BONUS BLUEPRINT

Biological lenses using gene prints

Annual report 2015

Lead Institution	University of Copenhagen, Denmark
Partner Institutions	KTH Royal Institute of Technology (KTH), Sweden Stockholm University (SU), Sweden Linnaeus University (LNU), Sweden University of Helsinki (UH), Finland Leibniz Institute for Baltic Sea Research Warnemünde (IOW), Germany University of Tartu (UT), Estonia

Preface

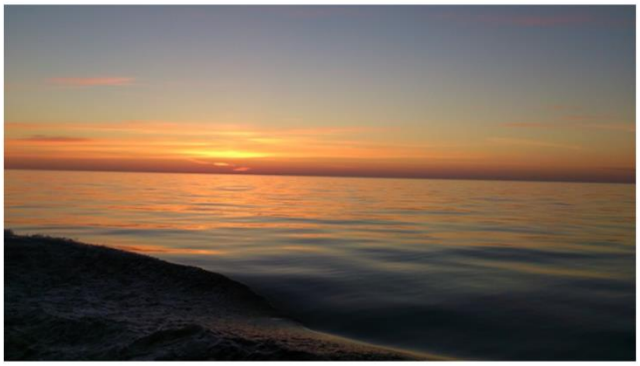
The BONUS BLUEPRINT (Biological lenses using gene prints) project started in January 2014 and will be running for four years. BONUS BLUEPRINT is funded by the BONUS programme through the European Community's Seventh Framework Programme (FP/2007-2013) under implementation agreement R&I/I3/2012/BONUS made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

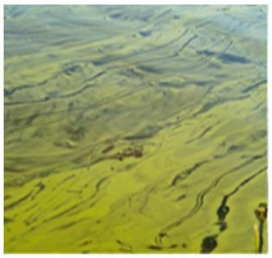
In the second year of BONUS BLUEPRINT we have reached major milestones, including the successful sequencing of our first metagenomes & transcriptomes from field- and experimental samples, the generation of the BARM reference genome, the measurements of diverse process rates describing important biogeochemical turnovers in the Baltic Sea, crucial advancements in improving the BALTSEM model, and the finalized development and testing of the AFIS sampler. Our work has benefited from intensified interactions between the different work packages, and from reaching out to HELCOM monitoring experts during our second discussion forum during the BSSC meeting in Riga in June. We shared and discussed our results during the first annual project meeting in Gustavsberg, Sweden, in March 2015. This meeting was also marked by fruitful discussions with members of the advisory board, which helped shedding light on the strengths and weaknesses of our project approach and the necessary path forward. BONUS BLUEPRINT members have disseminated their work in additional publications, student theses, and at various conferences. This year's report provides an extensive overview of our activities and documents that BONUS BLUEPRINT progresses well according to the project plan.

February 2016
Lasse Riemann (project coordinator)
Deniz Bombar (secretary).

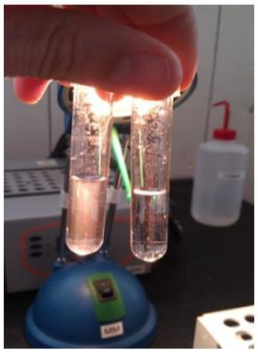
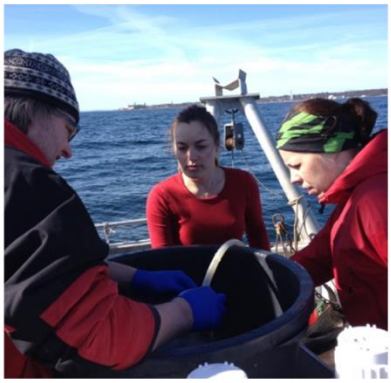
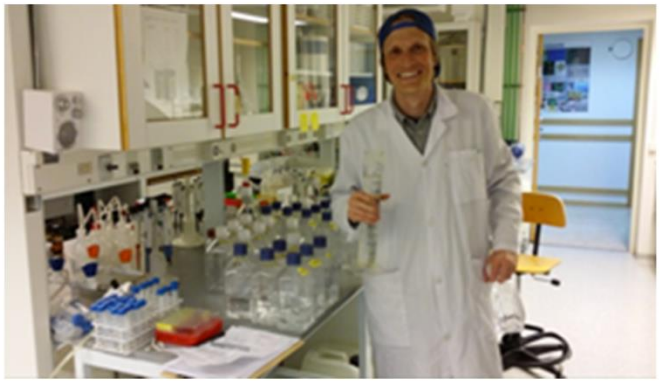
Table of contents

1. Some highlights from year 2







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. Thus, this 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 political implementation. We have implemented co-supervision of student and PostDoc projects 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 meeting, meetings, and the project website (http://blueprint-project.org/). Scientific and organizational decisions are made by the Management Board. The independent international Advisory Board will continue to provide input on project progress, management, and dissemination. For further details on project management in year 2 please see section 10.

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)

Ryan W. Paerl, postdoc

Demeng Tan, postdoc

Deniz Bombar, postdoc (not 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 Larsson (last name Sundh from 2015), 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. 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 goal of activities in WP1 is to analyze the transcriptional response of Baltic microbial communities along temporal and spatial gradients in order to link certain environmental parameter with the functional profile of prokaryotes and specific microbial activities. The major environmental gradients present in Baltic Sea environments are vertical oxygen depletion and horizontal changes in the salinity.

4.1. Field work and mixing experiment

Our first extensive field cruise in June 2014 with RV Alkor enabled us to obtain a large set of data describing these steep abiotic gradients and fundamentally different subsystems of the Baltic Sea. Apart from physicochemical, nutrient and pigment data, we have finalized analyses of dissolved organic carbon and nitrogen (DOC/DON) for 31 stations across the Baltic Sea, including spectrophotometric measurements that allow for identifying different components within the DOM pool, such as humic/terrestrial signals or amino acid like products from other sources (Fig. 1 A, B). We have further completed rate measurements representing key processes within the Baltic Sea biogeochemical cycles, including primary production and N_2 fixation at 18 stations (Fig. 1 C, D), and for the anoxic basins profiles of nitrification rates as well as the N-loss processes denitrification and Anammox. Together with metagenomic and metatranscriptomic data, which have successfully been sequenced by now and are analyzed in 2016 (WP6, Fig. 20), we will not only examine linkages between genetic BLUEPRINTS and

environmental conditions, but also illuminate if and in which way transcription levels of selected genes are mirrored in directly measured biogeochemical activity.

During 2015 we have also continued sampling from a coastal oxygen depleted zone at the Boknis Eck (Eckenfoerde Bay). We have furthermore compiled a list including approximately 1400 nucleic acid samples and corresponding metadata that were obtained during sampling activities from all BONUS BLUEPRINT partners (Deliverable D1.2). For networking and the planning of possible metastudies it is essential to keep an overview on the sampling activities of the different work packages within the BONUS BLUEPRINT project. The sample list created for this deliverable is therefore available for all BONUS BLUEPRINT members in Excel format on the internal page of the project website (http://blueprint-project.org/) and will regularly be updated to include the most recent samplings.

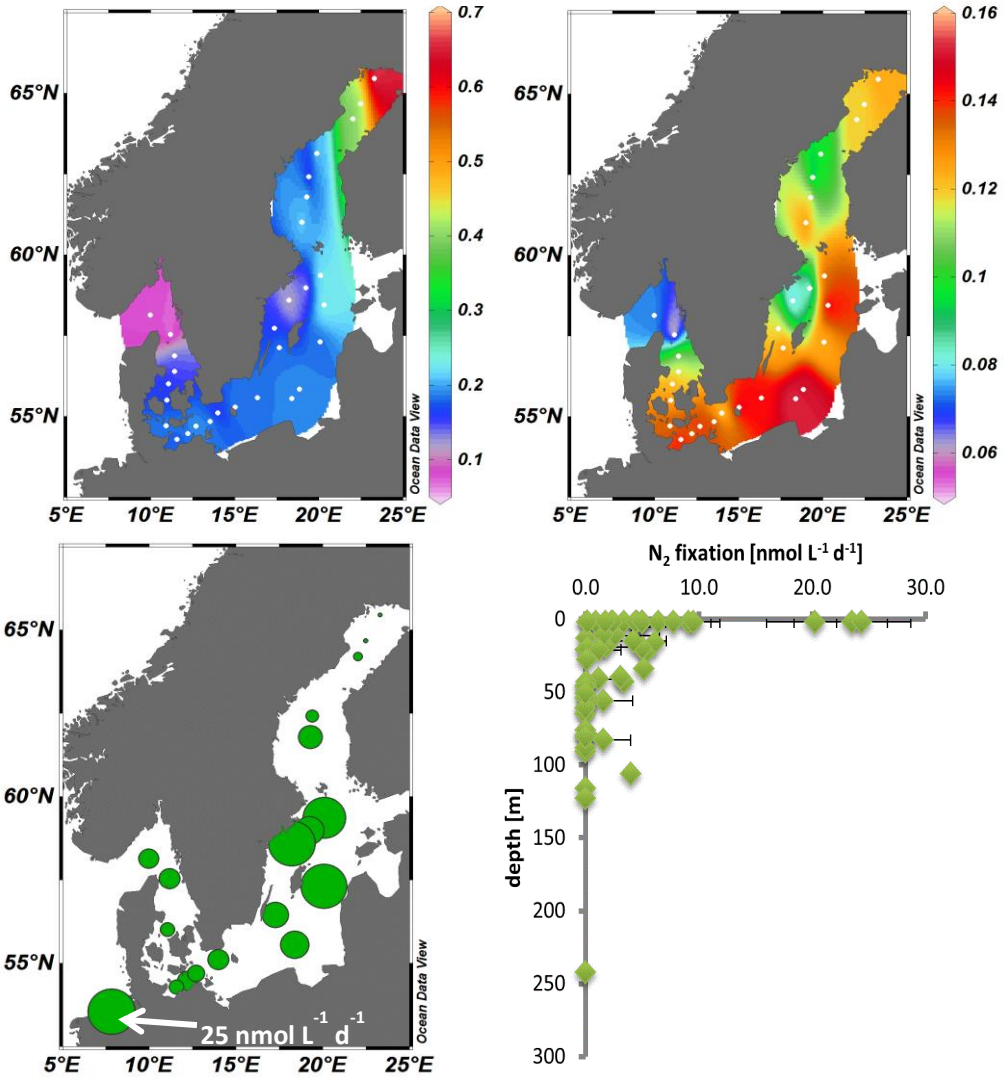


Figure 1: An example for the contrasting distribution of different components of dissolved organic matter (DOM) within the Baltic Sea: A) humic substances/terrestrial DOM, B) amino acid like substances / non-humic labile matter (relative units). White dots represent sampling stations. Lower panels: The C) horizontal and D) depth distribution of N_2 fixation in the Baltic Sea, highlighting the importance of cyanobacterial N_2 fixation within the Baltic proper and in surface waters, respectively. Data from cruise with RV Alkor in June 2014.

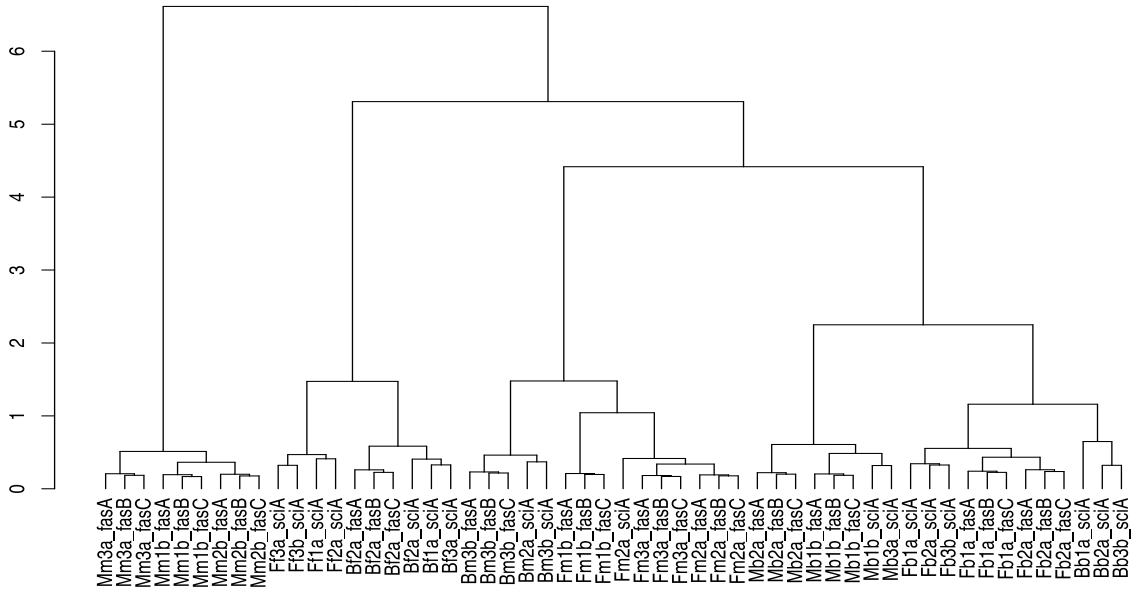


Figure 2: Dendrogram delineated from metatranscriptome data from technical as well as biological replicates that were mapped on metagenome contigs. M/B/F: marine, brackish or freshwater medium; m/b/f marine, brackish or freshwater inoculum; 1/2/3: biological replicates; a/b RNA extraction from filter a or b; fas/sci: library preparation at Fasteris (https://www.fasteris.com/dna/) or SciLife Lab (https://www.scilifelab.se/); A/B/C: replicate sequence runs. Library preparation for all replicates of the treatment Mf failed.

Nucleic acid samples from two experiments that were performed in 2014 (mixing experiment: time series sampling after mixing of two water bodies featuring anoxia and suboxic conditions; transplant experiment: full-factorial setup with bacterial communities originating from three salinity regimes of the Baltic Sea that were reciprocally incubated under each other's environmental conditions) and from several environmental samples were extracted and sent for metagenome and metatranscriptome sequencing.

Metagenome as well as metatranscriptome data from the mixing experiment and several environmental samples have been quality trimmed. Partners from WP4 currently assemble the processed metagenome sequence reads together with metagenome data from WP1 in order to construct a common Baltic Sea master assembly for downstream annotation of sequence data.

16S rRNA gene amplicon sequences, metagenome as well as metatranscriptome data from the transplant experiment have been processed and annotated. A more detailed description of the experimental setup and some first results are given below.

4.2. Transplant experiment

Little is known about how locally adapted bacteria cope with changes in salinity and which ecological strategies they employ in response to such changes. We have performed 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 and harvested after 4 days. All treatments were setup in triplicates. Metagenome data from each treatment were assembled and the resulting contigs were annotated to reference genomes from the RefSeq database as well as constructed genomes from Baltic Sea metagenomes that were provided by colleagues from WP4. Raw sequence reads were mapped on the obtained contigs.

Metatranscriptome sequencing was performed for each replicate. Because of problems during library preparation, samples for metatranscriptome sequencing have been processed at different sequencing facilities. Beside the biological replicates, also technical replicates for several samples were prepared in order to assess comparability between sequence data that were obtained from different

sequencing platforms. Metatranscriptome raw reads were mapped on the assembled metagenome contigs for annotation. The evaluation of all technical and biological replicates demonstrated that at the contig level, all replicates from the same treatment were more similar to each other than to replicates from any other treatment (Fig. 2).

Analyses of 16S rRNA amplicons have revealed that all three inocula contained taxa (defined as operational taxonomic units - OTUs) that featured preferential growth in marine, brackish or freshwater medium (Fig. 3) or alternatively were characterized by equal growth in the different media. In order to extract metatranscriptome data from taxa that belonged to the groups of marine, brackish and freshwater responders, with preferential growth in the respective medium, or to salt tolerant taxa with equal growth in all media, we applied the following rules to match contigs to representative OTUs from each group.

1. Pearson correlation of the relative abundance of an OTU and metagenome derived genome bins across the treatments > 0.9
2. The predicted genus from the extracted data is identical to the genus /genera represented by the first 10 Blast Hits of the respective OTU against RefSeq RNA sequences.

By applying these rules 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. Analyses concerning common transcriptional patterns among the representatives of each ecological group are currently in progress.

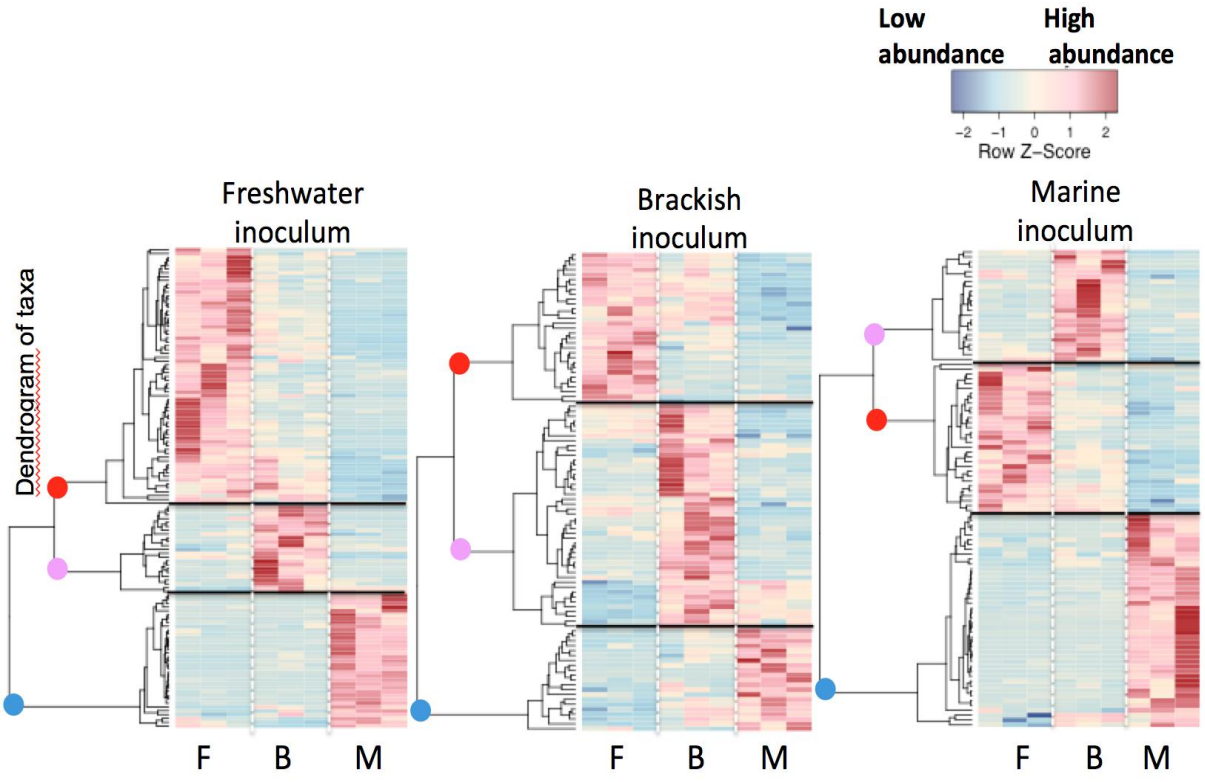


Figure 3: Heatmaps displaying representative OTUs from three inocula with preferential growth in different media (F: freshwater medium, B: brackish medium, M: marine medium).

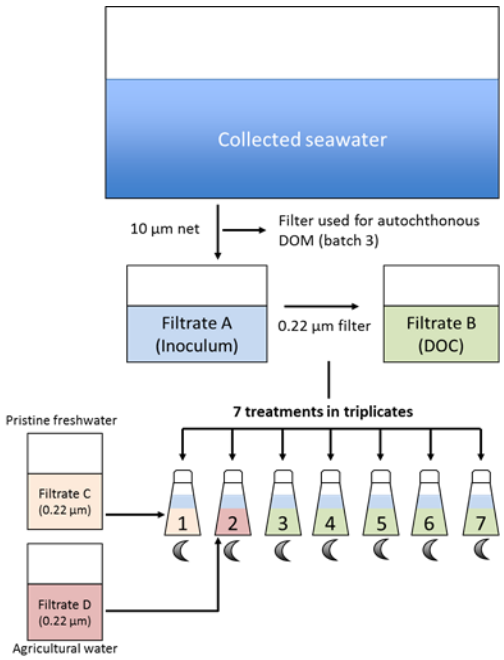
4.3. Outlook 2016

Our focus in 2016 will be on finishing analyses for the transplant experiment as well as the mixing experiment (oxic-anoxic interface central Baltic Sea). One manuscript based on data from the transplant experiment with a focus on taxa with different ecological strategies is in preparation and will be submitted in spring 2016. A second manuscript from the transplant experiment with a focus on functional redundancy between the treatments is planned. We further aim to prepare a manuscript for the mixing experiment towards the end of 2016.

5. WP2: Effects of environmental change on the microbial genetic BLUEPRINT

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

In year 2015, the planned batch experiments were conducted at two sites using natural bacterial communities from the Gulf of Finland and Øresund to examine the effects of changes in environmental condition on the activities and functional and genetic properties of the microbial communities. The experiments were thus designed to experimentally identify key genetic and functional changes in natural microbial assemblages exposed to defined stressors (e.g., hypoxia, inorganic nutrient additions, toxic cyanobacteria, and increased input of various sources of DOM) influencing the environmental status of the Baltic Sea. Increased input of DOM derived from increased river discharge is predicted as a consequence of climate change due to increased precipitation. Frequency and magnitude of cyanobacterial blooms, especially of toxin producing species, and hypoxia events are expected to increase



Treatments (incubation in the dark):

1) + Humic DOM (from river, Finland) (3 replicates)

2) + Agricultural DOM (3 replicates)
3) + Autochthonous DOM (3 replicates)
4) + Toxic algae lysate (3 replicates)
5) + inorg. Nutrients (N+P) (3 replicates)
6) Reduced oxygen (3 replicates)
7) Control (6 replicates)

Figure 4: Design of two batch experiments. Batches were set up in 10 L volume incubations in the dark and at in situ temperature; Triplicate treatments of the following stressors: increased input of various sources of DOM from rivers, N limitation leading to N2-fixing cyanobacterial blooms and lowered oxygen levels, and controls).

Figure 5: Upper left – sampling on Øresund; upper right – view on Lapväärtti river, the sampling site for pristine and humic rich river water in Finland. Bottom left – view to Storfjärden (Gulf of Finland) from Tvärminne Zoological Station; bottom right – Dr. Trine M Markussen working hard in the 7 C° climate room.

in the Baltic Sea. The environmental factors mentioned above affect the fundamental biogeochemical process modulated by microbes and therefore the links between environmental conditions, genomic BLUEPRINTs of microbial communities and biogeochemical rates were measured in controlled experiments. Both experiments were set up according to the same experimental design developed in previous test experiments. One part of seawater inoculum was diluted with 5 parts of bacterial free natural water (Fig. 4) manipulated with respect to environmental conditions and the activity, composition, and abundance of the bacterial community was followed over 3-4-day re-growth experiments.

5.1. Experiments

Test experiments carried out in 2014 indicated that 10L batches are well suited for small scale re-growth experiments with duration from 3 to 6 days.

The first experiment (Exp I) was carried out from 20.04 – 26.04.2015 at the Marine Biology Section, University of Copenhagen, Helsingør, Denmark. The second experiment (Exp II) was carried out from 27.07 – 02.08.2015 at the Tvärminne Zoological Station, Finland. In both experiments teams from UCPH, UH and UT participated, representing WPs 2 and 3 (Fig. 5). Natural seawater samples (300 L) were collected by boat from Øresund or Storfjärden (Gulf of Finland), respectively, from the upper 10 m of the water column. The seawater was filtered in two ways: (i) to prepare inoculum for batch incubations, water was sieved through 10 µm plankton net; (ii) and for preparation of the bacterial free growth medium, this water was subsequently filtered through 0.22 µm capsule filters (Fig. 4). Bacteria-free water served as medium for treatments, which were subsequently manipulated with respect to nutrient concentration, DOM sources, and oxygen concentration. The filtered sea water alone was used as controls, whereas the other incubations were amended with DOM from rivers, inorganic nutrients, or with lysate from planktonic organisms (e.g. cyanobacteria). For the DOM treated manipulations, agricultural river water was collected from agriculturally polluted Lielupe river in Latvia and humic rich river water was collected from Lapväärtti river in Finland. River water was filtered through 0.22 µm capsule filters and kept in the dark at +4 C° immediately after collection. Lowered oxygen treatment was set up as the blank control but oxygen concentration was reduced to ~15 % saturation by bubbling with N_2-gas.

The microcosms (7 treatments in triplicate 10 L containers) were then incubated at in situ temperature for 3-4 days. In situ collected samples and the manipulated batch treatments were sub-sampled for measurements of bacterial abundance (BA) and production (BP), O_2 consumption, inorganic nutrient and organic matter concentrations, activity of bacterial extracellular enzymes during the course of

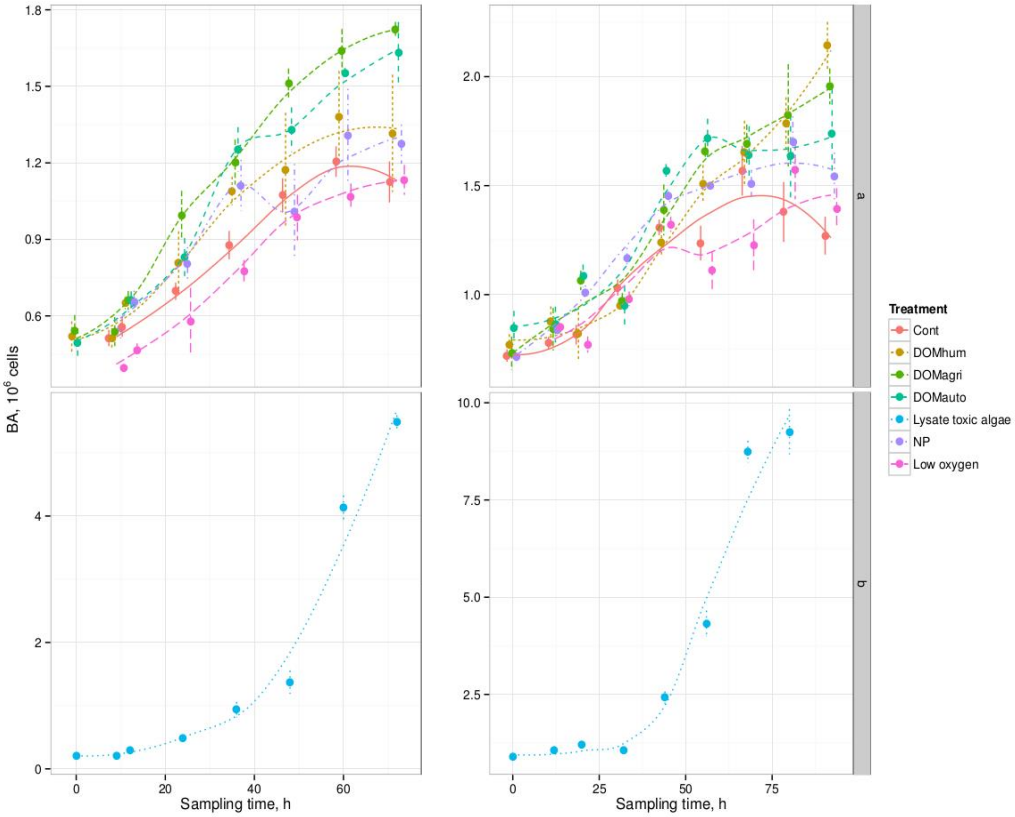


Figure 6: Time course of bacterial abundance in batch culture. Left panel – Exp I (Helsingør). Right panel Exp II. (Tvärminne) Cont - controls in the dark; DOMhum – fresh water from humic rich river; DOMagri – fresh water from polluted river influenced by agriculture; DOMauto – autochthonous dissolved organic matter; Lysate toxic algae – cell lysate from cyanobacterial culture; NP – inorganic nutrient addition; Low oxygen – reduced O2 concentration.

experiments with 6 to 12 h intervals. For analysis of bacterial community composition, samples were collected on 0.22 µm filters. DNA and RNA was extracted in October 2015 and will be exposed to 16S rDNA gene fragment sequencing and metagenomics.

5.2. Preliminary results

Overall the experiments went as planned with a sampling frequency and duration of the experiments that allowed high resolution measurements of the developments in rates and pool sizes. The addition of cyanobacterial lysates constituted a large input of labile DOM in both experiments, which was reflected in increased BA and BP (Fig. 6, Fig. 7). The other manipulations had less pronounced influence but in general the various DOM amendments affected BA and BP, especially in Exp II (Tvärminne). In addition, the functional properties of the communities (e.g. physiological fingerprints, and enzymatic activities) showed significant differences between treatments and indicated that discrete microbial communities differ in responses to environmental perturbations. The rate and pool measurements for the two experiments are all worked up and analyzed.

5.3. Outlook 2016

The coming year we will focus on two major tasks: Firstly, preparing the sequencing libraries for DNA sequencing from Experiment I and II. The samples will be analyzed for changes in community composition and presence of specific genes, and expression of specific genes related to carbon and nitrogen metabolism will be quantified by qPCR and compared to environmental manipulations in the experiments. Secondly, a large scale mesocosm experiment is planned in Kalmar, Sweden. This is anticipated to be with active participation by partners within WPs 1, 2 and 3, and later participation of partners in WP 4 and 5. It will be an expansion of the small scale experiments where we will: (i) Examine effects of different DOM sources on bacterial composition, changes of gene abundances in metagenome and mRNA abundance in metatrascriptome in a different geographical location compared to previous

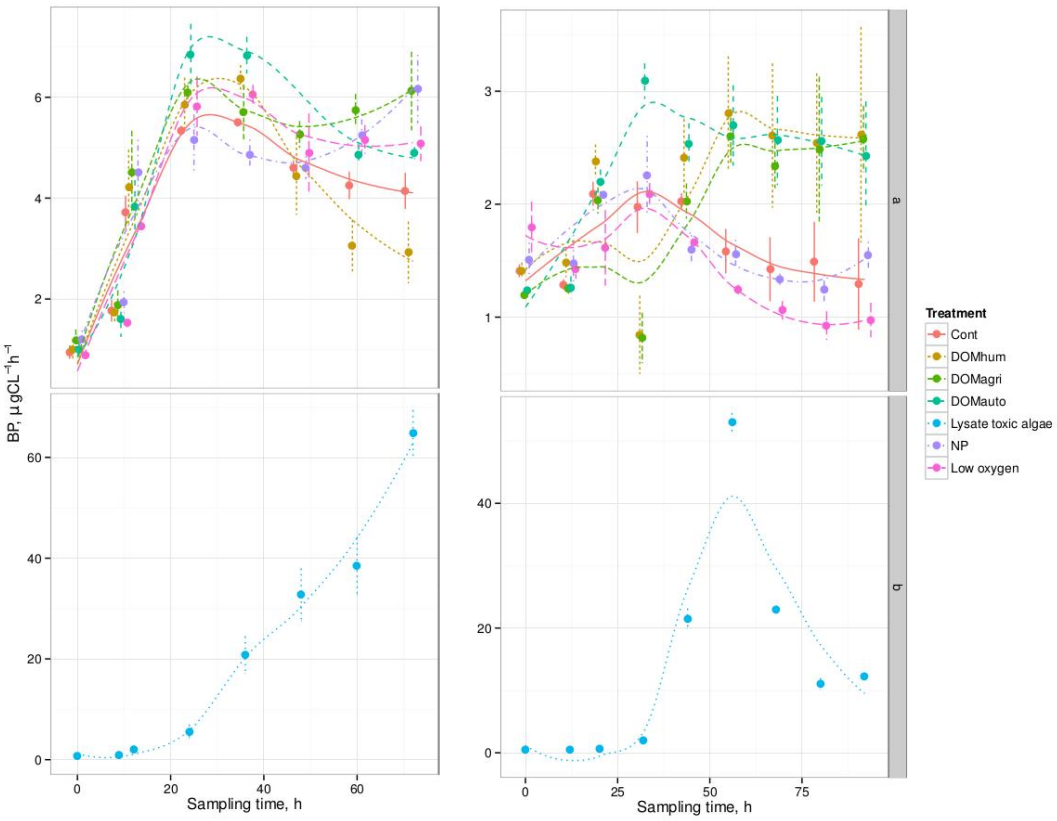


Figure 7: Time course of bacterial production (BP) in batch cultures. Left panel – Exp I, (Helsingør). Right panel Exp II (Tvärminne).

experiments; (ii) Examine to what extent the effects of DOM input in small scale experiments are affected/modified by food web structure and processes; and (iii) Assess the diversity and dynamics of viral populations in relation to environmental conditions. This experiment is planned to take place in June 2016 and will be carried out with ca 200L of Baltic seawater collected from 10 km off the east coast of Öland, Sweden, at 2 m depth at the Linnaeus Microbial Observatory (LMO) (N 56°55.851, E 17°03.640), sieved <90 µm to remove larger zooplankton but retain microzooplanton, in situ light and temperature, manual stirring, duration up to 10-12 days.

6. WP3: Impact of environmental stressors on the BLUEPRINT of model bacteria

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

The aim of WP3 is to find linkages between external drivers/stressors and the expression of specific genes in model microbial organisms representative of Baltic Sea microbiota. Microorganisms typically respond to changes in their environment by adjusting their metabolism through rearrangement of gene expression patterns. Thus, work with specific model heterotrophic bacteria and photosynthetic cyanobacteria has the potential to provide insight into key genes associated with responses to e.g. organic and inorganic nutrient loading, pollutants and changing salinity. Moreover, WP3 aims at finding relationships between key genes and different measures of microbial activity, to contribute to defining the role of microbes in biogeochemical cycles.

6.1. Growth performance experiments with Baltic Sea model bacteria

During the year, a series of experiments with Baltic Sea model heterotrophic bacteria has been carried out. The isolates used represent genome-sequenced bacteria from the major taxa Bacteroidetes (abundant following the spring phytoplankton bloom), Alphaproteobacteria (abundant in summer) and Gammaproteobacteria (forming opportunistic blooms). The isolates have been used to carry out growth performance experiments in different seawater media where bacterial production and respiration was measured. The technical setup (Fig. 8A) and measurements of respiration (Fig. 8B) using a Picarro δ13C carbon dioxide analyzer were optimized.

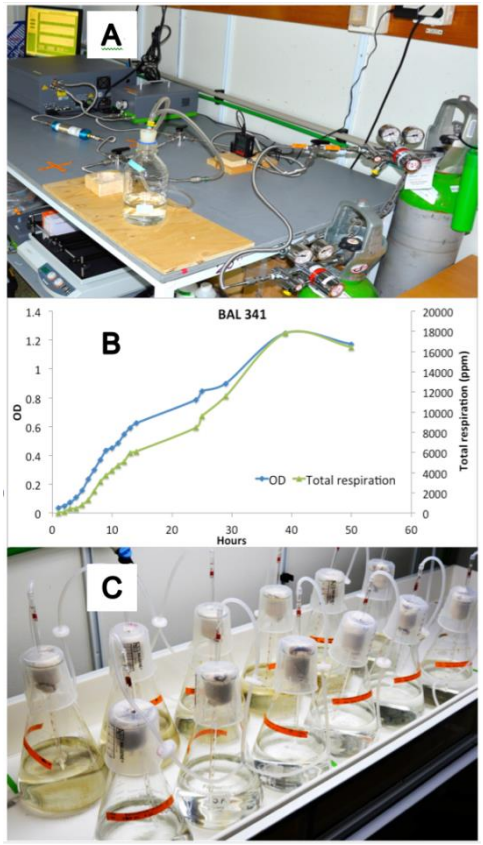


Figure 8: Technical setup for the Picarro δ13C carbon dioxide analyzer (A). Growth performance results for heterotrophic Baltic Sea model bacteria using Picarro (B). Model bacteria growing in organic matter from different phytoplankton species (C).

To explore bacterial responses to different organic matter sources relevant to the Baltic Sea, bacteria-free (axenic) cultures from three phytoplankton species typically forming blooms spring (diatom *Skeletonema marinoi*), summer (cyanobacteria *Nodularia spumigena*) and autumn (dinoflagellate *Prorocentrum minimum*) were established. Organic

Figure 9: Cyanobacterial isolates growing in different salinities.

matter from these cultures was harvested in early and late growth phase, and used as resources for growth in experiments with the model bacteria (Fig. 8C). Comparative experiments with protein and polysaccharides as representative organic macromolecules were done. Analyses of samples for biomass production, respiration and genome-wide gene expression (RNAseq) from these experiments is currently ongoing.

Laboratory experiments showed that Baltic Sea model cyanobacteria isolates can use the simplest forms of phosphonates as their sole phosphorus source. On the contrary, more complex compounds used as herbicides and detergents (such as glyphosate and phosphonobutanetricarboxylic acid) were growth inhibitory for the studied cyanobacterial isolates. To determine the genes responsible for phosphonate usage in these experiments, RNAseq analysis on collected samples will be carried out during 2016. In another experiment the role of interactions between diazotrophic cyanobacterium (Nodularia sp. AV2) and its bacteriophage (siphophage vB_NpeS-2AV2) on biogeochemical cycles (namely, the nitrogen cycle) and planktonic community dynamics was determined. The results indicated that nitrogen released from the cyanobacterial host as a result of phage-induced host cell lysis strongly supported plankton growth but that the effect was lost as a result of evolution of phage resistance. In a separate laboratory study, we determined the threshold salinity in which species distributions of cyanobacteria may shift from freshwater *Anabaena* to brackish water *Nodularia* (Fig. 9) - the crucial concentration was around salinity 3. In salinities below 3, *Anabaena* dominated strongly whereas in higher salinities *Nodularia* predominated. The molecular responses to salinity of these cyanobacteria will be analyzed using RNAseq. Moreover, novel *Anabaena* and *Nodularia* strains for future experiments were isolated during summer (Fig. 10).

In addition to this experimental work, participants in WP3 have participated in WP2 experiments (at Tvärminne and Helsingør), and are also directly involved in the bi-weekly work discussions developing in WP5. Moreover, planning has been ongoing of the mesocosm experiment at LNU, organized by WP2 and scheduled for June 2016. LNU members also contributed to WP1 and WP4 by bi-weekly sampling of DNA from the Linnaeus Microbial Observatory (LMO) station in the Baltic Sea.

6.2. Controls on heterotrophic N_2 fixation

During 2015, several experiments also tested the ability of abiotic surfaces to facilitate N_2 fixation by heterotrophic non-cyanobacterial isolates from the Baltic Sea under typical atmospheric levels of oxygen - concentrations of oxygen expected to occur in the environment. Particle types previously reported to stimulate N_2 fixation were used in the experiments, including intact and shredded glass fiber filters, plastic filters, transparent exopolymer (TEP) and Zostera (Eel grass) fragments. Intriguingly, no significant N_2 fixation activity (determined by acetylene reduction) was detectable in our particle treatments, relative to negative and positive controls (Fig. 11). Colonization of particles was evident in experiments based on microscopy (Fig. 12), despite the absence of notable N_2 fixation activity. The lack of N_2 fixation stimulation was unexpected, but at the same time intriguingly

Figure 10: In summer 2015 new cyanobacterial strains were isolated from a dense bloom in the coastal Baltic Sea. Both Anabaena and Nodularia strains able to grow with methylphosphonate as a sole phosphorus source were found. Purification of those strains is in progress.

points to a necessity for particle structure likely provided by other microorganisms for the N_2 fixing isolate to conduct N_2 fixation under full oxygen. The importance of microbial interactions in facilitating heterotrophic N_2 fixation is largely unknown; this may be one layer of important interactivity.

6.3. Outlook 2016

During the coming year, analysis of the experimental samples mentioned above and collected in 2015 will proceed. Complementary experiments to examine the influence of organic matter on bacterial growth and gene expression will be done. A new set of experiments will be carried out to investigate the potential effects of hazardous substances on the growth and gene expression profiles of Baltic Sea model bacteria. This will be done by using representative compounds of selected hazardous chemical classes in seawater culture experiments with our taxonomically distinct model bacteria. This will allow evaluating the influence of such substances on key bacterial genes and on central metabolic routes. The RNA sequencing and data analysis of the salinity, phosphorus/phosphonate experiments with cyanobacteria will be carried out early 2016. Growth and process rates in cyanobacteria grown in various conditions concerning the experiments (2015 and 2016) will be determined.

In 2016 we will also test the working hypothesis that heterotroph diazotroph isolates can fix N_2 under full oxygen when provided with particles pre-colonized with biofilm-forming microbes, or if the isolates are embedded in an artificial biofilm (e.g. agarose or alginate) on an abiotic particle. Lastly, we will investigate if *nifH* transcription by strains that are actively fixing N_2 is truly a useful indicator of N_2 fixation activity.

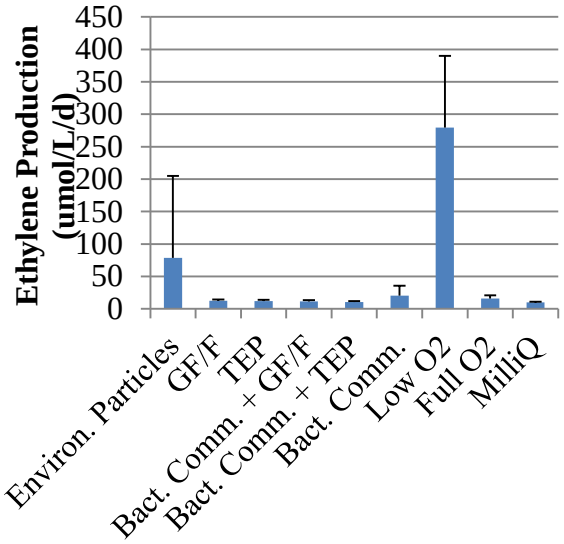


Figure 11: Addition of various particles to a N-limited culture of *Pseudomonas stutzeri* BAL361 does not stimulate significant N-fixing activity (as measured by acetylene reduction to ethylene; vertical axis).

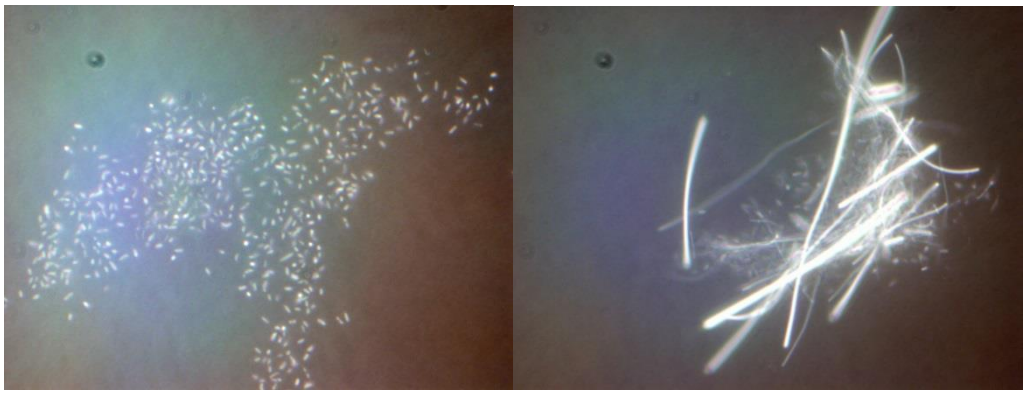


Figure 12: Particle colonization by strain BAL361 using dark-field microscopy. On the left are cell clumps formed in cultures supplemented with transparent exopolymer (TEP) material; On the right is a bundle of glass fiber filter (GF/F) pieces with associated bacterial cells formed in cultures supplemented with GF/F pieces. Bacteria photographed are white short rod-shaped cells.

7. WP4: The bioinformatics platform

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

7.1. Brief description of WP4

The BONUS 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.

7.2. Activities in 2015

One of the goals of WP4 is to create a Baltic Sea reference metagenome (BARM) onto which individual metagenome- or transcriptome samples can be mapped and their functional and/or taxonomic profile (BLUEPRINT) deduced. To reach this goal, shotgun metagenome sequencing (Fig. 13) has this year been conducted on 44 samples obtained in a Baltic Sea transect cruise of WP6 and WP1 (June 2015). These samples were taken at different depths at stations spanning the whole salinity gradient and they cover the major geochemical guilds of the Baltic Sea. The data was supplemented with metagenomes from 37 samples collected ~weekly at a single station east of Öland (the Linnaeus Microbial Observatory; LMO) by WP3. The short shotgun DNA sequences were

Figure 13: DNA sequencing instrument used for metagenome sequencing of the 81 samples that are the basis for BARM v1.

collectively assembled into hundreds- to hundreds of thousands base-pair long genome fragments (contigs) using a high-performance computer (Fig. 14). Following assembly, protein-encoding genes were identified on the contigs, and the proteins' functions were predicted by comparing the sequences to proteins and protein domains of known functions. In total the assembly resulted in 22 million contigs, of which 2.4 million were longer than 1000 bp. These encoded 6.7 million genes.

In order to provide the BONUS BLUEPRINT partners, and in the future other researchers, easy access to the metagenome data, a database (BalticMicrobeDB) has been designed and implemented where the processed metagenome data will be stored and accessed through a graphical user interface. Here the user will be able to select data for visualization and downloading by selecting specific functional gene(s), taxa or samples (e.g. based on environmental metadata).

In addition to assembling and annotating the BARM and developing the BalticMicrobeDB database, a large number of bacterioplankton genomes have been reconstructed from the metagenome data from the LMO time-series (Hugerth et al, 2015) through a bioinformatic procedure called binning. Binning the contigs into genomes allows us to investigate which functional genes co-exist in the same genome. This will likely lead to better predictions of existing metabolic pathways in the dataset.

7.3. Outlook 2016

During 2016 we will continue working on the BalticMicrobeDB-database with the goal to have it up and running before the summer. Together with WP1 and WP6 we will investigate spatiotemporal variability of the genetic BLUEPRINT relative to environmental conditions based on the data in BalticMicrobeDB, and participate in analyses of sequence datasets from the WP2 experiments. We will also continue the collaboration with WP5 to link enzymes and uptake systems identified in the metagenome to processes of the BALTSEM model.

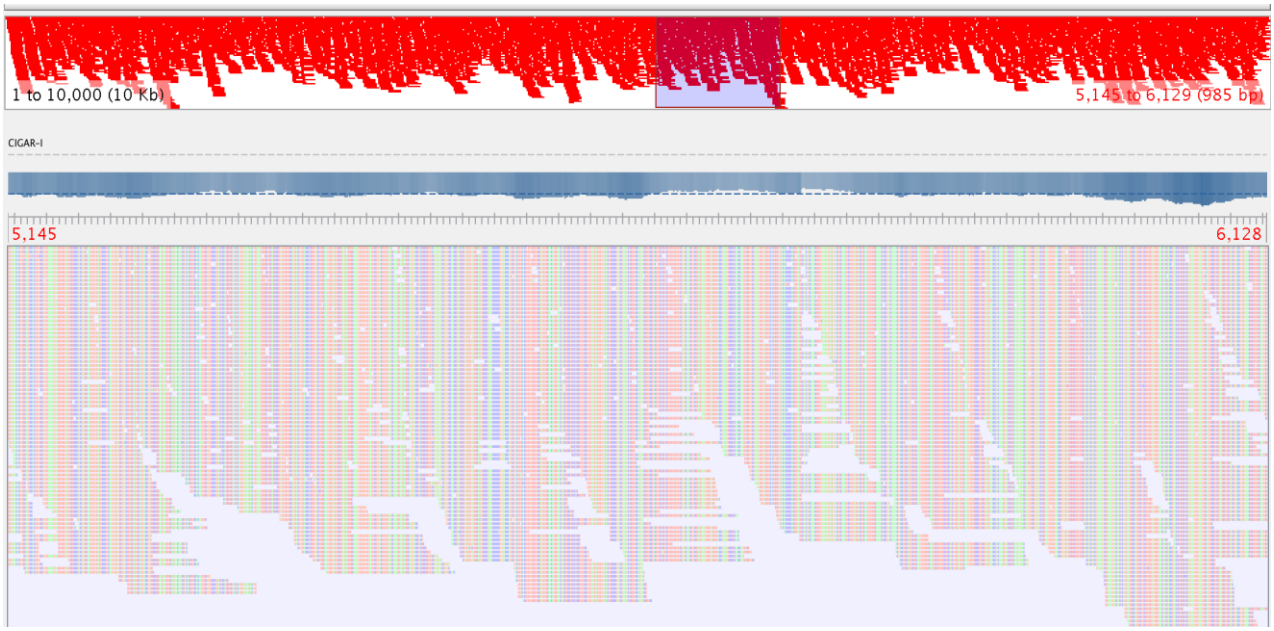


Figure 14: View of a BARM contig using the program Tablet. The lower panel is a zoom in of a ~1000 bp region of the contig where individual sequence reads that have been mapped to the contig are shown as horizontal lines, and where each position is coloured according to nucleotide (A,T,C or G).

8. WP5: Incorporation of BLUEPRINT in biogeochemical modeling

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

8.1. General work strategy

The aim of WP5 is to utilize the emerging wealth of genetic information to improve the possibility of making realistic validations of biogeochemical models. The microbial genomes present in the environment can thus be matched to biochemical processes depicted in the models. Throughout the second year we have continued to dig for common lines of how to read the vast amount of metagenome data in a functional context. This means continuous work towards the Deliverables 5.2 and 5.3. In the D5.2, we are matching the BALTSEM structure to different classes of metagenomic data. This will be a catalog consisting of a semi-quantitative matrix where the abundance of functional genes are aligned with the flow of matter generated by the model (Month 30). The final deliverable 5.3 will be improved biogeochemical model formulations where we are trying to translate the genomic topology and forcing response into biogeochemical model parameterizations (Month 47).

8.2. The Baltsem Model outline

The BALTSEM model has been expanded to represent the microbial food web in closer detail. Since the very large amount of genetic information cannot be aggregated in a meaningful manner the model structure has to portrait the food web in greater detail in order to match biochemical transfer. In Figure 15 an example is given on how the flow of PO4 has been diversified.

8.3. Linking mathematical modeling and genetics

As commented already in last year's report, in the initial plan for the BONUS BLUEPRINT project the flows of matter depicted in the model were regarded as structures parallel to the metabolic pathways in the biochemical map. However, the flow of matter in the model is primarily restricted to inorganic mineral salts whereas in the biochemical pathway the flow is mainly organic matter (carbon). While these entities in an overall scale can be converted to each other by applying a C:N:P ratio the comparison between model and biochemistry pathway map is not as

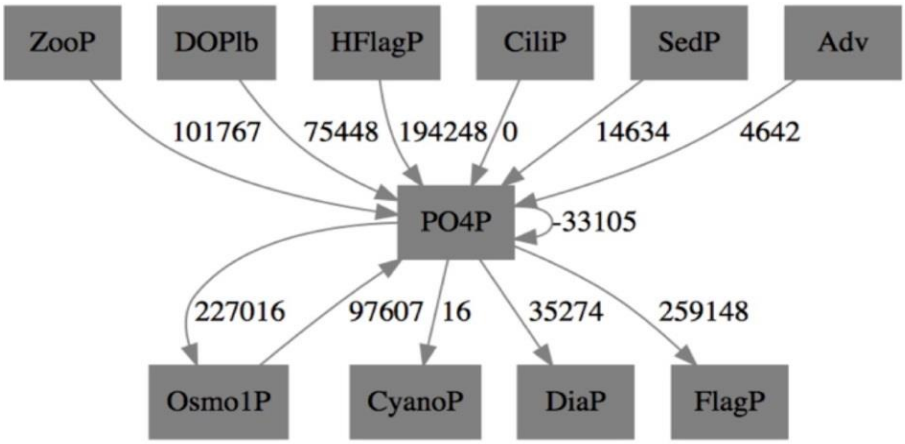


Figure 15: Flow of PO_4-P in the BALTSEM model. At steady state the flow of P through the model can be seen as the activity of each of the components. For example, Osmo1P (=heterotrophic bacterium taxa1) draws 227016 units of P while returning 97607 units to the P-pool. PO4 is also regenerated by the different grazers feeding on bacteria, seen in the upper row as ZooP, HFlagP and CiliP.

straightforward as first indicated. Instead the following modified strategy is now investigated. Since the model depicts transfer of matter between organisms/groups while most metabolic maps show transformation of matter through biochemical processes within the cell. We need to find connecting points where the flow of matter in quantitative terms can be extracted from model runs and correlated to the abundance of molecular elements.

A strong temporal signal has been detected in the distribution of transporters in the metagenomes obtained from the LMO time-series (please see Fig. 11 in last year's report), corresponding to the temporal signal seen in the model time-slices observed at the LMO station. The significance of this observation is that transporters are indicators of the uptake of small molecules. Since uptake and release of nutrients is an important description of the flow of matter in the BALTSEM model we therefore have a possible argument to test that would be a "true" connection between gene variation and model dynamics. This line of investigation is in close collaboration with WPs 2 and 3.

8.4. Further investigations into transporter identity

In 2015 we have identified the primary transporter types in the Baltic Sea surface waters. This is based on both metagenomic (DNA level) and metaproteomic (expressed protein level) data (Fig. 16 A, B). The microbial community appears to be focused mainly in the transport of carboxylic acids, carbohydrates (such as simple sugars) and amino acids. In addition, we find that transporters for organic phosphorous are surprisingly abundant in the microbial community. We are currently working to better define and quantify transporters in the datasets, linking the transporter abundances to nutrient shifts predicted by the BALTSEM model as well as relating transporter distributions to ecological processes in the Baltic Sea.

8.5. Outlook 2016

At present, the work in WP5 is concentrated on defining the particular phases of the year in the pelagic environment of the Baltic proper. Spring, early and late summer, autumn and winter. The significant environmental, taxonomic and genetic data for each phase are aligned in a table to be presented as deliverable 5.2 in mid-2016. From this table a textual and numerical description of the food web state during each phase will be composed in relation to the model output. In particular the dynamic behavior between dominant prokaryotic taxa will be explored both in the BALTSEM model and in an animation

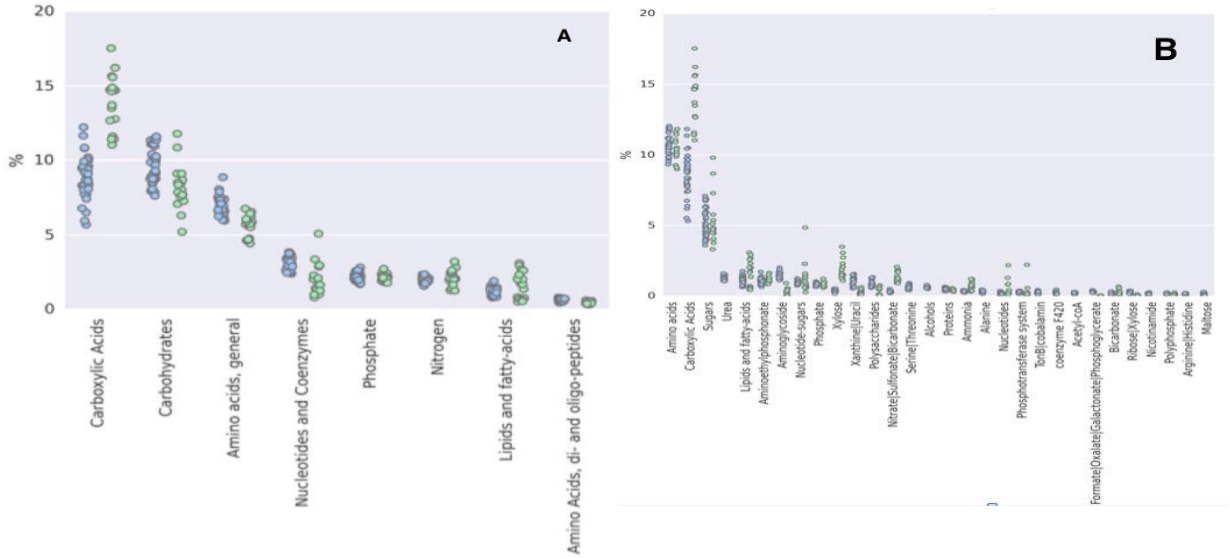


Figure 16: A) shows transporters grouped at a general level. Circles represent individual transporters and are grouped by predicted compound that they transport. Blue circles indicate mean metagenomic abundance in a 2012 time-series from the LMO station (March - December, 37 samples). Green circles indicate mean metaproteomic abundance in 7 samples also taken in 2012 (May - December). B) Abundance of different transporter types shown in finer detail. Circles represent individual transporters and are grouped by predicted compound that they transport. Blue circles indicate mean metagenomic abundance in a 2012 time-series from the LMO station (March - December, 37 samples). Green circles indicate mean metaproteomic abundance in 7 samples also taken in 2012 (May - December).

based on a population dynamics framework. In conclusion it is important to stress the close cooperation between the different WPs, primarily between WP 2 and 3, but increasingly also with WP1 as the spatial data from the Baltic Sea field cruises become available.

9. 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 BONUS BLUEPRINT into practical operability within the BLUEPRINT Competence Center (BCC).

Figure 17: Round-table discussion the 10th BSSC in Riga (18.06.2015). From left to right: Lena Avellan (HELCOM), Christin Bennke (IOW), Lasse Riemann (UCPH) and Matthias Labrenz (IOW). Photo: A. Eggert.

During the first year we established a discussion forum to discuss the approach of BONUS BLUEPRINT integrating microbial indicators into monitoring. Moreover, we set up a standardized protocol for nucleic acid sampling, extraction and sequencing together with WP4.

9.1. Monitoring discussion forum (stakeholder event)

The second BONUS BLUEPRINT discussion forum took place in combination with the 10th Baltic Sea Science Congress in Riga, Latvia (15.-19.06.2015). It was implemented in the round-table discussion "Development and implementation of novel indicators for Baltic Sea monitoring" on June, 18th 2015 (Fig. 17). Sectors represented during the meeting included research facilities (thirteen different research institutes/universities) and a monitoring agency (HELCOM). As a keynote speaker Lena Avellan from HELCOM was invited to give a presentation on "HELCOM core indicators on Baltic Sea environmental status". She provided an overview about HELCOM monitoring and assessment strategies, indicator definitions and core indicators, as well as GES-boundaries.

Figure 18: CTD with regular free-flow bottles (left) and new AFIS (right). Photo: C. Bennke

The round-table gave us the opportunity to get in close contact with a HELCOM representative discussing the approach of BONUS BLUEPRINT and its first results. The audience, consisting of more than 25 participants from 10 different countries, was involved in the open discussion on indicators. The need for new ones, but also the long road from the initial idea of a novel indicator to political decision and practical implementation in the Baltic Sea monitoring was discussed.

9.2. Standardization of BCC procedures

During the first project cruise (June 2014), standardized nucleic acid samples were taken using the old AFIS system (see first year report). However, the company Hydro-Bios (Kiel, Germany) developed a new AFIS sampler (Fig. 18), which was extensively

tested in the laboratory and at sea. This was done in close collaboration with the BONUS Innovation project "AFISmon", where an automatic water in-situ fixation sampling prototype is currently developed. The new AFIS sampler (Fig. 18) was used for the first time during a four day cruise to BSH-Marnet stations in April 2015 with the RV Elisabeth Mann Borgese (EMB). It turned out that the new AFIS worked perfectly and samples are ready for further laboratory analysis ashore.

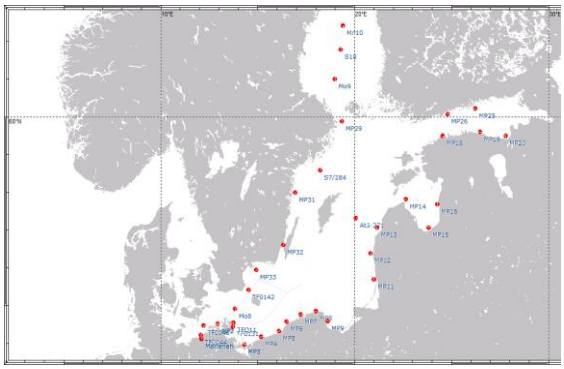


Figure 19: Station map of the BLUEPRINT cruise in August 2015. The circuit route started eastwards. Map was generated using the software eMission (D. Rüss, IOW).

The second BONUS BLUEPRINT cruise took place with the RV POSEIDON from 17.08.-04.09.2015 and concentrated on collecting samples close to shore, especially at the estuaries of the rivers Oder, Vistula, Neman, Daugava and Neva (Fig. 19). Furthermore, we were able to sample also in the Gulf of Riga and Gulf of Finland, which was omitted in June 2014.

The guest scientist Peeter Laas from the University of Tallin, Estonia, was able to gain bacterial abundances using flow cytometry directly on board. This cruise was performed in close collaboration with the Leibniz society financed microplastic project MIKROMIK. All samples with regard to metatranscriptomic analyses were taken with the new AFIS sampler.

Nucleic acid extractions (DNA and RNA) were performed from 10 stations of the Alkor cruise (June 2014, Fig. 20) and sent for sequencing to SciLife, Sweden (Metagenomes) and Eurofins, Germany (Meta-transcriptomes). With regard to metatranscriptomics, and the decision of sequencing total RNA or mRNA, the first set of samples was analyzed together with WP4. The results indicate that sequencing mRNA is more cost-effective than total RNA, which will guide our further sequencing efforts.

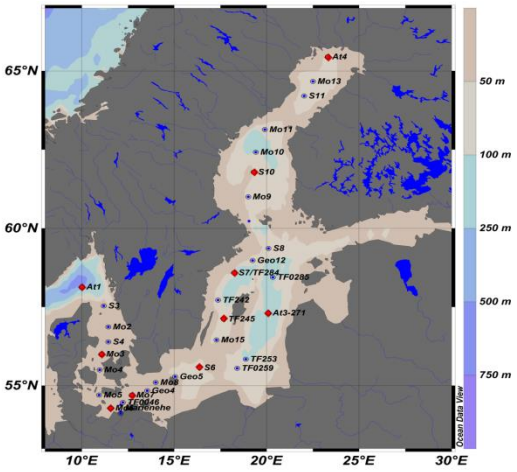


Figure 20: Cruise track of the ALKOR cruise in June 2014. Metagenomes and metatranscriptomes were obtained from those stations marked with the red diamonds.

The retrieved metagenomes alongside the salinity gradient of the Baltic Sea were implemented into the Baltic Sea reference metagenome (BARM), which will be used to map the metatranscriptome sequences against, and to link genetic functional information with measured activities (e.g. WP1, Fig. 1). In addition to the metagenome and metatranscriptome datasets, we have generated 16S amplicons from the same stations, targeting the V3/V4 region of the 16S rRNA and the 16S rRNA gene. Eventual analyses will show how the bacterial activity fingerprints, indicated by the 16S RNA, in combination with the succession of bacterial assemblages along the salinity gradient of the Baltic Sea, can be used as an indicator for a specific environmental state.

9.3. Outlook 2016

The upcoming third year will focus on further investigating the 16S amplicon dataset with respect to the question how close can we get to a state description by using molecular based analysis and can we use those genetic fingerprints as state indicators? Further on, we will focus on publishing the BARM together with WP4 and WP1. Also the mapping of the metatranscriptome dataset onto the BARM using the BONUS BLUEPRINT bioinformatics pipeline linking the presence of genes with their expression needs to be completed.

10. 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 second Skype meeting on 18th September 2015 was a valuable opportunity to brief all project partners on the progress in the different WPs, and to discuss *ad hoc* questions. The second discussion forum held in June 2015 at the BSSC in Riga again proved to be an extremely valuable exchange between BONUS BLUEPRINT scientists and monitoring experts. The 2. annual BONUS BLUEPRINT meeting will take place 9-11 May in Helsinki.

11. Original vs. Future working plan

We currently do not envision any major changes in the working plan, financial plan, or the timing or character of deliverables. However, we would like to mention that due to methodological difficulties with illumina sequencing and companies carrying out this service in Year 1 and parts of Year 2, the overall sequencing efforts in the project are delayed. The problems have now been resolved and several high-quality sequence datasets have been received based on DNA as well as RNA templates. Moreover, several planned project employments got initially delayed. Taken together this has caused a slight delay in use of finances resulting in that BONUS BLUEPRINT overall after Year 2 has spent 77.9% of the finances originally budgeted for Years 1 and 2.

12. Dissemination

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.

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., F. Baltar, J. M. González and J. Pinhassi. **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.
3. Hugerth LW, Muller EEL, Hu YOO, Lebrun LAM, Roume H, Lundin D, Wilmes P, Andersson AF. **2014**. Systematic Design of 18S rRNA Gene Primers for Determining Eukaryotic Diversity in Microbial Consortia. PLoS ONE 9(4): e95567.
4. Lindh MV, Sjöstedt J, Andersson AF, Baltar F, Hugerth L, Lundin D, Muthusamy S, Legrand C, Pinhassi J. **2015**. Disentangling seasonal bacterioplankton population dynamics by high frequency sampling. Environmental Microbiology. 17(7):2459–2476.
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. 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

Publications in preparation / submitted

1. Beier S, Shen D, Juergens K. et al. Functional redundancy along changing salinity. Manuscript in preparation.
2. Bennke CM, Pollehne F, Müller A, Hansen R, Kreikemeyer B, Labrenz M. The potential for plastidal rRNA as a useful indicator for state descriptions. Manuscript in preparation.
3. 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.
4. 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.
5. Larsson J, Müller-Karulis B, Karlsson C, Andersson A, Hugerth L, Alneberg J, Pinhassi J, Hagström Å. Seasonal shifts in transporter gene abundance and composition in Baltic Sea microbial communities. Manuscript in preparation.
6. 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.

7. 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.
8. 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.
9. Lindh MV, Sjöstedt J, Ekstam B, Casini M, Lundin D, Hugerth LW, Hue Y, Andersson AF, Andersson A, Legrand C, and Pinhassi J. Bimodal occupancy-frequency distributions uncover the importance of regional dynamics in shaping marine microbial biogeography. Submitted to PNAS.
10. 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.
11. Sundh J, Karlsson CMG, Bunse C, Müller-Karulis B, Alneberg J, Pinhassi J, Andersson AF, Hagström Å. Ecological significance of transporters in the Baltic Sea microbial foodweb. Manuscript in preparation.
12. Herlemann D, Lundin D, Andersson A, Labrenz M, Jürgens K. Phylogenetic signal of salinity and season on bacterial community compositions in the salinity gradient of the Baltic Sea. Submitted to Environmental Microbiology.
13. Bombar D, Paerl R, Riemann L. Non-cyanobacterial diazotrophs: a role in marine N_2 fixation? Review manuscript in preparation for Trends in Microbiology
14. 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.

Project 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**).

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. Bennke CM, Riemann L, Andersson A, Labrenz M. From mechnistic to functional monitoring - guiding microbial indicators into practical operability. Oral presentation 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.
33. 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
34. 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

Student theses

Cairns J. **2015**. Evolution in host-parasite interaction between novel cyanophage and filamentous, nitrogen-fixing cyanobacterium. Master’s thesis. University of Helsinki, Division of Microbiology and Biotechnology, June **2015**.

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**.

Participation as members or observers in stakeholder committees (performance statistic 3)

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

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	6	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