should be considered in global antibiotic resistance gene dissemination. In this process mobile genetic elements play the crucial role. Among them, integrons are suspected to be the most important.

The objective of this study was to investigate antibiotic resistance profiles in 774 *E. coli* isolates from two local wastewater treatment plants (WWTPs), their marine outfalls located in the Gulf of Gdańsk, the Baltic Sea (Poland), and from major tributary of the Baltic Sea - the Vistula River. In order to evaluate the role of the studied wastewater effluents and tributaries in dissemination of integrons and antibiotic resistance genes in anthropogenically impacted part of the Gulf of Gdańsk, prevalence of class 1 and 2 integrons among *E. coli* isolates resistant to at least one antimicrobial agent was analyzed using PCR. The association between resistance or multiresistance to tested antimicrobials and presence of integrons in *E. coli* isolates was also studied. To assess the diversity of gene cassettes in integron-positive isolates, 35 selected amplicons representing variable region of integrons were sequenced and annotated.

Among all tested isolates ampicillin-resistant *E. coli* were the most frequent, followed by amoxicillin/clavulanate (up to 32 %), trimethoprim/sulfamethoxazole (up to 20 %), and fluoroquinolone (up to 15 %)-resistant isolates. Presence of class 1 and 2 integrons was detected among tested *E. coli* isolates with rate of 32.06 % (n=84) and 3.05 % (n=8), respectively. The presence of integrons was associated with increased frequency of resistance to fluoroquinolones, trimethoprim/sulfamethoxazole, amoxicillin/clavulanate, piperacillin/tazobactam, and presence of multidrug-resistance phenotype. Variable regions were detected in 48 class 1 and 5 class 2 integron-positive isolates. Nine different gene cassette arrays were confirmed among sequenced variable regions, with predominance of *dfrA1-aadA1*, *dfrA17-aadA5*, and *aadA1* arrays.



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In conclusion, data obtained during this study indicate the potential of WWTP’s effluents in spreading of resistance genes in the environment and facilitating horizontal gene transfer of mobile genetic elements and MDR phenotypes in the studied area.



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### Wastewater, riverine estuary and anthropogenically impacted marine catchment basin as a source of drug-resistant and hospital-associated *Enterococcus faecium*

Ewa Sadowy<sup>1</sup>, Aneta Łuczkiwicz<sup>2</sup>

<sup>1</sup>National Medicines Institute, Warszawa, Poland; <sup>2</sup>Gdansk University of Technology, Gdańsk, Poland

**Abstract:** Background: Enterococci, ubiquitous colonizers of humans and other animals, play an increasingly important role in health-care associated infections (HAIs). It is believed that the recent evolution of two clinically relevant species, *Enterococcus faecalis* and *Enterococcus faecium* occurred in a big part in a hospital environment, leading to formation of high-risk enterococcal clonal complexes (HiRECCs), which combine multidrug resistance with increased pathogenicity and epidemicity. The aim of this study was to establish the species composition in wastewater, its marine recipient as well as a river estuary and to investigate the antimicrobial susceptibility of collected isolates. Molecular methods were additionally applied to test the presence of HiRECC-related *E. faecium*.

Results: Two wastewater treatment plants (WWTPs), their marine outfalls and Vistula river that influence significantly the quality of waters in Gulf of Gdansk were sampled to investigate the presence of *Enterococcus* spp. Four-hundred-twenty-eight isolates were obtained, including *E. faecium* (244 isolates, 57.0%), *E. hirae* (113 isolates, 26.4%) and *E. faecalis* (63 isolates, 14.7%); other species (*E. gallinarum/casseliflavus*, *E. durans* and *E. avium*) accounted for 1.9%. Antimicrobial susceptibility testing revealed the presence of isolates resistant to erythromycin, tetracycline, ampicillin, fluoroquinolones and aminoglycosides (high-level resistance), especially among *E. faecium*, where such isolates were usually characterized by multilocus sequence types associated with nosocomial lineages 17, 18 and 78 of this species representing HiRECC, formerly called CC17. These isolates not only carried several resistance determinants but were also enriched in genes encoding pathogenicity factors (Esp, pili) and genes associated with mobile genetic elements (MGE), a feature also typical for nosocomial HiRECC.

Conclusions: Our data show that WWTPs constitute an important source of enterococcal strains carrying antimicrobial resistance determinants, often associated with the presence of MGE, for the recipient water environment, thus increasing a pool of such genes for other organisms. The presence of HiRECCs in wastewaters and marine/river environment may indicate that adaptations gained in hospitals may be also beneficial for survival of such clones in other settings. There is an obvious need to monitor the release and spread of such strains in order to elucidate better ways to curb their dissemination.

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**Friday 21th November 2014**

**Plenary lecture:**

**Modeling microbial response to anthropogenic impacts in the Baltic Sea**

**Bärbel Müller-Karulis**

Baltic Sea Center, Stockholm University, Stockholm, Sweden

**Abstract:** Eutrophication, hypoxia as its direct consequence, climate change, enrichment of toxic pollutants, and food-web changes caused by fishing are major anthropogenic impacts that affect the Baltic Sea ecosystem. The microbial response to these anthropogenic impacts is often not incorporated directly into models in terms of buildup of microbial biomass or shifts in microbial community composition. Instead, Baltic biogeochemical models describe microbial processes implicitly by assuming an imaginary ubiquitous microbial community where substrate turnover depends mainly on substrate concentration, potential inhibitors and ambient temperature. Parameterizations of microbial processes in Baltic Sea ecosystem models also tend to focus on microbial transformations that affect major biogeochemical pathways of nutrients and carbon.

Nitrogen fixation by cyanobacteria, which is a significant nitrogen source in the brackish Baltic Sea ecosystem, is therefore described in detail in all biogeochemical models of the Baltic Sea and mimics the response of nitrogen fixers to temperature and salinity. Other microbial processes in the euphotic zone are treated with less detail and despite the importance of pelagic nutrient turnover for nutrient export from the Baltic Sea and for organic matter sedimentation, biogeochemical models of the Baltic Sea mainly rely on simplified descriptions instead of an explicit microbial foodweb.

The pelagic redoxcline that separates oxic surface from anoxic bottom waters has recently received more attention in Baltic biogeochemical models. Climate change and eutrophication scenario experiments suggest that pelagic denitrification is a major nitrogen removal pathway under increasingly anoxic conditions. In addition, metagenomic studies currently provide more details on ammonia oxidation, denitrification, sulfide oxidation and other microbial transformations at the oxic-anoxic boundary.

Simple implicit parameterizations of microbial processes also dominate model descriptions of organic carbon mineralization, oxygen consumption, nitrogen transformations and phosphorus storage in Baltic Sea sediments. Despite of the importance of sediment processes in Baltic biogeochemistry and even though models are extrapolated to a wide range of carbon supply and bottom water oxygen conditions, microbial processes are described with little detail.

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Metagenomics, metatranscriptomics and metaproteomics presently generate new insights into microbial community composition, their metabolic capacity and expressed metabolic pathways. To analyze the fluxes of metabolites in microbial communities a range of bioinformatics tools are developed. In parallel, first approaches are tested to predict the distribution of metabolic pathways in marine samples and to use genomics information as biogeochemical model validation data. However, a large gap still separates traditional microbial foodweb based models, marine biogeochemical models and bioinformatics tools from providing more realism in simulating Baltic microbial communities and their carbon and nutrient transformations.



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### Session 4 - “Metagenomics of Baltic microbes”:

#### Using metagenomics to find biological indicators and predict biochemical processes in the Baltic Sea

**John Larsson<sup>1</sup>, Bärbel Müller-Karulis<sup>2</sup>, Anders Andersson<sup>3</sup>, Luisa Hugerth<sup>3</sup>, Johannes Alneberg<sup>3</sup>, Jarone Pinhassi<sup>1</sup>, Åke Hagström<sup>1</sup>**

<sup>1</sup>Linnaeus University, Kalmar, Sweden; <sup>2</sup>Stockholm University, Stockholm, Sweden; <sup>3</sup>KTH Royal Institute of Technology, Stockholm, Sweden

**Abstract:** Microbial communities are the primary drivers of biochemical processes such as carbon and nitrogen fixation and the remineralization of nutrients in the sea. Today, high-throughput sequencing allows us to investigate the genetic makeup of these communities and thereby to infer the biochemical pathways at work in various environments. Furthermore, these microbial communities respond rapidly to environmental change and therefore the combined expressed and maintained gene-pools can be viewed as a biological indicators of environmental conditions.

In this project, metagenomic sequence data from samples collected in the Baltic Sea region are linked to environmental data in order to 1) infer a potential novel indicator of good environmental status according to the Marine Strategic Framework Directive (MSFD) named BPI (Biogeochemical Pathway Indicator) drawing on the genetic signal coupled to the nutrient biogeochemistry in the Baltic Sea and 2) improve parameterization of biogeochemical models by matching genetic information on underlying microbial processes.

Here we present results from a large-scale time-series study in the central Baltic Sea where biochemical processes were inferred both in microbial communities as well as in 30 assembled genomes of dominant prokaryotes in the environment. We also compared this dataset to a previous metagenomic survey of 11 sites in a Baltic Sea transect sampled three years earlier. Our results show that changes in several biochemical processes can be intimately linked to environmental conditions, with some processes forming a ‘core’ set of microbial pathways. Analysis of the assembled genomes show that these uncultivated prokaryotes separate into phylogenetic bins with different functional potential. These data increase our understanding of the underlying mechanisms in biogeochemical transformations in the Baltic Sea and can now be used in an effort to improve and tune parameters of Baltic Sea environmental models.

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**A metagenomic analysis of planktonic Actinobacteria and Bacteroidetes in the Baltic proper**

**Luisa Warchavchik Hugerth<sup>1</sup>, Joahannes Alneberg<sup>1</sup>, Anders F. Andersson<sup>1</sup>, Jarone Pinhassi<sup>2</sup>**

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**Abstract:** DNA fingerprinting technologies have previously suggested that Baltic bakterioplankton is composed of a mixture of fresh and salt water species. Here, we have assembled 30 nearly complete genomes from shotgun metagenomics of samples from the Linneaeus Microbial Observatory (LMO) Time-series. Of these, 9 are affiliated to Bacteroidetes and 9 to Actinobacteria, in each case covering several subdivisions. We notice that in these samples, the Actinobacteria are more closely related to freshwater species, while the Bacteroidetes are more closely affiliated to marine divisions. However, we also notice intrinsic brackish signatures connecting our genomes to metagenomes in geographically distant surface waters with similar salinity ranges. We also observe genomic markers of differences in life strategy between these two phyla, with Actinobacteria presenting more complete biosynthesis pathways, while Bacteroidetes are enriched for the transport of macromolecules. Interestingly, these typically free-living Actinobacteria are also enriched in phage and plasmid defence mechanisms as compared to the typically particle-attached Bacteroidetes.



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### Pico - to mesoplankton distribution along the 2000 km salinity gradient of the Baltic Sea

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**Abstract:** Microscopic plankton form the productive base of both marine and freshwater ecosystems and are key drivers of global biogeochemical cycles of carbon and nutrients. Plankton diversity is immense with representations from all major phyla within the three domains of life. So far, plankton monitoring has mainly been based on microscopic identification, which has limited sensitivity and reproducibility, not least because of the numerical majority of plankton being unidentifiable under the light microscope. Next Generation Sequencing (NGS) of taxonomic marker genes (typically rRNA genes) offers a means to identify taxa inaccessible by traditional methods; thus, recent studies have unveiled an enormous diversity of previously unknown plankton. Here, we conducted ultra-deep Illumina sequencing (average 105 sequences/sample) of surface water eukaryotic and bacterial plankton communities along a 2000 km transect following the salinity gradient of the Baltic Sea. The distribution of major plankton species followed expected patterns as observed in monitoring programs, but we also observed novel groups to the Baltic, such as relatives to the coccolithophore *Emiliana huxley* in the northern Baltic Sea. By comparison with microscopic counts of aliquot samples, a high correlation was generally observed between counts of species or genera identifiable under the light microscope and their rRNA gene sequences. Community composition was strongly correlated with salinity for both bacterial and eukaryotic plankton assemblages, highlighting the importance of salinity for structuring the biodiversity within this ecosystem. The deep sequencing also enabled accurate enumeration of highly resolved (>99% similarity) operational taxonomic units, which revealed contrasting distribution profiles also among closely related populations, reflecting niche partitioning into ecotypes. This study provides the first deep sequencing-based survey on both eukaryotic and bacterial plankton in the Baltic Sea.

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### Clustering Metagenomic Contigs using CONCOCT

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**Abstract:** Metagenomic sequencing can successfully produce genome fragments (contigs) from complex microbial communities through assembly, but since the assembly does not reconstruct entire genomes, binning of contigs is necessary. Traditionally, unsupervised metagenomic binning programs have used sequence composition, exploiting k-mer biases between species, and/or coverage differences within a sample. CONCOCT (Clustering cONTigs on COverage and ComposiTiOn) combines these two sources of information and extends them by using patterns of coverage distributions over multiple samples instead of a single one. Using Bayesian statistics to estimate the parameters of a Gaussian Mixture model, CONCOCT can automatically cluster contigs into genomes without the need to fix the number of clusters in beforehand. The performance of CONCOCT will be demonstrated on a real metagenomic data set from the Linneaeus Microbial Observatory time series in the Baltic Sea.

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### Vistula Lagoon a place where fresh and sea waters meet

**Anetta Ameryk, Janina Kownacka, Katarzyna Piwosz, Mariusz Zalewski**

National Marine Fisheries Research Institute, Department of Fisheries Oceanography and marine Ecology, Gdynia,  
Poland

**Abstract:** The Vistula Lagoon constitutes an estuary which is connected with the Gulf of Gdańsk Baltic Sea by a narrow inlet, Baltiysk Strait, allowing water exchange between these two water reservoirs. For this reason the water retention time in the lagoon is much longer than in the neighboring Gulf of Gdańsk. A limited water exchange results in a slight salinity gradient, which in turns provides conditions for co-occurrence of marine and freshwater species, e.g. herring and bream. A similar co-existence concerns also microorganisms but neither occurrence nor activity of planktonic bacteria has been studied in the Vistula Lagoon yet.

We have investigated four bacterial clades: freshwater *Limnohabitans*, *Betaproteobacteria*, LD12 and marine SAR11 in the salinity gradient in the Vistula Lagoon. The monthly sampling was carried out from April till October 2011 at three distant stations in Polish part of the lagoon. Additionally, a single set of sampling took place in the middle of winter from under the ice cover. In addition to the percentage contribution of mentioned bacterial groups (made by CARDFISH method) the environmental background (i.e. temperature, salinity, water transparency, nutrient concentrations, phytoplankton composition, chlorophyll concentrations, dissolved organic carbon, overall abundance, biomass and production of bacteria) has been described.

Bacteria activity, measured with application of  $^3\text{H}$ -leucine incorporation, showed the highest value in July when water temperature reached its maximum whereas total bacterial number and biomass had the highest values in September. The salinity ranged from 0.32 in April in the Elbląg Bay to 3.92 in October at the station located close to the borderline with Russia. Salinity together with seasonality explained most of the abundance variabilities of *Limnohabitans*, *Betaproteobacteria* and SAR11. However, LD12 did not follow such a pattern showing much more complicated dependences.

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### Poster 1:

#### Characterization of a lytic cyanophage that infects the bloom-forming cyanobacterium *Aphanizomenon flos-aquae*

**Sigitas Sulcius<sup>1</sup>, Eugenijus Simoliunas<sup>2</sup>, Juozas Staniulis<sup>3</sup>, Judita Koreiviene<sup>3</sup>, Paulius Baltrusis<sup>2</sup>, Rolandas Meskys<sup>2</sup>, Ricardas Paskauskas<sup>3</sup>**

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**Abstract:** *Aphanizomenon flos-aquae* (L.) Ralfs ex Bornet et Flahault is a filamentous diazotrophic cyanobacterium that causes harmful blooms in temperate fresh and brackish water ecosystems of the Baltic Sea. Although ecophysiology of *A. flos-aquae* is relatively well understood, little is known about the virus control of *A. flos-aquae* population.

We will present a novel cyanophage Vb-AphaS-CL131 that infects and lyses the harmful cyanobacteria *Aphanizomenon flos-aquae* isolated from the Curonian Lagoon (south-eastern Baltic Sea). We will describe general characteristics of the cyanophage, including morphology life cycle and host range. Genome sequence analysis of CL131 will be presented if the data is processed by the time. We will also show virus sensitivity to UV and temperature as well as effect of cyanophage infection on host biomass production and population phenotype.

### Poster 2:

#### Effects of a changing phytoplankton community composition during the spring bloom on biogeochemical cycles in the Baltic Sea.

**Tobias Lipsewers<sup>1</sup>, Maria Teresa Camarena Gomez<sup>2</sup>, Riina Klais<sup>3</sup>, Daniel Perez<sup>4</sup>, Kristian Spilling<sup>1</sup>**

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**Abstract:** The co-occurrence of diatoms and dinoflagellates in high abundances is characteristic for the Baltic Sea phytoplankton spring bloom. A shift from diatoms to dinoflagellates caused by e.g, changing climatic conditions was observed after the 1970's e.g. in parts of the Baltic. We hypothesize that this has direct consequences on biogeochemical fluxes. Diatoms are important in the fixation of atmospheric CO<sub>2</sub> and transport of organic matter (OM) to sediments. Dinoflagellates contribute only a minor fraction of sedimentation flux. Thus, the dominant group directly affects summertime nutrient pools in the pelagic zone and the availability of fresh OM for benthic organisms. Little is known about species specific effects on biogeochemical cycles. In order to investigate the effect of the phytoplankton community composition on primary/bacterial

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production, and bacterial community composition (Cruise 2013), as well as to quantify the carbon transferred to higher trophic levels by the microbial loop (Cruise 2014), two research cruises were conducted. Laboratory experiments have shown that the quality of OM released by phytoplankton is species specific and affects e.g. bacterial production. The aim of Cruise 2013 was to find out if this effect can be seen during the spring bloom in situ. Cruise 2014 was carried out to investigate how the phytoplankton community composition affects the release of dissolved organic matter (DOM) after the bloom and to what extent it is channeled up the food web by microorganisms. Therefore, dilution experiments were performed to estimate grazing rates of ciliates and heterotrophic nanoflagellates feeding on bacteria.

Preliminary results from 2013 have shown that the phytoplankton community was composed of the typical bloom-forming species in the GoF (*Thalassiosira baltica*, *T. levanderi*, *Achnanthes taeniata*, *Chaetoceros wighamii*, *C. holsaticus*, *Skeletonema costatum*, *dinoflagellate-complex*, and *Peridiniella catenata*) and that the dinoflagellate-complex, *Gymnodinium* spp., and cryptophytes dominated in the Baltic Proper. Generally, the autotrophic biomass was low and the dominant organisms were *P. catenata*, *Mesodinium rubrum*, and the dinoflagellate-complex. Due to a significantly higher biomass in the GoF, the gross primary production was also clearly higher compared to the Baltic Proper and Åland Sea in 2013. In the GoF, the size fraction below 10µm contributed 5-20% to the total production, whereas the smaller species were more important in the Baltic Proper (20-40%). Due to the low biomass the primary production was clearly lower in 2014. The microalgae below 10µm contributed majorly to the production (20- >50%) in 2013 as well. Smaller species seem to become more important upon N-depletion. In 2013, the bacterial production was relatively low and uniform amongst different stations. In 2014, the bacterial activity was significantly higher. This indicates that the still ongoing bloom in 2013 did not release bioavailable OM to enhance bacterial growth. At the end of the bloom in 2014, the excretion of fresh OM resulted in higher bacterial production. More detailed conclusions can be drawn once the samples for bacterial community composition and abundances (2013 & 2014), grazing rates (2014), and the phytoplankton community composition and abundances (2013) are analyzed.

### Poster 3:

#### Phycocyanin as a proxy for cyanobacterial biomass

Kevin Vikström<sup>1</sup>, Angela Wulff<sup>2</sup>, Bengt Karlson<sup>3</sup>

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**Abstract:** Cyanobacteria are a vital part of the ecological web of the Baltic Sea. Their innate ability to form massive annual blooms greatly affect the oxygen levels of the Baltic Sea and are therefore a high priority species group for monitoring programs.



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A common way of collecting data are buoys with fluorometers, where chlorophyll a is the most dominant. However, studies have shown chl a to be less effective when applied to cyanobacteria compared to eukaryotic species. This is possibly due to the shading effect of the phycobilisome phycocyanin attached to the chl a molecule. Phycocyanin is a specific pigment to cyanobacteria. This study has applied two phycocyanin fluorometers and has to some extent confirmed the effectiveness of phycocyanin in contrast to chlorophyll fluorometers when applied on cyanobacteria.

### Poster 4:

#### Seasonal changes in bacterial diversity and community structure in Vistula estuary

**Marcin Golebiewski<sup>1</sup>, Joanna Calkiewicz<sup>2</sup>, Simon Creer<sup>3</sup>, Katarzyna Piwosz<sup>2</sup>**

<sup>1</sup>Nicolaus Copernicus University, Torun, Poland; <sup>2</sup>National Marine Fisheries Research Institute, Gdynia, Poland; <sup>3</sup>Bangor University, Bangor, United Kingdom

**Abstract:** Bacterial diversity at three sites differing in salinity was studied with 16S rRNA fragments pyrosequencing. The first site had brackish waters (7-8 PSU), the second one lied in the mixing zone (2-4 PSU, and the third one was located on Vistula river (0 PSU). Samples were collected in April, July and October, 2011 and in February 2012 Diversity appeared to be highest in the mixing zone, freshwater samples were slightly less diverse, and marine diversity was lowest. Lowest diversity was observed in spring at the freshwater and mixing zone sites, and during summer at the brackish site. The values got higher later in the year reaching peak in winter. Proteobacteria and Cyanobacteria appeared to be the most numerous bacterial phyla, with Actinobacteria and Bacteroidetes as less frequent contributors.

### Poster 5:

#### Degradation of crude oil and PAHs by microbial communities of iron-manganese concretions and sediment in the northern Baltic Sea

**Anna Reunamo, Pirjo Yli-Hemminki, Jari Nuutinen, Jouni Lehtoranta, Kirsten S Jørgensen**

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**Abstract:** The ability of iron-manganese concretions to biodegrade crude oil and PAHs was studied for the first time and compared to that of sediment. The aim was to simulate the natural recovery of brackish Baltic Sea bottoms after oil contamination under oxic and anoxic conditions. Concretions and sediment had a very similar capacity to remove petroleum compounds and



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naphthalene. The disappearance rate of crude oil was faster in oxic conditions but half of the added crude oil disappeared also from the anoxic microcosms. Total PAH disappearance was significant, whereas mineralization of  $^{14}\text{C}$ -naphthalene was moderate in all non-autoclaved treatments. Copy numbers of PAH-RHD $\alpha$ -gene increased 10-1000 fold depending on the treatment, being highest in the concretion microcosms. Only few anoxic concretion incubations showed dissolution of Fe and Mn to extend where metal reduction may have been coupled to anaerobic degradation of crude oil compounds. Both concretions and sediment had a diverse and clearly different bacterial community prior to exposure to crude oil. Concretion bacterial sequences were affiliated to bacterial groups previously found from concretions and other metal rich environments whereas sediment bacterial sequences were similar to ones originating from sediments and oil contaminated sites.

### Poster 6:

#### Incidence of multidrug-resistant human-associated bacteria in anthropogenically impacted marine coastal waters

Ewa Kotlarska<sup>1</sup>, Katarzyna Tarasewicz<sup>2</sup>, Aneta Łuczkiwicz<sup>2</sup>, Anna Baraniak<sup>3</sup>

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**Abstract:** The safe, economical and reliable way of wastewater disposal is a principal problem in ecosystems, which are highly anthropogenically impacted, like the Gulf of Gdansk and its shallow western branch - Puck Bay. Due to the limited water exchange between these water bodies and open Baltic Sea, the introduced pollutants remain there for a long time. Antibiotic resistant bacteria and antibiotic resistance genes are considered as novel emerging pollutants in marine environments. Thus, the aim of this study was to achieve a more comprehensive assessment of the drug susceptibility of human-associated enteric bacteria isolated from wastewater treatment plant (WWTP) effluents and their role in dissemination of resistance genes and integrons in the Baltic Sea.

In this study bacteria associated with the human intestine were isolated from two local wastewater treatment plants and their receiving waters: Gulf of Gdańsk (Baltic Sea). Bacteria were isolated according to the procedure dedicated for fecal coliforms. All isolates were biochemically identified and their drug susceptibility was determined. Multidrug resistant isolates were tested for prevalence of integrons and antibiotic resistance genes using PCR. In all samples *Escherichia coli* was a dominant species. However, members of other genera (*Aeromonas*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Shigella*, *Plesiomonas* and *Vibrio*) were also isolated. *Vibrio cholerae* strains were detected only in Puck Bay waters. They were non-pathogenic, lacked the genes involved in cholera enterotoxin production, but they all possessed *int1AVch*. Two isolates of *E.*

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coli were found to produce extended-spectrum  $\beta$ -lactamases (ESBL). Their ESBL type was characterized as CTX-M  $\beta$ -lactamase type, that is produced by nosocomial and community strains of Enterobacteriaceae in different countries.

Data obtained in this study indicated that in general applied wastewater treatment level together with effective dilution of treated wastewater by marine outfalls were sufficient to protect coastal water quality from sanitary degradation (low number of *E. coli* detected in marine waters). Detailed analyses showed however, that human-associated bacteria, even potential pathogens and bacteria carrying antibiotic resistance genes of clinical significance can survive in wastewater and marine water conditions. These findings highlight that further studies are needed to understand the dissemination, stability and transmission of resistance genes in water ecosystems.

### Poster 7:

#### Presence of antimicrobial agents in sediments of Polish coastal zone

**Grzegorz Siedlewicz, Ksenia Pazdro**

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**Abstract:** In the course of last decade antimicrobial agents like antibiotic residues have been recognized as relevant environmental contaminants. These bioactive compounds are detected in low concentrations but their continuous input to the environment and prolonged exposure may strongly affect microbial populations and induce biological effects in nontarget organisms, potentially disrupting ecosystem processes. The main sources of antibiotics in environment are animal farms, agriculture, urban/municipal and hospital wastewaters. Due to the fact that some of the active pharmaceutical compounds are not degraded during waste water treatment, they are released into the environment with effluents. Antibiotic residues may be transported to groundwaters, rivers, and finally to the sea. Seas can be seen as the final sink of the most persistent antibiotic residues, however the availability of data on pharmaceuticals concentration and ecotoxicological properties in marine or estuarine water is still very limited. In our study 14 compounds were identified in samples at concentration levels of a few to tens ng g<sup>-1</sup> d.w. of sediment. The occurrence frequency differ significantly among the compounds. The most frequent compounds were: sulfametoxazol, oxytetracycline, trimethoprim and sulfachloropyridazine. These compounds revealed also the highest concentrations in the sediments collected in the investigated area. Occurrence of the identified antibiotics was characterized by high spatial and temporal variability. The highest concentrations were measured in the Szczecin Lagoon and in the Gulf of Gdańsk.

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In conclusion, the results of performed chemical analyses show contamination of coastal baltic sediment by target antibiotic residues and indicate that these contaminants may affect sedimentary microbial community.

### Poster 8:

#### Short-time fluctuations of diazotrophic cyanobacteria in the Gulf of Gdańsk

Justyna Kobos, Ilona Złoch, Anna Krakowiak, Hanna Mazur-Marzec

Institute of Oceanography, University of Gdańsk, Gdynia, Poland

**Abstract:** Diazotrophic cyanobacteria which dominate the annual summer blooms in the Baltic Sea (*Aphanizomenon flos-aquae*, *Nodularia spumigena* and *Dolichospermum* spp.) cause environmental and sanitary problems. In the 2012, the vertical distribution of the three bloom-forming, filamentous cyanobacteria were studied at two offshore sampling stations and at several costal stations in the Gulf of Gdańsk and Puck of Bay (Baltic Sea). At the same stations the sediment samples were collected and checked for the presence of akinetes. The cyanobacterial biomass, occurrence of heterocysts and akinetes, the shape and length of trichomes as well as environmental parameters were monitored.

Solitary filaments of *A. flos-aquae* and *N. spumigena* were observed in the Gulf of Gdańsk throughout the whole year, being most abundant in summer. *Dolichospermum* spp. were found only during the warmest summer season. The highest biomass of diazotrophic cyanobacteria (1,284  $\mu\text{g dm}^{-3}$  and 1,250  $\mu\text{g dm}^{-3}$ ) in surface water were recorded at station off Hel (12.06.12) and off Sopot (06.07.12). In these samples, *A. flos-aquae* dominated and accounted for over 80% of the cyanobacterial biomass. The large-scale occurrence of filamentous cyanobacteria was recorded in the costal zone only at the beginning of July. During the bloom, *N. spumigena* biomass ranged from 87,140  $\mu\text{g dm}^{-3}$  to 197,110  $\mu\text{g dm}^{-3}$ . Some morphological features of the filamentous cyanobacteria (e.g. the length of filaments, number of heterocysts in filament) depended on the depth and season of the year. In a single filament of *A. flos-aquae*, 1-5 heterocysts were found most frequently. During summer, *A. flos-aquae* with a higher number of heterocysts per filament prevailed in the surface layers of water column. From autumn to early spring, only cyanobacteria devoid of heterocysts were observed, regardless of the depth. The three filamentous cyanobacteria are capable of producing akinetes, but only in *D. flos-aquae* and *D. lemmermannii* the resting cells were often observed. In *N. spumigena*, akinetes were seldom produced in bloom samples. However, under the culture condition used in our experiments, they were able to germinate and form vegetative cells.

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### Poster 9:

#### Limited influence of IS elements in genetic adaptations of Baltic Sea bacteria

**Carl Theoden Vigil-Stenman**

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**Abstract:** Insertion Sequences (ISs) are simple ~1000 bp Mobile Genetic Elements found in bacterial genomes and may occupy up to 40% of some bacterial genomes. ISs are facilitators of genomic rearrangements and gene duplications, thereby providing a mean for an organism to adapt to environmental stresses/changes, and be highly beneficial and support survival. Conversely, the transpositions of ISs within the genome may disrupt crucial genes and threat survival, especially in organisms with already small or streamlined genomes. The ISs multiply in genomes via (occasional irregular) transposition events, as the IS gene consists of an ORF encoding a Transposase, flanked by imperfect repeats. The Transposase protein excises the IS gene from its position and reinserts it in a new location in the genome. Transposition events are particularly common during genome replication and stress. Our recent surveys show that bacteria (particularly cyanobacteria) with larger genomes and in particular symbionts, exposed to extreme living conditions, tend to be heavily colonized by ISs (1). In contrast, tiny or stream-lined bacterial genomes are almost or completely devoid of ISs (2). To get a deeper understanding of to what extent the abundance and activity of ISs in bacteria subject to the unique environmental conditions offered by the Baltic has shaped their existence Sea, we recently initiated a survey of ISs in the massive metagenomic and transcriptomic dataset from microorganisms generated in the MiMeBS program\*. These represent microbes from locations along the entire Baltic Sea salinity gradient.

The expression of the transposase gene (i.e. transcripts) suggests a low activity as these occupy only ~1.5 ‰ of the sequenced transcriptome (781 of the investigated 529910 sequences). Identified transcriptome sequences also ranged considerably in length from below 100 to 1298 bp. Only five transcripts had lengths over 1000 bp and appeared to produce full length transposases. Hence, our data so far imply a low activity of ISs in Baltic Sea bacteria, which in turn suggests that their utility in rapid adaptation to changes (such as climate changes) may be limited, and explains the survival of genomes crowded with ISs. Analyses are however currently being carried out to correlate IS abundance and activity with environmental conditions and species composition.

\*) MiMeBS, Microbial Metagenomics of the Baltic Sea

(1) Ran L, Larsson J, Vigil-Stenman T, Nylander JAA, Ininbergs K, et al. (2010) Genome Erosion in a Nitrogen-Fixing Vertically Transmitted Endosymbiotic Multicellular Cyanobacterium. PLoS ONE 5(7)

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(2) Vigil-Stenman et al. Local hopping mobile DNA implicated in pseudogene formation and reductive evolution in an obligate cyanobacteria-plant symbiosis. (submitted manus)

### Poster 10:

#### Relations between autotrophic, heterotrophic and mixotrophic plankton during spring-summer succession in the Baltic Sea

Inga Lips<sup>1</sup>, Peeter Laas<sup>1</sup>, Madis Metsis<sup>2</sup>, Kadri Antik<sup>1</sup>, Elina Šatova<sup>1</sup>

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**Abstract:** During last 20 years, numerous molecular tools became valuable in plankton diversity studies, and especially for identification and quantification of pico- and nano-sized microorganisms. The dynamics in plankton biodiversity that are often masked by difficulties in species identification when using light microscopy only, can have a different pattern when using the molecular approach. In spring and summer 2012 spatial and temporal variability of bacteria and eukaryotes were analysed from 45 samples taken along cross-section between Estonia and Finland in the Gulf of Finland at 5 m depth. We used massively parallel sequencing of ribosomal small subunit gene and internal transcribed spacer regions on Illumina MiSeq platform. In addition, flow cytometry was used to enumerate different ‘morphotypes’ of pico- and nanoplankton and light microscopy was used to perform the phytoplankton sample analysis in a “traditional way”. From environmental parameters, changes in temperature, salinity and inorganic nutrient concentration were measured. The aim of the study was to characterize the seasonal succession of plankton (bacteria and phytoplankton) from spring to summer in the changing physicochemical environment and to have deep insight to the overall biodiversity of surface layer microorganisms in the study area. The used approach also helped us to identify and quantify the members of heterotrophic eukaryotes (very often neglected during phytoplankton analysis) and to follow the changes between autotrophic, heterotrophic and mixotrophic compartments.

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### Poster 11:

#### Chemical, biological and taxonomical characterization of the Baltic cyanobacteria of *Lyngbya* genus

Anna Pietrasik, Anna Toruńska-Sitarz, Agata Błaszczuk, Justyna Kobos,  
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**Abstract:** Introduction: Most studies on *Lyngbya* genus (Oscillatoriales) were focused on the species occurring in tropical waters. These cyanobacteria were found to be of interest due to the



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production of many bioactive metabolites, including toxins and potential pharmaceuticals. Some of the metabolites are used in clinical trials as antimicrobial, anticancer, cytotoxic, immunosuppressing agents or inhibitors of key metabolic enzymes.

In the Baltic Sea, *Lyngbya* belongs to the unexplored component of the phytoplankton community. So far, no studies into the diversity, ecology or ecotoxicology of the cyanobacterium in the Baltic Sea have been conducted.

In the current work, four *Lyngbya* strains isolated from Puck Bay (southern Baltic Sea) were characterized with respect to their taxonomic position, metabolic diversity and biological activity

**Materials and methods:** Cyanobacteria were isolated from surface waters of Bay of Puck. The four isolates were identified based on phenotypic and genetic features. To characterize the metabolic diversity, the profiles of metabolites were determined using liquid chromatography combined with tandem mass spectrometry (LC-MS/MS). The activity against aquatic organisms was assessed using heterotrophic bacteria (*Enterococcus faecalis*, *Vibrio cholerae*, *Klebsiella pneumoniae*) and the brine shrimp *Artemia franciscana*. In addition, the potential biotechnological application of the Baltic *Lyngbya* was tested using key metabolic enzymes (serine proteases and protein phosphatases).

**Results:** The cyanobacterial strains were identified as *Lyngbya aestuari*. In antimicrobial assays, both stimulatory and inhibitory effects were observed. The extracts from *Lyngbya* CCNP1314 inhibited the growth of the Gram-negative bacterium *V. cholera* and Gram-positive *E. faecalis*. *Lyngbya* CCNP1324 was active against the same strains, but in the case of *E. faecalis* strong stimulation, instead of inhibition, was observed. The effects on the shrimp *A. franciscana* were negligible.

Enzymatic assays showed that *Lyngbya* extracts were inactive against phosphatases, but inhibited the activity of some proteases. The LC-MS/MS profiles revealed high similarity of the strains CCNP1315 and CCNP1316. Generally, cyanobacterial strains with similar profile of metabolite exhibited similar activity in the performed assays.



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