



BONUS BLUEPRINT

Biological lenses using gene prints

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1. Brief description of the project's overall goal(s) and expected final results

The countless planktonic microbes are the principal drivers of carbon and nutrient cycles in the Baltic Sea. Yet, they are not included among natural indicators used to describe the environmental status of the Baltic Sea as part of the EU water framework directive. This flaw has been highlighted by HELCOM and OSPAR in their work to coordinate the development of indicators and determining the “good environmental status” in the Baltic and North Sea areas. Through tremendous technological advancements in the last decade, we now have the methodological capacities to retrieve and process the genetic information from these microbes in a cost-efficient manner. Thereby we can gain mechanistic understanding of how the microbes drive food-web processes and how they are affected by the environment. Based on such knowledge, the BONUS BLUEPRINT project aims to develop a framework that allows us to determine the ecological status of the Baltic Sea based on microbial genetic indicators. The expected final results are a set of indicators reflecting the biodiversity and genetic functional profiles of microbes in a given seawater sample.

2. Work performed since the beginning of the project

The main challenges of BONUS BLUEPRINT are

- i) to describe and understand the links between environmental parameters and the genetic information carried by microorganisms
- ii) to establish easily reproducible and automated methods of obtaining and analyzing this genetic information, and
- iii) to use the genetic functional profiles to improve ecosystem models, which are needed to predict future scenarios of the Baltic Sea.

To address the first point, the first two years of BONUS BLUEPRINT included elaborate field sampling as well as experimental manipulations with natural samples and laboratory model microorganisms. We have obtained roughly 1400 nucleic acid samples and corresponding metadata, covering all different subsystems of the Baltic Sea and representing the responses of microbial communities to additions of well-known stressors such as eutrophication or hypoxia (Fig. 1). This sample set is crucial to assess how the microbial genetic profiles vary along environmental gradients and in response to conditions that represent deviations from “good environmental status”. Field sampling and manipulation experiments are ongoing in 2016 since we aim to effects of environmental perturbation on the microbial genetic information.

Addressing point ii) involves the development of standardized sampling procedures and bioinformatics pipelines for processing the wealth of genetic sequence data. Since 2014 we have developed and extensively tested an automatic water in-situ fixation sampler in collaboration with the BONUS Innovation project BONUS AFISmon. This sampler is optimized for obtaining undisturbed gene expression (transcriptional) profiles of the microbes. Further, we have made great progress in developing the bioinformatics pipelines for the extensive sequence handling. This includes the elaborate and computationally extensive steps of assembling and annotating obtained sequences (what genes & functions are encoded?), generating the Baltic Sea Reference Metagenome (BARM) which functions as the sequence “backbone” against which all future sequence datasets can be mapped, and designing a database where the processed metagenome data are stored and can be accessed via a graphical user interface.

point iii), the integration of sequence data into biogeochemical models, is particularly challenging since it requires finding connecting points between the ecosystem model (flow of matter, mainly inorganic mineral salts) and genetic sequence information (genes representing biochemical pathways of organic matter within cells). Since the beginning of the project, project researchers have succeeded in improving the existing model formulations, and in identifying said connecting points in the form of transporter genes. Transporter genes link processes within the cell with nutrient concentrations in the surrounding water. Various examples of such genes indeed exhibit changing relative abundances across seasons, and these changes correspond with the concentration changes of their respective substrate. Further investigations

into transporter identities and how they are linked to nutrient shifts will improve the simulations of aquatic ecological processes in the Baltic Sea.

3. Main results achieved during the reporting period, including Potential impact and use envisaged by the results noted (including the socio-economic impact and the wider societal implications)

Our project combines cutting edge technologies including next-generation sequencing, bioinformatics, and mathematical modeling. The results achieved so far open up new avenues for studying aquatic ecosystems and provide new conceptual frameworks for the use of meta-omics data and ecosystem modeling. The multitude of obtained environmental data, in combination with genetic functional profiles, as well as the development of standardized sampling procedures, are important prerequisites for the development and integration of new microbial indicators into existing monitoring procedures. The development of functioning bioinformatics pipelines and the Baltic Sea reference metagenome (BARM) are invaluable contributions towards being able to quickly process and interpret accumulating genetic field data. The BARM can be compared to the generation of a fixed and extremely detailed background picture onto which investigators can in the future easily post and assemble new sets of puzzle pieces (new metagenomes/transcriptomes) that match the background. Thus, we have reached important milestones for fulfilling the overarching project objective, i.e. to reliably deduce environmental status based on indicators reflecting the biodiversity and genetic functional profiles of microbes in seawater samples. The development of new and improved ecosystem models will serve as an essential tool in designing environmental management actions required to reach specific environmental targets or statuses in the future Baltic Sea.

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