Project acronym: BONUS BLUEPRINT

Project title: <u>B</u>iological <u>L</u>enses <u>U</u>sing g<u>E</u>ne <u>PRINT</u>s

Period covered: from January 2016 to December 2016

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Title and Organisation:

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Preface

The BONUS BLUEPRINT (Biological lenses using gene prints) project started in January 2014 and is running for a total of four years. BONUS BLUEPRINT is funded by the BONUS programme through the European Community's Seventh Framework Programme (FP/2007-2013) under implementation agreement R&I/I3/2012/BONUS made with BONUS, the joint Baltic Sea research and development program.

For the third year of BONUS BLUEPRINT we can again document good progress in all work packages. This includes the finalization of microbial response experiments to salinity, oxygen, and pollutants, experiments on the ecology of N_2 fixing microbes, the successful run of the large scale mesocosm experiment, finalization of various sequencing efforts, the generation of the bioinformatics user interface, as well as the further improvement of integrating sequence information with biogeochemical modeling.

Our work keeps on being highly interactive among work packages. We shared and discussed our results during the second annual project meeting in Helsinki, Finland, in May 2016, which again included members of the advisory board who helped navigating the project into its final phase. BONUS BLUEPRINT members have disseminated their work in further publications and at various conferences. It remains challenging to guide some of the most ambitious project goals and concepts into reality, but the progress documented in this year's report gives us confidence that BONUS BLUEPRINT will ultimately proof highly beneficial for all partners involved as well as the wider community of marine microbial ecologists and monitoring experts.

February 2017
*Lasse Riemann (project coordinator)*
Deniz Bombar (secretary)

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1. Some highlights from year 3

- **Controls = tank 1, 2, 3:** 160 L 100 µm filtered LMO water + 40 L of 0.2 µm filtered LMO water
- **Treatment N+P+Si = tank 4, 5, 6:** 160 L 100 µm filtered LMO water + 40 L of 0.2 µm filtered LMO water + inorganic N, P and Si
  - 12 µM N (NaNO3), addition will be 0.5 L
  - 0.8 µM P (Na2HPO3), addition will be 5 mL
  - 24 µM Si (NaSiO3), addition will be 0.5 L
- **Treatment Humic River = 7, 8, 9:** 160 L 100 µm filtered LMO water + 40 L of 0.2 µm filtered humic river water
- **Treatment Agriculture River = 10, 11, 12:** 160 L 100 µm filtered LMO water + 40 L of 0.2 µm filtered agriculture river water
- **Treatment Cyanobacteria = 13, 14, 15:** 160 L 100 µm filtered LMO water + 40 L of 0.2 µm filtered LMO water + 50 ml Cyanobacterial lysate (Nodularia)

2. Background and Objectives

Prokaryotic microbes are principal drivers of carbon and nutrient biogeochemistry and account for a major fraction of pelagic biomass and productivity in the Baltic Sea. Still, these organisms are neither included among the indicators of environmental status currently in use nor considered as functional entities in biogeochemical models. This flaw has been highlighted by HELCOM and OSPAR in their work to coordinate the development of indicators and determining good environmental status (GES) in the Baltic and North Sea areas. The last decade has witnessed a tremendous increase in the capacity of high-throughput technologies for retrieving and processing genetic information from environmental samples; this has given insights into microbially driven food-web processes and how microbes are affected by environmental conditions. Therefore, for the first time we now have the potential to make integrated use of this analysis capacity in a cost-efficient manner for developing a conceptual and methodological framework for the assessment of ecological status of the Baltic Sea ecosystem based on genetic information related to microbial functions and processes. The BONUS BLUEPRINT project will combine field studies, experiments, next-generation sequencing, bioinformatics and modeling to achieve the overarching objective: to establish a capacity to reliably deduce Baltic Sea environmental status based on indicators reflecting the biodiversity and genetic functional profiles of microbes in seawater samples.

3. Management and Organization

3.1 Management of BONUS BLUEPRINT

The BONUS BLUEPRINT project is organized as an interdisciplinary and multi-national consortium, which combines researchers with exceptional expertise in microbial oceanography and extensive experience with studying the Baltic Sea pelagic ecosystem. BONUS BLUEPRINT researchers merge the competence, geographical distribution around the Baltic Sea, and infrastructure needed to meet the complex challenges associated with the research, output coordination and communication. Co-supervision of students and PostDocs is implemented in order to promote cross-disciplinary communication. Continuous involvement of stakeholders and end-users allows for underway project adjustments and efficient knowledge transfer from the project. Progress and internal communication is ensured by the coordination staff (UCPH) through travel, Emails, Skype meetings, meetings, and the project website (http://blueprint-project.org/). Scientific and organizational decisions are made by the Management Board. The independent international Advisory Board provides input on project progress, management, and dissemination.

3.2 Staff affiliated with the BONUS BLUEPRINT project

University of Copenhagen (UCPH), Denmark

Lasse Riemann, project coordinator, work package 7 leader

Mathias Middelboe

Trine Markussen, postdoc (maternity leave 29.11.2015-23.9.2016)

Marianne Saietz, technician

Ryan W. Paerl, postdoc

Sachia J. Traving, postdoc

Deniz Bombar, postdoc (partly BLUEPRINT)

Elisabeth M. Happel, PhD student

KTH Royal Institute of Technology (KTH), Sweden

Anders Andersson, work package 4 leader

Johannes Alneberg, PhD student

Luisa W Hugerth, PhD student (not BLUEPRINT)

Yue Hu, PhD student (not BLUEPRINT)

Stockholm University (SU), Sweden

Christoph Humborg

Bärbel Müller-Karulis, postdoc

Linnaeus University (LNU), Sweden

Jarone Pinhassi, work package 3 leader

Åke Hagström, work package 5 leader

John Sundh, postdoc

Daniel Lundin, postdoc (not BLUEPRINT)

Christofer Karlsson, PhD student

University of Helsinki (UH), Finland

Kaarina Sivonen

Jonna Teikari, PhD student

Hao Wang, postdoc

Leibniz Institute for Baltic Sea Research Warnemünde (IOW), Germany

Klaus Jürgens, work package 1 leader

Matthias Labrenz, work package 6 leader

Christin Bennke, postdoc

Sara Beier, postdoc

University of Tartu (UT), Estonia

Veljo Kisand, work package 2 leader

Vimala Huchaiah, postdoc

4. Scientific and/or technological results achieved during the reporting period

4.1 WP1: Spatiotemporal variations in the microbial BLUEPRINT of the Baltic Sea

Responsible partner: Klaus Jürgens, Leibniz Institute for Baltic Sea Research (IOW). Email: klaus.juergens@io-warnemuende.de

The overall aim of WP 1 is to characterize the linkages between different environmental conditions and the genetic blueprint (including the taxonomic profile, the functional potential and the specific activities) of microbes in the Baltic Sea along temporal and spatial gradients. Understanding the impact of natural environmental gradients on the microbial activities and functions is a prerequisite to identify later anthropogenic disturbances. The focus in this WP is therefore on the gradients in oxygen (mainly vertical and small-scale) and salinity (mainly horizontal and large scale), two of the most important environmental factors of the Baltic Sea, shaping the bacterioplankton communities.

Whereas the large horizontal salinity gradient of the Baltic Sea has been extensively sampled and analyzed in WP1, we also performed experimental investigations in order to obtain insights into short-
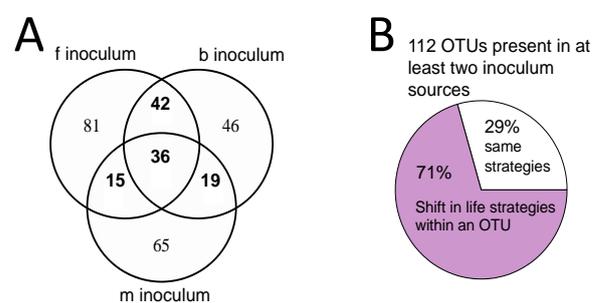

Figure 1: A) Venn diagram illustrating the overlap in Operational Taxonomic Units (OTUs) in the communities that derived from different inocula (freshwater (f), brackish (b), marine specialists and habitat generalists (m). B) proportion of the shared OTUs that changed their strategies or displayed the same strategy among three inoculum sources.

term responses and adaptations to fluctuations in salinity and oxygen concentration.

4.1.1 Microbial response to shifts in salinity

We have proceeded in the analysis of a full-factorial transplant experiment with bacterial communities originating from three salinity regimes of the Baltic Sea (freshwater, brackish, marine), which were reciprocally incubated under each other's environmental conditions.

By clustering abundance patterns of OTUs ("operational taxonomic units"), we identified four discrete life strategies in the experiment: freshwater, brackish and marine specialists (that is, OTUs found with high absolute abundance in one salinity treatment but lower in other two treatments), and habitat generalists (equal abundance in all three salinity treatments).

Life strategies varied among dominant bacterial classes; some classes showed similar strategies independent of inoculum source, while others did not. For example, most β-Proteobacteria were freshwater specialists, whereas Flavobacteria were primarily freshwater specialists (35%, 12 out of 34) if they originated from the freshwater inoculum but generalists if originated from the marine inoculum (42%, 10 out of 24).

We also examined whether specific OTUs were recruited following transplant or whether they varied in their strategies depending on the inoculum source. Of 304 OTUs in total, that were identified as habitat specialists and generalists found in all microcosms, 112 were present in microcosms receiving at least two inoculum sources (Fig. 1a). The remaining OTUs (81 from f inoculum, 46 from b inoculum and 65 from m inoculum) were either absent in other inoculum sources, or too few to be detected according to our filtering criteria. Of these 112 OTUs identified in at least two of three inoculum sources (either f, b or m source), 71% were assigned to a different strategy if they originated from one inoculum source than if they originated from other inoculum sources (Fig. 1b).

Metagenome data from each treatment were assembled and the resulting contigs were annotated using reference genomes from the RefSeq database as well as constructed genomes from Baltic Sea metagenomes that were provided by WP4's Baltic Sea Reference Metagenome (BARM) assembly. Transcriptome sequence reads were mapped on the obtained contigs.

For linking metatranscriptome data to dominant taxa of the different ecological groups, with preferential growth in the respective medium (marine, brackish, freshwater), or to euryhaline taxa with equal growth in all media, we correlated the relative abundance of an OTU and metagenome derived genome bins. By this we could extract contigs that could be assigned to 29 representative OTUs for the different ecological strategies which were identified by growth preference in different media.
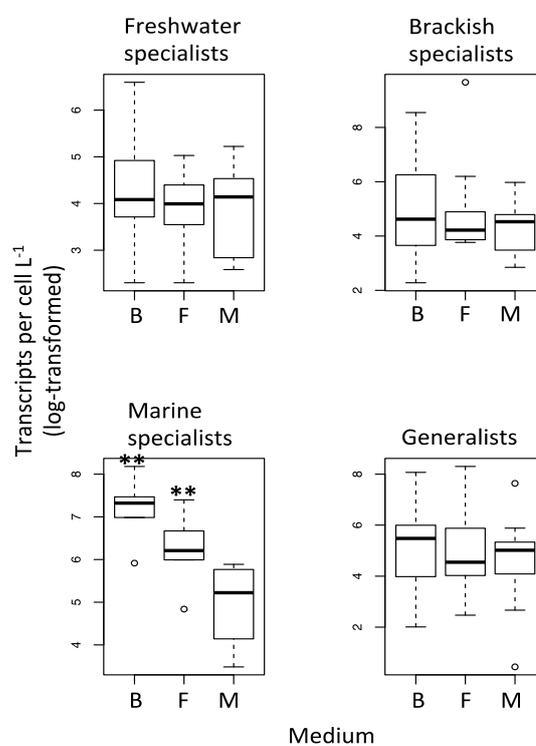
Figure 2: Transcripts per cell of selected OTUs belonging to each of the different "life strategy" groups. F, B and M refer to freshwater, brackish and marine incubation media, respectively.

We hypothesized that cell-specific transcriptional level should be higher in the preferred growth medium. The observed results did not support this hypothesis. In the case of marine specialists, transcriptional activities were significantly higher when grown under the brackish and freshwater conditions compared to marine incubation conditions (ANOVA, P <0.01, Fig. 2). These results demonstrated that taxa with a specific

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habitat preference remained transcriptionally active under adverse conditions.

Future work will focus on taxon- specific response at the gene level. Few representatives from each life strategy group will be selected, and their gene expression profile (functional genes annotated using KEGG database as reference) will be screened across three salinity treatments. This enables us to gain insight into physiological adaption of individual representatives to different salinities.

4.1.2 Microbial response to shifts in oxygen and redox condition

Small-scale mixing events, e.g., due to lateral intrusions or internal waves, occur regularly in the redox transition zones of the central Baltic Sea and presumably affect microbial activities and important biogeochemical transformations (e.g., sulfide oxidation and denitrification).

We continued analyzing an experiment in which we have mixed adjacent oxic (O_2: 0.93 mg L^{-1}) and sulfidic layers (O_2: not detected; H_2S: 0.92 mg L^{-1}) of the oxygen minimum zone in the Gotland Deep during a ship expedition. The treatments were sampled at three different time points after the mixing event (T1: ~1h; T2: ~4h; T3 ~10h) in order to analyse the metatranscriptomes. This should elucidate multiple activity shifts that are predicted to occur due to the mixing of anoxic/sulfidic with oxic water.

The development of total bacterial transcripts (Fig. 3) revealed that bacterial activities in the mixing treatment as well as in the anoxic control significantly increased already after 1 h. Presumably, the anoxic control experience a slight oxygen contamination during the sampling process and therefore behaved relatively similar to the mixing treatment.

We focused the metatranscriptomic analysis on the functional genes related to nitrogen and sulfur

transformations and chemoautotrophic activities. For many genes a significant up- or down-regulation became visible (Fig. 4), indicating a high sensitivity of these metabolic pathways to small-scale mixing events. We are currently extending this analysis to other metabolic pathways in order to obtain a more comprehensive picture of how the mixing of different water masses within the redoxcline is changing the physiology and performance of the bacterial key players.
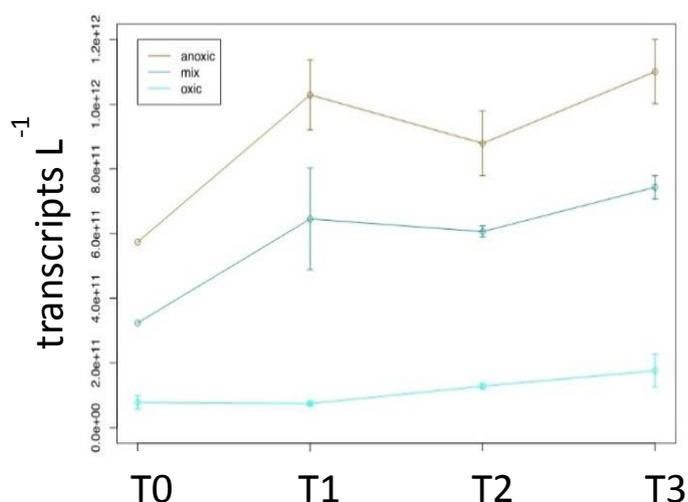
Figure 3: Development of total transcripts in the different treatments of the mixing experiment (mean and range of 2 replicates).

4.1.3 Outlook 2017

In 2017 focus will be on finishing the transcriptomic analyses of the transplant and mixing experiments and on revising and finishing manuscripts based on data from the transplant experiment (2 manuscripts in revision after receiving reviewer comments) and on the mixing experiment (manuscript in preparation).
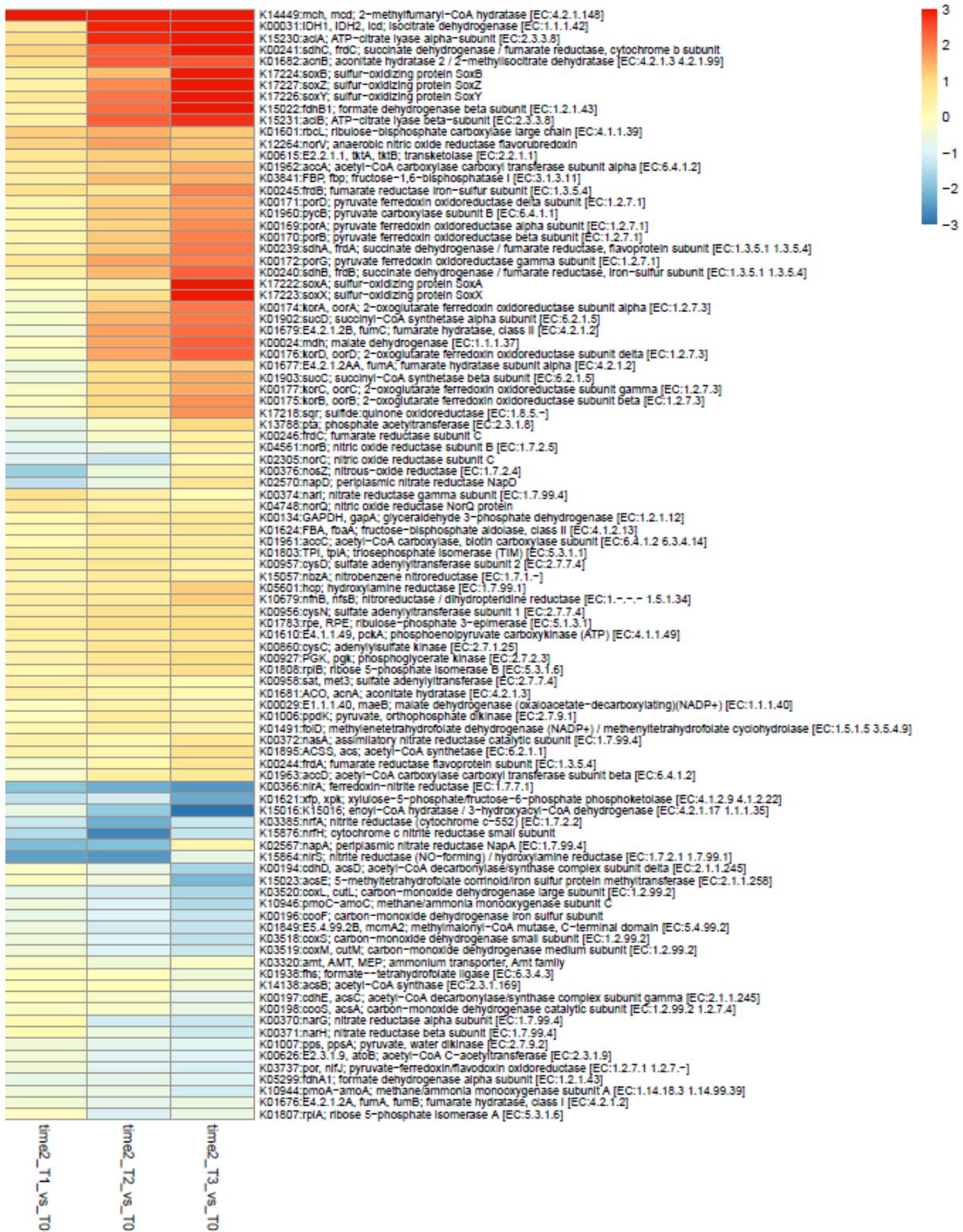
Figure 4: Heatmap of temporal changes in expression for representative functional genes. Expression levels at times T1, T2 and T3 versus T0 are compared. Blue colors refer to down-regulation, orange/red colors to upregulation of genes.

4.2 WP2: Effects of environmental change on the microbial genetic BLUEPRINT

Responsible partner: Veljo Kisand, University of Tartu, Estonia. Email: kisand@ut.ee

4.2.1 Achievements in 2016

In 2016, we completed a number of important milestones for WP2:

(i) the large scale mesocosm (volume 200 L) experiment at Linneaus University (LNU) in Sweden, hosted by Prof. Jarone Pinhassi (WP3 leader)

(ii) finalization of the metagenome sequencing of 54 samples from the 2015 microcosm experiments

(iii) sequencing of samples for microbial community analysis using 16S DNA amplicons; 348 samples in total from triplicate time point samples from both microcosm experiments

(iv) analysis of metagenomes by mapping sequences onto the Baltic Reference Metagenome Database (BARM) and annotating genes using COGs, Pfam, EggNOG, dbCAN and KEGG EC

(v) analysis of community data using the Operational Taxonomic Units (OTU) approach
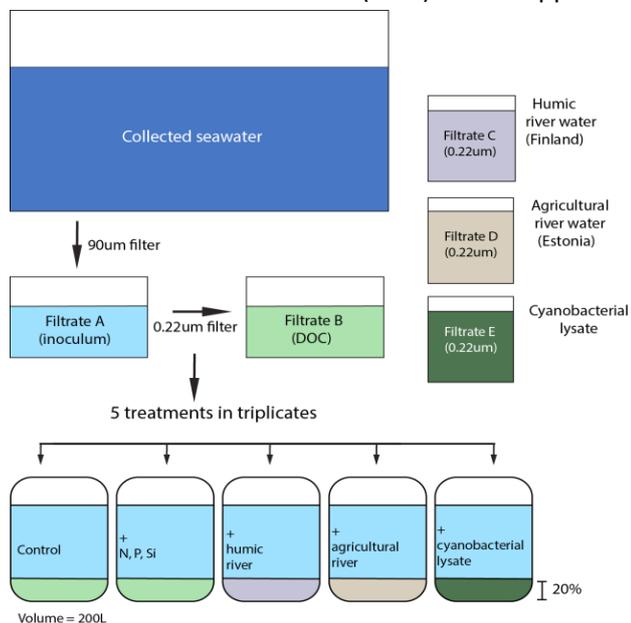
Figure 5: Design of the mesocosm experiment. 200 L batches were incubated in a climate controlled room under simulated *in situ* conditions including a natural light:dark cycle. All treatments and controls were set up in triplicates.

(vi) preparation of a manuscript for publication in a peer reviewed journal: "Biogeochemical process rates in relation to microbial blueprints in indigenous microbial communities under GES/sub-GES conditions"

4.2.2 The mesocosm experiment

The overall aim of this experiment was to link environmental changes due to specific stressors (riverine inputs, phytoplankton blooms, toxic cyanobacteria growth) with changes in bacterial activity (rate measurements), DNA based community composition, and gene abundance and activity (metagenomes and metatranscriptomes). More specifically, the objectives were to:

- Examine if microbial taxa, analysed by 16S rDNA amplicon sequencing, reflect specific environmental conditions.
- Examine effects of environmental drivers on nitrogen fixation (*nifH* gene expression + nitrogen fixation rates) and nitrification (*amo*A gene expression + nitrification rates).
- Examine effects of environmental drivers on bacterial metabolism (C, N, P transporter and metabolic genes, extracellular enzymatic activity).
- Examine to what extent the effects of environmental drivers in small scale bacterial regrowth experiments (i. e. previous microcosm experiments) are affected/modified by food web structure and processes.
- Determine environmental drivers of viral communities and subsequent effects on bacterial community composition.
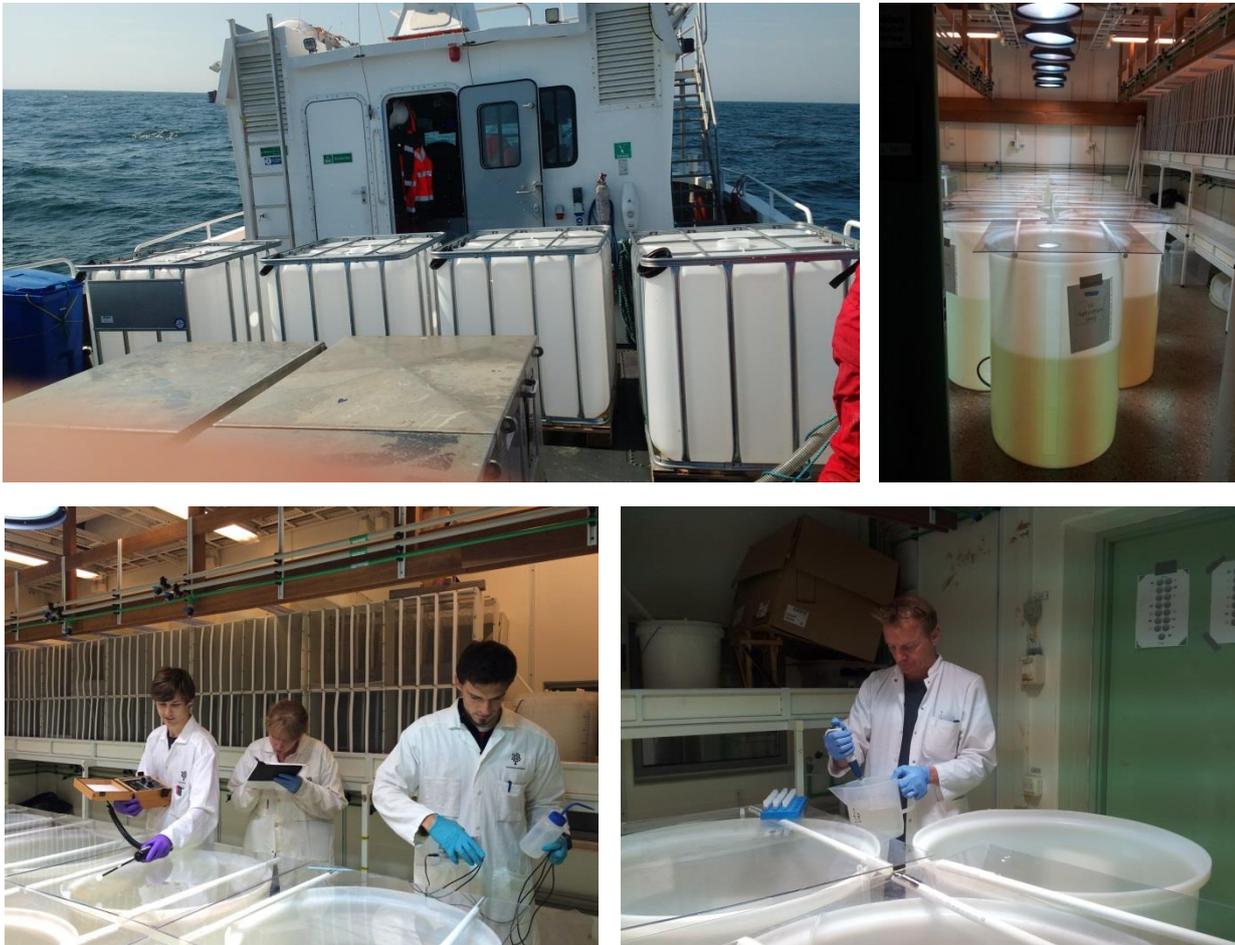
Figure 6: Empty tanks to be filled (upper left); Experiments "ready to roll" (upper right); a young team of PhD students from the host institution monitors irradiance levels (bottom left); Prof. Mathias Middelboe setting up bacterial activity measurements (bottom right).

The experiment was performed from May 30 – June 8 2016 at Linneaus University (LNU), Kalmar, Sweden with participation of 15 people representing 4 work packages. Seawater (ca. 4 m^2) was collected on May 30 at the Linnaeus Microbial Observatory (LMO) site in the Baltic Sea (5655'.51.24"N, 17°3'38.52"E), from 2 m depth, using a peristaltic pump, and transported in several 1 m^3 containers to the LNU within few hours. On May 31, the 15 indoor mesocosm tanks containing ~200 L were set up (Fig. 5+6).

The mesocosm experiment was designed to mimic an algal bloom (NP+Si amendment) in pico-, nano- and microplankton (size fraction <90 um) from the Central Baltic Sea (LMO station). Defined stressors, similar to those chosen in previous small-scale microcosm experiment carried our earlier, were simulated in triplicate mesocosms during the experiment (Fig 5). These included addition of 0.22 μm filtered water from the pristine and agriculturally influenced rivers Lapväärti and Lielupe, respectively. Also, lysate from toxic cyanobacteria was added to one set of triplicate mesocosms.

During the 8 days incubation under simulated *in situ* conditions, sub-samples were collected every 24 h for measurements of bacterial abundance (BA) and production (BP), O_2 consumption, inorganic nutrient and organic matter concentrations, activity of bacterial extracellular enzymes, and a physiological fingerprint (BIOLOG) of the communities. Further, samples were collected to analyze the viral metagenome and specific virus-host systems were isolated at specific time points. For analysis of bacterial community composition, total community metagenomes and metatranscriptomes, the samples were filtered onto 0.22 um filters for later DNA and RNA extraction. RNA extraction was performed immediately after the experiment, whereas the remaining DNA extractions were performed within 2 months. All the DNA samples have since been sequenced and are currently being analyzed.
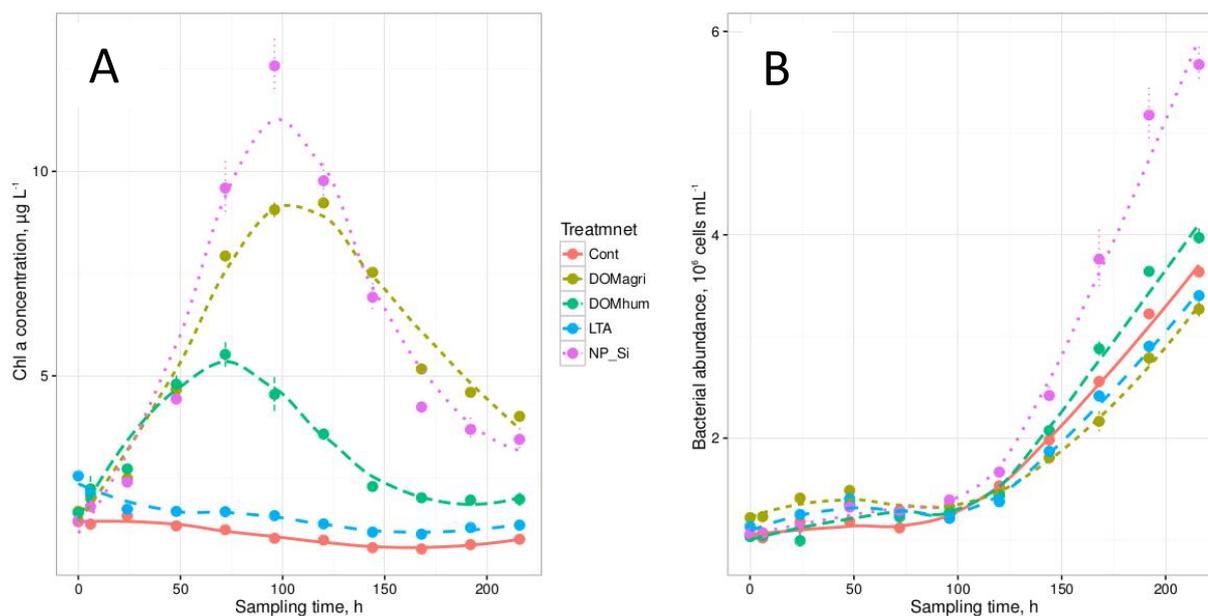
Figure 7: Time course of basic variables in triplicate mesocosms: A) Chlorophyll a concentration; B) bacterial abundance. Cont - controls; DOMhum – fresh water from humic rich river; DOMagri – fresh water from polluted river influenced by agriculture; LTA – cell lysate from cyanobacterial culture; NP_Si – inorganic nutrient addition including silica).

4.2.3 Preliminary results

The algal bloom in the mesocosm with nutrient amendment (directly as nutrient additions or indirectly with river water) developed as predicted (Fig. 7A): the Chl a concentrations reached a maximum within 4 days in mesocosms amended with NP_Si or agricultural river water, while in the mesocosms receiving humic rich river water, the bloom peak occurred a bit earlier. At the same time, no bloom development was observed in the control mesocosms, which did not receive any additional nutrients compared to *in situ* conditions. Bacterial abundances and activities responded to the decaying algal blooms with elevated bacterial density (Fig. 7B), especially in the mesocosms amended with NP_Si.

4.2.4 Outlook 2017

In the coming year, we will focus on finalizing the DNA/RNA sample analysis from the mesocosm experiment, with particular emphasis on the changes in the microbial metagenomes and metatranscriptomes in response to the different stages of algal bloom development and decay as well as to the additions of various DOM sources. All samples for community analysis (PCR amplicons, n=145) will be sequenced at the beginning of 2017. Basic bioinformatic analyses of 15 metagenomes are completed (quality filtering and basic annotation of gene functions) – more detailed analyses of these data will continue. Metatranscriptome samples (n=45) are in the pipeline for sequencing and results are expected within 1-2 months. A production of 3-5 manuscripts based on the mentioned experiments is envisioned for the coming year.

4.3 WP3: Impact of environmental stressors on the BLUEPRINT of model bacteria

Responsible partner: Jarone Pinhassi, Linnaeus University, Sweden. Email: jarone.pinhassi@lnu.se

Work in WP3 uses model marine bacteria to experimentally investigate genetic responses to selected environmental drivers/stressors. The model bacteria are isolated strains of heterotrophic bacteria and photosynthetic cyanobacteria that represent major bacterial lineages in the Baltic Sea. This work package thus complements WP1, WP2 and WP4 in identifying key diagnostic genes indicating principal microbial processes in the Baltic Sea, and works toward experimentally validating the BLUEPRINT approach for assessing environmental status.

4.3.1 Experiments with pollutants

During the third year, experiments on hazardous substances and their effect on Baltic Sea model bacteria have been carried out. Briefly, the bacterial isolates were exposed to hazardous chemicals, namely persistent organic pollutants (POPs), representing classes of compounds widely distributed in marine environments. These included a mix of selected model molecules representing each of the classes polycyclic aromatic hydrocarbons (PAHs),

alkanes (hydrocarbons) and organophosphates (OPEs), perfluorooctanesulfonic acids (PFOSs) and perfluorooctanoic acids (PFOAs). Monocultures of genome-sequenced Baltic Sea model bacteria belonging to the major taxa *Bacteroidetes*, *Alphaproteobacteria* and *Gammaproteobacteria* were grown in artificial seawater medium. The response in bacterial production and abundance were measured after 1 and 2 hours exposure to nanomolar concentrations of the pollutants in the exponential growth phase and the stationary phase (as compared to controls without pollutants) (Fig. 8A). As an example, results showed a significant decrease in bacterial production for the *Alphaproteobacteria* isolate BAL450 upon exposure to mixes of the non-fluorates (PAHs, alkenes and OPEs) as well as to mixes of the fluorates (PFOSs/PFOAs) in the exponential phase after 1 hour and to the non-fluorates after 2 hours (Fig. 8B). In stationary phase, no statistical differences following exposure to pollutants were recorded. These findings indicate that actively
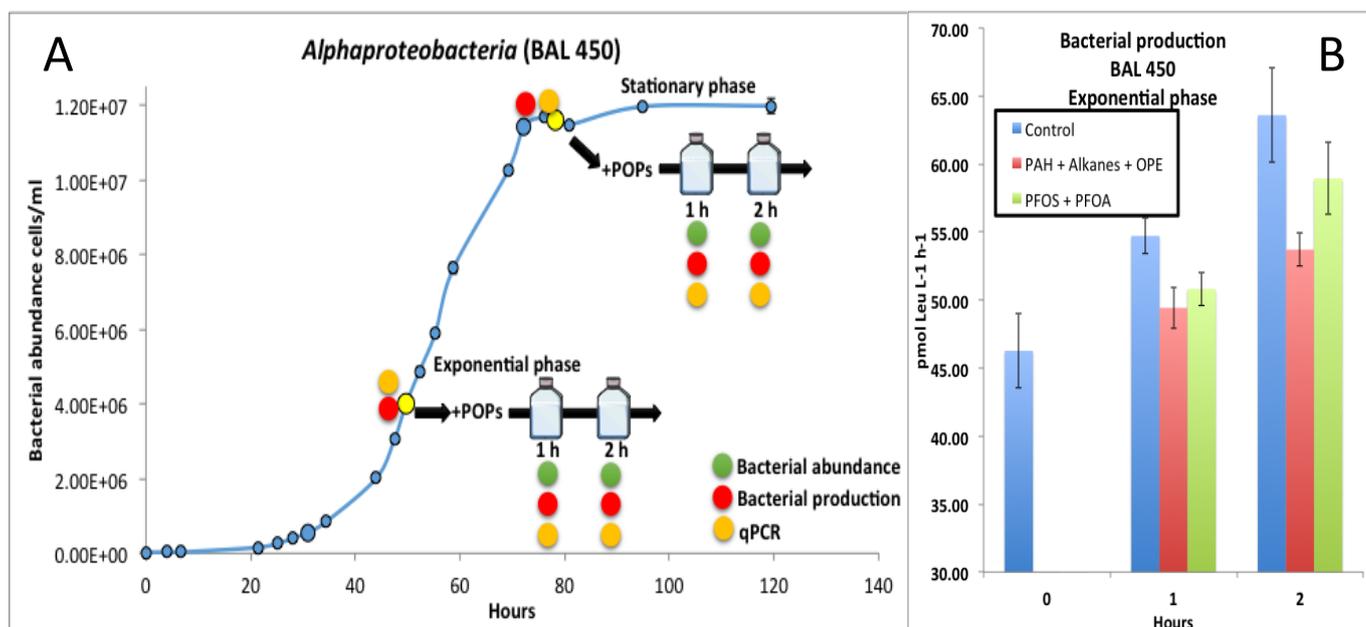

Figure 8: A) Experimental setup for assessing the influence of persistent organic pollutants (POPs) on Baltic Sea model bacteria. B) Bacterial production results from an Alphaproteobacteria isolate (BAL450) in the exponential growth phase after 0,1 and 2 hours exposure to different classes of POPs.

growing cells are more sensitive to hazardous substances than cells adapted to starvation/stress conditions in stationary phase.

To identify indicator genes for the studied pollutants we assayed gene expression patterns in these exposure experiments using two separate approaches. First, samples of RNA where collected to quantify responses in genes related to oxidative stress and different degradation routes for alcohol and ketones (products derived from metabolism of the pollutants) using gene-specific primers in quantitative PCR (qPCR). Second, RNA samples for genome-wide gene expression analysis in the *Gammaproteobacteria* isolate BAL341, in both exponential and stationary phase, were collected and sent for sequencing. Each of these samples generated on average 22 millions reads, and sequencing results are currently under analysis.

4.3.2 Experiments with DOM

To provide information about the results of internal nutrient loading, we carried out experiments on responses of the model bacteria to phytoplankton dissolved organic matter (DOM). This is important since phytoplankton blooms and their associated release of DOM critically determine bacterioplankton species composition as well as bacterial activities. Accordingly, organic matter collected from axenic (bacteria-free) cultures of the diatom *Skeletonema*

Figure 9: Sampling from a short-term bioassay experiment.

marinoi, cyanobacteria *Nodularia spumigena* and dinoflagellate *Prorocentrum minimum* - i.e. the principal bloom forming microalgae in the Baltic Sea - was provided as growth resources to the Baltic Sea model bacteria. This was done in both short- and long-term bioassay experiments (1 h and 80 h growth with different DOM, respectively). Growth and process rate measurements and RNA-seq samples were taken during both experiments and extractions of RNA from these experiments are currently ongoing (Fig. 9). Analysis of the gene expression patterns will provide necessary detail to interpret genetic responses of bacterial communities influenced by environmental stressors and disturbances in algal bloom dynamics.

The availability of phosphorus plays a key role in regulating cyanobacterial blooms in the Baltic Sea Proper. Thus, it is important to investigate the potential to use microbial phosphorus metabolism genes as indicators of environmental status. Previously we have shown that cyanobacteria isolated from the Baltic Sea are able to utilize phosphonates as an alternative phosphorus source. Our recent RNA-sequencing analysis now shows that methylphosphonate is a good source of phosphorus for cyanobacteria. Importantly, presence of methylphosphonate induced expression of a small set of genes mainly related to phosphorus metabolism. Among differentially expressed genes, a gene cluster responsible for phosphonate transport and utilization (*phnC-N*) was highly upregulated in the medium where phosphonate was the sole source of phosphorus. Further analysis using RT-qPCR approach showed that *phnD*, a gene participating in phosphonate transport, was upregulated also under inorganic phosphate scarcity whereas *phnJ* (gene encoding a C-P lyase) was substrate specific and its induction required presence of phosphonate. In addition, based on headspace technique and gas chromatography analysis (Fig. 10), methane, an organic remnant of methlyphosphonate, was released to the environment which may partially explain methane supersaturation in the upper water column in the Baltic Sea. These findings show that analyses of cyanobacterial phosphorus metabolism genes can provide important information to guide assessments of good environmental status.

Figure 10: Analysis of methane concentrations in experiments.

A threshold salinity of 3 PSU shifted the species distributions of cyanobacteria from freshwater *Dolichospermum* sp. to brackish water *Nodularia spumigena*. By analysing transcriptomes in unfavourable salinities we found that, in addition to general stress responses, low salinity triggered reconstruction of cell wall structure and glycogen synthesis in *N. spumigena*. On the contrary, high salinity promoted protein and lipid metabolism in *Dolichospermum* as well as arrested nitrogen fixation. In addition, microcystin production increased in high salinity suggesting its protective role against oxidative stress caused by high salinity. Altogether, the WP3 work on model bacteria shows that environmental stressors are strong triggers of changes in the BLUEPRINT of Baltic Sea bacterioplankton.

The LNU team in WP3 has successfully hosted co-workers from the other BLUEPRINT teams that participated in the WP2 mesocosm experiment in June 2016. Further, the pollutants used in the WP3 work have been used in the frame of WP2 to find links in key indicator genes between the model bacteria and natural communities. The continued bi-weekly sampling of DNA and RNA from the Linnaeus Microbial Observatory (LMO) station in the Baltic Sea has contributed information to WP1 and WP4. Together with WP4 we have shown a strong genetic coherence between genomes assembled from Baltic Sea metagenomic datasets (MAGs) and genomes obtained by the single amplified genome (SAGs) sequencing method; this is important for identifying key diagnostic genes in the Baltic Sea bacteria. We have also continued with bi-weekly discussions with WP5 to provide input for the modeling, to also include novel results from the mesocosm experiment.

4.3.3 Experiments with BAL361

One of the primary targets for the nitrogen (N) - focused research within WP3 is to improve the understanding of N_2 fixation – a key source of new N in the Baltic Sea. We specifically used a non-cyanobacterial N_2-fixing *Pseudomonas stutzeri* BAL361
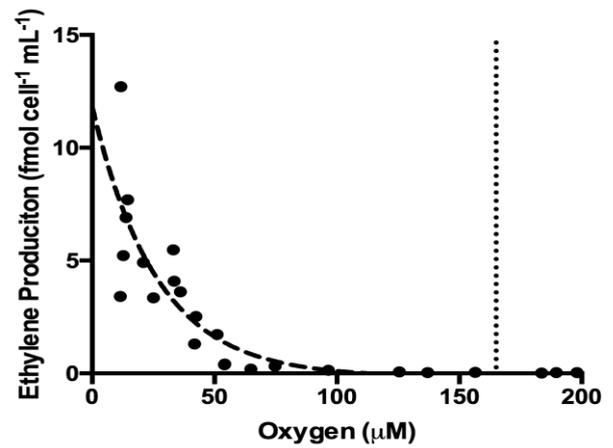
Figure 11: Nitrogenase activity (Ethylene production) of BAL361 cultures is inhibited by oxygen starting ~55 uM. The dashed line marks the previously measured oxygen threshold for ethylene production using washed BAL361 cells initially grown on nutrient rich medium (Bentzon-Tilia et al. 2015).

strain to learn more about oxygen limitation of N_2 fixation by non-photosynthetic N_2 fixers, as well as about interactions between non-cyanobacterial N_2 fixers and organic particles (putative sites of microoxic conditions, acting as an ecological niche for heterotrophic diazotrophs). Thus far very little physiological information is available for non-cyanobacterial N_2 fixers, despite their ubiquity in the environment. We recently reviewed knowledge about this (Bombar et al. 2016).
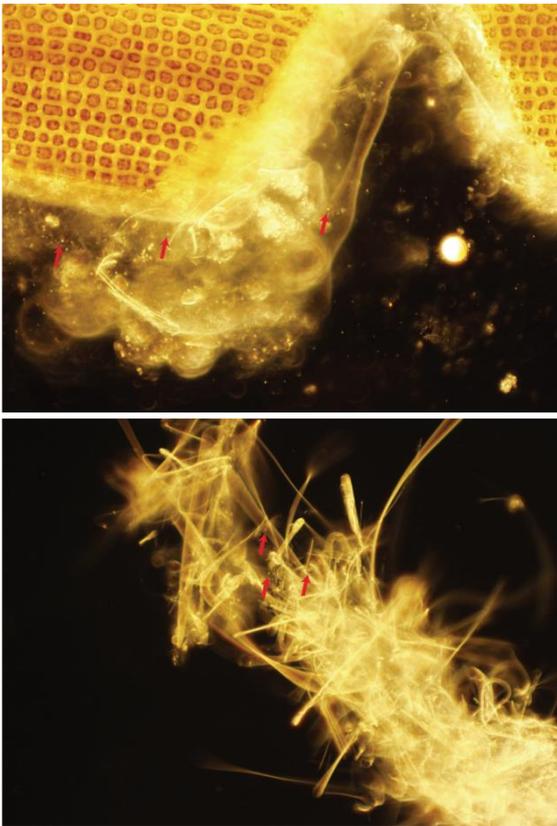
Figure 12: Microscopic images of BAL361 cells (marked by red arrows) associated with a Zostera particle (upper picture) and GF/F particles (lower picture).

Experiments revealed that when BAL361 cultures were maintained as planktonic in low-concentration, large (~1 mm diameter) clusters did not form as observed previously (Bentzon-Tilia et al. 2015) and they exhibited a lower oxygen threshold for nitrogenase activity (proxy for N_2 fixation), ~50-60 µM O_2, than the previously reported threshold (>165 µM O_2) for BAL361 cultures containing clumps (Fig. 11).

The lower than expected oxygen threshold means BAL361 has a reduced capacity to run nitrogenase (and fix N_2) under oxygen exposure than previously recognized. Microbes can generate microoxic zones using particles or surfaces – possibly this strain can advantageously utilize available particle surfaces to drive N_2 fixation under fully oxic conditions. We tested if addition of particles would stimulate nitrogenase activity in fully oxygenated BAL361 cultures using particle types previously shown to stimulate N_2 fixation in natural communities, e.g. whole GF/F filters, shredded GF/F particles, transparent exopolymer particles (TEP) (Fig. 12); surprisingly, no additions stimulated nitrogenase activity (Fig. 13).
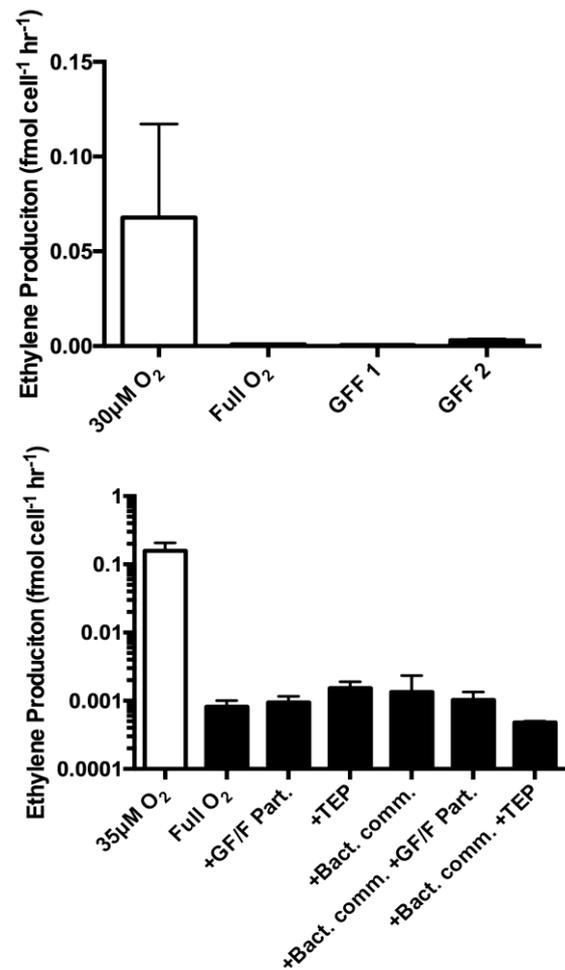
Figure 13: A range of experiments showed that addition of different surfaces or particles did not stimulate nitrogenase activity of BAL361 under fully oxic conditions.

However, when BAL361 was concentrated on GF/F filters (F) and covered with 1% agarose (F+A) – nitrogenase activity could be stimulated under fully oxic conditions (Fig. 14), directly demonstrating that BAL361 cells within a proper 'biofilm' or association with other cells can facilitate nitrogenase activity under fully oxic conditions. We lastly tested if addition of pre-colonized natural *Zostera* (Eel grass) particles could stimulate nitrogenase activity of BAL361 cultures. Results were mixed, in that bulk nitrogenase activity sometimes was stimulated while at other times bulk nitrogenase activity was reduced – speculatively due to competitive or antagonistic interactions with co-occurring bacteria (data not shown). Overall, our results reveal the true complexity of conditions likely needed for BAL361, and potentially its ubiquitous diazotroph relatives, to

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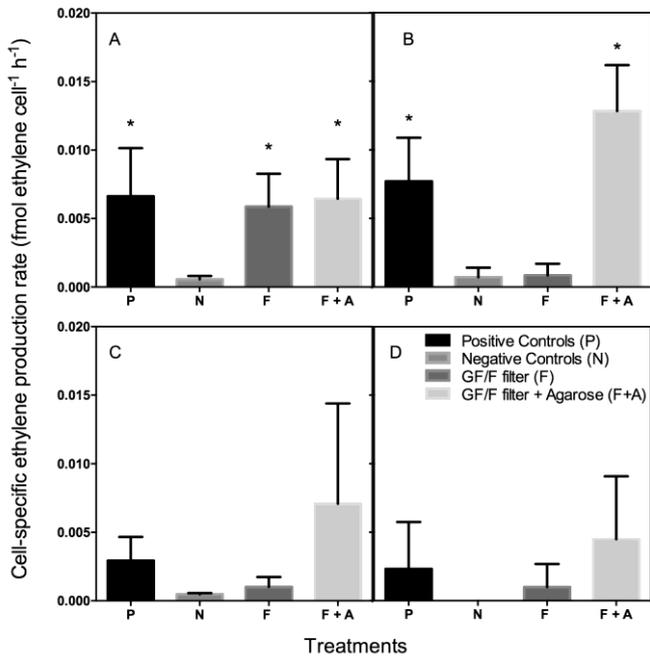
Figure 14: Elevated nitrogenase activity by BAL361 under fully oxygenated conditions occurs only after cells accumulate on a GF/F filter (F) or on a filter covered with a layer of agarose (F+A).

perform N_2 fixation in the water column of oxygenated brackish and marine waters.

4.3.4 Outlook 2017

Analyses of the sequences from the Baltic Sea bacteria experiments with hazardous substances and phytoplankton DOM will be carried out. Statistical analyses of gene expression data will uncover responsive genes in metabolic pathways sensitive to disturbances. For cyanobacteria, two manuscripts will be written and submitted to scientific journals. One manuscript will explore the genomics, physiology and gene expression to changing salinities in *N. spumigena* UHCC 0039 and *Dolichospermum* sp. UHCC 0315. A second manuscript will cover the linkage between phosphorus metabolism and the genetic blueprint in *N. spumigena*. In addition, a PhD thesis entitled "New knowledge on gene expression in toxic and bloom forming Baltic Sea cyanobacteria under changing environmental conditions" will be finalized and defended.

In regards to BAL361 experiments – all experimental data has been collected and is being synthesized into a manuscript for submission to a peer-reviewed journal in Spring 2017.

References

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4.4 WP4: The bioinformatics platform

Responsible partner: Anders Andersson, KTH Royal Institute of Technology, Sweden. Email: anders.andersson@scilifelab.se

4.4.1 Brief description of WP

The Blueprint project aims at predicting the environmental status of a sample based on the taxonomic and functional properties of its microbial community. Work package 4 is responsible for building bioinformatic solutions for converting the metagenomic- and metatranscriptomic sequences into suitable data structures for downstream analysis, for storing this data in a database, and for predicting the environmental status of a sample from the data.

4.4.2 Activities during 2016

One of the resources that the Blueprint project will deliver to the scientific community is a database with processed meta-omics data for the Baltic Sea. Implementing this database and a user-friendly web interphase (deliverable D4.2 *Database containing data on blueprints and environmental data*) was a major task for WP4 during 2016. The data populating the database is generated by mapping metagenome and metatranscriptome samples (i.e. millions of short DNA sequences from these) onto the Baltic Sea Reference Metagenome (BARM) that we generated in 2015. By doing so our bioinformatics pipeline calculates the abundance distribution of functional genes and taxonomic groups in the sample (i.e. the

sample's "BLUEPRINT"). This data is stored in the database that we call BalticMicrobeDB. From its graphical user interphase the user can search for and select one or several functional genes or taxonomic groups and retrieve their abundance profile across a set of samples. The user can chose to visualise the data in a basic graphical layout or in tabular form (Fig. 15). The user can also download the data. The public version (http://barm.scilifelab.se/) only includes data from a published dataset - a time-series from the Linnaeus Microbial Observatory (Hugerth et al. 2015) - but more datasets will be included here as they are being published.

In addition to developing BalticMicrobeDB, WP4 has mapped a number of meta-ome datasets against BARM and calculated their BLUEPRINTS. At this point we have calculated BLUEPRINTS for 149 metagenome and 27 metatranscriptome samples, representing both *in situ* samples and micro- and mesocosm experiments from WP1, 2 & 6. We have also assisted WP2 in conducting more refined functional annotation for selected functional genes, and in retrieving specific BARM sequences for designing primers etc. We have continued the collaboration with WP5 to link enzymes and uptake systems identified in BARM to processes of the BALTSEM model.

Besides the above work related to BARM and the BalticMicrobeDB we have conducted the first study where metabarcoding was used to map overall plankton diversity across the salinity gradient of the Baltic Sea. We simultaneously sequenced bacterioplankton and eukaryotic plankton sampled at 21 stations from Kattegat to the Bay of Bothnia. We could verify previously observed patterns in bacterioplankton community structure along the salinity gradient, but with a higher resolution due to the ultra-deep sequencing applied. For eukaryotes, we verified distribution patterns observed in microscopy-based monitoring programs, but also identified a wealth of previously undetected eukaryotic diversity in the Baltic Sea (Hu et al. 2016).

4.4.3 Outlook 2017

During 2017 we will continue to develop the BalticMicrobeDB including more options in the graphical user interphase and we will populate the database with more datasets. Major goals will also be deliverables D4.3 *Report on signature genes for environmental status diagnosis* and D4.4 *Establishment of software for standardized environmental status monitoring*. In D4.3 the meta-omics data will be used together with statistical and machine-learning approaches to find genes that are indicative of different environmental conditions, and D4.4 software will be developed that takes meta-omics data as input and predicts environmental conditions of the sample. Both of these deliverables will be a joint effort of most WPs within Blueprint and a dedicated workshop for D4.3 will be held in Helsingør, Denmark in May 2017.

BARM - Baltic Sea Reference Metagenome

Filtering Options

General Settings

Function Class
All

○ Filter by a search term
○ Filter by typing in individual annotation identifiers

Start searching by typing here

Number of rows
20

Type Identifier

Add another type identifier   Remove last type identifier

Choose sample group

Sample Group

lmo
transect
redox

View Results   Gene List   Download

Show Annotation Description   Show Sample Information

Type identifier	120314	120322	120328	120403	120416	120419	120423	120507	120516	120521	120531	120604	120613	120619
PFAM00005 external link	3444.1192	3708.7009	1385.3497	1170.5838	1415.8868	2777.0666	1647.2912	2580.1062	1260.1025	3194.2239	1579.4617	2230.1629	1445.6304	2504.8521
PFAM12796 external link	3478.6661	4088.2171	2636.6771	1664.527	1173.3683	2934.3483	1231.2834	748.1689	935.9701	823.2072	1394.3375	1468.026	1416.3713	873.3027
PFAM00106 external link	2418.9052	1633.0429	702.746	595.5408	710.5988	1259.3779	763.2616	1337.4773	628.3598	1485.6915	770.4913	1288.2343	724.9679	1300.7211

Figure 15: An example view of the web interface of BalticMicrobeDB.

4.5 WP5: Incorporation of BLUEPRINT in biogeochemical modeling

Responsible partner: Åke Hagström, Linnaeus University, Sweden. Email: ake.hagstrom@lnu.se

4.5.1 Comment to the 2015 report

From the response to last year's report, given to us by the BONUS secretariat, we will comment in this report on the WP5 deviation from the original work plan and promise. The past year has resulted in major advancements towards the goals of the BONUS Blueprint project. First, the connection between biogeochemical modeling output and metagenome markers has been strengthened. Secondly, a number of molecular indicators of Good Environmental Status (GES) have been outlined. In the following we will present some data and the arguments behind these advancements.

4.5.2 Modeling the Baltic Sea biogeochemistry

Understanding the biogeochemistry of the Baltic Sea is an important platform for the governance of the environment. Thus, any steps that may improve the predictive power of the models used to foresee the results of initiatives such as the Baltic Sea Action Plan (BSAP) are of great value. Therefore, the existing BALTSEM model has been modified to include a more comprehensive role of the bacterial community in particular in the surface layer of the Baltic Sea and the redoxcline of its deep basins.
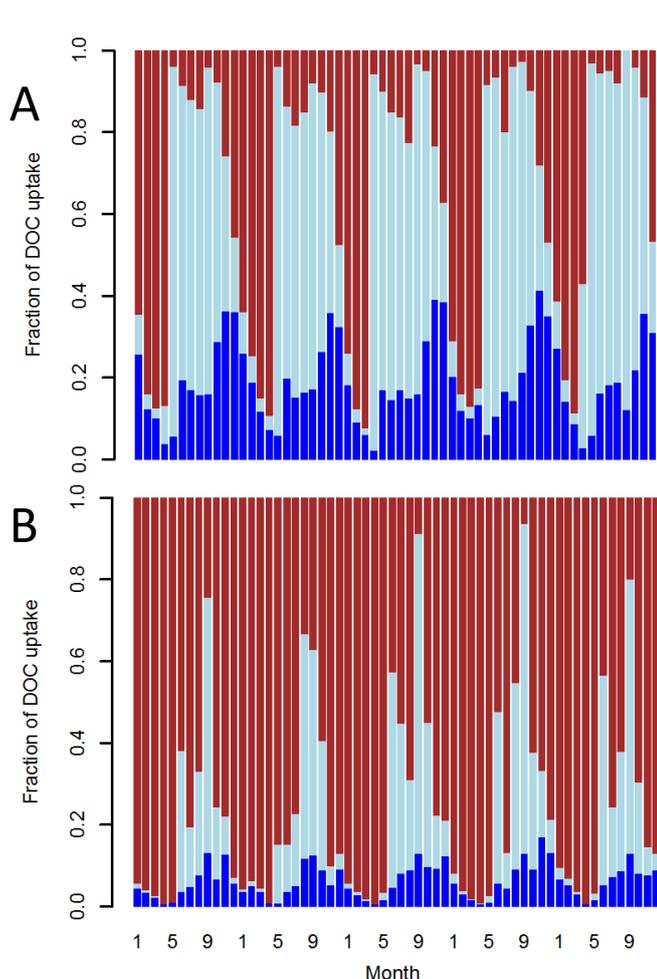
Figure 16: Simulated share of different dissolved organic carbon (DOC) sources in bacterial metabolism in the Gotland Sea and Bothnian Bay (dark blue – protein-nucleotide DOC, light blue – carbohydrate, brown – terrestrial DOC).

The current BALTSEM includes four microbial groups: two groups of organotrophs - aerobs and denitrifiers - and two chemolitoautotroph groups – nitrifiers and sulfide oxidizing denitrifiers. Except for sulfate reduction, all pelagic microbial processes that were parameterized implicitly in the original BALTSEM model, are now explicitly coupled to bacterial metabolism and growth. Microbial growth stoichiometry, growth yields and maximum growth rates were derived from a simplified energetic representation of electron flow in bacterial metabolism.

With these revised features, the BALTSEM model simulates seasonal patterns in the uptake of different organic matter types (Fig. 16), and we have further focused on finding connecting points between the geochemical model output and metagenomic temporal
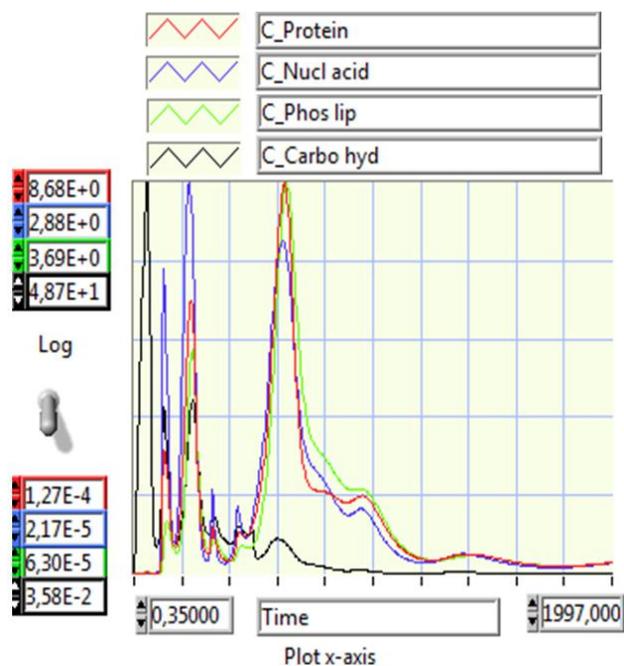
Figure 17: Simulation output showing consumption of a pulse of organic matter in the form of four macromolecules exposed to four different kinds of bacteria having different affinity for the respective transporters handling the monomer of the different macromolecules.

and spatial markers. These represent gene abundance and expression of the surface water biochemistry.

This is in essence the promise made in the original BLUEPRINT proposal, except the specific molecular markers have been extended and modified. The overall priority of WP5 for the final year of the BLUEPRINT project will be to implement Task 5.4."Microbial food web parameterization" by a) adjusting BALTSEM model formulations to the METABALTSEM metabolic landscape, and by b) modifying the response to environmental forcing in BALTSEM according to the response of the blueprint metabolic networks.

4.5.3 Transporter proteins: the link between organism and environment

In a review of the "Ecological significance of transporters in the Baltic Sea microbial foodweb" we have used the Baltic Sea reference metagenome (BARM) produced by WP4 to identify proteins that mediate transfer of organic and mineral nutrients between the environment and the microorganisms, described in a public GitHub repository (https://github.com/johnne/transporters). Briefly, it involves 1) pattern matching of protein family
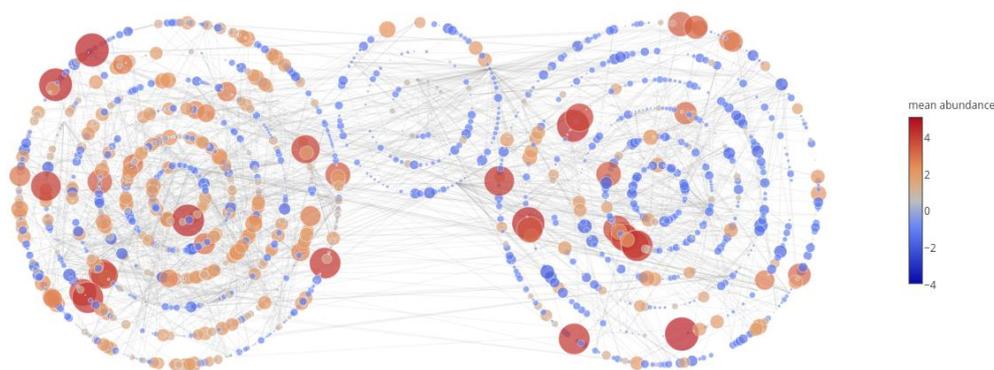
Figure 18: Two-dimensional representation of the metacyc database with abundance values overlaid from a metatranscriptomic sample taken from the LMO sampling site in August 2012. The three large circles each contain pathways (nodes) involved in biosynthesis (left), energy metabolism (center) and degradation (right). Grey lines show connections between pathways. Both the radius and color of the pathway nodes indicate the abundance of the pathway. github.com/johnne/biovisualize/tree/master/metacyc

descriptions to find transporter families in three databases (COG, PFAM and TIGRFAM) and 2) merging of the identified protein families based on cross-referencing and gene operon predictions.

Transporter abundances were analyzed in both metagenomic and metatranscriptomic time-series sampled at the Linnaeus Microbial Observatory (LMO) site, thereby giving estimates of both transporter potential (metagenomes) and usage (metatranscriptomes). The major substrate categories across the analyzed samples were 1) carbohydrates, 2) cations and iron, 3) nitrogen, 4) amino acids and 5) phosphorus compounds. In the P category phosphonate transporters dominated both the metagenomic and metatranscriptomic proportion of the bacterial community transporters. The high-affinity *pst* inorganic phosphate transporter was also among the most abundant in the bacterial community, but mainly so in mid- to late summer. There were apparent taxonomic differences in transport of nitrogen, with a focus on ammonium and nitrite transport among eukaryotes and urea transport in bacteria and cyanobacteria.

Using transporter abundance profiles we divided samples into "sample groups" that reflected conditions before, during and after the typical spring and summer blooms in Baltic Sea surface waters and identified sets of transporters with large differences in abundance between these groups. For instance we found that

different phosphonate transporters were overrepresented during and after the spring bloom as well as during the summer bloom, with different bacterial taxa expressing these transporters. The same was true for TonB-dependent transporters with some transporters being highly expressed during the spring bloom (with expression then dominated by gammaproteobacteria), others mainly during the summer bloom (with expression then dominated by bacteroidetes). We also found that sample clustering based on transporter abundances was largely similar to clustering based on environmental data, which supports the idea that the presence of transporters in the microbial community is tightly linked to the external cellular environment. Correlations between transporter abundance and environmental data showed that most of the strong pair-wise correlations were between individual transporters and temperature and phosphate.

Thus we have managed to find a solution to the 2015 problem of matching metagenomic markers to the biogeochemical transfer of nutrients.

4.5.4 Transporter affinity simulation and basis for annual succession of bacteria

Based on the above compilation of metagenome data on transporters we have initiated a module for simulation of the interaction of bacteria exerting different transporter affinities. In Fig. 17 a pulse of organic matter in the form of four macromolecules is
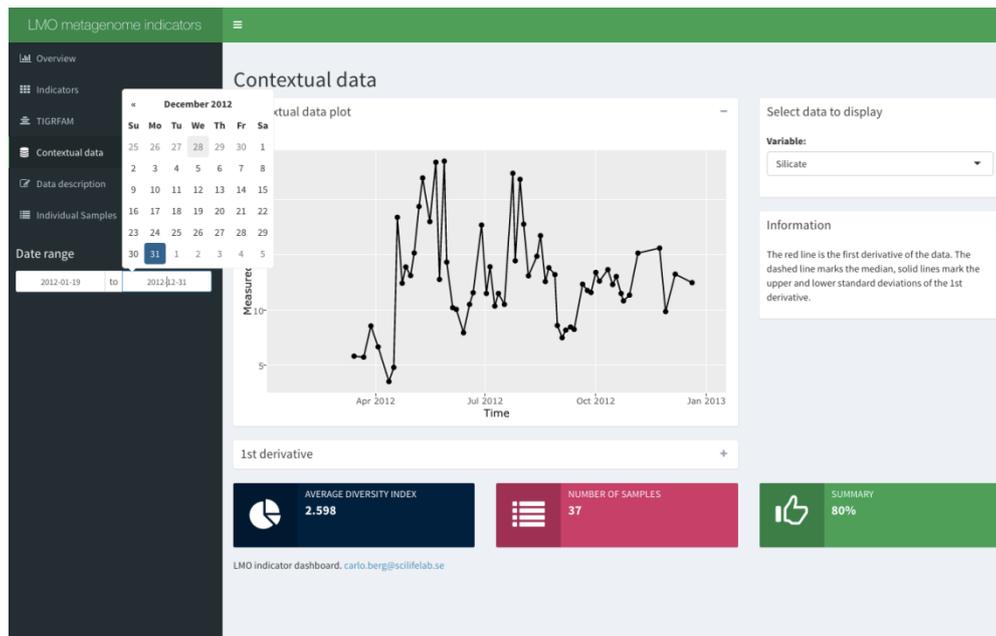
Figure 19: Shiny App webpage interface for assessing ecological indicators. The website is running R Server in the background for computing specific indicator values. This example showing a possible indicator parameter "Average Diversity Index".

exposed to four different kinds of bacteria having different affinity for the respective transporters. The pattern shows a successional utilization of the organic input gradually being respired.

In a review paper "Culture the Marine Microbiome" we have further examined the different strategies of bacteria to proliferate in seawater and the effects of temperature as a major regulator of growth rate. We have demonstrated the importance of the phylogenomic affiliation of bacteria. The different temperature optima for different phylogenetic bacterial groups together with the temporal variation of transporter proteins indicated above adds to the understanding of the annual succession of bacteria in the Baltic, where temperature is a major factor structuring the microbial food web.

4.5.5 Metabolic pathway indicators (MPI)

The work to identify metagenome indicators of GES has focused on two alternative strategies. In the first strategy *"Metabolic pathway visualization"*, the relative weight of the metabolic network is divided into anabolic, katabolic and basic energy metabolism as defined in the Metacyc database (metacyc.org).

In Fig. 18, individual metabolic pathways are represented as nodes and ordered with the most interconnected (or 'central') pathways in the middle of each of the three categories. The pathways were then quantified in the sampled meta-genomes/-transcriptomes and the abundances overlaid onto the corresponding nodes in the visualization. This way, the 'weight' of a particular sample in the 2d-network can be viewed, either interactively to gain information on the exact abundance of specific pathways or exported to flat images as seen above.

The second strategy "*Ecological Indicator Dashboard"* is a more general approach since this is a system for accessing and calculating specific parameters, derived from the metagenomic data, that might be used as GES indicators. This approach provides an easy to use interface, which can be accessed by stakeholders or decision-makers. The data can be browsed and selected and subsets can be downloaded: interactive graphs, heatmaps, networks support visualization, and comparison of different time-points. A current version is available at shinyapps.io: http://cberg.shinyapps.io/indicators/ An example of a suggested indicator parameter is Average Diversity Index based on a large set of metabolic markers (Fig. 19).

4.5.6 Outlook 2017

During the final phase of the BLUEPRINT project we will evaluate candidate indicators based on the performance in a temporal and spatial matrix. In addition the perturbations that have been investigated in the meso and microcosm experiments will be analyzed through the use of the candidate indicators in order to evaluate the final selection of one or more Metabolic Pathway Indicator(s).

4.6 WP6: From mechanistic to functional monitoring – guiding microbial BLUEPRINT into practical operability

Responsible partner: Matthias Labrenz, Leibniz Institute for Baltic Sea Research (IOW). Email: matthias.labrenz@io-warnemuende.de

WP6 focuses on the transfer and integration of new microbial descriptors into existing monitoring procedures. We will merge these aspects by improving, developing, evaluating, and standardizing general and specific workflows to guide BLUEPRINT into practical operability within the BLUEPRINT Competence Center (BCC). The deliverable 6.1 produced in 2016 describes the detailed workflow of the BCC and reports on sampling, laboratory and bioinformatics procedures. It also provides a cost overview per sample for community (taxonomic), metagenome and metatranscriptome analyses (Fig. 20).

During the third year no discussion forum was held, since we focused on the analysis of the amplicon and metatranscriptomic data retrieved from the 2014 cruise.

4.6.1. Using 16S rRNA gene data for analyzing phytoplankton communities

During the 3rd year the 16S rRNA gene (DNA-based) and 16S rRNA transcript (RNA-based) amplicon libraries were analyzed with respect to the usage of a general 16S rRNA primer system to identify phytoplankton in the Baltic Sea as a non-specific molecular tool for monitoring. The bioinformatics analysis was performed using the SILVAngs-pipeline (Quast et al. 2013) as an independent and less laborious tool which is accessible via web interface. All 16S rRNA gene and transcript sequences which were identified as chloroplasts were re-analyzed with the recently published database phytoREF by Decelle and coworkers (2015).

The aim of our study was to determine whether, using phytoREF, the occurrence and distribution of phototrophic eukaryotes could sufficiently be described by 16S rRNA gene and 16S rRNA transcript analysis of *in situ* samples. To examine if the molecular identification of phototrophic eukaryotes is in accordance with microscopic identification phytoplankton > 20 µm were analyzed with both methods, allowing a comparison and evaluation of these different approaches.
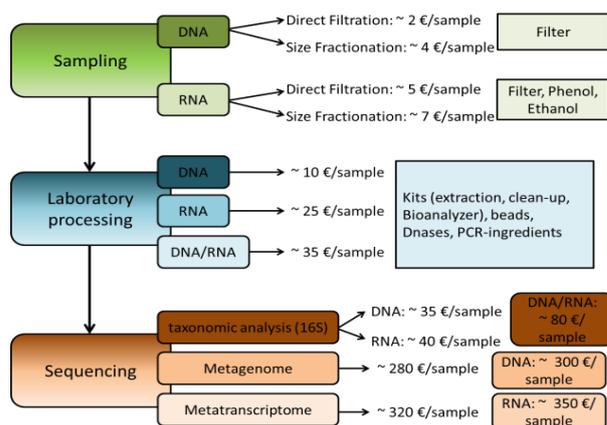

Figure 20: Overview of costs per sample in EURO. Prices include all consumables but no equipment and personal costs and are given separately for each step within in the Blueprint pipeline. Sequencing is usually carried out by companies and prices therefore where obtained from LGC Genomics.
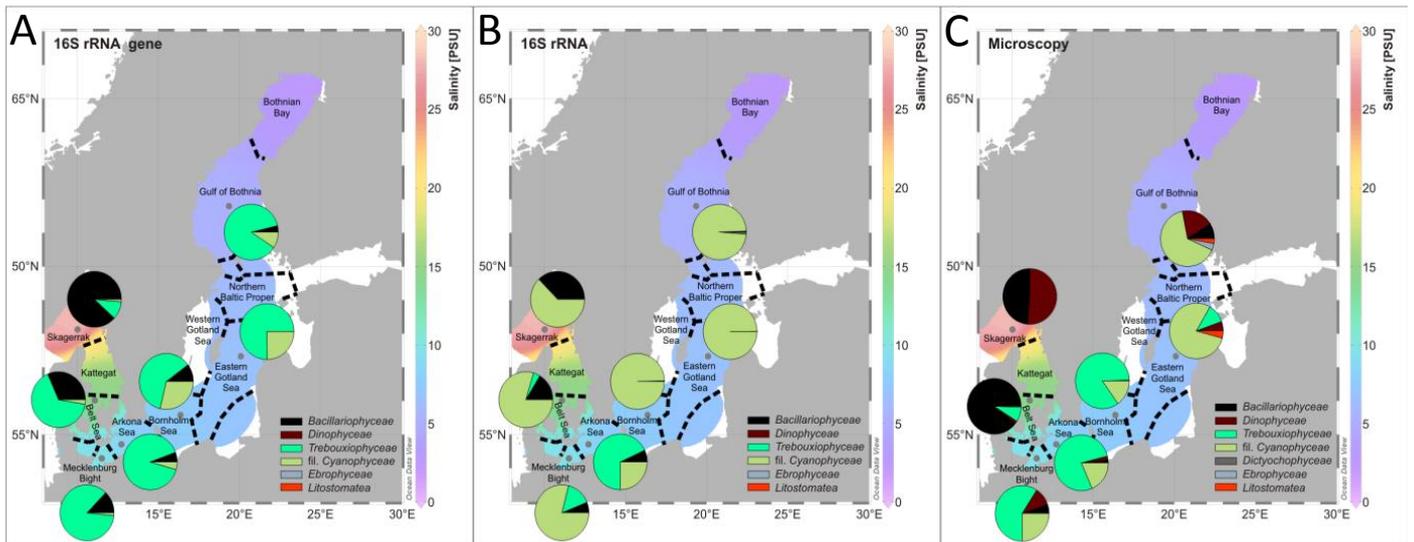
Fig. 21: Overview of the sampled stations, salinity gradient, and phytoplankton throughout the Baltic Sea in June 2014. The dotted lines indicate the borders of specific Baltic Sea regions, and the grey dots the sampled stations in that region. The pie charts indicate the phytoplankton community > 20 µm in size as determined by: 16S rRNA gene analysis (A), 16S rRNA transcript analysis (B), and microscopy (C).

In general molecular and microscopic investigations revealed similar distribution patterns of the phytoplankton > 20 µm. Diatoms were mainly present in the western Baltic Sea, whereas green microalgae predominated the central Baltic and filamentous Cyanobacteria prevailed in the northern part of the Baltic Sea (Fig. 21). Molecular identification of dinoflagellates suffered from uncertain plastid allocation by some species and from underrepresentation within the phytoREF database. Whether all cryptophyte sequences detected in both 16S rRNA library types truly belong to cryptophytes is unclear, since some members of the dinoflagellates and *Mesodinium rubrum* "steal" chloroplasts from their prey, mainly cryptophytes. This annexation of plastids is referred to as kleptoplastidy (Park et al. 2014).

As both DNA-based and RNA-based amplicon libraries were prepared, the ratio of 16S rRNA transcripts to 16S rRNA genes can probably serve as an activity indicator since the 16S rRNA transcripts serve as the active fraction of a population (Foesel et al. 2014). In our study, the ratios for chloroplasts were all below 1 except of the cryptophytes. At the Belt and Arkona Sea their ratio exceeded 2. In contrast, the ratio for the filamentous cyanobacteria *Aphanizomenon* spp. *and Nodularia* spp. ranged between 0.1 and 12. *Aphanizomenon* had their highest ratios (3.9/5.4) at areas with salinities <7. In contrast, the rRNA transcript to gene ratios of *Nodularia* was highest at salinities of ~15, indicating that *Nodularia* is more

active at these salinities than *Aphanizomenon*. The ubiquitous distributed unicellular cyanobacteria *Synechococcus* had over all stations similar numbers of 16S rRNA transcripts and 16S rRNA gene sequences, revealing ratios of around 1 (Fig. 22).

This study was presented as poster at the VAAM annual meeting (Vereinigung für Allgemeine und Angewandte Milrobiologie) from the 13th to 16th of March 2016 in Jena (Germany). Further on, this study is submitted for publication to L&O Methods.
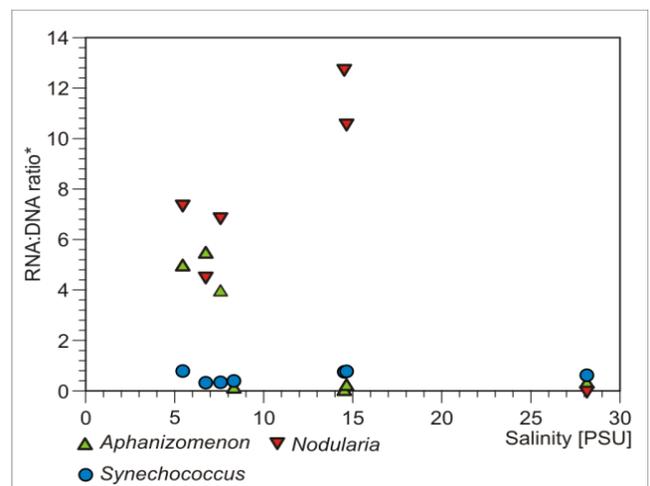
**Figure 22:** The graph depicts the 16S rRNA transcripts to 16S rRNA gene ratios of different *Cyanobacteria* displaying distinct activity levels alongside the salinity gradient of the Baltic Sea. *RNA:DNA ratios were normalized to the average number of 16S rRNA operons per genera. Average operon number for *Aphanizomenon*: 5, *Nodularia*: 4 and *Synechococcus* 1.75 (www.rrnadb.umms.med.umich.edu/search/).

Together with WP4 all surface samples from the POS488 cruise (34 stations at around 2 m depth) were extracted according to the BLUEPRINT protocol for DNA samples (see deliverable 6.1) and sequenced (metagenomes) at the SciLife institute in Stockholm, Sweden. Six out of 34 stations were already sampled in 2014 during the AL439 cruise (AT3, S7, Mo9, S10, Mo10, and Mo8). The remaining 28 stations were coastal stations and will help to complete the BARM assembly.

4.6.2 Outlook 2017

The upcoming fourth year will focus on further investigating the metatranscriptome dataset with respect to the question what metatranscriptomics can tell us about organic matter degradation in the Baltic Sea. Moreover, work will continue on further elaborating the Blueprint Competence Center (BCC) and communication of the main outputs to the scientific community, stake holders and the public. Within Blueprint, synthesis of the BCC will be discussed at a workshop in Helsingør, Denmark in May.

References

Decelle et al. 2015 Mol Ecol Res 15 (6) 1435-1445.

Foesel et al. 2014 EMI 16 (3) 658-675.

Park et al. 2014 Acta Protozool 53 (1) 39-50.

Quast et al. 2013 Nuleic Acids Res 41 (D1) D590-D596.

4.7 WP7: Project management and political implementation

Responsible partner: Lasse Riemann, University of Copenhagen, Denmark. Email: lriemann@bio.ku.dk

We have strictly followed our chosen mechanisms to ensure effective internal communication, progress evaluation, and discussion of pertinent questions. Our project website (http://blueprint-project.org/) as well as the official BONUS website continues to list the latest updates from our project activities. The website features an internal section which can only be accessed by BONUS BLUEPRINT members and contains method protocols, minutes and presentations from meetings, non-public deliverables, and other valuable information. In addition to regular communication by email, the third Skype meeting on 24th November 2016 was a valuable opportunity to brief all project partners on the progress in the different WPs, and to discuss *ad hoc* questions. The 2. annual BONUS BLUEPRINT meeting took place 9-11 May in Helsinki.

The coming year will encompass several deliverables touching upon the essential aims of the overall BONUS BLUEPRINT project. To facilitate efficient communication between partners about main project results a workshop is scheduled for 15-16 May 2017 in Helsingør, Denmark. The final and 4th year annual meeting is scheduled for 30th October – 1st November 2017 in Helsingør, Denmark.

5. Summary of the produced scientific and technological foreground capable of industrial or commercial application and measures taken for its protection

Nothing to report this year

6. Promoting an effective science-policy interface to ensure optimal take up of research results

6.1 Participation as members or observers in stakeholder committees

1. Bärbel Müller-Karulis (SU) contributed to the ICES/HELCOM Working Group on Integrated Assessments of the Baltic Sea in and its DEMO workshop (DEMOnstration exercise for Integrated Ecosystem Assessment and Advice of Baltic Sea fish stocks). Bärbel provided model scenario output for testing fish stock management strategies and model development and limitations were discussed. Cadiz, Spain, 9–13 March 2015

2. Bärbel Müller-Karulis (SU) presented the description of the microbial loop parameterization within the BALTSEM model (as developed in BLUEPRINT) as well as the overall BLUEPRINT modelling approach to 30 experts working at the Swedish Agency for Marine and Water Management, who deal with Baltic Sea management and especially BSAP issues. Göteborg, Sweden, 30. April 2015

Nothing to report for 2016

7. Collaboration with relevant research programs and the science communities in the other European sea basins and on international level

Nothing to report

8. List of peer-reviewed publications arising from the project research and defended PhD dissertations

8.1 Publications in press / published (peer-reviewed publications arising from the project research with authors from, at least, two different participating states)

1. Alneberg J, Bjarnason BS, de Bruijn I, Schirmer M, Quick J, Ijaz UZ, Lahti L, Loman NJ, et al. **2014**. Binning metagenomic contigs by coverage and composition. Nature Methods advance online publication.
2. Logue JB, Stedmon CA, Kellerman AM, Nielsen NJ, Andersson AF, Laudon H, Lindström ES, Kritzberg ES. **2015**. Experimental insights into the importance of aquatic bacterial community composition to the degradation of dissolved organic matter. *ISME J.* 2015 Aug 21. doi: 10.1038/ismej.2015.131. [Epub ahead of print]
3. Teikari J, Österholm J, Kopf M, Battchikova N, Wahlsten M, Aro EM, Hess WR, Sivonen K. **2015**. Transcriptomic and proteomic profiling of *Anabaena* sp. strain 90 under inorganic phosphorus stress. Appl Environ Microbiol 81:5212–5222.
4. Hu Y, Karlson B, Charvet S, Andersson AF. Diversity of Pico-to Mesoplankton along the 2000 km Salinity Gradient of the Baltic Sea. **2016**. Front Microbiol.; 7: 679.
5. Vaquer-Sunyer R, Reader HE, Muthusamy S, Lindh MV, Pinhassi J, Conley DJ, and Kritzberg ES. **2016**. Effects of wastewater treatment plant effluent inputs on planktonic metabolic rates and microbial community composition in the Baltic Sea. Biogeosciences. 13:4751–4765. doi: 10.5194/bg-13-4751-2016

8.2 defended PhD theses
Nothing to report

9. Progress in comparison with the original research plan and the schedule of deliverables

9.1 Deliverables accepted by BONUS
Deliverable 7.1: Launching of the BLUEPRINT webpage (**Delivery month 3**)

Deliverable 3.1: Compilation of signature genes indicative of GES/sub-GES conditions from literature on model microorganisms and natural seawater samples (**Delivery month 6**); available at http://blueprint-project.org/publications/eliverables/

Deliverable 4.1: Establishment of a bioinformatics toolbox for functional analysis of sequences from experimental work on isolates and natural microbial communities (**Delivery month 12**).

Deliverable 5.1: Compilation of available model time slices presented in a protocol showing the steady state values of individual processes and evaluation of sample range (**Delivery month 12**). Model time-slices can be extracted and analyzed online at http://130.237.91.172:3838/BALTSEM_TS_finder/

Deliverable 7.2: Annual BLUEPRINT year 1 scientific report (**Delivery month 14**)

Deliverable 1.1: Insights into linkages between blueprint genomic information and environmental status in selected samples (**Delivery month 18**).

Deliverable 1.2: Establishment of a complete Baltic Sea sample set (**Delivery month 24**).

Deliverable 7.3: Annual BLUEPRINT year 2 scientific report **(Delivery month 26)**

Deliverable 5.2: Matching the BALTSEM structure to three classes of metagenomic data **(Delivery month 30)**

Deliverable 4.2: Database containing data on blueprints and environmental data (BalticMicrobeDB) **(Delivery month 32)**

Deliverable 1.3: New knowledge on spatiotemporal variability of the genetic blueprint relative to environmental conditions in the Baltic Sea **(Delivery month 36)**

Deliverable 2.1: Biogeochemical process rates in relation to microbial blueprints in indigenous microbial communities under GES/sub-GES conditions **(Delivery month 36)**

Deliverable 3.2: New knowledge on gene expression in Baltic Sea cyanobacteria relative to growth under changing environmental conditions **(Delivery month 36)**

Deliverable 6.1: Report on BCC development **(Delivery month 36)**

10. Progress in comparison with the original financial plan (more detailed explanation requested if reported costs differ more than 20% of the valid budget of the reporting period)
Nothing to report

11. Amendments to the description of work and schedule of deliverable

We currently do not envision any major changes in the working plan, financial plan, or the timing or character of deliverables. As mentioned in previous reports, methodological difficulties with Illumina sequencing and companies caused a delay of some project parts. Consequently, the due date of a few of the central deliverables was postponed. All of these rearrangements have been approved by BONUS, and we do not foresee problems in fulfilling the current schedule for these deliverables.

12. Other dissemination

12.1 Publications in press / published that acknowledge BONUS and BONUS BLUEPRINT (but do not have authors from at least two different participating states)

1. Muthusamy S, Baltar F, González JM, and Pinhassi J. **2014**. Dynamics of metabolic activities and gene expression in the Roseobacter clade bacterium *Phaeobacter* sp. MED193 during growth with thiosulfate. Applied and Environmental Microbiology. 80(22):6933-6942.
2. Hugerth LW, Wefer HA, Lundin S, Jakobsson HE, Lindberg M, Rodin S, Engstrand L, Andersson AF. **2014**. DegePrime, a Program for Degenerate Primer Design for Broad-Taxonomic-Range PCR in Microbial Ecology Studies. Applied and Environmental Microbiology 80(16):5116-5123.
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5. Lindh MV, Figueroa D, Sjöstedt J, Baltar F, Lundin D, Andersson A, Legrand C, Pinhassi J. **2015**. Transplant experiments uncover Baltic Sea basin-specific responses in bacterioplankton community composition and metabolic activities. Frontiers in Microbiology. 6:Article 223
6. Leisner JJ, Jørgensen NOG, Middelboe M. **2015**. Predation and selection for antibiotic resistance in natural environments. Evolutionary Applications. doi:10.1111/eva.12353
7. Hagström Å, Azam F, Berg C, and Zweifel UL. Culture the Marine Microbiome. Journal of Aquatic Microbial Ecology SPECIAL ISSUE 6: Progress and perspectives in aquatic microbial ecology: Highlights of the SAME 14 conference, Uppsala, Sweden, **2015**. Editors: Paul A. del Giorgio, Fereidoun Rassoulzadegan, Eva Lindström. In press.
8. Bunse C, Lundin D, Karlsson CMG, Akram N, Vila-Costa M, Palovaara J, Svensson L, Holmfeldt K, González JM, Calvo E, Pelejero C, Marrasé C, Dopson M, Gasol JM, Pinhassi J. **2016**. Response of marine bacterioplankton pH homeostasis gene expression to elevated CO_2. Nature Climate Change. In Press. http://www.nature.com/nclimate/journal/vaop/ncurrent/full/nclimate2914.html
9. Bombar D, Paerl R, Riemann L. **2016**. Marine Non-Cyanobacterial Diazotrophs: Moving beyond Molecular Detection. Trends Microbiol. 24(11): 916-927. doi: 10.1016/j.tim.2016.07.002
10. Lindh MV, Sjöstedt J, Casini M, Andersson A, Legrand C, and Pinhassi J. **2016**. Local environmental conditions shape generalist but not specialist components of microbial metacommunities in the Baltic Sea. Frontiers in Microbiology. 7:Article 2078. doi: 10.3389/fmicb.2016.02078

11. Traving SJ, Bentzon-Tilia M, Knudsen-Leerbeck H, Mantikci M, Hansen JLS, Stedmon CA, Sørensen H, Markager S and Riemann L. **2016**. Coupling bacterioplankton populations and environment to community function in coastal temperate waters. Frontiers in Microbiology, 7: 1533

12. Lindh MV, Sjöstedt J, Ekstam B, Casini M, Lundin D, Hugerth LW, Hue Y, Andersson AF, Andersson A, Legrand C, and Pinhassi J. **2017**. Metapopulation theory identifies biogeographical patterns among core and satellite marine bacteria scaling from tens to thousands of kilometers. Environmental Microbiology. In Press. DOI: 10.1111/1462-2920.13650

12.2 Publications in preparation / submitted

1. Bombar D, Bennke C, Labrenz M, Riemann L et al.: Assessment of microbially-mediated nitrogen gain and loss terms in Baltic Sea redoxclines using metatranscriptomics and stable isotope rate measurements. Manuscript in preparation.

2. Bombar D, Labrenz M, Sørensen SJ, Morberg S, Riemann L et al.: Metagenomic characterization of uncultivated heterotrophic N_2-fixing microorganisms (diazotrophs) isolated from the Baltic Sea Redoxcline using flow cytometry cell sorting. Manuscript in preparation.

3. Larsson J, Müller-Karulis B, Karlsson C, Andersson A, Hugerth L, Alneberg J, Pinhassi J, Hagström Å. Mapping gene expression to reconstructed microbial genomes reveals key players in Baltic Sea nutrient transformations. Manuscript in preparation.

4. Muthusamy S, Lundin D, Branca RM, Baltar F, González JM, Lehtiö J, Pinhassi J. Comparative analysis of exponential and stationary phase proteomes in three marine model bacteria (Alpha- and Gammaproteobacteria and Bacteroidetes). Manuscript in preparation.

5. Cairns J, Coloma S, Sivonen K, Hiltunen T. Evolving interactions between diazotrophic cyanobacterium and phage mediate nitrogen release and host competitive ability. Manuscript submitted to Royal Society Open Science.

6. Muthusamy S, Karlsson CMG, Akram N, Lundin D, González JM, Branca J, Lehtiö J, Pinhassi J. Improved light-mediated survival during starvation in a proteorhodopsin-containing marine bacterium involves pronounced rearrangements of the proteome. Manuscript in preparation.

7. Bartl I, Happel EM, Riemann L, Voss M. In situ nitrification rates and activity of present nitrifiers in the bottom water layer of two Baltic coastal zones affected by different riverine nutrient loads. In prep.

8. Sundh J, Berg C, Andersson AF, Müller-Karulis B, Alneberg J, Karlsson CMG, Pinhassi J, and Hagström Å. Ecological significance of transporters in the Baltic Sea microbial food web. In prep.

9. Traving SJ, Rowe O, Jakobsen NM, Sørensen H, Dinasquet J, Stedmon CA, Andersson A, Riemann L. The effect of increased loads of dissolved organic matter on estuarine microbial community composition and function. In press at Frontiers in Microbiology

10. Paerl R, Riemann L. Nitrogen fixation by a model marine non-cyanobacterial diazotroph *Pseudomonas stutzeri* BAL261 – an oxygen limited process not readily promoted by customary particle addition

11. Markussen T, Happel EM, Teikari J, Huchaiah V, Alneberg J, Andersson A, Sivonen K, Riemann L, Middelboe M, Kisand V. Biogeochemical process rates in relation to microbial blueprints in indigenous microbial communities under GES/sub-GES conditions. In prep

12. Bennke CM, Pollehne F, Müller A, Hansen R, Kreikemeyer B, and Labrenz M. The use of a general 16S rRNA primer system to identify phytoplankton throughout the Baltic Sea. Submitted to Limnology and Oceanography Methods

13. Shen DS, Jürgens K, and Beier S. Changes in salinity affect the community assembly of bacteria with different life strategies. Submitted

12.3 Conference talks and posters

1. Lindh MV, Sjöstedt J, Ekstam B, Legrand C, Baltar F, Hugerth L, Lundin D, Nilsson E, Andersson AF, Pinhassi J. Ecological patterns within spatio-temporal fluctuations of the bacterioplankton consortium in the Baltic Sea. ASLO/AGU Ocean Sciences Meeting. Honolulu, Hawaii, 23-28 February, **2014**. Oral presentation.

2. Pinhassi J, Palovaara J, Akram N, Baltar F, Forsberg J, Bunse C, Pedrós-Alió C, González M, González J. Regulation of proteorhodopsin phototrophy in the flavobacterium Dokdonia sp. MED134. ASLO/AGU Ocean Sciences Meeting. Honolulu, Hawaii, 23-28 February, **2014**. Oral presentation.

3. Branca R, Zhu Y, Muthusamy S, Andersson AF, Pinhassi J, Lehtiö J. Sample fractionation by high resolution isoelectric focusing and database fragmentation by peptide pI allows efficient marine metaproteomics of seasonal dynamics in Baltic Sea microbiomes. 10th Siena Meeting. From genome to proteome: 20 years of proteomics. Siena, Italy, 31 Aug - 4 Sept, **2014**. Oral presentation.

4. Lindh MV, Figueroa D, Sjöstedt J, Lundin D, Andersson AF, Legrand C, Pinhassi J. Baltic Sea transplant experiments uncover distinct water mass-dependent responses in bacterioplankton community composition and activities to changes in salinity and dissolved organic matter. Swedish Marine Sciences Conference, Umeå, Sweden, November **2014**. Oral presentation.

5. Riemann L. Biological lenses using gene prints: BLUEPRINT introduction. Kick-off meeting of the BONUS projects, 26-27 August **2014**, Riga, Latvia. Oral presentation.

6. Riemann L. N_2 fixation by heterotrophic diazotrophs in temperate estuarine waters. Microbes in the Baltic: Small things, small sea, big questions; 18-21 November **2014**, Gdynia, Poland. Oral presentation.

7. Riemann L. The BLUEPRINT project – an overview. First BLUEPRINT Discussion Forum, 30. November -1. December **2014**, Warnemuende, Germany. Oral presentation.

8. Larsson J, Müller-Karulis B, Andersson AF, Hugerth L, Alneberg J, Pinhassi J, Hagström Å. Using metagenomics to find biological indicators and predict biochemical processes in the Baltic Sea. Microbes in the Baltic: Small things, small sea, big questions; 18-21 November **2014**, Gdynia, Poland. Oral presentation.

9. Karlsson C. Exploring genetic responses of marine bacteria to environmental challenges in the Baltic Sea: Biological lenses using gene prints (BLUEPRINT). Poster presentation at Computational molecular analysis summer school, 29 Sept-3 Oct **2014**, Wilhemshaven, Germany.

10. Hugerth LW, Alneberg J, Larsson J, Pinhassi J, Andersson AF. NGS of the Baltic Sea microbiome - from diversity patterns to genome-ecology links. Microbes in the Baltic: Small things, small sea, big questions; 18-21 November **2014**, Gdynia, Poland

11. Hugerth LW, Alneberg J, Pinhassi P, Andersson AF. A metagenomic analysis of planktonic Actinobacteria and Bacteroidetes in the Baltic Proper. Microbes in the Baltic: Small things, small sea, big questions; 18-21 November **2014**, Gdynia, Poland

12. Hu Y, Karlson B, Andersson AF. Pico - to mesoplankton distribution along the 2000 km salinity gradient of the Baltic Sea. Microbes in the Baltic: Small things, small sea, big questions; 18-21 November **2014**, Gdynia, Poland

13. Alneberg J, Bjarnason BS, Hugerth LW, Larsson J, Pinhassi J, Schirmer M, Ijaz UZ, Loman N, Andersson AF, Quince C. Clustering Metagenomic Contigs using CONCOCT. Microbes in the Baltic: Small things, small sea, big questions; 18-21 November **2014**, Gdynia, Poland

14. Kisand V. Microbial food webs and carbon cycle in the Baltic, any significant changes in a discourse during the last decades? Microbes in the Baltic: Small things, small sea, big questions; 18-21 November **2014**, Gdynia, Poland.

15. Larsson J, Müller-Karulis B, Andersson AF, Hugerth L, Alneberg J, Pinhassi J, Hagström Å. Using metagenomics to find biological indicators and predict biochemical processes in the Baltic Sea. Microbes in the Baltic: Small things, small sea, big questions; 18-21 November **2014**, Gdynia, Poland

16. Labrenz M. The BONUS projects BLUEPRINT and AFISmon. Presented at "Rendez-Vous de Concarneau: where Industry meets Science in marine Biotechnology" as well as the Biological Institute Helgoland colloquium of the Alfred-Wegener Institute. Concarneau, France, 9-10th October and Helgoland, Germany, 9th July **2014**.

17. Teikari J, Kisand V, Mattila A and Sivonen K. Impact of major environmental stressors on bacterial community in the Baltic Sea. 10th Baltic Sea Science Congress. Riga, Latvia, 15-19 June, **2015**. Oral presentation.

18. Hugerth LW, Alneberg J, Larsson J, Pinhassi J, Andersson AF. Reconstruction of Baltic Sea bacterioplankton genomes from time-series metagenomes uncovers a global brackish microbiome. ASLO **2015**, February 22-27, Granada, Spain.

19. Andersson AF. Next-generation sequencing analysis of the Baltic Sea microbiome: from billions of short DNA sequences to genome-ecology links. The annual Swedish OIKOS meeting **2015**, February 4-6, Umeå, Sweden.

20. Alneberg J, Hu Y, Hugerth LW, Andersson AF. Linking Metagenome Assembled Genomes with 16S OTUs to Uncover Spatio-temporal Distribution Patterns. EMBO Conference on Aquatic Microbial Ecology (SAME-14), 23-28 August **2015**, Uppsala, Sweden.

21. Hu Y, Karlson B, Andersson AF. Diversity of pico- to mesoplankton along the 2000 km salinity gradient of the Baltic Sea. EMBO Conference on Aquatic Microbial Ecology (SAME-14), 23-28 August **2015**, Uppsala, Sweden.

22. Hugerth LW, Larsson J, Alneberg J, Lindh MV, Legrand C, Pinhassi J, Andersson AF. Metagenome-assembled genomes uncover a global brackish microbiome. EMBO Conference on Aquatic Microbial Ecology (SAME-14), 23-28 August **2015**, Uppsala, Sweden.

23. Bunse C, Lundin D, Dopson M, Karlsson CMG, Palovaara J, Vila-Costa M, Pelejero C, Marrasé C, Gasol JM, and Pinhassi J. Ocean acidification causes a community wide bacterial pH stress response. ASLO Aquatic Sciences Meeting. 22-27 February **2015**, Granada, Spain. Oral presentation.

24. Pinhassi J. Fitness benefits of proteorhodopsin phototrophy in marine bacteria. American Society for Microbiology (ASM), General Meeting. 30 May-2 June **2015**. New Orleans, USA. Oral presentation - Invited speaker.

25. Hagström Å. Re-discovering marine microbial biology through metagenomics. Second EMBO conference on Aquatic Microbial Ecology: SAME-14. August 23-28, **2015**. Uppsala, Sweden. Oral Presentation - Invited Key Speaker.

26. Muthusamy SD, Karlsson CMG, Akram N, Lundin D, González JM, Branca R, Lehtiö J, Pinhassi J. Improved light-mediated survival during starvation in the proteorhodopsin-containing marine bacterium involves pronounced rearrangements of the proteome. Second EMBO conference on Aquatic Microbial Ecology: SAME-14. August 23-28, **2015**. Uppsala, Sweden. Oral Presentation.

27. Larsson J, Hugerth LW, Müller-Karulis B, Karlsson CMG, Alneberg J, Muthusamy SD, Branca R, Lehtiö J, Pinhassi J, Andersson A, Hagström Å. Meta-omic analysis of Baltic Sea microbial communities and the link to biogeochemical models. Second EMBO conference on Aquatic Microbial Ecology: SAME-14. August 23-28, **2015**. Uppsala, Sweden. Oral Presentation.

28. Bombar D, Andersson A, Hagström Å, Humborg C, Jürgens K, Kisand V, Labrenz M, Middelboe M, Pinhassi J, project. Oral Presentation. Annual Science Conference of the International Council for the Exploration of the Sea (ICES). Copenhagen, Denmark, 21-25 September 2015

29. Bombar D, Münster-Happel E, Sørensen S, Milani S, Bennke C, Labrenz M, and Riemann L. **2015**. Elucidating the ecology of heterotrophic nitrogen-fixers in chemocline waters of the Baltic Sea. Poster. Second EMBO Conference on Aquatic Microbial Ecology: SAME-14. Uppsala, Sweden, 23-28 August 2015

30. Happel EM, Andersson B, Nahar N, and Riemann L. **2015**. Nitrogen fixation and nifH gene expression in heterocystous and non-heterocystous cyanobacteria – vertical and diurnal patterns in the Baltic Sea proper. Poster. Second EMBO Conference on Aquatic Microbial Ecology: SAME-14. Uppsala, Sweden, 23-28 August 2015

31. Riemann L. **2015**. "Development of molecular microbial indicators: the BONUS project BLUEPRINT". Oral presentation at and chairing round-table discussion on indicators of marine environmental status at the 10th Baltic Sea Science Congress "Science and innovation for future of the Baltic and the European regional seas", Riga, Latvia, 15-19 June.

32. Riemann L. **2015**. Presenting BLUEPRINT and being moderator of "MOLECULAR MICROBIAL ECOLOGY AND THE BALTIC SEA: A PANEL DISCUSSION". Second EMBO Conference on Aquatic Microbial Ecology: SAME-14. Uppsala, Sweden, 23-28 August 2015

33. Paerl R, Larsson J, Hugerth L, Andersson A; Bouget FY, Palenik B, Azam F; Pinhassi J, Riemann L. **2015**. Culture experiments and metagenomes emphasize that a variety of vitamin sources sustain vitamin B1 auxotrophic plankton. Oral presentation. Second EMBO Conference on Aquatic Microbial Ecology: SAME-14. Uppsala, Sweden, 23-28 August 2015

34. Sjöqvist C, Alneberg J, Bennke C, Labrenz M & Andersson AF. **2016**. Population genomic analysis of microbial communities along environmental gradients in the Baltic Sea. *16th International Symposium on Microbial Ecology 21-26 August 2016, Montreal, Canada*

35. Paerl RW, Bouget FY, Lozano JC, Vergé V, Schatt P, Allen EE, Palenik B, and Azam F. **2016**. Key marine picoeukaryotic phytoplankton exhibit novel conditional growth using vitamin B1 precursors. Poster presentation. *16th International Symposium on Microbial Ecology 21-26 August 2016, Montreal, Canada*

36. Teikari J, Fewer D, Leikoski N, Mäkelä M, Simojoki A, and Sivonen K. **2016**. Are phosphonates contributing to formation of massive cyanobacterial blooms in the Baltic Sea? Poster presentation. *16th International Symposium on Microbial Ecology 21-26 August 2016, Montreal, Canada*

37. Jonna Teikari, Shengwei Hou, Wolfgang R. Hess and Kaarina Sivonen. **2016**. Comparative genome analysis of toxic and bloom-forming Baltic Sea cyanobacteria Nodularia spumigena UHCC 0039 and Dolichospermum sp. UHCC 0315. Genomic Applications in Biotechnology (natural products) Genomics Workshop, 13-15 December 2016, Porto, Portugal

12.4 Outreach and Media output

1. Jarone Pinhassi: "Bakterier kan motverka klimatförändringarna": Interview in Swedish regional TV-news "Smålandsnytt" and Interview in Swedish regional newspaper "Barometern" on 19 August **2014**

2. Lasse Riemann: Article in the regional newspaper "Nordsjælland". November **2013**

3. Lasse Riemann: Interview and article on the BLUEPRINT project, its visions and potential impact with the regional newspaper "Helsingør Dagblad". November **2013**

4. Lasse Riemann at Rotary club Helsingør. Molecular tools for monitoring environmental status in the Baltic Sea; the BLUEPRINT project. 16 June **2014**

5. Christofer Karlsson. Exploring genetic responses of marine bacteria to environmental challenges. GENECO Winter Meeting 2015, February 10, **2015**. Lund, Sweden.

6. Christofer Karlsson. Marine bacteria - a tool for environmental monitoring. Scientific speed networking. Workshop on Genomics. January 12, **2015**. Cesky Krumlov, Czech Republic.

7. Christofer Karlsson. Research blog. Started 17 February **2015**. http://www.bonusprojects.org/bonusprojects/blogs/the_model_bacteria

8. Åke Hagström. Så ska forskarna rädda Östersjön. Interview in the Swedish regional newspaper "Uppsala Nya Tidning" on 23 August **2015**.

9. Jarone Pinhassi. "Rhodopsin light harvesting empowering microbial oceanography." Symposium for 50 years of molecular biology at Umeå University, Sweden. 19 August **2016**.

10. Åke Hagström. "Idealized microbial food web or Frede's stepping stones." International symposium honoring Prof. T. Frede Thingstad. Bergen University, Norway. Dec **2016**.

11. Jarone Pinhassi and Christofer Karlsson. Försurning hindrar havsbakterier fylla sin funktion. Interview in the program "Vetandets värld" Swedish national radio P1. 26 Feb **2016**.
12. Jarone Pinhassi. Havsbakterier störs av försurning. Interview in the Swedish journal Kemivärlden. 14 Jan **2016**.
13. Kaarina Sivonen. Cyanobacterial mass occurrences, toxins and bioactive compounds. Interview in the national newspaper, Helsingin Sanomat, science pages B10-B11; Published 27. 6. **2016**.

13. Distribution of the project's research staff involved

Age group	PhD students		Post-docs		Assistants, lecturers, instructors and eq		Associate professors and eq		Professors and eq	
	F	M	F	M	F	M	F	M	F	M
<= 24	0	0	0	0	0	0	0	0	0	0
25 - 49	4	3	4	7	2	2	0	6	0	1
50 - 64	0	0	0	0	0	0	0	0	1	2
>= 65	0	0	0	0	0	0	0	0	0	1