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Spatiotemporal Niche-Partitioning of Bacterioplankton Community across Environmental Gradients in the Baltic Sea

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Declaration:

Hereby I declare that this doctoral thesis, my original investigation and achievement, submitted for the doctoral degree at the Tallinn University of Technology has not been submitted for doctoral or equivalent academic degree.

/Peeter Laas/

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Bakterplanktoni koosluse ajalis-ruumiline nišijaotus läbi keskkonnagradientide Läänemeres

PEETER LAAS

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1. LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on three papers, in the following referred to by their Roman numerals:

I Laas, P., Simm, J., Lips, I., Metsis, M. (2014). Spatial variability of winter bacterioplankton community composition in the Gulf of Finland (the Baltic Sea). *Journal of Marine Systems*, 129, 127 - 134.
II Laas, P., Simm, J., Lips, I., Lips, U., Kisand, V., Metsis, M. (2015). Redox-specialized bacterioplankton metacommunity in a temperate estuary. *PLoS ONE*, 10(4), e0122304
III Laas, P., Šatova, E., Lips, I., Lips, U., Simm, J., Kisand, V., Metsis, M. (2015). Near-bottom hypoxia impacts dynamics of bacterioplankton assemblage throughout water column of the Gulf of Finland (Baltic Sea). *PLoS ONE,* 11(5), e0156147.

Author's Contribution to the Publications

I – The author was responsible for collecting and processing the samples, doing the molecular analyses, doing some of the statistical analyses and writing the manuscript.
II – The author was responsible for collecting and processing the samples, doing the molecular analyses, doing some of the bioinformatics analyses, doing the statistical analyses and writing the manuscript.
III – The author was responsible for collecting and processing the samples, supervising the molecular analyses, supervising the flow cytometry analyses, doing most of the bioinformatics analyses, doing the statistical analyses and writing the manuscript.

INTRODUCTION

Studying aquatic microbiology is like drilling into the very core of life on earth. For about two billion years, aquatic microbes were the single living organisms on the planet. The evolution and environmental impact of these microbes have started a beginning for the existence of complex multicellular terrestrial organisms that can breathe oxygen. However, despite having to share the planet with these "new kids on the block", the importance of aquatic microorganisms has not disappeared. They are still essential mediators of biogeochemical transformations that most other living beings are dependent on. One can consider microbes as a foundation for an ecosystem. We stand on the shoulders of tiny giants.

Ironically, along the investigations into aquatic ecosystems the outlines of "the full picture" of prokaryotic life in these ecosystems has started to emerge just recently. This fact that of microbiologists are late to the scene has been mainly caused by methodological limitations in the past – the technological capability to tap into the structural and functional diversity of microbial life has just gotten into the reach of researchers past couple of decades. Especially the emergence of molecular methods that enable cultivation-independent investigations of microorganisms. What a treat it has been - to find such multiverse of diversity and complexity. However, a long road lies ahead making sense of it all in an ecological context.

This thesis is about microbial life along gradients. The Baltic Sea offers many. On the evolutionary scale, the Baltic Sea just appeared with the melting of a giant ice block in North Europe some eight thousand years ago. For macro-organisms it is a limited period to adapt, and this is reflected by low diversity in mesohaline regions of the sea. In contrast, it cannot be said about microbial diversity in the Baltic Sea, as is discussed in subsequent chapters. The gradients of environmental parameters (e.g. salinity, temperature, concentrations of inorganic nutrients and dissolved oxygen) and seasonal changes make this interesting study area to investigate spatiotemporal dynamics of bacterioplankton community composition (BCC) in relation to these changes.

ABBREVIATIONS

BCC – bacterioplankton community composition
BLAST – Basic Local Alignment Search Tool
CFU – colony forming units
DNA - deoxyribonucleic acid
DOC – dissolved organic carbon
DOM – dissolved organic matter
HELCOM - The Baltic Marine Environment Protection Commission, also known as Helsinki Commission
HMW – high-molecular-weight
LMW – low-molecular-weight
NCBI – National Center for Biotechnology Information
NGS – next-generation sequencing
NMDS - non-metric multidimensional scaling
OMZ – oxygen minimum zone
OTU – operational taxonomic unit
PCR – polymerase chain reaction
POM – particulate organic matter
rDNA - ribosomal ribonucleic acid gene
rRNA – ribosomal ribonucleic acid
TEA – terminal electron acceptor

1. REVIEW OF THE LITERATURE

1.1 Bacterioplankton cell size and abundance

In marine biology plankton is a very general term for a large variety of organisms suspended in the water column that are incapable of swimming against large-scale currents. Nekton - another very broad term for organisms that do have sufficient power for such locomotion (Lalli & Parsons, 1997). Bacterioplankton is the fraction of plankton contributed by members of domain *Bacteria*. In some cases, also *Archaea* are included in the bacterioplankton. Therefore, it is important to emphasize that at the present dissertation only bacteria are considered under the term. A logarithmic scale is used to divide plankton into size categories, ranging from femtoplankton (<0.2 μm) to megaplankton (>20 cm); most of the bacterioplankton fall into picoplankton (0.2-2.0 μm). Although, some bacteria, like many cyanobacteria or some sulphur-oxidizing bacteria, occur in larger categories (Munn, 2011). In this thesis 0.2 – 5.0 μm fraction is studied, so also part of nanoplankton (2.0 – 20.0 μm) was included.

In microbial world size not only is important, moreover, it also has many different aspects tied to it. As most of the ocean is oligotrophic (low nutrient concentration), uptake of nutrients is critical. Small cells can take up nutrients more efficiently than a larger ones – surface area/volume ratio is determinative (Munn, 2011). On the other hand, certain range of cell size can put cell populations under grazing pressure, depending on the predators (Jürgens & Matz, 2002). Likewise, filamentous bacteria and those attached to particles can be more resistant to predation (Jürgens & Matz, 2002; Šimek *et al.*, 2001).

In contrast to terrestrial ecosystems, microbes contribute the main bulk of biomass in the oceans. Every drop of "the clear blue water" contains millions of microbes (bacteria, archaea, viruses and protists) - out-ranking metazoan in abundance, biomass, metabolic activity, and structural and functional genetic diversity (Pomeroy *et al.*, 2007). The estimated number of prokaryotes (bacteria and archaea) in the aquatic habitats is little over 1.1×10^{29}, majority of which is contributed by marine ecosystems (Whitman *et al.*, 1998). About two-third of this number is contributed by bacteria.

1.2 Importance of bacteria through their functional roles in aquatic ecosystems

There is a saying that "money makes the world go round", there are many who would disagree, after all, the earth has been spinning for billions of years without a scent of a penny. However, it can be stated with full confidence that "micro-

organisms make the biogeochemical cycles go round" and following chapter is dedicated to summarizing this process in confines of domain *Bacteria*.
No other domain of life withholds so many types of metabolism than *Bacteria* (Gottschalk, 2012), this broad range of capabilities makes their role in biogeochemical cycles so pivotal. These types of metabolisms can be divided into two main groups by sources of energy: phototrophic bacteria that use light (Table 1); and chemotrophic bacteria that use chemical reactions (Table 2).

Table 1. Types of phototrophic metabolism (Gottschalk, 2012).

Type	Electron donor	Carbon source	Examples
Photolithotrophy	H_2O	CO_2	*Cyanobacteria*
	H_2S, S^0, H_2	CO_2	*Chromatiaceae*, *Chlorobiaceae*
Photoorganotrophy	Organic substrates	Organic substrates	*Rhodospirillaceae*

Cyanobacteria are the first and still important drivers of light-driven primary production (CO_2 fixation; Figure 1). Phytoplankton is accountable for little over half of the net primary production on planet Earth (Behrenfeld *et al.*, 2001), 10% (~4 PgC y^{-1}) of which is contributed by cyanobacteria (Rousseaux & Gregg, 2014). The proliferation of ancient cyanobacteria carrying out oxygenic photosynthesis brought fourth redox-revolution on the planetary scale about 2.5 billion years ago (Hamilton *et al*., 2015). *Cyanobacteria* are also among few groups of organisms that are capable of fixing N_2, giving them an important role in the nitrogen cycle. Genus *Trichodesmium* is responsible for fixation of 240 Tg N_2 per year, which is over 40% of the total global nitrogen fixation (Berman-Frank *et al.*, 2003). Two filamentous cyanobacterium genera, *Aphanizomenon* and *Nodularia,* carry out most of the nitrogen fixation in the Baltic Sea (Larsson *et al*., 2001).

The average depth of the ocean is 4000 m and light can reach up to only 300 m. Therefore, the body of water that is aphotic – is not penetrated by light – is massively larger (1.27×10^{18} m^3) than the euphotic zone (3.0×10^{16} m^3), where light-dependent primary production by phytoplankton can take place (Orcutt *et al.*, 2011). For a long time, it was believed that organic carbon reached this "dark ocean" only via biological and microbial carbon pump (Figure 1). However, in the 1970s when technology became advanced enough to overcome the extreme pressure to study these depths, species-rich ecosystems were discovered around hot springs on the Galapagos volcanic Rift at a depth of 2.5 km (Corliss *et al.*, 1979; Lonsdale, 1977). These ecosystems are supported by chemolithoautotrophic bacteria (and archaea) that fix CO_2 using energy derived from oxidation of reduced inorganic compounds (like ammonia, iron, sulphur, sulphide, manganese; Table 2). Moreover, these microbes can be divided as free-living and those in symbiosis with macro-organisms (Cavanaugh *et al.*, 2006).

Table 2. Types of chemotrophic metabolism and some specific examples (Gottschalk, 2012; Orcut *et al.*, 2011).

Type	Electron donor	Electron acceptor	Carbon source	Examples, comments
Chemoorganotrohy	Organic substrate	O_2	Organic substrate	Heterotrophs, '*Candidatus* Pelagibacter'
	Organic substrate	NO_3^-	Organic substrate	Denitrifying bacteria, *Comamonadaceae*
	Organic substrate	SO_4^{2-}	Organic substrate	Sulphate reducers, *Desulfobacula*
	Organic substrate	Organic substrate	Organic substrate	Clostridia, lactic acid bacteria
Chemolithotrophy	H_2	O_2	CO_2	Hydrogen-oxidizing bacteria
	H_2S	O_2	CO_2	*Thiothrix*
	H_2S	NO_3^-	CO_2	*Sulfurimonas*
	Fe^{2+}	O_2	CO_2	*Acidimicrobium*
	NO_2^-	O_2	CO_2	*Nitrospina*
	NH_3	O_2	CO_2	Ammonia oxidizing bacteria
	NH_3	NO_3^-	CO_2	*Planctomycetes* (anammox)

Chemoorganotrophic (or heterotrophic) bacteria have been investigated till the early days of microbiology. However, their crucial role in marine ecosystems as degraders of particulate and dissolved organic matter (POM and DOM, respectively) was only fully accepted at the end of last century (Azam *et al.*, 1983; Pomeroy, 1974). The importance of degradation process is two-fold: firstly, inorganic nutrients are released (remineralization), which can be utilized again by primary producers; secondly, the organic matter is assimilated into bacterial biomass and thereby re-entered into the "classical food-web" via bacterivorous organisms – a pathway for POM and DOM referred as microbial loop (Azam *et al.*, 1983; Figure 1).

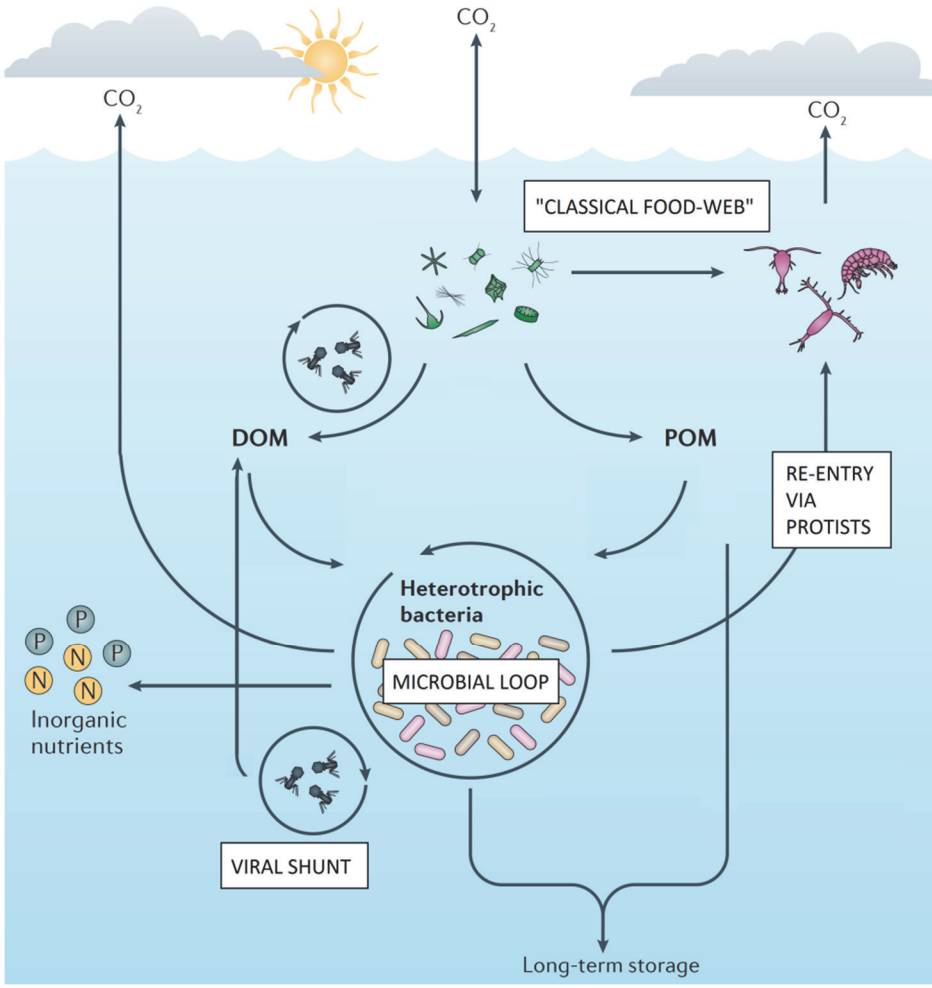

Figure 1. A simplistic visualization of the microbial loop. Photosynthetic phytoplankton species (green) produce organic carbon using CO_2. Dissolved organic matter (DOM) and particulate organic matter (POM) is released by phytoplankton (e.g. via the viral shunt - viral-mediated cell lysis) and other organisms (e.g. sloppy feeding). A fraction of the heterotrophic bacteria is consumed by zooplankton (purple) directly or via protists and thereby carbon is transferred up in the food web. In addition, heterotrophic bacteria contribute to the remineralization of DOM and POM; thereby released inorganic forms can be taken up by phytoplankton. The biological pump refers to the export of phytoplankton-derived POM from the surface oceans to deeper depths via sinking. The microbial carbon pump refers to the transformation of organic carbon into recalcitrant DOC (dissolved organic carbon) that is highly resistant to further degradation and is sequestered in the ocean for thousands of years (modified from Buchan et al., 2014)

1.3 Microbial ecology – theory in the making

Theories of animal and plant ecology are well established. However, applying these same theories on microorganisms is in many cases questionable due unique aspects of their biology, as small size, vast abundance, rapid growth, short generation time, resting stages, great dispersal, and parasexuality (Prosser *et al.*, 2007). Even the concept of species promoted by Mayr (1957) is not suitable for asexual bacteria that are capable of homological recombination. Cohan (2002) proposed ecological species or "ecotypes", which are populations of organisms occupying the same niche and exhibiting behavioural, structural or physiological differences from other members of the species (Cohan, 2002).

The ecological theory of microorganisms on community level becomes even more complicated. However, there is an increasingly popular concept of metacommunities that has provided useful improvements on existing ecological thinking (Leibold *et al.*, 2004; Prosser *et al.*, 2007). Metacommunities are defined as a set of communities that are linked by dispersal of multiple potentially interacting species (Leibold *et al.*, 2004). There are four paradigms that each emphasizes different processes of potential importance in metacommunities: the species-sorting view, the mass effects view, the neutral view and the patch-dynamic view (Figure 2; Leibold *et al.*, 2004; Logue *et al.*, 2011). The latter approach assumes that habitat patches are environmentally homogeneous, and a relatively low dispersal of species occurs, but this is not well suited for the microscale marine environment and microorganisms. Therefore, only three paradigms are discussed below.

Species-sorting by the local environment has been shown to be one of the key processes driving assembly of bacterioplankton community (Jones & McMahon, 2009; Lindström & Langenheder, 2012; Logares *et al.*, 2013; Logue & Lindström, 2010; Östman *et al.*, 2010). In essence, the species sorting assumes that community composition is determined by the competition of species with different adaptions (niche requirements) to local abiotic and biotic conditions. This concept is linked to Baas-Beckin's (1934) famous statement "everything is everywhere, but the environment selects" and is an ecological concept considered for bacterial communities. The "everything is everywhere" part requires that bacteria due their minute size and high level of plasticity are able to spread globally, which is true, in fact, both *Bacteria* and *Archaea* have been shown intercontinental dispersal with transpacific winds (Smith *et al.*, 2013). Species sorting is, in fact, the perspective that most often explains assembly of bacterioplankton community (Lindström & Langenheder, 2012).

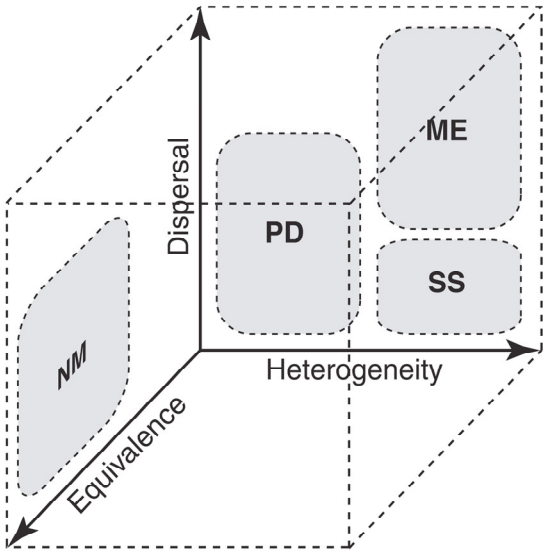

Figure 2. The depiction of the four metacommunity paradigms and areas of potential advances. The four formulated paradigms of metacommunity theory placed within a common framework, depicting their differences and overlaps along axes of the rate of dispersal, of the heterogeneity of habitat patches with regard to local environmental and biotic characteristics, and of the equivalence among species regarding niche and fitness. Abbreviations: ME, mass-effects; NM, neutral model; PD, patch-dynamic; SS, species-sorting (modified from Logue et al., 2011)

However, species sorting alone is insufficient, in some cases, another theory connected with dispersal and migration provides a better explanation - mass effect (Crump *et al.*, 2007; Lindström *et al.*, 2006). By this theory, demising bacterial populations can be stabilized via continuous inflow by migration (Shmida & Wilson, 1985). The vast and continuous spread of bacteria help explain why some bacterial populations can contribute a significant fraction of BCC in environments that they are not well adapted to. Nevertheless, there is some doubt cast upon mass effect being relevant in natural communities (Lindström & Östman, 2011; Logue & Lindström, 2010). Moreover, studies lacking data on dispersal (rates and/or frequencies) and community composition in connected systems have a hard time distinguishing between species sorting and mass effect (Logue *et al.*, 2011).

In some cases the community assembly mechanism can be better explained by neutral model; which assumes that species do not differ in their fitness or niche and local community assembly is a random process (Hubbell, 2001; Sloan *et al.*, 2006; Woodcock *et al.*, 2007). Along these investigations, there have been results which indicate a combination of several mechanisms (Eiler *et al.*, 2011; Langenheder & Székely, 2011; Ofiţeru *et al.*, 2010) and the relative importance of these mechanisms may be temporally variable (Langenheder *et al.*, 2012).

In summary, ecological theory suitable for microbial communities is much needed as its applications would be incredibly beneficiary (e.g. in fields of ecosystem management and conservation biology). However, this theory is still in the making and without a doubt offers a compelling intellectual challenge for ecologists.

1.4 Microbial structuring of marine ecosystems

1.4.1 Ribosomal RNA as marker for BCC determination and overall bacterial diversity

Previous chapters encapsulate what can be an overwhelming realization to any marine microbiologist or microbial ecologist, the ocean – largest living space on Earth (>95%) – is literally packed with tiny organisms that are basically running the planet (cumulative influence on biogeochemical cycles).
The traditional way to identify microbes is to cultivate them, for example using plate count agar (Massa *et al.*, 1998). On Petri dish, the colony forming units (CFU) can be enumerated and isolated into pure culture (containing only one bacterial strain), which is then used for downstream analyses to identify phylogenetic and genotypic properties of the bacterium. However, direct counts (using a microscope or flow cytometer) have constantly produced much higher results, about the order of magnitude, than CFU counts using cultivation when analysing environmental samples, it was called "the great plate count anomaly" (Staley & Konopka, 1985). Consequently, Pace and colleagues (1985) developed the approach that would eventually offer the solution to the phenomena, about the same time. It involved cloning polymerase chain reaction (PCR) products from whole community amplicons, thereby bypassing the cultivation step entirely (Pace *et al.*, 1985; Schmidt *et al.*, 1991). Small ribosomal subunit RNA gene (16S rDNA in case of bacteria and archaea; 18S rDNA in case of eukaryotes; Figure 3) was used for phylogenetic comparison as suggested by Woese and colleagues (Woese, 1987; Woese & Fox, 1977). These "environmental clone sequences" act as a marker for the organism when assigned to a location on a phylogenetic tree. This approach is now used in *Bergey's Manual of Systematic Bacteriology* – leading reference for prokaryotic taxonomy (George *et al.*, 2001).

With the introduction of next-generation sequencing technologies (Azam *et al.*), similar approach became much more high-throughput (Jones, 2010; Margulies *et al.*, 2005; Vieites *et al.*, 2009). Consequently, the databases have been piling with ribosomal rDNA sequences (National Center for Biotechnology Information, NCBI, nucleotide database http://www.ncbi.nlm.nih.gov/). In such cultivation-independent phylogenetic studies, operational taxonomic units (OTUs) are generated via clustering sequences based on a certain similarity threshold. Usually, 97% similarity is used to generate species level units. In such sequencing based analyses, an OTU is tentatively assumed to be a valid taxon. It is estimated that the total number of bacterial species in the world could range from 10^7 to 10^9, around 2×10^6 species of which could be associated with the marine environment (Curtis *et al.*,

2002; Dykhuizen, 1998; Pedrós-Alió, 2006). However, only around 1% of bacteria can be readily cultivated *in vitro* (Amann *et al.*, 1995; Hugenholtz *et al.*, 1998). Out of 61 bacterial phyla level taxa, based on 16S rRNA, 31 have no cultivable representatives (Hugenholtz, 2002).

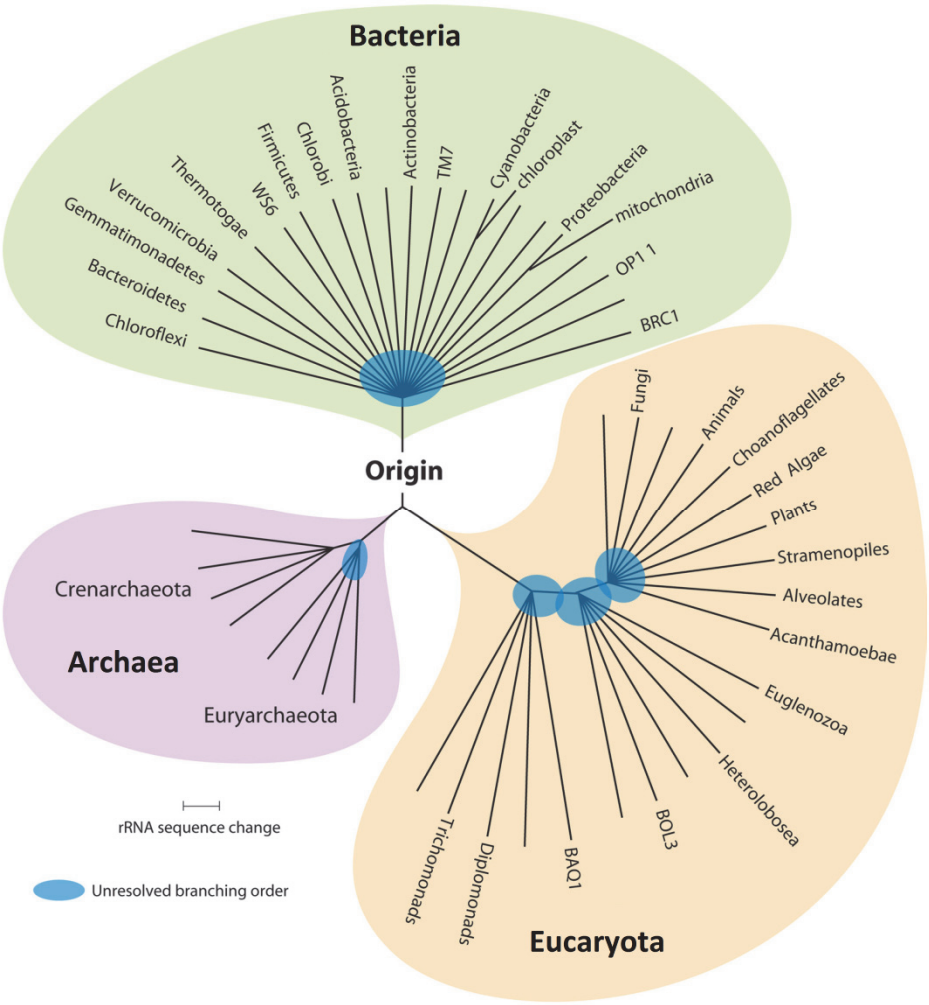

Figure 3. The phylogenetic "tree of life" based on rDNA sequence. Only few of the known lines are displayed (modified from Pace, 2009)

1.4.2 BCC between the scales and gradients

Microbial interactions take place on the micrometer scale. Thus, there can be significant differences in community composition even within millimetres (Long & Azam, 2001). Seawater may deceptively seem like a homogeneous environment;

however marine habitats can be tremendously heterogeneous with a broad suite of discrete microniches divided on temporal and spatial scales (Azam & Malfatti, 2007). Nevertheless, on the other side, microbial ecologist seeks to understand the impact of microbial processes on the ocean biogeochemical cycles on a global scale (Falkowski *et al.*, 2008). Therefore, marine microbiology is a field that is caught between extremities of sales, and one of the end games is bringing these two together.

The ocean is filled with different physical and/or chemical gradients that force shifts in BCC: among others the oxic–anoxic interfaces, salinity, and the availability of inorganic nutrients, temperature, especially over oceanic fronts where different water masses "collide" (Baltar *et al.*, 2015). Most dramatic shifts in plankton and also macro-organisms are produced by the availability of terminal electron acceptors (Crump *et al.*), out of which oxygen is the only option for most of the marine organisms; they either die or leave the area (such as fish) (Stramma *et al.*, 2008). Therefore, oxygen-starved regions of the global ocean – oxygen minimum zones (OMZ) - are sometimes referred as "dead zones". These, often eutrophication-associated zones, have been expanding over the last half century and have been reported in 400 systems (Diaz & Rosenberg, 2008).

However, calling these regions of the ocean "dead" is very misleading, since these areas provide many niches for micro-organisms that can use alternative TEAs. There is a hierarchy of terminal electron acceptors by Gibbs free energy potential ($O_2 \rightarrow NO_3^- \rightarrow Mn^{IV} \rightarrow Fe^{III} \rightarrow SO_4^{2-} \rightarrow CO_2$) and favoured microbial processes vary in the presence of different electron acceptors (Schlegel & Bowien, 1989). Therefore, oxygen, the most energetically favourable electron acceptor, is located at the top of this "electron tower" and CO_2 yields the least energy of all the electron acceptors. Thereby, major biogeochemical transformations are taking place in OMZ.

The depletion of TEAs alters ecosystem energy flow. Energy is increasingly diverted away from higher trophic levels into microbial community metabolism causing changes in carbon and nutrient cycling (Figure 4; Hawley *et al.*, 2014). Under oxygenated conditions 25–75% of the energy generated via oceanic primary production is transferred to mobile predators (Rosenberg, 1977). Prolonged periods and lower oxygen concentration result energy shift towards microorganisms. Over the last century decreased oxygen conditions in the ocean severely reduced valuable services that coastal ecosystems provide to humans. Expansion of oxygen-deficient waters cause the reduction of the potential fish yield and favoured noxious algal blooms (Carstensen *et al.*, 2014).

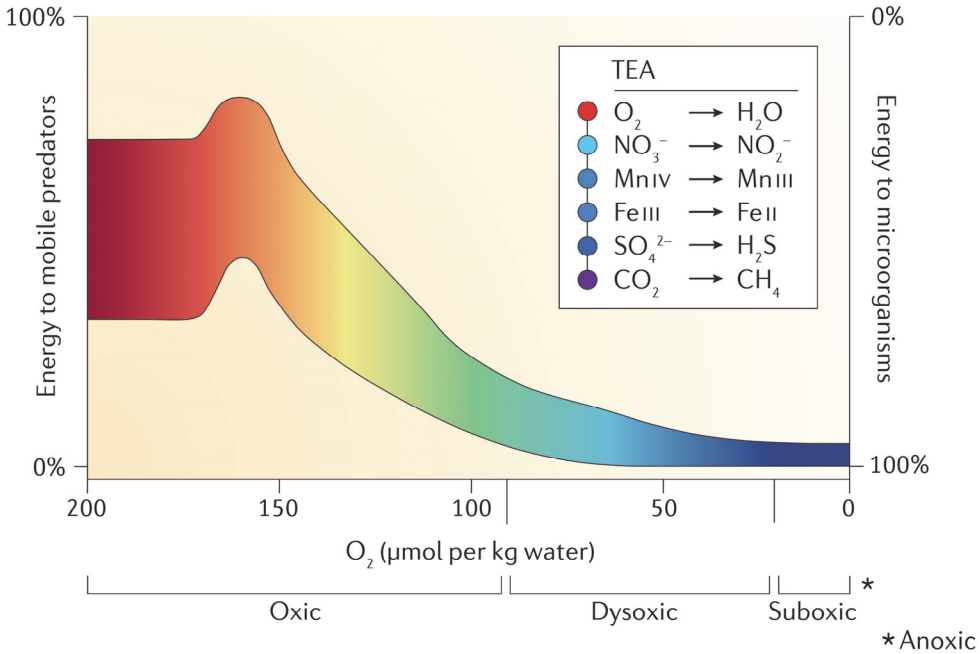

Figure 4. Conceptual view of how hypoxia affects ecosystem energy flow. The red–orange area indicates the range of energy transferred from the benthos to higher-level predators under oxic conditions (normoxia). The yellow–green–blue area indicates O_2 levels decline resulting in the suspension of higher-level predation, the continuance of benthic predation, and rapid increase in the proportion of benthic energy transferred to microorganisms. This energy is generated via microbial respiration using a defined order of terminal electron acceptors (TEAs) (Wright et al., 2012).

The zonation of different TEAs using bacteria is called redox-driven niche partitioning (Ulloa *et al.*, 2013; Wright *et al.*, 2012). Co-occurrence network analysis was carried out using 16S rRNA gene sequences from the Hawaii Ocean Time-series, the northeast subarctic Pacific, the Saanich Inlet and the eastern tropical South Pacific (off Peru and Chile), and non-random patterns of co-occurrence were identified among bacterial populations associated with oxic, dysoxic, suboxic or anoxic environmental conditions (Wright *et al.*, 2012).

Salinity is another important environmental parameter that strongly impacts bacterial community composition across different ecosystems (Lozupone & Knight, 2007). Estuaries are systems where freshwater and marine bacterioplankton communities mix and exhibit drastic spatiotemporal variability in community structure (Bernhard *et al.*, 2010; Bouvier & del Giorgio, 2002; Crump *et al.*, 2004; Herlemann *et al.*, 2011; Hewson & Fuhrman, 2004; Selje & Simon, 2003; Troussellier *et al.*, 2002). The spatial variability of BCC from river to estuary to ocean has been demonstrated to overwhelm seasonal shifts (Fortunato et al., 2012).

Moreover, salinity gradient also produces shifts in functional capacities of bacterioplankton (Dupont *et al.*, 2014).

Salinity, however, is not the only factor for influencing bacterial populations along estuaries. There are covarying parameters, including temperature, dissolved inorganic nutrients, and concentrations of dissolved and particulate organic matter, contributing to the variation among communities (Bernhard *et al.*, 2010; Bouvier & del Giorgio, 2002; Stepanauskas *et al.*, 2003). Marine particles have been identified as "hotspots" of high microbial abundance and activity (Azam *et al.*, 1994; Smith *et al.*, 1992).

Fortunately, the 16S rRNA-based investigations have unveiled wide distribution of certain bacterial clades, like candidate order "Pelagibacterales" (SAR11 clade), *Prochlorococcus*, *Synechococcus, Roseobacter* and SAR86 clade (of *Gammaproteobacteria*). While all these phylogenetic groups are divided between specific ecotype the unique traits possessed by these clades help to seek mechanistic explanations for spatiotemporal structuring of BCC (Buchan *et al.*, 2005; Fuhrman *et al.*, 2008; Mazard *et al.*, 2012; Brown *et al.*, 2014; Pommier *et al.*, 2007). For example, members of the SAR11 clade represent 25% of the ocean surface layer (up to 200 m) bacterioplankton cells worldwide (Giovannoni & Stingl, 2005; Morris *et al.*, 2002). Ocean surface waters are characterized by oligotrophic conditions, and experimental evidence suggests that possession of proteorhodopsin (light-driven proton pump) gene provides adaptation for carbon starvation (Steindler *et al.*, 2011).

These revolutionary advances in DNA sequencing technologies (described above) helped to extract community composition in higher resolution (or sequencing depth) and thereby uncovered a "rare biosphere" that is formed by large amount of low abundance taxa and accounts for most of the observed phylogenetic diversity of bacterioplankton community (Galand *et al.*, 2009; Gibbons *et al.*, 2013; Sogin *et al.*, 2006). This reservoir of genetic and functional diversity acts as a "seed bank" out of which resuscitation of certain bacterial populations can be recruited when environmental conditions become favorable for them; however some of these bacterial populations may stay transiently rare (Figure 5; Lennon & Jones, 2011; Aanderud *et al.*, 2015; Lynch & Neufeld, 2015). The threshold between the "rare" and "abundant" is subjective, criterion most commonly used is 0.1% of the sequence counts in generated dataset (Lennon & Jones, 2011; Lynch & Neufeld, 2015). Most common and abundant bacterial OTUs have temporally defined niches, so they emerge from rare-biosphere (Gilbert *et al.*, 2012).

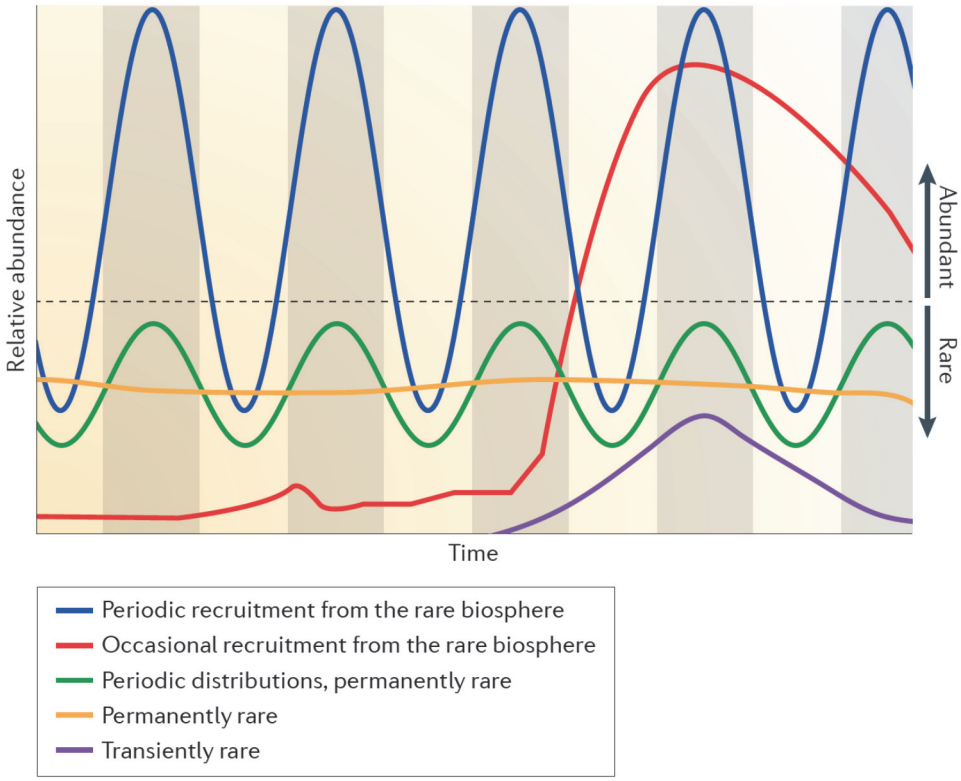

Figure 5. Hypothetical scenarios for microorganisms part of the rare-biosphere (Lynch & Neuman, 2015).

1.5 The Baltic Sea and the Gulf of Finland

1.5.1 The Baltic Sea

Following the Black Sea, the Baltic Sea is the second largest brackish water body in the world. It is semi-enclosed, water exchange with the North Sea is restricted through narrow and shallow Danish Straits; therefore, the Baltic Sea is characterized by long retention time (>25 years). It is a large estuary-like water body with both horizontal and vertical salinity gradients (Fennel & Seifert, 2008; Stigebrandt, 2001). The catchment area of the Baltic Sea is over 1.7 x 10^6 km^2 and habitat for over 85 million people (Commission, 2009). Both, the partly enclosed geography and anthropogenic influence, amplify effects of interconnected environmental problems such as eutrophication, oxygen depletion and climate

change (Andersson *et al.*, 2015; Carstensen *et al.*, 2014). Influence of higher temperature and anthropogenic forcing have resulted in a 10-fold increase of hypoxia in the near-bottom layer over past 115 years (Figure 6). Decrease in oxygen concentrations in lower layers impacts the whole ecosystem through loss of secondary production (Diaz & Rosenberg, 2008), it has been estimated that about 30% of secondary production is lost in the Baltic Sea because of oxygen deficiency (Karlson *et al.*, 2002).

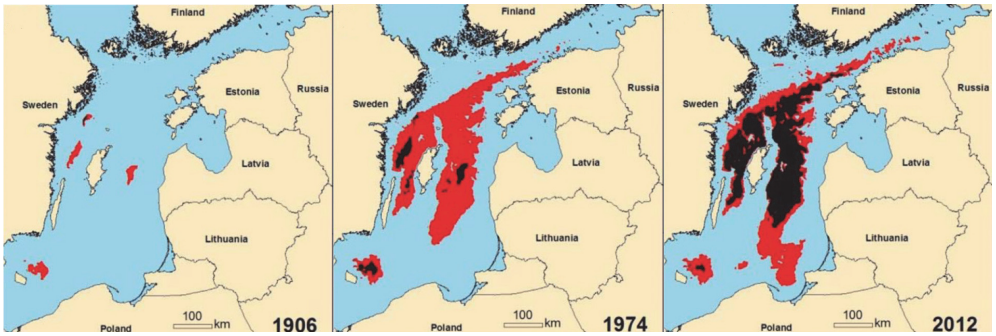

Figure 6. Snapshots of spread of hypoxia (red, $[O_2]<2$ mg L^{-1}) and anoxia (black, $[O_2]=0$ mg L^{-1}) over time, based on yearly averages (modified from Carstensen et al.*, 2014)*

There is a repeating seasonal variation in inorganic nutrient concentrations in the Baltic Sea upper layer: minimum values are measured in summer and maximum in winter. The winter level of inorganic nutrients impacts the phytoplankton spring bloom during which major bulk of annual primary production is carried out. The spring bloom in the Baltic Sea is co-dominated by diatoms and dinoflagellates, the relative abundance of these groups varies between years (Klais *et al.*, 2011). After the spring bloom, inorganic nutrients are mostly depleted in the euphotic layer, and strong stratification due the formation of the thermocline in the summer prevents vertical mixing with nutrient-rich deeper layers (Liblik & Lips, 2011). These conditions give an advantage to diazotrophic cyanobacteria (able to fix N_2) and dinoflagellates able to migrate vertically in the water column (Jephson & Carlsson, 2009; Lips & Lips, 2008; Lips *et al.*, 2011). Over the autumn, the water column is mixed down to the halocline or down to the bottom in shallow areas. In winter, the low light levels inhibit the growth of phytoplankton and due to that the nconcentrations of inorganic nutrients increase again in the upper layer.

1.5.2 The Gulf of Finland

The Gulf of Finland is an elongated estuarine-like basin. The salinity increases from east to west being about 2–6 in the surface layer and about 5–9 in the deep layer (Figure 7; Kullenberg & Jacobsen, 1981; Andrejev *et al.*, 2004). The residual circulation in the gulf consists of an outflow of fresher waters (originating from

river discharges, mainly the River Neva) in the northern part and an inflow of saltier waters from the Northern Baltic Proper along the southern coast (Alenius *et al.*, 1998). Vertical stratification of the water column and water exchange with the Northern Baltic Proper is characterized by high variability both in long-term (Liblik & Lips, 2011) and at synoptic to monthly scales (Elken *et al.*, 2003). The topography of the Gulf of Finland is complicated, in general, the northern slope of the gulf is shallower, and the bottom slope is about two times lower than at the southern side (Laanemets *et al.*, 2009).

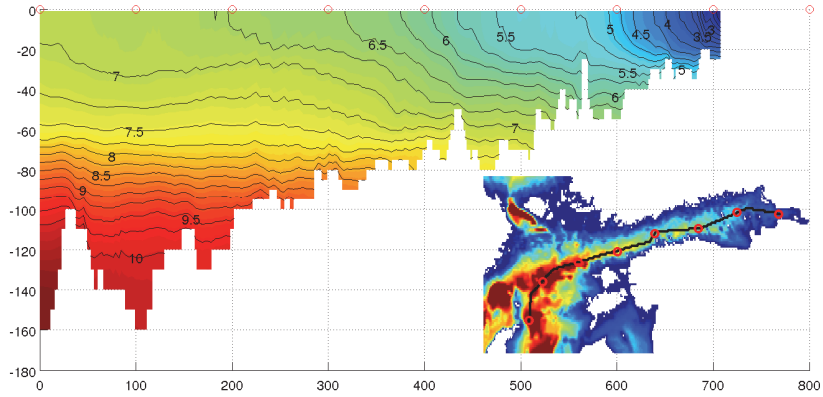

Figure 7. A transect from central Baltic Sea to the Gulf of Finland, the salinity values (g kg^{-1}) are based on forty-year averages. The dark blue to dark red colors on the map give an indication of the topography(Maljutenko et al., Unpublished).

1.5.3 Bacterioplankton community via next generation sequencing technologies

Molecular-based approaches had helped to expand knowledge of BCC in the Baltic Sea long before application of NGS technologies and revealed strong influence of freshwater phyla (Kisand *et al.*, 2005; Pinhassi & Hagstroem, 2000; Pinhassi *et al.*, 1997; Riemann *et al.*, 2008). The first study that utilized pyrosequencing approach uncovered contrasting seasonal shifts and interannually repeating patterns in BCC (Andersson *et al.*, 2010). Even greater complexity and occurrence of short-lived "pulse-populations" were demonstrated via high-frequency sampling approach (Lindh *et al.*, 2015). Investigations of spatial variability of BCC in the Baltic Sea have shown structural and functional niche partitioning along the salinity (Dupont *et al.*, 2014; Herlemann *et al.*, 2011) and dissolved oxygen gradients (Herlemann *et al.*, 2011; Koskinen *et al.*, 2011; Thureborn *et al.*, 2013). However, until undertaking current Ph.D. study, there were no spatiotemporal datasets available.

2. AIMS OF THE STUDY

This Ph.D. study was undertaken to investigate spatial and temporal structuring of BCC in relation to main physicochemical parameters in the Gulf of Finland, to identify spatiotemporal niche partitioning of BCC in the context of metacommunity theory and regional invariance on a global scale in brackish estuaries with seasonal or permanent redoxcline. Following aims were set to meet this goal.

1) To develop methodology to collect whole-community DNA (metagenome) samples cost-effectively and minimize the probability of contamination.

2) To determine spatial variability of the BCC both vertically (different depths) and horizontally (different stations).

3) To determine temporal dynamics of BCC in entire water column;

4) To identify the key bacterial populations in the region and track isolation sources of their closest database affiliations, to resolve biogeography of these phylogenetic lineages.

5) To compare BCC structuring in the Gulf of Finland to community profiles recorded in other estuaries or estuary-like water bodies.

3. MATERIAL AND METHODS

3.1 Study area

Water sampling aboard the RV Salme was performed on three transects in the Gulf of Finland from December of 2010 to October of 2012 (Figure 8; **I**, **II**, **III**). West-east coverage was provided by NS-transect, north-south coverage by AP- and the KERI-transect, latter is located near the Island of Keri, which is the deepest location (>110 m) in the central area of the gulf.

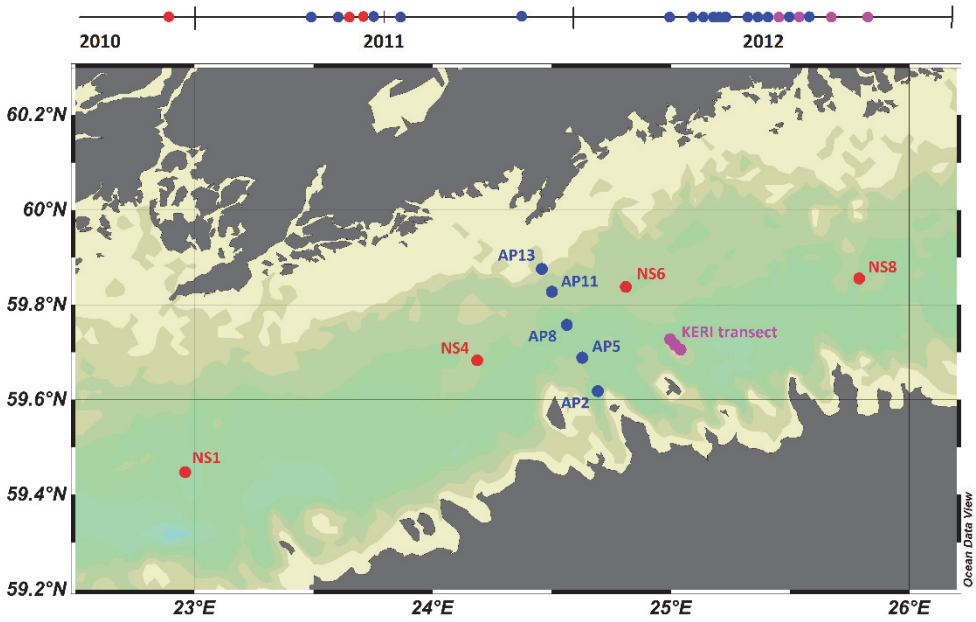

Figure 8. Map of the study area and sampling stations in different years. The timeline of the sampling cruises is provided with corresponding color-codes.

3.2 Sample collection and filtration

A rosette sampler (M1018, General Oceanics) equipped with Niskin water samplers (volume 1.7 l) was used for sampling (**I**, **II**, **III**). The design of filtration system is presented in Figure 9. The target sample volume was 1.0 liters, but in some cases system clogged beforehand and therefore sample volumes varied between 0.5 and 1.0 liters.

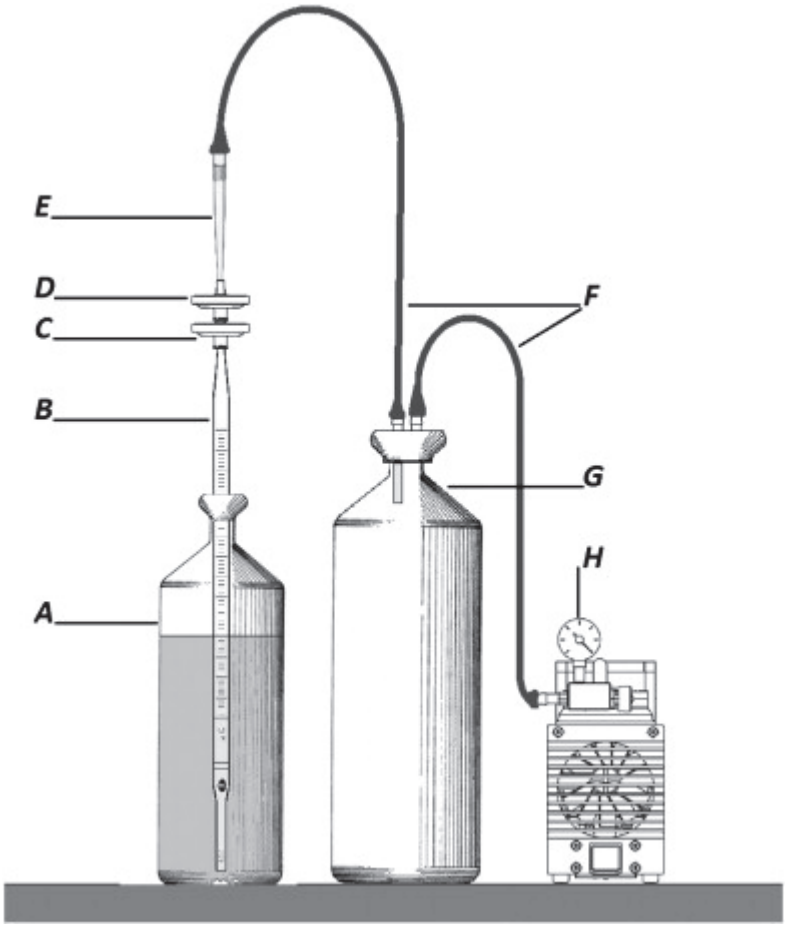

Figure 9. Closed filtration system used in the present study. Sterile bottle (A) and serological pipette (B; upside down); pre-filter (5 µm cellulose acetate syringe filter; C) and filter (0.2 µm cellulose acetate syringe filter; D); tubing (F) with a sterile tip (E); and collecting tank (G) connected to a vacuum source (H).

3.2 DNA extraction and 16S rDNA amplification

Whole bacterioplankton community (0.2–5.0 µm fraction) was extracted from 0.2 µm filters using commercial kits (**I**, **II**, **III**). The bacterial 16S rRNA genes V1-V2 hypervariable regions (using universal bacterial primers BSF8 and BSR357) were amplified in one reaction in case of publication **III** and in two polymerase chain reactions (PCR) in case of publications **I** and **II**.

3.3 Sequencing

Next generation HTS technologies were used to analyse amplicons that were indexed with barcodes (Hamady *et al.*, 2008). 454 pyrosequencing on was used in publications **I** (Roche GS Junior System) and **II** (Roche GS FLX). Illumina MiSeq platform was used in publication **III**.

3.4 Bioinformatics

OTUs were defined using the average neighbor-clustering algorithm of MOTHUR (Schloss et al., 2009; **I**, **II**, **III**). The Ribosomal Database Project naïve Bayesian Classifier was used for taxonomic assignments (Wang *et al.*, 2007) and BLAST searched (Altschul *et al.*, 1997) against a NCBI nucleotide database (http://www.ncbi.nlm.nih.gov/) (**I**, **II**, **III**). Statistical analyses and data visualizations were carried out with different packages in program R (**I**, **II**, **III**), mainly the „vegan“ package (Oksanen *et al.*, 2007).

4. RESULTS AND DISCUSSION

4.1 Physicochemical structuring in the study area

The sampling took place along three transects in the western and central part of the Gulf of Finland (Figure 8). The NS-transect from west to east provided horizontal salinity gradient of few g kg^{-1} (**I**, **II**). At the same time the impact of the horizontal salinity gradient was overwhelmed by vertical gradient through permanent halocline in the study area (**I**, **II**, **III**). The salinity values below halocline varied between 8.5–12.6 g kg^{-1}, and at the surface layer (5 m depth) between 4.8 and 6.6 g kg^{-1} along the transects, lower values during spring and highest in winter (**I**, **II**, **III**). The expansion of the hypoxic and anoxic area in the central Baltic Sea has a direct impact on the near bottom oxygen conditions in the Gulf of Finland. Due to the absence of a sill at the gulf entrance area and a direct inflow of saltier permanently hypoxic/anoxic Baltic Proper waters to the Gulf of Finland (Figure 6; Carstensen *et al.*, 2014) the oxygen conditions remained hypoxic for most of the study period, reaching as low as 0.1 mg L^{-1} at the near-bottom layer along the KERI-transect in 2012 (**I**, **II**, **III**). Strong negative correlation between oxygen concentration and salinity remained for the whole period (**I**, **II**, **III**).

The temperature variation was minor below the halocline (3.5-5.8 °C) and contrarily, strong seasonal changes were observed in the surface layer above 40 m, ranging between 0.1 and 18.7 °C (**I**, **II**, **III**). In both studied years the thermocline started to form at the beginning of summer (at a depth of 10-25 m), and a well-pronounced temperature stratification was established by July (e.g. the year 2012, Figure 10; **II**, **III**). Hence, from mid-summer to mid-autumn, the gulf was strongly stratified into three layers – upper mixed layer followed by the seasonal thermocline, cold intermediate layer followed by permanent halocline in deep (over 60 m) areas, and water layer below the halocline (**II**, **III**).

A large fraction of the primary production in the Baltic Sea is carried out during spring bloom, also reflected well in the chlorophyll *a* measurements (Figure 10; **II**, **III**). Annual spring blooms are terminated due to the depletion of inorganic nutrients at the well-lit surface layer, and the summer phytoplankton bloom commences after the temperature rise high enough for the proliferation of diazotrophic cyanobacteria (Vahtera *et al.*, 2007).

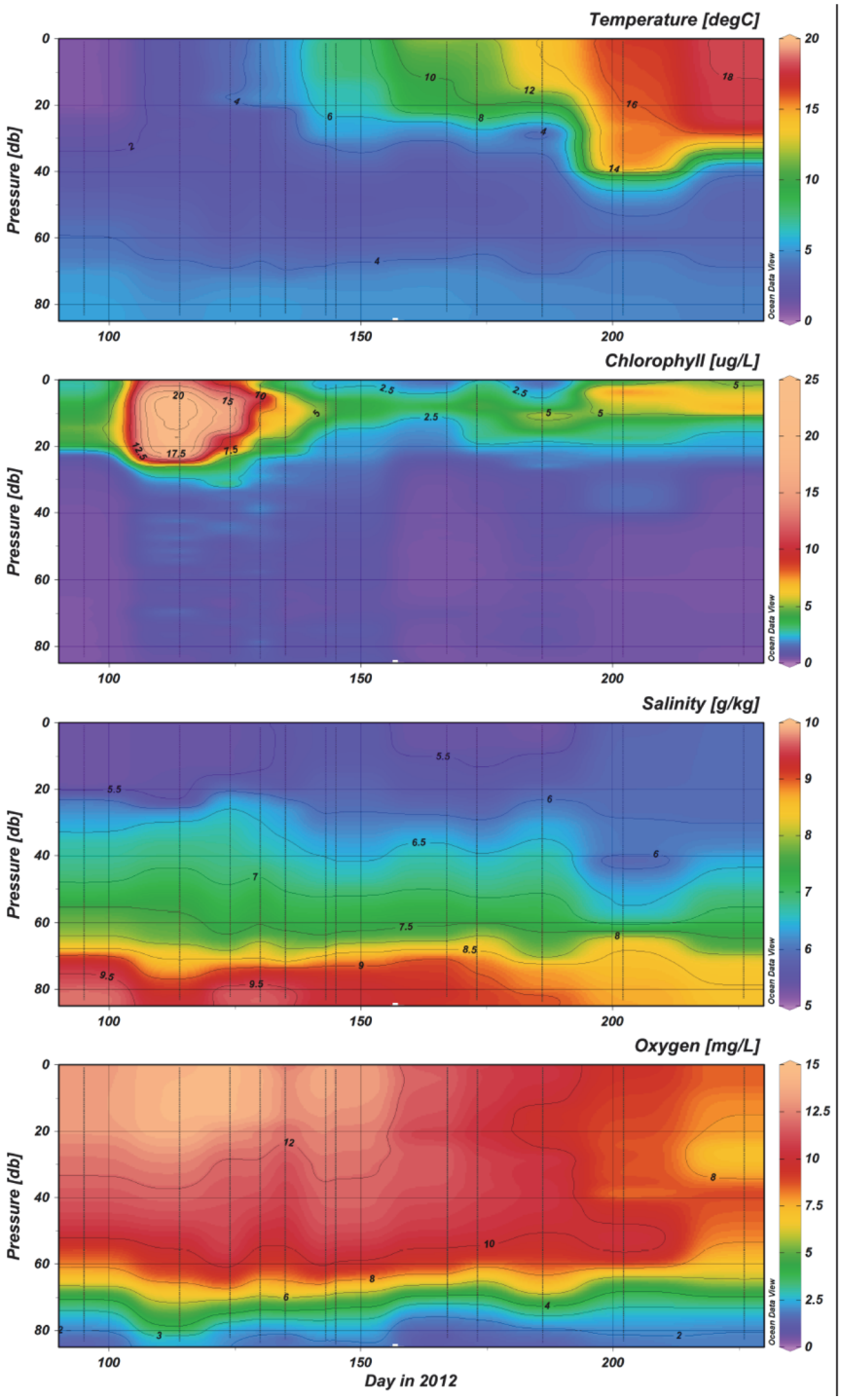

Figure 10. Time series of temperature, chlorophyll a, salinity and oxygen concentration at station AP5 in 2012. Vertical scattered lines indicate the times of the measurements.

4.2 Bacterioplankton community composition

4.2.1 Overview of dynamics of BCC on bacterial class level

In the framework of studies carried out for this dissertation, a total of 240 samples were analysed using 1,058,888 partial rRNA gene sequences (30,371 in publication **I**; 73,494 in publication **II**; 955,023 in publication **III**). *Alphaproteobacteria*, *Actinobacteria*, *Betaproteobacteria*, *Cyanobacteria*, *Epsilonproteobacteria*, *Gammaproteobacteria*, *Flavobacteria* and *Sphingobacteria* contributed 85.2% of total sequences (Table 2). The brackish environment and long retention time of the Baltic Sea enable assembly of unique bacterioplankton community structure. The bacterioplankton community lacks some typical marine taxa and, on the other hand, the growth of typical freshwater groups is enabled, like *Actinobacteria*, *Betaproteobacteria* and *Verrucomicrobia* (Kisand et al., 2005; Riemann et al., 2008; Bergen *et al.*, 2014; Herlemann *et al.*, 2011). Baltic Sea environment offers habitat for bacterial populations that are specialized for the brackish environment (Herlemann *et al.*, 2011; Hugerth *et al.*, 2015a). These trends are reflected from results obtained in the framework of the present dissertation (**I**, **II**, **III**).

Table 2. Relative abundance (%) of predominant bacterial classes in each study.

	Publication I	**Publication II**	**Publication III**	**Mean**
Alphaproteobacteria	28,9	31,2	40,8	33,6
Actinobacteria	14,6	17,8	12,8	15,1
Betaproteobacteria	5,1	8,9	6,7	6,9
Cyanobacteria	2	9,3	2,5	4,6
Deltaproteobacteria	0,7	0,5	0,7	0,6
Epsilonproteobacteria	25,9	5,8	11,1	14,3
Flavobacteria	3,2	8,7	5,7	5,9
Gammaproteobacteria	3,3	2,5	3,7	3,2
Sphingobacteria	1,1	1,6	0,4	1,0
Other	15,2	13,7	15,6	14,8

Throughout the sampling period, *Alphaproteobacteria* and *Epsilonproteobacteria* contributed the largest fraction of BCC above and below the oxycline, respectively (**I**, **II**, **III**). Members of *Delta-* and *Gammaproteobacteria* and a group of unclassified *Bacteriodetes* also were mainly found in the hypoxic zone (**I**, **II**, **III**). Other relatively abundant bacterial classes displayed seasonal variability. The representatives of *Betaproteobacteria* and *Flavobacteria* were more predominant in spring, whereas relative abundance of *Actinobacteria* increased in late summer and peaked in autumn (**II**, **III**). Similar seasonal shifts have been demonstrated by

several previous temporal studies on dynamics of BCC in the Baltic Sea (Andersson *et al.*, 2010; Hugerth *et al.*, 2015b; Lindh *et al.*, 2015).

Members of *Cyanobacteria* displayed a strong correlation with temperature and became considerably more abundant in summer above the thermocline (**II**, **III**). The distribution of picocyanobacteria during summer bloom was patchy even along the same transect. However, class level analyses provide very low-resolution description of BCC, because species within one bacterial class can occupy different niches as was demonstrated by non-metric multidimensional scaling (NMDS) analysis (Figure 11; **III**). In general, the observed phylogenetic structure of the bacterioplankton community on the class level composed of groups routinely found in the Baltic Sea. Although, *Gammaproteobacteria* and *Verrucomicrobia* contributed considerably lower fraction compared to some other studies (Riemann *et al.*, 2008; Andersson *et al.*, 2010; Herlemann *et al.*, 2011; Thureborn *et al.*, 2013; Lindh *et al.*, 2015).

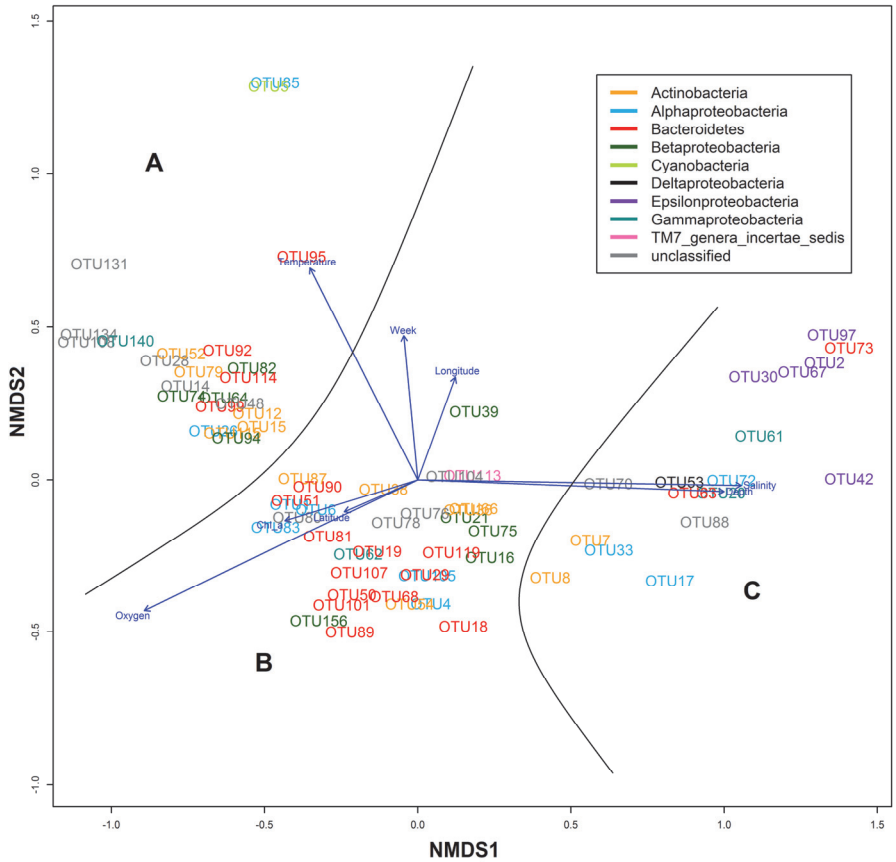

Figure 11. The non-metric multidimensional scaling (NMDS) analysis plot of abundant and common OTUs fitted with environmental parameters (stress score: 0.124). The black lines divide OTUs into three general groups.

4.2.2 Niches occupied core populations in the ecosystem

At species level (OTUs clustered with 97% similarity), the complexity of bacterioplankton community structure and variability was much higher. In studies **I**, **II** and **III** were identified 609, 1,557 and 4,692 of such OTUs, respectively. The observed number of OTUs in a sample was strongly correlated with a number of sequences used to analyse the sample (sequencing depth). Most of these OTUs could be categorized as part of the rare biosphere and occurred in low abundances. Less than a hundred OTUs could be considered relatively common or abundant (**I**, **II**, **III**). In 2012 only 17 OTUs could be referred as 'relatively abundant' (accounted more than 1% of total dataset each), cumulatively adding up 76% of the entire dataset (Figure 12; **III**). This trend of around twenty key populations could also be seen in first two studies (**I**, **II**) and therefore the sequences were unevenly distributed between these thousands of OTUs. Most of these relatively abundant OTUs were restricted to a distinct seasonal occurrence when they became abundant, in contrast, some remained transiently abundant throughout the investigated period. Corresponding OTUs represent the core populations of the studied ecosystem and are therefore also best adapted to most broad niches in this system.

Most of the *Alphaproteobacteria* were contributed by OTUs classified as "*Candidatus* Pelagibacter" (SAR11 clade, candidate order "*Pelagibacterales*"), these OTUs were transiently abundant in the oxygenated upper layer throughout all seasons, although relative abundance of OTUs varied (**I**, **II**, **III**). The candidate order "*Pelagibacterales*" is the most abundant bacterial order in ocean surface waters (Carlson *et al.*, 2009; Morris *et al.*, 2002; Rappe & Giovannoni, 2003). It has been demonstrated that there is a salinity-driven niche partitioning of representatives of SAR11 clade which results in overlapping populations throughout horizontal salinity gradient of the Baltic Sea (Herlemann *et al.*, 2011; Herlemann *et al.*, 2014). Results of the present study demonstrate clear seasonal shifts in relative abundance of populations specialized for certain salinity range (**I**, **II**, **III**). For example, the presence of fresh water associated (LD12 clade related) OTU only in the surface layer in spring (**III**). Moreover, the most prevalent members of SAR11 had their closest database affiliations to annotations isolated from brackish estuaries; however, this is further discussed in the following chapter.

Another alphaproteobacterial order, *Rhodobacterales,* was transiently present in oxygenated waters throughout all seasons (**I**, **II**, **III**). However, one OTU classified as *Rhodobacteraceae* contributed considerably larger fraction during spring blooms (**II**, **III**; Figure 12). This OTU (BSNS3149 in publication **II** and OTU4 in publication **III**) had an identical match with a sequence isolated from a coastal North Sea diatom bloom (Teeling *et al.*, 2012) strongly suggesting that this population occupies an important niche in degradation of spring phytoplankton bloom derived organic matter. There was also one *Rhodobacteraceae* (OTU85) that co-localized with picocyanobacteria, indicating another possible specialization (**III**).

Both groups, *Rhodobacteraceae* and "*C.* Pelagibacter", are known for photoheterotrophic lifestyle, which gives them a distinct advantage in carbon

limited waters (Brown *et al*., 2014). Especially, when generated ATP can be in turn used to mediate the uptake of small molecules (Buchan *et al.*, 2014). These groups have been shown to possess and express ATP-dependent and independent transporters for low-molecular-weight (LMW) organic compounds (Teeling *et al.*, 2012; Hugerth *et al.*, 2015b; Voget *et al.*, 2015). Members of SAR11 clade withhold some traits (such as small cell size) that confer a competitive advantage in nutrient uptake (Giovannoni *et al.*, 2005). During spring bloom in the North Sea, the *Rhodobacteraceae* out ranked all other groups in expression of LMW transporters. Both these groups are also known to contribute to the conversion of dimethylsulfoniopropionate to dimethylsulfide, which is especially relevant to phytoplankton blooms (Howard *et al.*, 2006). Aerobic oxidation of LMW organic molecules is fast and high-yield energy source, and the prominent presence of these two phylogenetic lineages suggests they are well adapted to occupy this niche in the studied ecosystem.

Although the dominant picocyanobacterial population exhibited strong seasonal variability in abundance, it still should be accounted as a core population. Picocyanobacteria were present for the whole observed period in the euphotic layer, contributing about 3% in winter (**I**), but reaching up to 88.7% of BCC in the summer of 2011 (**II**). This OTU (NSD007, BSNS2840, and OTU5 in publications **I**, **II**, and **III**; respectively) was classified as *Synechococcus* and had an identical match to a sequence isolated from laminae of the Gulf of Finland sediments dating back 3000 years (to the Late Litorina Sea; Lyra *et al.*, 2013), hence this population is likely endemic to the Baltic Sea area (**II**, **III**). The *Synechococcus* populations in the Baltic Sea have also been demonstrated to possess unique clustering of the phycobiliprotein genes (type IIB, Larsson *et al.*, 2014). Unlike filamentous cyanobacteria, unicellular picocyanobacterial are not grazing resistant and provide a food source for higher trophic levels.

The oxic-anoxic interface in the near-bottom layer provides a niche for chemotrophic bacteria that utilize reduced substrates produced from "lower steps of the redox-tower" from the sediments (e.g. CH_4 and H_2S) and terminal electron acceptors from the oxygenated upper layer (e.g. O_2 and NO_3). Throughout the investigated period hypoxic water was overwhelmingly dominated by representatives of genus *Sulfurimonas* (**I**, **II**, **III**). Most abundant of which had a nearly identical match to *Sulfurimonas gotlandica* (**II**). *S. gotlandica* is a key chemolithoautotrophic (H_2S oxidizing and nitrate reducing) taxa in the central Baltic Sea (Glaubitz *et al.*, 2010; Grote *et al.*, 2008). Investigations along the NS-transect (west-east) in the Gulf of Finland revealed that this population exhibited strong negative correlation with longitude and therefore it is likely that this population is dispersed into the Gulf of Finland with the deep inflow from the Baltic Proper (**I**, **II**). *S. gotlandica* is probably the main nitrate reducing (denitrifying) and sulphide oxidizing group in the Gulf of Finland (as it is in the central Baltic Sea).

Methane, a powerful greenhouse gas, holds a global significance, and there is a widespread release of methane from the seafloor of the Baltic Sea (Schmale *et al.*,

2010). The abundance and activity of methane-oxidizing bacteria in the water column regulates methane emissions to the atmosphere. Results of the present study reveal vertical oxygen-driven niche partitioning of two dominant C1 substrate utilizing taxa. The relative abundance of *Methylobacter* (genus of *Gammaproteobacteria*) increased towards oxygen depleted seafloor (**II**, **III**), where the methane concentrations are potentially highest (Schmale *et al.*, 2012; Schmale *et al.*, 2010). On the other hand, *Methylophilus*, a non-methane-oxidizing bacterial group, which can utilize other C1 substrates, was abundant also in the intermediate and surface layers (Figure 12; **I**, **II**, **III**; Kalyuzhnaya *et al.*, 2008). The members of *Methylobacter* and *Methylophilus* have been shown to cooperate in methane utilization (Beck *et al.*, 2013; Hernandez *et al.*, 2015). Experiments with such methane-consuming communities suggested similar oxygen-driven niche partitioning between the two taxa, confirmed by the *in situ* results of the current study (Hernandez *et al.*, 2015; **II**, **III**). These C1 substrate utilizing bacterial populations contributed a significant fraction of BCC throughout the observed period and therefore be considered as core populations.

Trace metals, like iron and manganese, are important carriers of oxidation-reduction potential, in the central Baltic Sea, Fe(III)/Fe have been shown as dominant redox couple (Meyer *et al.*, 2014). In the Baltic Sea "Mn and Fe shuttle" is generated at the interface between euxinic and oxic water (Dellwig *et al.*, 2010). When oxygenated, insoluble compounds form insoluble aggregates, which sink below the redoxcline, where these can be used as electron acceptors by bacteria or reduced chemically (Scholz *et al.*, 2013; Tebo *et al.*, 2004; Trouwborst *et al.*, 2006; Weber *et al.*, 2006). The results of present investigation points towards bacterial populations classified as *Ilumatobacter* (Figure 12; **I**, **II**, **III**), members of *Acidimicrobiaceae* (family of *Actinobacteria*) that presumably are involved in iron cycling (Itoh *et al.*, 2011; Matsumoto *et al.*, 2009). The occurrence patterns exhibited by two prevalent populations were different, which indicates separate survival strategies. One *Ilumatobacter* population peaked in abundance after the spring bloom at the near bottom layer (**II**, **III**), this group has been demonstrated to be involved in degradation of diatom bloom derived organic material in hypoxic conditions (Zakharova *et al.*, 2013).

4.2.3 Temporal dynamics above and below the oxycline

The main bulk of photoautotrophic primary production in the Baltic Sea (and other temperate coastal ecosystems) is carried out during spring phytoplankton bloom, and the fate of corresponding organic matter is essential to biogeochemical cycles of the ecosystem; which gives occurrence and activities of bloom-associated 'specialists' a great significance (Figure 12). The bacterial populations responsible for degradation of corresponding POM and DOM clustered together (group B in figure 11; figure 9 in publication **II**; **III**). The spring is also the period characterized by a large inflow of freshwater and successful immigration of freshwater bacterial populations (Kisand *et al.*, 2005; Riemann *et al.*, 2008).

Consequently, prevalent spring bloom-associated OTUs, mainly members of *Actinobacteria*, *Betaproteobacteria,* and *Bacteroidetes*, had their closest database affiliations to annotations isolated from freshwater ecosystems and coastal ecosystems impacted with similar freshwater inflow (**II**, **III**).

The massively parallel sequencing of tagged 16S rDNA amplicons has revealed contrasting seasonal dynamics of BCC in the surface layer of the central Baltic Sea, especially in relation to phytoplankton community (Andersson *et al.*, 2010; Lindh *et al.*, 2015). High temporal resolution enables capturing short-lived "opportunistic pulse populations" that would be otherwise missed. However, without the context of community composition in the deeper layers, the transitions in assemblage in response to environmental parameters and shifts due exchange of water masses (e.g. vertical mixing) cannot be differentiated (Lindh *et al.*, 2015). Therefore, the first spatiotemporal studies of BCC of the Baltic Sea undertaken in the the present study have provided new insight into phytoplankton bloom related shifts in deeper layers, especially in the hypoxic zone (**II**, **III**).

One powerful approach for unravelling the niche partitioning is analysing the co-localization of OTUs (Ruan *et al.*, 2006; Wright *et al.*, 2012). With this goal, NMDS analysis was used (**III**; Figure 11) and OTUs were clustered using correlation matrix (Pearson; **II**). The efficiency of approach applied was proven by the fact that OTUs related with database matches or phylogenetically close, clustered together, for example by isolation source (Table 4 in publication **II**). This approach is also complimentary to analyses using just environmental parameters because it helps to discriminate groups that occur in both extremities in relation to main structuring factors, for example, depth and oxygen concentration.

The occurrence of a dominant representative of *Rhodobacteraceae* (discussed in the previous chapter as a core population) was heavily influenced by spring phytoplankton bloom (Figure 12 and 13; **II**, **III**). It contributed with a large fraction at the surface, but also in the intermediate layer (40 m), in 2012 it reached high abundance in the hypoxic zone (Figure 12 and 13; **II**, **III**). However, the presence of *Rhodobacteraceae* in oxygen depleted waters sharply dropped at the beginning of summer (Figure 13). In contrast, some OTUs classified as *Flavobacteraceae* (*Bacteroidetes*) and *Comamonadaceaen* (*Betaproteobacteria*) reached maximum abundance by the end of spring bloom (Figure 13). These high abundances after the spring bloom could occur because of sedimentation and detachment from lysing phtoplankton cells in the hypoxic layer at the beginning of summer. However, members of *Comamonadaceae* are capable of denitrification (nitrates as TEA) in the hypoxic zone (Khan *et al.*, 2002; Li *et al.*, 2012). Interestingly, at the same time, the relative abundance of *Sulfurimonas* decreased, which could indicate substrate competition.

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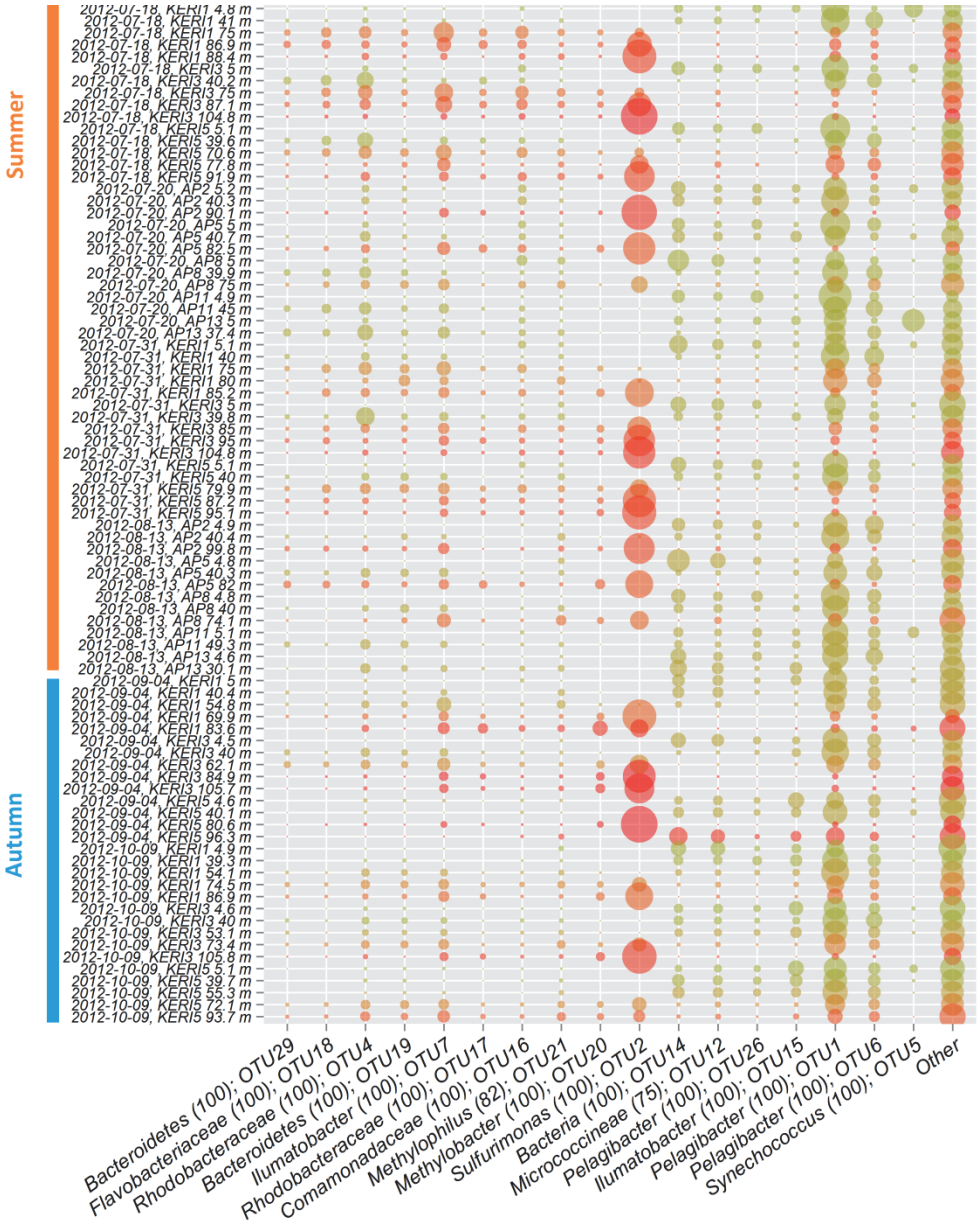

*Figure 12. Occurrence patterns of top 17 OTUs (>1% of the dataset). OTUs are ordered by their co-localization and supplemented with oxygen concentrations. Adopted from publication **III**.*

Certain representatives of *Bacteroidetes* are specialized degrading diatom-derived organic matter and often prefer particle-attached lifestyle (DeLong *et al.*, 1993; Kisand & Wikner, 2003; Pinhassi *et al.*, 2004; Woebken *et al.*, 2007). During the spring bloom in the North Sea, this group was shown to be in the central role in

the conversion of high molecular weight (HMW) compounds into LMW compounds (Teeling *et al.*, 2012). Some of the OTUs classified as *Bacteroidetes* in the present study occurred only in the hypoxic zone (**III**). Likewise, unclassified *Flavobacteraceae* contributed a large fraction of BCC in a deep and hypoxic layer of Byfjord, a Swedish fjord, before it was oxygenated followed by the drastically reduced abundances (Forth *et al.*, 2015).

Although the overall fraction of *Actibobacteria* increased in the autumn (**III**), certain OTUs classified as *Actinomycetales* and *Micrococcineae* were mainly found during spring bloom (**II**, **III**). Some of these were closely related to the freshwater AcI lineage (**II**). AcI group is characterized by small cell sizes, and their presence suggests indication of grazing pressure by bacterivorous nanoflagellates. A metagenomic study carried out suggests that populations of *Mycobacterium* are involved degradation of polyaromatic hydrocarbons (e.g. cellulose and chitin) in the near-bottom layer of Landsort Deep, the deepest location of the Baltic Sea in the Baltic Proper (Thureborn *et al.*, 2013).

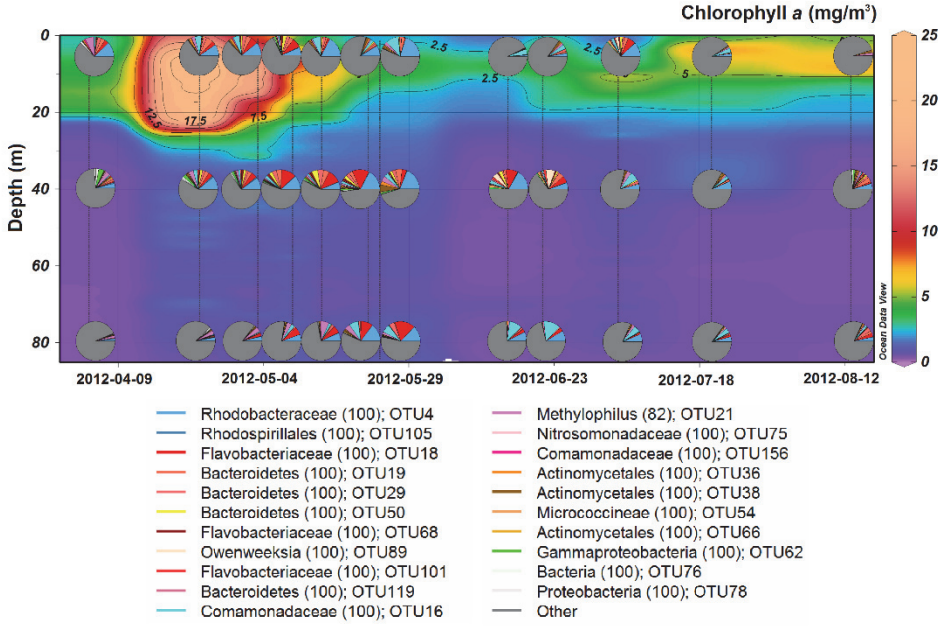

Figure 13. Time series of chlorophyll a at station AP5. Pie charts indicate the relative abundance of OTUs that were associated with spring phytoplankton bloom. Note that the second surface community (2012-04-3, 5 m depth) was recorded at station AP2, not AP5. Scattered vertical lines indicate the sampling times.

Certain bacterial populations were associated with summer phytoplankton bloom, both in the surface and near-bottom layers (**II**, **III**). However, the important conclusion drawn from these observations is that presence of hypoxic zone provides a specific niche for phytoplankton bloom associated bacteria that are capable of

switching to another TEA. Thereby combined effect of substrate- and redox-driven niche partitioning on the BCC was observed. As the hypoxic and anoxic zones are expanding in the Baltic Sea, the relative abundance and dispersal of these populations will probably increase.

4.5 Comparison with other similar ecosystems

One effective approach to better understand the processes underlying the assembly of BCC is to consider similarities between communities (Östman *et al.*, 2010). The OMZs in the ocean are globally inhabited by same cosmopolitan phylogenetic lineages. They occupy similar functional niches and together cover the whole redox-tower – so the community assembly is divided by the redox-driven niche partitioning (Ulloa *et al.*, 2012; Ulloa *et al.*, 2013; Wright *et al.*, 2012). Occupants along the redox gradient are involved in metabolite exchange and substrate competition forming a complex web of interactions (Hawley *et al.*, 2014; Wright *et al.*, 2012). Biogeochemically most important exchanges are concerning C-, N- and S-cycles. The niches in the oxygenated overlaying water are usually dominated by photoheterotrophic "Canditatus Pelagibacteria" and *Rhodobacterales*, with members *Synechococcus* being the most relevant picocyanobacterial taxon (Ulloa *et al*., 2013). The deeper layers usually have representatives of *Methylophilales*, SUP05 and SAR324 clades present (Wright *et al.*, 2012; Ulloa *et al.*, 2013; Fuchs *et al.*, 2005).

It was found that some of the more relevant (by abundance) OTUs had their closest database affiliations isolated from geographically distant, but hydrographically close (temperate climate, brackish water, and partially oxygen depleted) estuaries, like the Chesapeake Bay and the Saanich Inlet (Crump *et al.*, 2007; Zaikova *et al.*, 2010). Also, the overall phylogenetic composition was similar to those microbial communities, except *Sulfurimonas* being dominant sulfur oxidizer in the Gulf of Finland (and in the Baltic Sea in general) and SUP05 clade members in those other estuaries. On the other hand, like in the Baltic Sea, *Epsilonproteobacteria* have been shown as dominant chemoautotrophic group of the Black Sea, the largest OMZ of the world (Grote *et al.*, 2008). Both ecosystems have characteristic particulate Mn–Fe–P-shuttle at the redoxcline, which probably offers similar niches for trace metal oxidizing bacterial populations (Dellwig *et al.,* 2010). Same phylogenetic linages in similar ecosystems thousands of kilometers apart match well the species-sorting paradigm in metacommunity theory, and it is proposed that these local estuarine communities can be viewed as globally spread salinity- and redox-driven metacommunity (**II**, **III**).

The evidence connecting globally distant communities has been accumulating, genome level comparisons have revealed that some of the bacterial populations inhabiting the Chesapeake Bay and the Baltic Sea have a high genome-wide similarity (Hugerth *et al*., 2015). The highest similarity was displayed by spring phytoplankton bloom associated *Flavobacteriaceae* (Hugerth *et al.*, 2015), which indicates very specific niche (combination of salinity-, substrate- and redox-driven

niche partitioning) and also cosmopolitan lineage occupying it. The phylogenetic matches displayed that Pelagibacteria could also be only salinity-driven, at the same time the impact of hypoxic conditions should not be underestimated. In Chesapeake Bay SAR11 members also persisted in hypoxic layers (Crump *et al.*, 2007), and their metabolic adaptations for anaerobic growth remain to be uncharacterized (Wright *et al.*, 2012).

Over past few years, the compelling amount of evidence has been published that supports an emerging pattern of cosmopolitan specialists. Similar bacterioplankton community structures have been reported all over the world, for example in Byfjord in Sweden (Forth *et al.*, 2015), Sendai Bay in Japan (Sakami et al., 2015), Pearl Estuary in China (Liu *et al.*, 2015) and Sydney Harbour in Australia (Jeffries *et al.*, 2015). Estuaries are coastal ecosystems that often suffer from anthropogenic impact, eutrophication and also oxygen depletion; this is a global problem in the context of ecosystem management. The fact, that there are similarities in BCC between distant ecosystems holds great significance because their impact on biogeochemical cycles and energy flow of the entire ecosystem (Diaz & Rosenberg, 2008). Therefore, the results of oxygenation experiment carried out in Byfjord can give a good estimate for sifts in BCC in other estuaries, where similar natural or unnatural changes in oxygen concentration take place (Forth *et al.*, 2015).

CONCLUSIONS

This Ph.D. thesis investigated the spatiotemporal niche-partitioning of the bacterioplankton community composition in the western and central area of the Gulf of Finland, the easternmost sub-basin of the Baltic Sea. The Gulf of Finland is an estuary-like waterbody providing multiple natural gradients of physical, chemical and biological conditions; which all affect the bacterioplankton community assembly. Massively parallel tag sequencing of 16S rDNA was deployed to identify bacterioplankton community structure. The produced community profiles were compared to those recorded in similar ecosystems around the world to advance metacommunity theory in aquatic microbial ecology.

The main results of the present thesis can be summarised as follows:

- The unique closed filtration system (with syringe filters) was developed and used for sample collection.
- The core bacterioplankton populations transiently abundant or occupying an important seasonal niche, like members of "*Canditatus* Pelagibacteria" (SAR11 clade), *Rhodobacterales*, *Methylophilales*, *Ilumatobacteria Synechococcus* and *Sulfurimonas* were identified for the area.
- The vertical physicochemical structuring of the gulf had an immense impact on the bacterioplankton community assemblage. The covarying trio of depth, salinity, and oxygen concentration were put into a central role by all different analyses approaches. However, the community composition shifted according to redox-driven niche partitioning (users of alternative TEA in the hypoxic zone), thereby oxygen concentration could be considered as a main environmental factor shaping community structure.
- Present studies covered all seasons, including the first description of winter-time pelagic bacterioplankton communities in the Baltic Sea. The seasonal changes of different interconnected physical, chemical and biological parameters were combined to determine their impact on a succession of BCC. Heterotrophic bacteria are substrate dependent, and organic matter produced by phytoplankton vary seasonally in quantity and chemical composition. Consequently, substrate-driven niche partitioning influenced bacterioplankton assemblage significantly. It is well established that freshwater associated bacterial lineages (members of *Bacteroidetes*, *Betaproteobacteria,* and *Actinobacteria*) have an important role in degrading high molecular weight organic substances during spring bloom, and there are shifts in BCC from start to collapse of the bloom. However, the present investigation demonstrated that in combination of substrate-driven niche partitioning there is also redox-driven shifts taking place as POM sediments into the hypoxic near-bottom layer. The TEAs used to

decompose POM and DOM have an important role in biogeochemical cycles of the ecosystem.

- The investigated area remained into mesohaline salinity range, and the horizontal salinity gradient was only a few g kg^{-1}, so only a small fraction of surface water gradient of the Baltic Sea was covered. The effect of salinity on the BCC was noted mostly indirectly, as a large fraction of relatively abundant bacterial populations had their closest database affiliations to annotations isolated from brackish and freshwater ecosystems. Naturally, some of the key populations could be identified as endemic to the Baltic Sea. Most notably, *Sulfurimonas gotlandica* that is well-established species in the central Baltic Sea was found to be abundant chemolithoautotroph also at the redoxcline of central part of the Gulf of Finland. The dominant picocyanobacterial population – *Synechococcus sp.* - produced an identical match to annotation isolated from 3000-year-old sediments of the gulf that agrees with previous findings, which suggests this population to be endemic to the area.
- Some of the most predominant OTUs had their closest database affiliations isolated from geographically distant estuaries, but with similar physicochemical structuring – brackish, (periodically) hypoxic and in temperate climate. Similarities in core bacterioplankton phylogenetic lineages were found with many other estuaries that also suffer from heavy anthropogenic influence and oxygen deficiency. These similar ecosystems provide specific niches and bacterial populations that thrive in have to be adapted to lower salinity range, be capable of temporarily persisting hypoxia/anoxia and in temperate climate be resilient to seasonal changes. These factors mentioned above create a highly specific environment that suits with the species-sorting paradigm of metacommunity theory. Therefore, a proposition to view habitats of these ecosystems as a metacommunity, which is defined as a set of local communities that are linked by dispersal of multiple potentially interacting species. This pattern has already been emerging in oxygen minimum zones, where closely related bacterial lineages figure and interact. Thereby, oxygen depleted estuaries can be considered as a salinity-driven subsystems of global redox-specialized (covering all functional niches across the redox-tower) metacommunities.

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Spatial variability of winter bacterioplankton community composition in the Gulf of Finland (the Baltic Sea)

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ABSTRACT

The microbial communities in the Baltic Sea are extremely diverse and highly dynamic and undergoing shifts in dominant phylotypes in response to spatial and temporal environmental gradients. We present the first study of wintertime bacterioplankton with a high-throughput sequencing approach. Using barcoded pyrosequencing of the 16S rRNA gene we investigated the bacterial diversity and spatial distribution of winter picoplankton communities in the Gulf of Finland.

We analyzed ten samples and identified 609 OTUs (operational taxanomic units), mostly members of Proteobacteria, Actinobacteria, Bacteroidetes and Cyanobacteria. Most abundant OTUs of the dataset were identified as Sulfurimonas and Pelagibacter, which dominated the near-bottom and surface layers, respectively. Surface communities yielded about the same species richness estimates than a previous study conducted in same area during springtime.

1. Introduction

The world's oceans are filled with enormous quantities of microbes (Whitman et al., 1998). Microbes are key components of the structure and function of aquatic ecosystems and global biogeochemical processes (Azam et al., 1983; Falkowski et al., 2008). Culture-independent community analysis methods have dawned a new era in microbial ecology by enabling identification of the not-yet-cultured majority of microbes. The bacterioplankton community composition (BCC) has been observed to possess great variability on a global and even millimeter scale (e.g., Long and Azam, 2001; Pommier et al., 2007). Proliferation of high-throughput sequencing technologies continues to expand our understanding of microbial diversity and community structure, mostly via deep sequencing and comparative analysis of the 16S rRNA gene, which has revealed the vast diversity of rare microbes in different marine environments (Andersson et al., 2010; Galand et al., 2009; Gilbert et al., 2009; Kirchman et al., 2010; Sogin et al., 2006).

The Baltic Sea is one of the largest brackish water basins in the world. It is under considerable anthropogenic pressure as it serves as a drainage area for over 85 million people in 14 different countries. The Baltic Sea is characterized by long retention time (>25 years) and horizontal and vertical salinity gradients (Stigebrandt, 2001). The Gulf of Finland is the easternmost sub-basin of the Baltic Sea. It is directly connected, without a sill, to the Baltic Proper at its western end and to the Neva River's mouth at its eastern end. The salinity increases from east to west. It is about 2–6 in the surface water and about 5–9 in the deep water (Kullenberg, 1981). The strong stratification in the central Gulf of Finland due to the seasonal thermocline and permanent halocline hinders mixing in the water column (Alenius et al., 1998). This, combined with eutrophication driven high primary production, leads to oxygen depletion in the near bottom layers. Environmental gradients are reflected in spatial variability of the bacterioplankton assemblage due to the influence of a complex matrix of biotic and abiotic environmental variables (Fortunato et al., 2012).

Salinity is one of the major environmental factors influencing microbial communities in estuaries (Crump et al., 2004; Fortunato et al., 2012; Lozupone and Knight, 2007; Troussellier et al., 2002; Wu et al., 2006) and in brackish water environments like the Baltic Sea (Herlemann et al., 2011). Salinity is also a main selective environmental parameter shaping the Baltic Sea ice bacterial community structure (Kaartokallio et al., 2005), which mainly consists of representatives of *Alpha-*, *Beta-*, *Gammaproteobacteria* and *Bacteroidetes* (also known as *Cytophaga-Flavobacteria-Bacteroides*) phyla, that differ markedly from wintertime microbial communities of underlying water (Kaartokallio et al., 2005, 2008; Petri and Imhoff, 2001).

Pelagic BCC in the Baltic Sea is characterized by a unique combination of typical freshwater phylogenetic groups (mostly representatives of *Actinobacteria*, *Betaproteobacteria*, *Verrucomicrobia*) together with a lack of typical marine groups and a presence of autochthonous brackish microbial populations (Andersson et al., 2010; Hagström et al., 2000;

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Herlemann et al., 2011; Kisand et al., 2005; Riemann et al., 2008). In the central Baltic Sea about a third of the 454 sequence reads were observed to be related to bacterial phyla characteristic to freshwater ecosystems (Andersson et al., 2010). Recently, the high spatial dynamics of Baltic Sea BCC is demonstrated by pyrosequencing of the 16S rRNA gene (Herlemann et al., 2011; Koskinen et al., 2011). Interestingly, the bacterial diversity is not changing significantly along the Baltic Sea salinity gradient (Herlemann et al., 2011), whereas reduced diversity of higher eukaryotic organisms can be observed in intermediate salinity ranges (reviewed by Telesh and Khlebovich, 2010). In temperate basins the microbial communities underlie a seasonal succession. In the Baltic Sea high seasonal dynamics of BCC were demonstrated with cultivation, denaturing gradient gel electrophoresis, clone libraries and pyrosequencing methods (Andersson et al., 2010; Pinhassi and Hagström, 2000; Riemann et al., 2008), excluding winter months.

Temperature is an important factor influencing bacterial abundance, production and specific growth rate (Shiah and Ducklow, 1994; White et al., 1991). Andersson et al. (2010) used deep sequencing to study eight samples collected over an annual cycle (May to October of 2003 and May of 2004) and observed contrasting temporal dynamics with a total of 4624 OTUs (operational taxonomic units) identified, out of which, less than 2% were present in all samples. Global predictions of bacterial diversity in marine surface waters suggest that global marine bacterial diversity peaks are at high latitudes in winter (Ladau et al., 2013). The aim of this study was to examine spatial variability and diversity of wintertime BCC in the Gulf of Finland using next-generation sequencing method. Based on earlier studies, we presumed that winter BCC would be considerately different from spring and autumn microbial communities.

2. Materials and methods

2.1. Sample collection and extraction of community DNA

Water sampling aboard the RV Salme was performed on 8–9 December 2010 from three stations. Samples were collected from the central part of the Gulf of Finland (stations NS6 (59° 50,29′ N, 24° 48,63′ E) and NS8 (59° 51,36′N, 25° 47,38′E)) and from the entrance area of the Gulf of Finland (station NS1 (59° 26,84′ N, 22° 57,69′; Fig. 1)). The length of transect was about 170 km. Samples were collected from three depths: at 5 m, 40 m (only in station NS8) and 8–13 m above the seafloor (Table 1). A rosette sampler (M1018, General Oceanics) equipped with 8 Niskin water samplers (volume 1.7 l) was used for sampling. For background information the depth profiles of conductivity and temperature were obtained with a SBE19plus CTD probe (conductivity–temperature–depth probe, Sea-Bird Electronics), chlorophyll *a* fluorescence with WETStar fluorometer (WETLabs), dissolved oxygen with SBE 43 probe (Sea-Bird Electronics), and turbidity with ECO-NTU Turbidity Meter (WETLabs). Chemical analyses were carried out by the Estonian Marine Institute (Tartu University) according to EVS-EN standard methods.

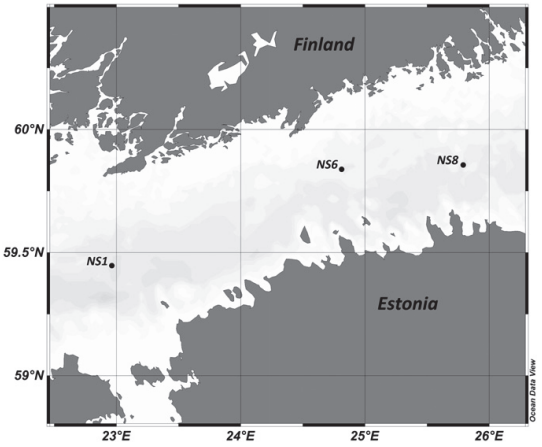

Fig. 1. Map of the sampling stations in the Gulf of Finland, Baltic Sea.

Water samples for microbial community extraction were collected into sterile bottles (Nalgene) and immediately filtered on 0.22 μm filters (Millipore, Millex-GP50) after preliminary filtration through 2.0 μm prefilters (Millipore, Millex-AP 50). The scheme of filtration system (except improvement by using Whatman syringe filters) is demonstrated in Supplementary Fig. 1. The sample volume varied between 0.5 and 1.0 l. Filters were kept frozen at −20 °C until community DNA was extracted with a PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc.). Few modifications were made to the protocol: syringe filters were incubated with the lyses buffer in the casing at 60 °C for 30 min and then eluate was removed.

2.2. Amplification of bacterial 16S rRNA gene sequences

The bacterial 16S rRNA gene V1–V2 hypervariable regions were amplified in two polymerase chain reactions (PCR). In the first reaction universal bacterial primers BSF8 and BSR357 (in italics; McKenna et al., 2008) were complemented with 8 nt barcode (marked as nnnnnnnn; Hamady et al., 2008) and partial adapter sequences (underlined): F8 5′ TTGGCAGTCTCAGnnnnnnnn*AGTTTGATCCTGGCTCAG* 3′ and R357 5′ GTCTCCGACTCAGnnnnnnnn*CTGCTGCCTYCCGTA* 3′. PCR was performed with Smart-Taq Hot Red 2 PCR Mix (Naxo, Estonia), 1 μl of extracted DNA, 0,2 μM each primer, using the following cycling parameters: 15 minute denaturation followed by 3 cycles (30 s at 95 °C, 30 s at 50 °C, 60 s at 72 °C), 28 cycles (30 s at 95 °C, 30 s at 65 °C, 60 s at 72 °C) and a final extension at 72 °C for 7 min. To achieve full length sequencing adapters, second PCR amplification was performed (A 5′-CCATCTCATCCCTGCGTGTCTCCGACTCAG-3′ and B 5′-CCTATCCCCTGTG TGCCTTGGCAGTCTCAG-3′). The reaction was run with Smart-Taq Hot Red 2× PCR Mix, 1 μl of 10× diluted amplicon, 0.2 μM each primer; using the following cycling parameters: 15 minute denaturation followed by 5 cycles (30 s at 95 °C, 30 s at 62 °C, 60 s at 72 °C), 20 cycles (30 s at 95 °C, 60 s at 72 °C) and a final extension at 72 °C for 10 min. PCR reactions were run in a thermal cycler 2720 (Applied Biosystems). Each PCR product was gel purified from a 1.5% agarose gel. DNA was isolated using the QIAquick® Gel extraction kit (Qiagen, Inc.). DNA concentrations were measured with a Qubit fluorometer (Invitrogen Corporation). 454 pyrosequencing (Margulies et al., 2005) was performed on a Roche GS Junior System (Biotap LLC, Estonia, Tallinn).

2.3. Bioinformatics

Reads with low quality and shorter than 150 bp (base pairs) were removed from the dataset. OTUs were defined using the average neighbor-clustering algorithm of MOTHUR 1.19.1 (Schloss et al., 2009) with 97% similarity threshold. Reference sequences were selected from the SILVA ribosomal RNA database (Pruesse et al., 2007). The PyroNoise algorithm was used to discard homopolymer-derived errors (Quince et al., 2011) and UCHIME to remove chimeric DNA sequences caused by PCR errors (Edgar et al., 2011). Taxonomic assignments were processed by the Ribosomal Database Project (RDP) naïve Bayesian Classifier (Wang et al., 2007) and BLAST (Altschul et al., 1997) searched against a local NCBI (National Center for Biotechnology Information) nucleotide database (released on the 29th of January 2011, http://www.ncbi.nlm.nih.gov/). Sequences matching eukaryotic DNA were discarded from the dataset. Statistical analyses were carried out with program R version 2.14.0 (http://www.r-project.org), ACE (Abundance-based Coverage Estimation; Chao and Lee, 1992) and Chao1 (Chao, 1984)

Table 1
Variation of environmental parameters in different stations and depths.

Station	Sample depth (m)	Total depth (m)	Temperature (°C)	Salinity	Oxygen (mg l^{-1})	Chl a (mg m^{-3})	NH_4^+ (μM)	NO_2^-/NO_3^- (μM)	$N_{tot.}$ (μM)	PO_4^{3-} (μM)	$P_{tot.}$ (μM)	SiO_4 (μM)
NS1	5	90	3.3	6.3	12.6	3.5	0.3	3.3	22.8	1.0	0.9	12.1
NS6	5	74	3.0	6.0	12.5	3.7	0.3	2.8	16.8	0.7	1.1	14.8
NS8	5	82	2.6	5.6	12.5	4.4	0.5	5.2	23.8	1.1	1.4	17.8
NS8	40	82	4.9	6.5	9.4	2.4	0.3	5.2	22.5	1.3	1.6	18.0
NS1	77	90	5.8	10.2	0.96	1.75	5.4	2.7	20.3	3.4	3.9	35.2
NS6	66	74	5.6	8.7	4.4	1.9	5.0	1.8	20.9	2.4	2.8	28.2
NS8	74	82	5.5	9.0	2.7	1.98	3.8	2.0	16.9	2.2	2.6	29.2

richness estimates were calculated using VEGAN package (Oksanen et al., 2009). The sequences have been deposited to GenBank with accession numbers: KC971255–KC972610.

3. Results and discussion

3.1. Physicochemical characteristics of the water column

According to the CTD profiles (Fig. 2) the water temperature ranged from 2.6 — 4 °C in the upper layer and 5–6 °C in the near-bottom layer. Halocline was present approximately at 40–65 m depth in different stations dividing the surface mixed layer with salinities of 5.6–7 and deep water with salinities up to 10.2. The halocline was more pronounced at station NS1. The upper layer above halocline was oxygen-saturated (oxygen concentrations between 11.3 and 12.6 mg l^{-1}), but at all the stations the oxygen concentrations were remarkably lower at the near-bottom layer (2.3–7.2 mg l^{-1}). At the deepest station (NS1) the near bottom environment was hypoxic with the oxygen concentration of 0.5 mg l^{-1}. The dissolved inorganic nutrient concentrations varied markedly between the oxygen rich and depleted zones of the water column and less between stations (Table 1), except significantly higher nitrate–nitrite concentrations were measured at 5 m depth at station NS8 compared with the same depth of the other stations. Ammonia, phosphate and silicate silicon dioxide exhibited higher and nitrate–nitrite lower concentrations in the oxygen-depleted parts of the water column.

3.2. Bacterioplankton community diversity

A total of 30,371 partial rRNA sequences were obtained. The mean length of used sequences was 271 bp (after removing primers). Total

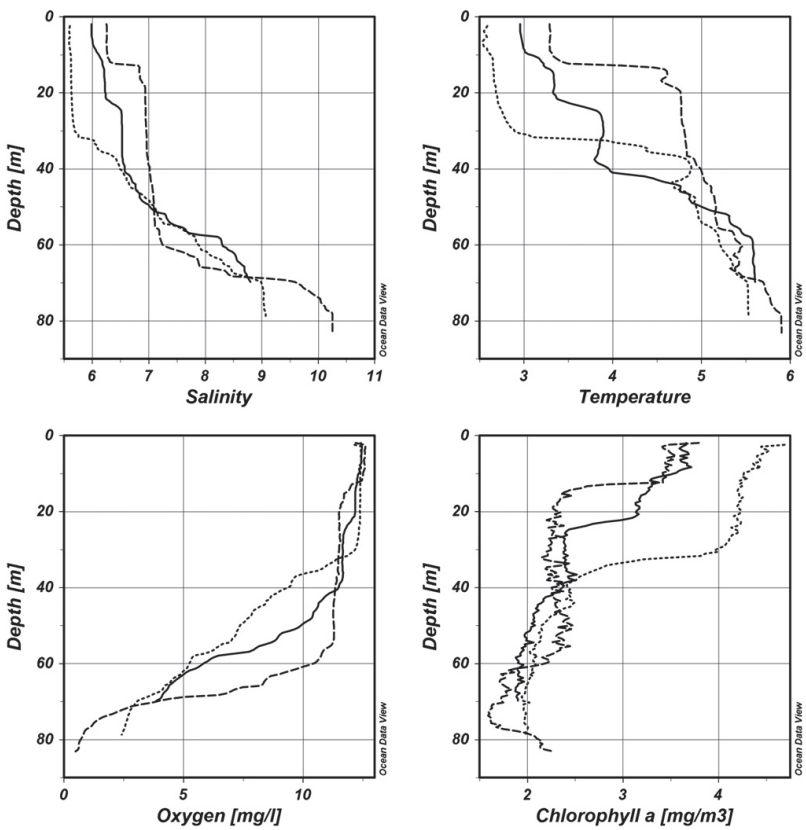

Fig. 2. Salinity, temperature (°C), dissolved oxygen and chlorophyll *a* profiles for stations NS1 (dashed), NS6 (solid) and NS8 (dotted). The near-bottom samples were collected at 77 m (NS1), 66 m (NS6) and 74 m (NS8) depth. Surface samples were collected at 5 m depth from all the stations. 40 m sample was collected only from station NS8.

of 1356 unique OTUs were identified with 97% similarity threshold. After removing the OTUs with only one sequence (which could have resulted from sequencing errors) the number of total OTUs dropped to 609. 34 OTUs were shared between all the samples and 177 and 76 OTUs were shared in surface samples and near-bottom samples, respectively. The sequences were matched against NCBI nucleotide database using BLAST (Altschul et al., 1997). Of 649 OTUs, 33% had more than 99% similarity to sequences in the database, whereas 22% had less than 95% similarity, and may represent novel bacterial genera or higher taxa. 8% of OTUs could be identified only as *Bacteria*, when using RDP naïve Bayesian Classifier (50% confidence threshold; Wang et al., 2007). This is a relatively high percentage compared to previous studies of pelagic bacterioplankton in the Baltic Sea area, so we classified these sequences alternatively with SINA webserver tool (Pruesse et al., 2012). In the case of 45% of these sequences the classification was improved, which rendered the 'unclassified *Bacteria*' fraction into size that would be presumed by high-throughput 454 sequencing of pelagic bacterioplankton in the Baltic Sea. The SINA webserver tool was also used to check the classification of relatively abundant OTUs of our dataset and the results were nearly identical with output of RDP naïve Bayesian Classifier.

The number of OTUs retrieved from each sample, as well as Chao1 and ACE species richness estimates are reported in Table 2. Species richness estimates were strongly affected by the number of sequences analyzed, as discussed elsewhere (Schloss and Handelsman, 2005). Our results indicate that species richness in surface water communities (Chao1 mean = 773, SD = 301) was not significantly ($p = 0.19$, two tailed *t*-test) higher than in previously analyzed spring pelagic surface water communities in station LL7, LL12 at the same area and TPDEEP (Chao1 mean = 478, SD = 73; Koskinen et al., 2011), here we have to consider that filamentous and aggregated bacterial cells larger than 2.0 μm were likely excluded from samples by prefiltration used. Comparing samples obtained with similar filtration system and same stations in spring and summer of 2011 (Chao1 mean = 250, SD = 96 Laas et al., unpublished) the difference was more pronounced ($p < 0.01$, two tailed *t*-test). Although the number of OTUs per sample was lower than in some of the previous studies of pelagic bacterioplankton of the Baltic Sea (Andersson et al., 2010; Herlemann et al., 2011), we have to consider that rarefaction curves outlined from each sample did reach a curvilinear phase in most cases (Supplementary Fig. 2), but the sequencing effort was not sufficient for the rarefaction curves to reach an asymptote. This indicates that higher diversity could have been observed if sample volume and amplicon size had been increased. Even though wintertime bacterioplankton is characterized by lower cell counts (e.g., Shiah and Ducklow, 1994), in a recent study species distribution modeling of bacteria in global marine surface waters indicates that diversity actually peaks globally in the temperate latitudes in the winter (Ladau et al., 2013). This pattern is contrasting to marine and terrestrial microorganism diversity, but this trend has been confirmed in bacterioplankton studies conducted in high latitude sampling sites (Ghiglione and Murray, 2012).

Table 2
Number of retrieved sequences, operational taxonomic units and calculated species richness estimates.

Sample ID	Number of sequences	Including singletons	Excluding singletons	Chao1	s.e.	ACE	s.e.
NS1_5m_A	3207	308	183	488	45	476	12
NS1_5m_B	3816	406	206	794	81	746	16
NS6_5m_A	3458	412	221	705	60	699	16
NS6_5m_B	7382	597	284	1273	114	1221	21
NS8_5m	3017	326	163	607	64	604	15
NS8_40m	2301	280	151	456	44	477	13
NS1_77m_A	1996	162	64	388	74	383	12
NS1_77m_B	1501	196	83	379	52	414	13
NS6_66m	2810	360	133	1051	157	951	19
NS8_74m	1557	236	103	423	47	479	14

s.e. — standard error.
A/B — differentiate subsamples from same site.
ACE (abundance-based coverage estimation; Chao and Lee, 1992).
Chao1 (Chao, 1984).

3.3. Bacterioplankton community composition

Most bacteria identified in our dataset were representatives of four phyla: *Proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Cyanobacteria*. 9 classes represented about 85% of bacteria identified: *Alphaproteobacteria* (28.9%) *Epsilonproteobacteria* (25.9%), *Actinobacteria* (14.6%), *Gammaproteobacteria* (3.3%), *Betaproteobacteria* (5.1%), *Flavobacteria* (3.2%), *Cyanobacteria* (2.0%), *Deltaproteobacteria* (0.7%), *Sphingobacteria* (1.1%).

The relative abundances of dominant classes and most abundant OTUs (>1% of dataset) at different stations and depths are shown in Figs. 3 and 4, respectively. Principal component analyzes revealed that BCC varied more between depths than the stations (data not shown). To analyze how different phylogenetic groups correlated with individual environmental parameters, the abundance profile of the most abundant classes and OTUs (top 20) were correlated with each of the measured environmental parameters (Figs. 5 and 6). The dendrograms represent clustering of phylogenetic groups and environmental parameters based on similarities in Pearson r-values, which revealed division into roughly two groups, bacteria associated with surface layer or near-bottom layer communities. Surface samples collected at station NS6 exhibited influence of bacterial groups that were dominant in the deeper layers and vice versa. This mixing may have been caused by convective mixing of the water column and restratification in that area. Cross-contamination can be ruled out because parallel samples were taken from the surface layer and same sampling technique was used in all stations.

In the surface layer *Alphaproteobacteria* constituted about a quarter of all bacteria identified with most dominant representatives belonging to the cosmopolitan SAR11 clade (order *Rickettsiales*). Ribotypes NSD002, NSD004 and NSD011 were classified as '*Candidatus* Pelagibacterb ubique' with very close matches to '*Ca.* P. ubique' strains HTCC1062 (NSD002, 87%; NSD011, 100%) and IMCC9063 (NSD004, >98% similarity), the last of which was isolated from the Arctic Ocean. '*Ca.* P. ubique' is widely distributed and represents the most abundant microbial species on earth (Carlson et al., 2008; Morris et al., 2002; Rappé and Giovannoni, 2003). The SAR11 clade also shows pronounced winter peaks in relative abundance in the Arctic and Antarctic (Ladau et al., 2013). Partly overlapping populations of SAR11 representatives have been identified throughout the Baltic Sea in summer (Herlemann et al., 2011) and at the same stations in spring and summer (Laas et al., unpublished). This indicates presence of '*Ca.* P. ubique' populations throughout seasonal succession. Alphaproteobacterial ribotypes NSD003 and NSD022 were both classified as *Rhodobacteraceae*, but their prevalence pattern was reversed. NSD003 was more abundant in the surface layer and had highest similarity (97%) to *Rhodobacter* sp. R7K3Z5 isolated from sewage (Genbank accession (GB): EU604756). The *Rhodobacter* spp. are metabolically highly diverse and able to perform anoxygenic photosynthesis (Arai et al., 2008; Verméglio and Joliot, 1999). Representatives of *Rhodobacteraceae* were relatively abundant in spring (Koskinen et al., 2011) and summer (Herlemann et al., 2011), which could mean their year-round presence in BCC of the Baltic Sea. NSD022 had the closest match (98.5%) to representative of genus *Parvularcula* (GB: HQ326293).

The near-bottom bacterioplankton communities were dominated by *Sulfurimonas* ribotype (OTU) NSD001, which had a high similarity (>99.3%) to *Sulfurimonas* subgroup sequences previously retrieved from Gotland Deep (GB: AJ810529, EF405797, EF405798; Brettar et al., 2006; Grote et al., 2007). This subgroup catalyzes chemolithotrophic denitrification and has been demonstrated to be most dominant CO_2-fixing group in anoxic interfaces of Gotland and Landsort Deeps (Glaubitz et al., 2009; Grote et al., 2008). This suggests that populations dominant in anoxic zones of Gotland Deep are also abundant in other

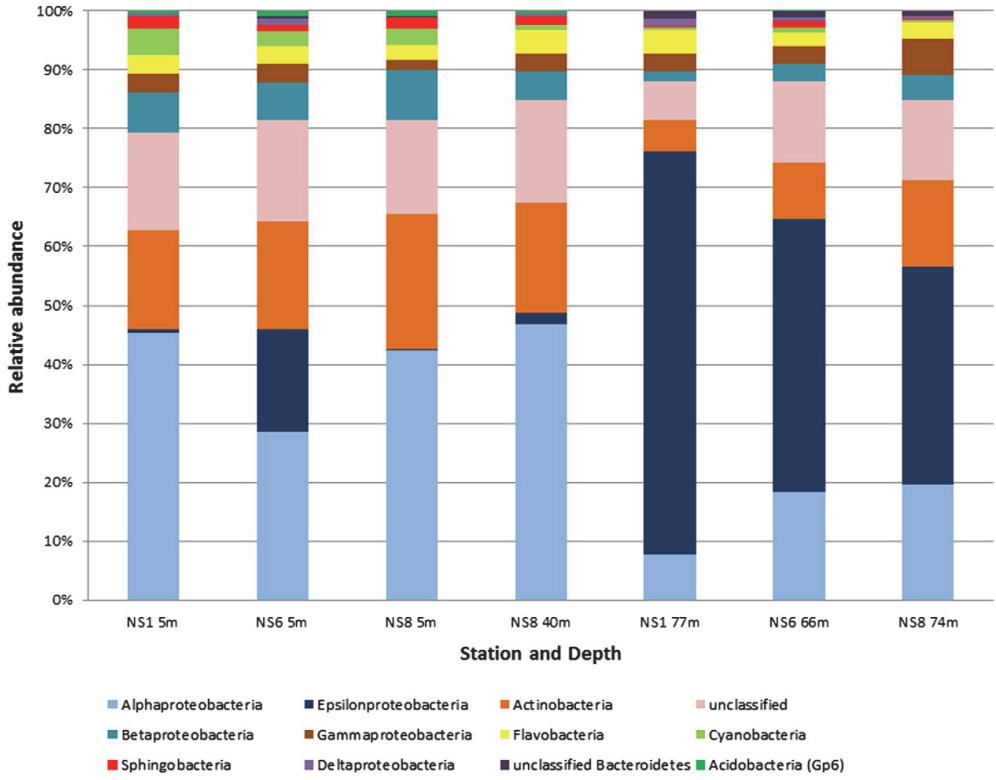

Fig. 3. Relative abundance of bacterial classes at different sampling stations and depths. Parallel samples from one site have been averaged.

sub-basins, where temporary oxygen depletion occurs. Genomic analysis of *S. denitrificans* has revealed that it has all genes necessary for complete oxidation of reduced sulfur compounds to sulfate (Sox pathway) and reductive citric acid cycle for carbon fixation (Sievert et al., 2008). Ribotypes NSD044 and NSD047, also classified as *Sulfurimonas*, had high similarities with sequences isolated from locations other than Baltic Sea (Fuchsman et al., 2011; Schmidtova et al., 2009) and were less abundant.

Actinobacteria contributed 19% of the surface community and 10% of the near-bottom bacterial community. Members of *Actinobacteria* were also demonstrated as an abundant and diverse component of bacterioplankton in the Baltic Proper and Gulf of Bothnia in previous studies (Andersson et al., 2010; Holmfeldt et al., 2009; Riemann et al., 2008). *Actinobacteri*a are mostly associated with freshwater and the increase in abundance is well correlated with the decrease in salinity in the Baltic Sea (Herlemann et al., 2011). In our studies representatives of *Actinobacteria* also demonstrated a negative correlation with salinity and strongest positive correlation with longitude of all phyla. Relatively abundant ribotypes NSD006, NSD008 and NSD027 were classified as *Ilumatobacter* sp. of *Acidimicrobiales* (Matsumoto et al., 2009). Although members of *Actinobacteria* are mostly heterotrophic, the nearest known relative to *Ilumatobacter* sp. is *Acidimicrobium ferrooxidans*, which is capable of oxidation of ferrous iron and autotrophic growth usually in iron-, sulfur- and mineral–sulfide rich environments (Clum et al., 2009). Ribotype NSD026 had an identical match with sequences isolated from a lake in China (GB: EU801245).

Members of *Betaproteobacteria* are mostly associated with freshwater and were represented by orders *Burkholderiales*, *Methylophilales*, *Nitrosomonadales*, with the methylotrophic genus *Methylophilus* (ribotypes NSD010, NSD028) identified as most common genera. This could mean elevated single-carbon metabolism in the microbial community. Ribytype NSD010 had a slightly higher presence in the surface layer, but its relative abundance increased towards the east — less saline waters. So far the presence of *Methylophilus* strains has been reported in the northern Baltic Sea (Kisand and Wikner, 2003).

Representatives of phylum *Bacteroidetes* were reported as being abundant in the Baltic Sea (Andersson et al., 2010; Hagström et al., 2000; Herlemann et al., 2011; Kisand et al., 2005; Pinhassi and Hagström, 2000; Riemann et al., 2008). In our observations, the representatives of phylum *Bacteroidetes* were split between classes *Flavobacteria* and *Sphingobacteria* or remained identified on phylum level. Relatively abundant ribotype in surface communities NSD015 remained classified on phylum level, with closest matches to members of genus *Marinoscillum* (of *Sphingobacteriales*, GB: FQ032830) isolated from North Atlantic Ocean. Ribotype NSD017 contributed 3.8% of the sequences in the near-bottom community of station NS1 and could be identified as *Flavobacteriaceae* with identical match to sequences retrieved from pelagic redoxcline of the central Baltic Sea (GB: KC492867, Glaubitz et al., 2013).

Aggregated and filamentous bacteria were caught on pre-filters, so from cyanobacteria only single cell pico-size groups were included in our analyses. The *Cyanobacteria* accounted for on average 3% in the surface layer. Ribotype NSD007 was identified as a freshwater species *Synechococcus* (>99% similarity), a common picocyanobacteria in the Baltic Sea. Ribotype NSD036 remained unclassified, but had the closest similarity (97%) with *Snowella litoralis* associated with lakes (Rajaniemi-Wacklin et al., 2006).

Deltaproteobacteria, mostly represented by the order *Desulfobacterales*, perform dissimilatory sulfur and sulfate reduction. Their representatives were detected in the near-bottom community samples, which was

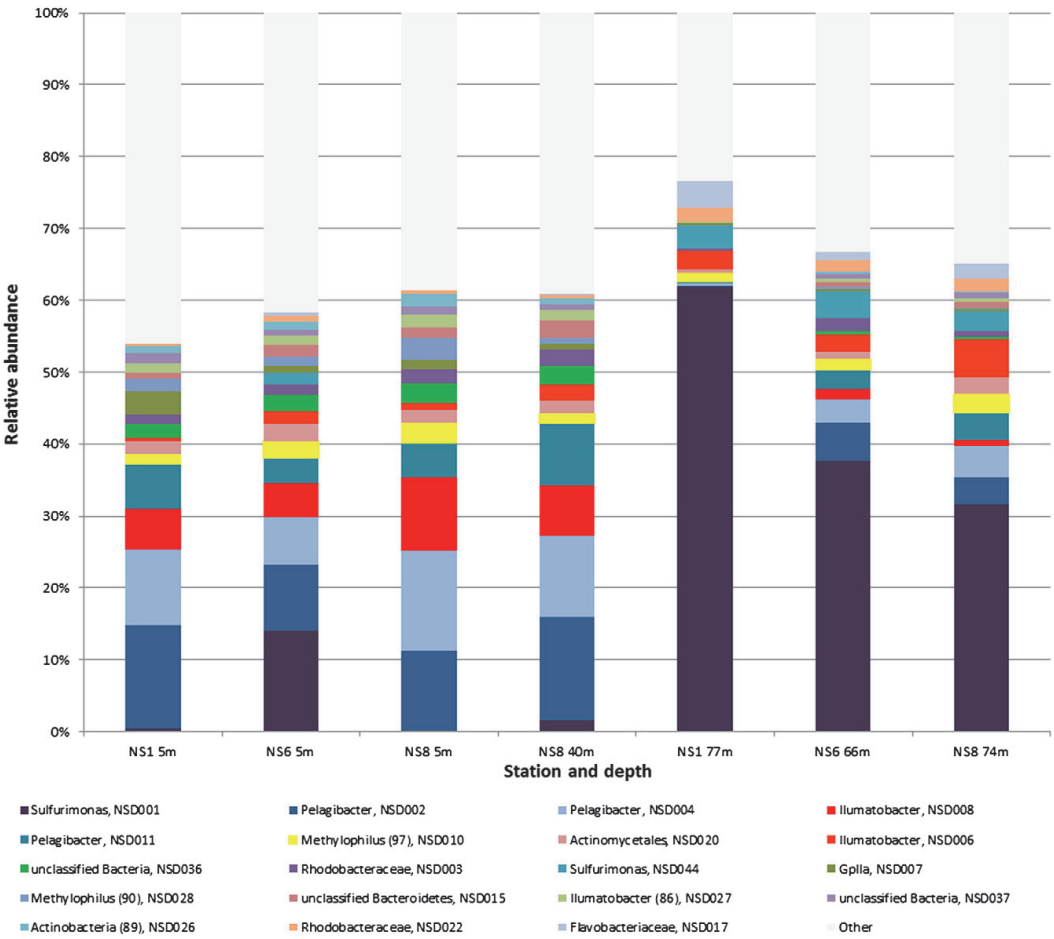

Fig. 4. Operational taxonomic units (OTUs) with highest relative abundance (>1% of total dataset) and their classification. GpIX is a genus of Cyanobacteria Family II. Parallel samples from one site have been averaged. Taxonomic assignments by the Ribosomal Database Project (RDP) naïve Bayesian Classifier (Wang et al., 2007) were added. Lower than 100% classification confidence is given in parentheses.

probably caused by diffusion of sediment microbiota. The most notable difference with results by Koskinen et al. (2011) is the low relative abundance of *Gammaproteobacteria* overall, but specially *Pseudomonas*, which constituted 45% of their dataset, while only a negligible part of ours. *Gammaproteobacteria* have been also demonstrated to account for considerable percentage of sea ice bacteria in the Baltic Sea (Kaartokallio et al., 2008). Almost absent were OTUs related to *Verrucomicrobia*, while during summer their abundance has been demonstrated to be considerable in the BCC (Andersson et al., 2010; Herlemann et al., 2011).

3.4. Conclusions

The wintertime BCC in the Gulf of Finland was dominated by representatives of *Proteobacteria*, *Actinobacteria* and *Bacteroidetes*, with '*Candidatus* Pelagibacter', *Sulfurimonas*, *Ilumatobacter* and *Methylophilus* as the most abundant genera. The seasonal redoxline in the gulf expands the distribution of bacteria permanently abundant under the redoxcline of the central Baltic Sea, mostly *Sulfurimonas* strains. This impacts important biochemical processes, like dark CO_2-fixation, denitrification and H_2S-oxydation. The redoxcline is also a main factor why changes of BCC are more contrasting vertically in the water column than horizontally between the stations.

There were certain phylogenetic groups that were observed by previous studies during different seasons, but also notable differences, as was expected. Interestingly, only about a fifth of the sequences belonged to bacterial groups usually affiliated with freshwater environments. One cause of this was a high relative abundance of alphaproteobacterial SAR11 cluster representatives in the surface layer.

Based on our limited dataset, we cannot suggest that the diversity of bacterioplankton in the Gulf of Finland peaks regularly during wintertime, as expected from the results of Ladau and colleagues. Nevertheless, comparisons with our new data (unpublished) and previous studies indicate, that the wintertime BCC has at least the same level of species diversity compared to other seasons. The rarefaction curves didn't reach an asymptote and significantly a higher number of sequences per sample (deep-sequencing approach) should be used to verify this hypothesis.

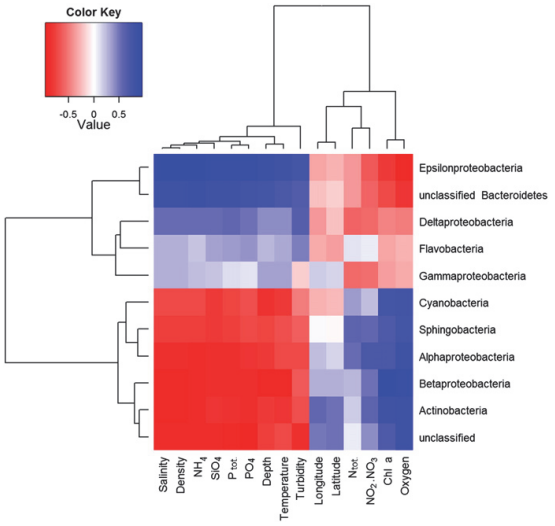

Fig. 5. Correlations between environmental parameters and classes of bacteria. Colors indicate r-values of Pearson correlations between environmental parameters (columns) and classes of bacteria (rows). The dendrogram represents clustering based on similarities in r-values.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jmarsys.2013.07.016.

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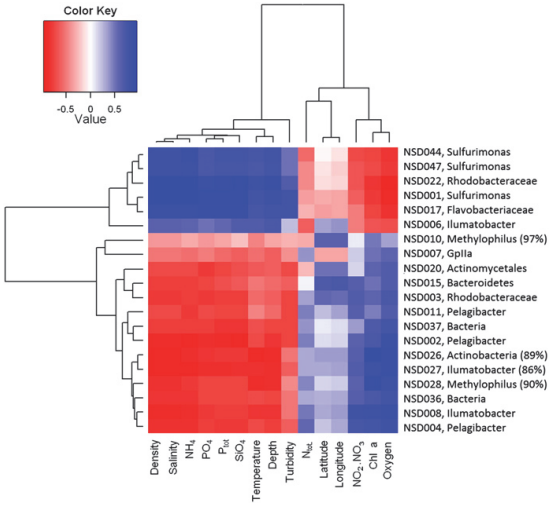

Fig. 6. Correlations between environmental parameters and top 20 most abundant individual operational taxonomic units (OTUs). Colors indicate r-values of Pearson correlations between environmental parameters (columns) and OTUs (rows). The dendrogram represents clustering based on similarities in r-values. Taxonomic assignments by the Ribosomal Database Project (RDP) naïve Bayesian Classifier (Wang et al., 2007) were added. Lower than 100% classification confidence is given in parentheses.

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Paper II

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PLOS ONE

RESEARCH ARTICLE

Redox-Specialized Bacterioplankton Metacommunity in a Temperate Estuary

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Abstract

This study explored the spatiotemporal dynamics of the bacterioplankton community composition in the Gulf of Finland (easternmost sub-basin of the Baltic Sea) based on phylogenetic analysis of 16S rDNA sequences acquired from community samples via pyrosequencing. Investigations of bacterioplankton in hydrographically complex systems provide good insight into the strategies by which microbes deal with spatiotemporal hydrographic gradients, as demonstrated by our research. Many ribotypes were closely affiliated with sequences isolated from environments with similar steep physiochemical gradients and/or seasonal changes, including seasonally anoxic estuaries. Hence, one of the main conclusions of this study is that marine ecosystems where oxygen and salinity gradients co-occur can be considered a habitat for a cosmopolitan metacommunity consisting of specialized groups occupying niches universal to such environments throughout the world. These niches revolve around functional capabilities to utilize different electron receptors and donors (including trace metal and single carbon compounds). On the other hand, temporal shifts in the bacterioplankton community composition at the surface layer were mainly connected to the seasonal succession of phytoplankton and the inflow of freshwater species. We also conclude that many relatively abundant populations are indigenous and well-established in the area.

Introduction

The world's oceans are the cradle of life; hence, the evolution of aquatic microorganisms for 3.5 billion years has produced enormous diversity and functional plasticity, only recently assessed by the sequencing of metagenomic DNA (pioneered by [1]). There are many aspects of microbial life that make the ecology of microorganisms different from that of macroorganisms [2], including intercontinental dispersion by winds [3] and the capability to persist in environmentally hostile conditions over a long period of time [4]. Aquatic microbes are essential for life on Earth [5,6] and therefore unveiling the mechanisms underlying the spatiotemporal dynamics

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of bacterioplankton community composition (BCC) remains the one of the most important issues in aquatic microbial ecology.

Over the last few decades, advances in sequencing technologies have revolutionized the power of the identification process for microorganisms and thereby revealed tremendous microbial diversity and plasticity in aquatic environments [7]. 16S rRNA gene-based investigations have contributed a massive number of sequences to databases and have revealed a comprehensive uncultured microbial diversity [8,9]. The ease of microbial community profiling has been effectively utilized to determine the biogeographic patterns of the most numerous and cosmopolitan marine bacterioplankton clades and, ultimately, to determine the functional traits that make them so successful [10–12]. More recently, high-throughput sequencing technologies have allowed for increasing the depth of investigation and thereby unveiled a rare biosphere that accounts for most of the observed phylogenetic diversity of bacterioplankton community [13,14]. This acts as a "seed bank" from where new dominant species can emerge when the environmental conditions change [4,15].

Consequently, species-sorting by the local environment has been demonstrated to be one of the main driving processes behind shaping the BCC [16–19]. However, in some cases, the assembly mechanism can be well explained by neutral models [20–23], by mass effects [24,25], or by the combination of several mechanisms [26–28]; the relative importance of these mechanisms may change over time [29].

In addition to unique environmental conditions, similarities to other communities have to be considered in order to identify processes underlying the assembly of local microbial communities [23]. Hence, in this study, we combined environmental factors with phylogenetic affiliations of relatively abundant populations for that purpose. Hence, special attention was paid to associations within between ribotypes, because these interactions can have stronger correlative relationships compared to relationships between bacteria and eukaryotes, or bacteria and abiotic environmental factors [30]. The co-occurrence of networks of dominant bacterial ribotypes isolated from the marine oxygen minimum zone (OMZ) throughout the world has revealed a pattern of cosmopolitan key species filling redox-driven niches [31]. These niches revolve around functional capabilities to utilize different electron receptors and donors [32]. Next important step towards a better understanding of these microbial communities inhabiting OMZ is to define shared or specialized metabolic subsystems in different oceanic provinces [31]. Our results contribute to this effort.

The Baltic Sea is one of the largest brackish basins of the world, characterized by a long residence time. Therefore, it is not just a mixing zone for fresh water and marine species, but a habitat for microbes specialized for brackish water, which has been illustrated by the spread of different bacterial populations throughout the salinity gradient of the Baltic Sea [33–35]. Unlike the diversity of macro-organisms, the BCC does not decline within a salinity gradient [35].

The Gulf of Finland is the easternmost sub-basin of the Baltic Sea. The strong stratification in the central part of the gulf due to the seasonal thermocline and permanent halocline often hinders mixing in the water column [36]. Eutrophication-driven phytoplankton production leads to increased sedimentation of organic matter and hence increased consumption of oxygen for which atmospheric and photosynthetic re-oxygenation cannot compensate [37].

Furthermore, the Gulf of Finland is directly connected to the Baltic Proper, where the anoxic zone is permanent and therefore inhabited by well-established anaerobic ecotypes typical of the Baltic Proper [36,38,39]. During oxygen deficiency, certain microbes are capable of using terminal electron acceptors other than oxygen (e.g. NO_3^-, SO_4^{2-} and metal oxides). Epsilonproteobacterial *Sulfurimonas gotlandica* clade GD1 has been demonstrated to be one of the most numerous chemolithoautotrophic bacteria present in the OMZ of the central Baltic Sea [40,41]. This clade has been shown to spread into the anoxic zone of the Gulf of Finland [42].

As a temperate estuary, the Gulf of Finland undergoes many seasonal changes in environmental parameters such as ice coverage, water temperature, solar radiance, inorganic nutrients, etc., which contribute to the recurring succession of phytoplankton. Variation in biotic and abiotic factors also leads to the recurring succession of bacterioplankton in the Baltic Sea [34,43,44].

The goals of present study were to investigate the spatial and temporal dynamics of the BCC of the Gulf of Finland during the spring to summer transition in order to determine the main factors driving the bacterioplankton community assembly. To these ends, we used pyrosequencing of 16S rRNA genes from community DNA samples, which were collected in parallel with monitoring of physicochemical parameters and phytoplankton community composition. Many annually recurring environmental shifts took place during the study period, mainly the formation of a thermocline, the depletion of oxygen in the deeper layers and the shift from the spring to summer phytoplankton community.

Materials and Methods

Ethics statement

No specific permits were required for the described field studies. Our study area is not privately owned or protected in any way. The study did not involve endangered or protected species.

Sample collection and extraction of community DNA

Water sampling was performed aboard the RV Salme in the spring and summer of 2011 on two transects in the central part of the Gulf of Finland (Fig 1). The coordinates of sampling stations are listed in Table 1. Samples were collected from three depths: at 5 m, 40 m and about 5 m above the seafloor; detailed information about each sample is provided in Table 2. A rosette sampler (M1018, General Oceanics) equipped with Niskin water samplers (volume 1.7 l) was used for sampling. For background information, the depth profiles of conductivity and temperature were obtained with a SBE19plus CTD probe (conductivity-temperature-depth probe,

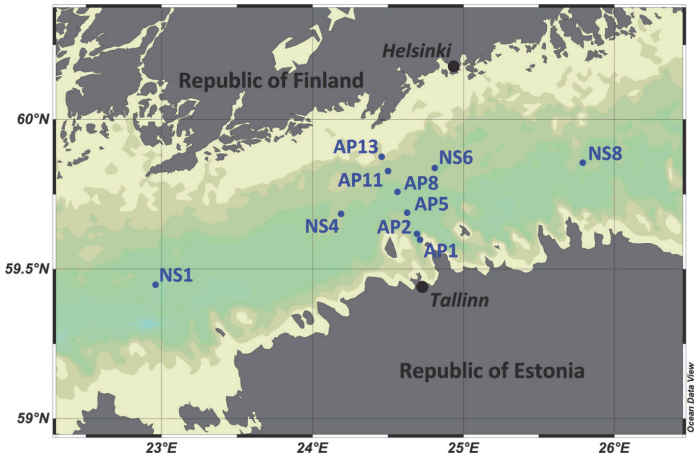

Fig 1. Map of the study area with sampling stations.

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Table 1. Coordinates of the sampling stations.

Station	Longitude	Latitude
AP1	24.71337	59.59792
AP2	24.69253	59.61823
AP5	24.62698	59.68858
AP8	24.56268	59.75820
AP11	24.50003	59.82780
AP13	24.45807	59.87580
NS1	22.96157	59.44745
NS4	59.68333	24.18667
NS6	24.81060	59.83822
NS8	25.78967	59.85605

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Sea-Bird Electronics), chlorophyll *a* fluorescence with WETStar fluorometer (WETLabs) and dissolved oxygen with SBE 43 probe (Sea-Bird Electronics).

The samples for nutrient (NO_2^- + NO_3^- and PO_4^{3-}) analysis were deep-frozen at −20°C after collection and analyzed at a shore-based laboratory using a Lachat QuikChem 8500 Series 2 automatic nutrient analyzer (Lachat Instruments, Hach Company). The nutrient analyses were performed according to the ISO 15681–1 method for PO_4^{3-} and ISO 13395 method for NO_2^- + NO_3^-. The lower detection ranges for PO_4^{3-} and NO_2^- + NO_3^- were 0.02 and 0.03 μmol l^{-1}, respectively.

Water samples for microbial community extraction were collected into sterile bottles (Nalgene) and immediately filtered through 0.2 μm filters (Whatman, Puradisc FP 30) after preliminary filtration through 5.0 μm prefilters (Whatman, Puradisc FP 30). The scheme of the filtration system was described by Laas *et al*. (2014) [42]. The sample volume varied between 0.5 and 1.0 liters. Filters were kept frozen at -20°C until community DNA was extracted with a PowerSoil DNA Isolation Kit (MO BIO Laboratories, Inc.). A few modifications were made to the protocol: syringe filters were incubated with the lysis buffer in the casing at 60°C for 30 min and then the eluate was removed.

Amplification of bacterial 16S rRNA gene sequences

The bacterial 16S rRNA gene V1-V2 hypervariable regions were amplified in two polymerase chain reactions (PCR). For the first reaction, universal bacterial primers BSF8 and BSR357 were complemented with 8 nt barcode and partial adapter sequences (Table 3) [46]. PCR was performed with Smart-Taq Hot Red 2X PCR Mix (Naxo, Estonia), 1 μl of extracted DNA and 0.2 μM each primer, using the following cycling parameters: 15 min denaturation followed by three cycles (30 sec at 95°C, 30 sec at 50°C, 60 sec at 72°C), 28 cycles (30 sec at 95°C, 30 sec at 65°C, 60 sec at 72°C) and a final extension at 72°C for 7 min. To achieve full length sequencing adapters, second PCR amplification was performed.

The second reaction was run with Smart-Taq Hot Red 2X PCR Mix (Naxo, Estonia), 1 μl of 10 X diluted amplicon, 0.2 μM each primer; using the following cycling parameters: 15 min denaturation followed by five cycles (30 sec at 95°C, 30 sec at 62°C, 60 sec at 72°C), 20 cycles (30 sec at 95°C, 60 sec at 72°C) and a final extension at 72°C for 10 min. PCR reactions were run on a thermal cycler (model 2720, Applied Biosystems). Each PCR product was gel purified on a 1.5% agarose gel. DNA was isolated using the QIAquick Gel extraction kit (Qiagen, Inc.). DNA concentrations were measured with a Qubit fluorometer (Invitrogen). Sequencing was performed on a Roche GS FLX next generation sequencing platform (IMGM Laboratories).

Table 2. The physico-chemical properties of the sampling sites. NA–not analyzed.

Code	Date	Station	Depth (m)	Volume (ml)	Oxygen (mg/L)	Temperature (°C)	Chl_a (mg m^{-3})	Salinity	NO2/NO3 (μmol/L)	PO4 (μmol/L)
WA_000034	21.04.2011	AP2	5	750	16.0	0.8	5.8	6.4	0.1	0.8
WA_000035	21.04.2011	AP2	40	600	14.7	0.1	1.8	6.8	5.8	1.2
WA_000036	21.04.2011	AP2	95	750	1.7	4.9	0.2	9.5	4.8	2.5
WB_000038K	21.04.2011	AP5	40	900	13.8	0.5	0.4	6.9	6.0	1.1
WA_000039	21.04.2011	AP5	83	700	1.4	5.1	0.2	9.7	4.7	2.9
WA_000040	21.04.2011	AP8	5	700	15.5	0.9	6.8	6.5	0.1	0.7
WB_000040K	21.04.2011	AP8	5	700	15.5	0.9	6.8	6.5	0.1	0.7
WB_000041K	21.04.2011	AP8	40	850	15.3	0.1	0.6	6.7	3.9	0.8
WB_000042K	21.04.2011	AP8	76	900	4.7	4.0	0.2	8.7	4.3	1.8
WB_000043K	4.05.2011	AP2	5	800	13.9	1.6	8.6	6.1	1.2	0.6
WA_000044	4.05.2011	AP2	41	950	9.3	1.5	0.6	7.2	4.5	1.2
WA_000049	4.05.2011	AP8	5	500	15.0	2.4	4.5	6.3	0.0	0.7
WA_000054	4.05.2011	AP13	5	750	14.7	3.1	5.7	6.2	0.3	0.6
WA_000057	10.05.2011	NS1	40	1000	14.5	1.2	3.1	7.0	NA	NA
WA_000058	10.05.2011	NS1	80	950	0.1	5.7	0.2	10.4	NA	NA
WA_000059	10.05.2011	NS4	5	650	16.0	3.2	4.2	6.1	NA	NA
WB_000060	10.05.2011	NS4	40	1000	14.1	1.3	2.2	6.9	NA	NA
WB_000061	10.05.2011	NS4	63	950	5.9	3.4	0.9	8.5	NA	NA
WB_000062	10.05.2011	NS6	5	750	15.8	5.5	4.4	5.9	NA	NA
WB_000063	10.05.2011	NS6	40	1000	12.8	0.5	1.7	6.7	NA	NA
WB_000064	10.05.2011	NS6	70	1000	2.9	4.5	0.5	9.2	NA	NA
WA_000065	10.05.2011	NS8	5	800	15.1	0.6	12.5	4.8	NA	NA
WA_000066	10.05.2011	NS8	40	850	9.3	1.9	1.5	7.0	NA	NA
WB_000068	3.06.2011	NS6	5	750	12.3	7.9	1.1	5.9	NA	NA
WB_000069	3.06.2011	NS6	40	850	11.2	0.8	0.7	6.8	NA	NA
WB_000070	3.06.2011	NS6	72	1000	2.5	3.7	0.4	8.7	NA	NA
WA_000072	3.06.2011	NS8	40	950	10.6	1.3	0.6	6.9	NA	NA
WB_000073	3.06.2011	NS8	80	1000	2.0	4.3	0.3	8.9	NA	NA
WA_000082	3.06.2011	NS4	5	750	11.3	9.5	1.0	6.3	NA	NA
WB_000085	3.06.2011	NS1	5	800	11.6	9.4	0.5	6.6	NA	NA
WB_000086	3.06.2011	NS1	40	950	10.9	1.1	0.2	7.0	NA	NA
WB_000087	3.06.2011	NS1	84	1000	0.5	5.1	0.2	9.7	NA	NA
WB_000090	10.06.2011	AP2	40	800	11.6	2.2	0.2	7.0	1.1	0.6
WB_000091	10.06.2011	AP2	90	800	0.2	5.0	0.2	9.6	1.3	2.6
WB_000093	10.06.2011	AP5	40	850	12.8	2.8	0.2	6.9	0.4	0.3
WB_000095	10.06.2011	AP8	5	550	10.7	12.8	1.0	6.4	0.0	0.2
WB_000098	10.06.2011	AP11	5	600	10.9	12.6	1.2	6.4	NA	NA
WB_000100	10.06.2011	AP13	5	775	12.1	10.6	1.1	6.1	0.0	0.3
WB_000101	10.06.2011	AP13	31	900	12.2	4.0	0.5	6.4	0.3	0.6
WB_000102	14.07.2011	AP1	5	625	10.0	16.9	1.2	6.3	NA	NA
WB_000103	14.07.2011	AP2	5	850	10.0	17.1	1.9	6.2	0.0	0.2
WB_000106	14.07.2011	AP5	5	600	10.4	17.7	1.9	5.6	0.0	0.2

(*Continued*)

Table 2. (*Continued*)

Code	Date	Station	Depth (m)	Volume (ml)	Oxygen (mg/L)	Temperature (°C)	ChLa (mg m^{-3})	Salinity	NO2/NO3 (µmol/L)	PO4 (µmol/L)
WB_000107	14.07.2011	AP5	40	725	12.5	3.3	0.2	6.9	1.0	0.8
WA_000108	14.07.2011	AP5	84	850	0.2	5.0	0.2	9.7	0.2	4.4
WB_000109	14.07.2011	AP8	5	500	10.5	18.7	5.1	6.0	0.0	0.3
WB_000110	14.07.2011	AP8	40	800	12.6	4.0	0.2	6.8	0.6	0.9
WB_000111	14.07.2011	AP8	74	900	2.7	3.9	0.2	8.8	5.3	3.5
WB_000112	14.07.2011	AP11	5	675	10.1	18.5	4.0	6.2	NA	NA

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Bioinformatics and statistics

Reads with low quality and those shorter than 150 bp (basepairs) were removed from the dataset. The PyroNoise algorithm was used to discard homopolymer-derived errors [47] and UCHIME to remove chimeric DNA sequences caused by PCR errors [48]. OTUs (Operational Taxonomic Units) were defined using the average neighbor-clustering algorithm of MOTHUR 1.19.1 [49] with a 97% similarity threshold. Reference sequences were selected from the SILVA ribosomal RNA database [50]. Taxonomic assignments were processed by the Ribosomal Database Project (RDP) naïve Bayesian Classifier [51]. For database affiliations RDP Seqmatch [52] and BLAST [53] search algorithms were used against RDP and a NCBI (National Center for Biotechnology Information) nucleotide databases, respectively. Chloroplast and mitochondrial sequences were discarded from the dataset. Statistical analyses were carried out with the R program version 2.14.0 (http://www.r-project.org), ACE (Abundance-based Coverage Estimation) [54] and Chao1 [55] richness estimates; multivariate statistics were calculated using the VEGAN package [56]. The similarity matrices and clustering were generated using the gplots package [57]. The sequences have been deposited into GenBank (accession numbers from KM489611 to KM491167).

Results

Environmental parameters structuring the bacterial community

The sampling took place from April to July in 2011 and the bacterial 16S rRNA gene libraries were generated from three sampling depths: (i) surface water at 5 m, (ii) the intermediate layer

Table 3. Primers used in this study.

Primer name	Sequence 5'- 3'	Citation
F8	TTGGCAGTCTCAGnnnnnnnnAGTTTGATCCTGGCTCAG*	[45]
R357	GTCTCCGACTCAGnnnnnnnnCTGCTGCCTYCCGTA*	[45]
Adapter A	CCATCTCATCCCTGCGTGTCTCCGACTCAG	**
Adapter B	CCTATCCCCTGTGTGCCTTGGCAGTCTCAG	**

*-nnnnnnnn is the barcode

**-standard adapter for 454 Titanium chemistry

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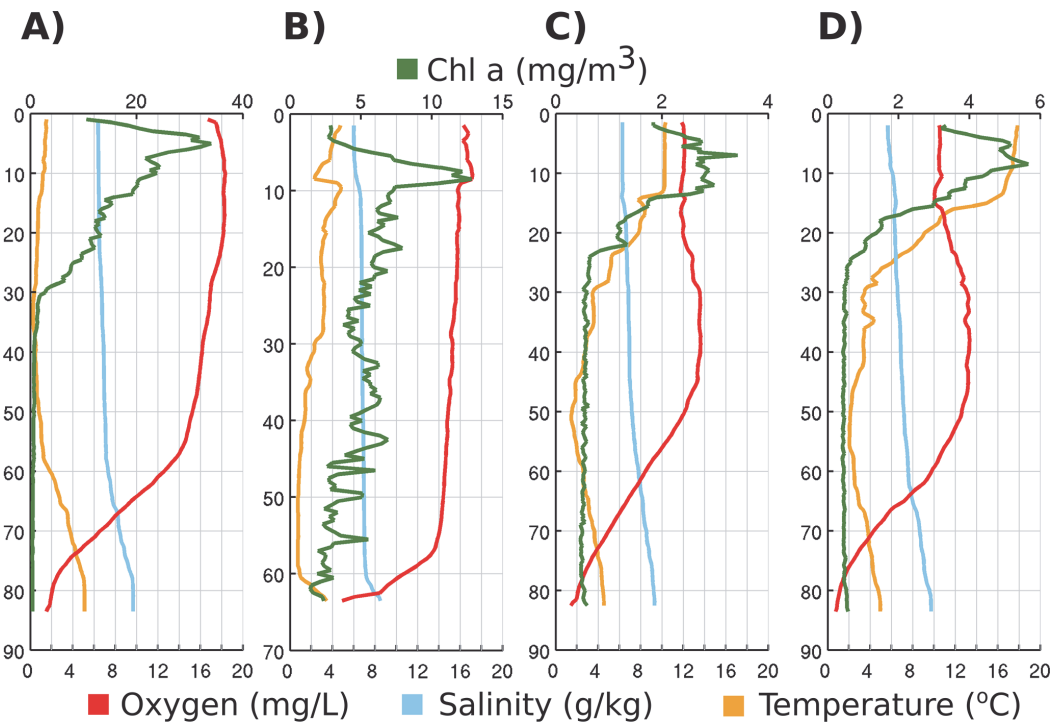

Fig 2. Water column profiles of station AP5 on 21th April (A), on 10th June (C), on 14th July (D) and station NS4 on 10th May (B).

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at 40 m and (iii) the near-bottom layer a few meters above the seafloor; depth varied between stations (63–95 m, Table 2). These depths were chosen because, in summer, the gulf is stratified into three layers. Four hydrographic profiles are visualized in Fig 2 to provide overview of changes of environmental parameters in the water column throughout the sampling period.

The 5 m and 40 m horizons always remained above the permanent halocline and at those depths the salinity varied in the range of 4.8–6.6 g kg^{-1} and 6.7–7.2 g kg^{-1}, respectively. Salinity decreased from west to east, with the lowest value recorded in the easternmost surface sample. The near-bottom layer samples were collected at the halocline or below it and the salinity ranged from 8.5–10.4 g kg^{-1}. The temperature varied little in the near-bottom layer (3.7–5.0°C in April and 0.1–4.0°C in July), but at the 5 m depth, warming was observed from 0.8–0.9°C in April to 17.1–18.7°C in July. The most remarkable change in temperature occurred between May and June, when a well-pronounced thermocline started to form at 10–25 m.

Oxygen conditions were hypoxic or anoxic in deeper sampling layer (> 63 m) throughout the sampling period. Oxygen concentrations at the 5 m depth were significantly ($p < 0.01$, Student's t-test) lower during the summer (10.0–12.3 mg l^{-1}) compared with the spring (13.9–16.0 mg l^{-1}). At 40 m depth, the overall tendency remained similar to the upper layer, but on two occasions in May, the oxygen concentrations were below 9.5 mg l^{-1}.

Chlorophyll *a* concentrations were highest in April (4.2–12.5 mg m^{-3}, Table 2), when phytoplankton was dominated by *Diatomphyceae* (data not shown). Also, concentrations of

inorganic nutrients at 5 m depth we higher during the spring months compared with June or July. Nitrites and nitrates remained at concentration levels from the detection limit to 1.2 μmol l^{-1} and phosphates varied between 0.6–0.8 μmol l^{-1}. In June-July, the concentrations of nitrites and nitrates remained below the detection limit and phytoplankton was dominated by diazotrophic *Nostocophyceae* (data not shown). The concentration of phosphates was also lower in the surface layer during the summer months (Table 2).

Bacterioplankton community diversity

A total of 73,494 partial rRNA gene sequences and 48 different samples were used in this analysis, on average 2,161 sequences per sample. The minimum number of sequences per sample was set to 350. In Table 2, the number of observed OTUs is accompanied with the Chao1 and ACE species richness estimates for each sample. Overall, the number of OTUs was significantly higher in the near-bottom layer (on average 144, SD = 34) than at 5 m and 40 m (98, SD = 44 and 90, SD = 43; respectively). Based on the rarefaction curves outlined from samples (S1 Fig), we assume that deeper sequencing (more sequences per sample) would have resulted in significantly higher estimates. Therefore, in this study, we could not study rare species and therefore concentrated on abundant members of the bacterioplankton community.

Bacterioplankton community composition

A total of 29 different bacterial classes were found. More than 85% of identified OTUs belonged to eight classes: *Alphaproteobacteria* (31.2%), *Actinobacteria* (17.8%), *Betaproteobacteria* (8.9%), *Cyanobacteria* (9.3%), *Epsilonproteobacteria* (5.8%), *Gammaproteobacteria* (2.5%), *Flavobacteria* (8.7%) and *Sphingobacteria* (1.6%). About 1/10 (11.6%) of the sequences remained unclassified at the bacterial class level. A total of 1,557 OTUs (97% cutoff) were obtained, out of which 839 were single-read OTUs. The most numerous OTUs with their database affiliations are listed in Table 4.

A descriptive multivariate statistical method, i.e. detrended correspondence analysis (DCA), was used to describe the overall OTU abundance and thereafter a vector-fitting procedure was applied to determine which environmental variables significantly related to BCC patterns (Fig 3, Table 5). As a result, oxygen, depth and salinity were distinguished as the most important co-varying environmental factors (r^2 and p values are presented in Table 5). Temperature, sampling time and Chl α could be identified as being less significant to the DCA space (in declining order). The geographic location (longitude and latitude) was rendered non-significant. The DCA also indicates how measured environmental parameters co-varied. To further investigate the dynamics of BCC, Pearson correlation analysis was carried out based on the relative abundance of OTUs, which was used to construct a similarity matrix (Fig 4). Organized according to clustering of the similarity matrix, all community profiles are visualized on the class level in Fig 5 for an overview and the OTU level (97% similarity) in Fig 6 to provide detailed insight into the relative abundance of dominant ribotypes. On a broader scale, the communities were divided into three groups: (i) surface communities during the summer months that were dominated by the unicellular cyanobacterium *Synechococcus* (OTU BSNS2840); (ii) hypoxic/anoxic near-bottom communities that contained a large fraction of chemolithotrophic *Sulfurimonas* (OTU BSNS3177); and (iii) a larger group with all remaining communities with notable subdivisions among them.

To examine how the BCC was related to environmental factors, each measured environmental parameter was correlated to the relative abundance of the dominant bacterial classes (Fig 7) of OTUs (Fig 8). These heatmaps illustrate how occurrence patterns of classes differ in relation to environmental conditions and that on OTU level there are subdivisions within these

Table 4. Database affiliations of the dominant OTUs (>300 sequences in the entire dataset).

OUT classification	OTU code	Isolation source (RDP)	Accession nr. Genbank (RDP)	%	Isolation source (BLAST)	Accession nr. Genbank (BLAST)	%
Bacteroidetes(100)	BSNS3145	deep-sea octacoral	DQ395498	90.9	coastal ocean	KC336890	99
Actinomycetales(100)	BSNS3157	lake epilimnion (Jimi Hendrix Bog Lake	EU117758	100	Gulf of Gdansk (Baltic Sea), 1 m	KF596574	100
Actinomycetales(100)	BSNS3107	lake epilimnion	EU117608	100	river water	GU641290	100
Flavobacteriaceae(100)	BSNS3079	marine biome, fjord, coastal water	FR686324	99.6	coastal ocean	KC336902	100
Rhodobacteraceae (100)	BSNS3149	culture collection: ATCC:17025	CP000661	97	marine bulk water	JX015552	100
Flavobacteriaceae(100)	BSNS3078	Lake Zurich, Spring Bloom 2009	HE574367	92.1	seawater, 2 m depth	FR648023	99
Bacteroidetes(100)	BSNS3163	coastal water	GU230419	94.8	Baltic Sea, 3m depth, Landsort deep St. BY31	EF627875	99
Polaribacter(100)	BSNS3072	Arctic sea water	EU330381	NC*	Arctic Sea water	FJ196065	99
Actinomycetales(100)	BSNS3110	lake epilimnion	EU117955	99.6	off-seep, ice-free (Alaska)	JN626833	99
Microbacteriaceae(100)	BSNS2531	lake water, West Lobe at 13 m	DQ015847	98.4	Arctic Ocean	JN976695	100
Flavobacteriaceae(100)	BSNS2870	oil-contaminated seawater	JQ712124	94.6	genomic DNA	AF388893	99
Comamonadaceae(100)	BSNS3044	mangrove	DQ234161	99.6	coastal ocean	KC336502	100
Pelagibacter(100)	BSNS3084	Chesapeake Bay, 8 m	EU800103	99.2	Gulf of Gdansk (Baltic Sea), 1 m	KF596598	99
Pelagibacter(100)	BSNS3076	deep-sea octacoral	DQ395535	100	300m depth water sample	JX530818	100
Pelagibacter(100)	BSNS3171	Chesapeake Bay, 25 m	EU801724	98.8	Qinghai Lake	HM127540	99
Bacteria(100)	BSNS3058	marine biome, fjord, coastal water	FR683807	96.2	surface water in the Northern Bering Sea	GQ452877	100
Ilumatobacter(100)	BSNS3154	Chesapeake Bay, 25 m	EU802230	100	Gulf of Gdansk (Baltic Sea), 1 m	KF596602	100
Flavobacteriales(99)	BSNS2920	Chesapeake Bay, 25 m	EU802220	97.6	genomic DNA	AF388893	99
Ilumatobacter(100)	BSNS2659	Delaware Bay, 8 m	EU800747	99.6	water of common carp culture pond	JQ305072	100
GpIIa(100)	BSNS2840	Synechococcus sp. isolate	AY151241	100	the Gulf of Finland (The Baltic Sea)	FR820441	100
Rhodothermaceae(100)	BSNS3172	deep-sea sediments	AB015587	95.5	Crambe crambe (sponge)	GU799618	98
Bacteria(100)	BSNS3174	marine sediment	FJ813551	93.6	municipal activated sludge wastewater treatment bioreactor	HQ509575	93
Rhodobacteraceae (100)	BSNS3179	ocean water from the Yellow Sea	HM057611	98.1	Crambe crambe (sponge)	GU799618	98
Alphaproteobacteria (100)	BSNS3143	strain of Reyranella massiliensis	EF394922	100	drinking water	KF515019	100
Gammaproteobacteria (100)	BSNS3168	Baltic Sea redoxcline, 119 m depth	JX974825	98.7	marine sample	JQ859404	99
Flavobacteriaceae(100)	BSNS3178	Baltic Sea redoxcline, 119 m depth	KC492867	100	Baltic Sea redoxcline, 119 m depth	KC492867	99
Methylobacter(100)	BSNS3164	cultured strain	AF152597	100	groundwater discharge zone sediment	KC922589	100
Ilumatobacter(100)	BSNS3169	hydrothermal vent waters	HM446118	99.6	Lubomirskia baicalensis (freshwater sponge)	JQ272709	100
Methylophilus(97)	BSNS3170	Kerguelen Plateau in the Southern Ocean, 120 m	EU005833	98.2	ocean surface water	JQ253996	99

(*Continued*)

Table 4. (*Continued*)

OUT classification	OTU code	Isolation source (RDP)	Accession nr. Genbank (RDP)	%	Isolation source (BLAST)	Accession nr. Genbank (BLAST)	%
Actinomycetales(100)	BSNS3032	deep-sea octacoral	DQ396268	99.6	Saanich Inlet, 10 m depth	GQ346797	99
Actinomycetales(100)	BSNS3133	bottom water in the northern Bering Sea	GQ850562	99.6	Saanich Inlet, 10 m depth	GQ346797	99
Actinomycetales(100)	BSNS3120	deep-sea octacoral	DQ396268	99.2	whole surface water from Chesapeake Bay	EF471727	100
Bacteroidetes(100)	BSNS3126	Baltic Sea redoxcline, 119 m depth	KC492874	100	Baltic Sea redoxcline, 119 m depth	KC492874	99
Sulfurimonas(100)	BSNS3177	Baltic Sea redoxcline, 119 m depth	KC492833	99.6	Baltic Sea redoxcline, 119 m depth	KC492833	100
Solirubrobacterales (100)	BSNS3175	soil sample above gas and oil field	GU056099	98.4	Baltic Sea brackish sediment, depth 0–1 cm	FN423884	99
Corynebacterineae (100)	BSNS3016	Lake Pavin (meromictic lake)	GU472705	96.9	the Gulf of Finland (The Baltic Sea) sediment	FR820412	100

Best matches found with the RDP Seqmatch tool and BLAST against the NCBI nucleotide database are listed and accompanied by the corresponding isolation source.

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groups. Interactions between microbes were as important as other environmental factors. Therefore, similar correlation analyses were carried out between dominant ribotypes (Fig 9). The clustering achieved in this way is slightly different and provides additional information. The efficiency of this approach is demonstrated by the fact that OTUs with related database matches (isolation source, etc.) clustered together. Therefore, relatively abundant ribotypes with the closest affiliations are given in the same order in Table 4.

Discussion

Connection between locally established bacterioplankton community and global redox-specific metacommunity

We used 16S rRNA gene-based community profiling to identify spatiotemporal patterns of bacterial picoplankton (including 0.22 to 5 μm fraction) in the Gulf of Finland. As discussed in detail below, the collected data demonstrate that the dynamics of the BCC in the Gulf of Finland exhibits striking parallels with other OMZ that are also characterized by salinity gradients and a temperate climate, like Chesapeake Bay and the Saanich Inlet [25,58]. This suggests that, in addition to the dynamics of different electron acceptors and donors, salinity also plays an important role.

In community ecology, there is an increasingly popular concept of metacommunities, defined as a set of local communities that are linked by the dispersal of multiple interacting species [59]. Considering the interconnectedness of aquatic ecosystems, we propose that bacterioplankton communities in the OMZ can be considered as a globally distributed redox-specialized metacommunity. In such a framework, OMZs occurring in freshwater and marine water mixing zones should form a salinity-dependent subsystem of this metacommunity. Due high selectivity by salinity- and redox-driven niche partitioning in these systems, species-sorting becomes the driving force behind community assembly in these systems.

The overall phylogenetic makeup of the bacterioplankton community observed on the bacterial class level resembled those routinely found in Baltic Sea pelagic waters using culture

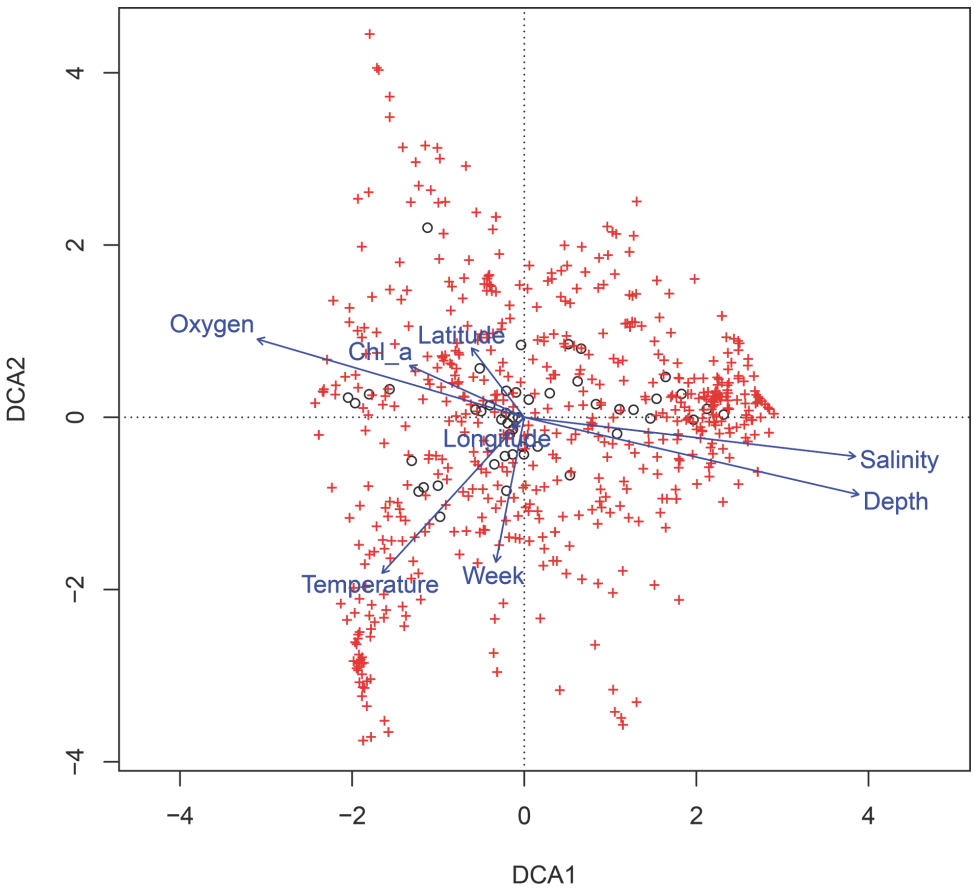

Fig 3. Detrended correspondence analysis of the bacterioplankton community composition on the operational taxonomic unit level (97% similarity). Axis 1 and axis 2 explain 11.1% and 9.5% of the variation, respectively.

doi:10.1371/journal.pone.0122304.g003

Table 5. Results of the detrended correspondence analysis of the bacterioplankton community composition.

	DCA1 (11.1%)	DCA2 (9.5%)	r^2	Pr(>r)
Oxygen	-0.95981	0.28064	0.4942	0.000999
Depth	0.97438	-0.22492	0.7527	0.000999
Temperature	-0.67420	-0.73855	0.2839	0.002997
Chlorophyll a	-0.91184	0.41055	0.1008	0.079920
Salinity	0.99312	-0.11714	0.7100	0.000999
Longitude	-0.75817	-0.65205	0.0015	0.964036
Latitude	-0.60613	0.79537	0.0483	0.316683
Week	-0.19239	-0.98132	0.1388	0.028971

P values based on 1000 permutations.

doi:10.1371/journal.pone.0122304.t005

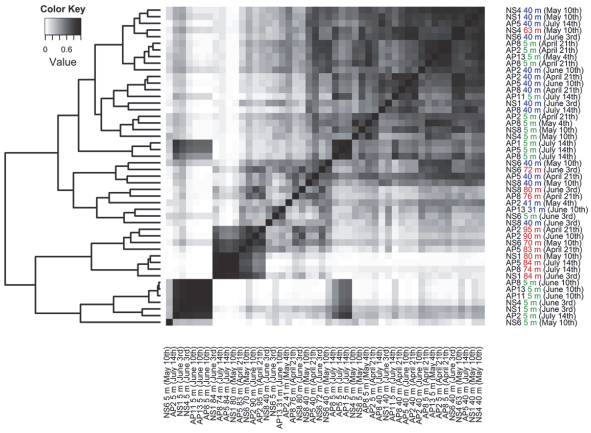

Fig 4. Pair-wise similarity matrix of the bacterial community based on relative abundances of OTUs. The dendrogram represents clustering based on pair-wise similarities in r-values (Pearson). Three different sampling depths are color-coded green (5 m), blue (30–40 m) and red (near-bottom).

doi:10.1371/journal.pone.0122304.g004

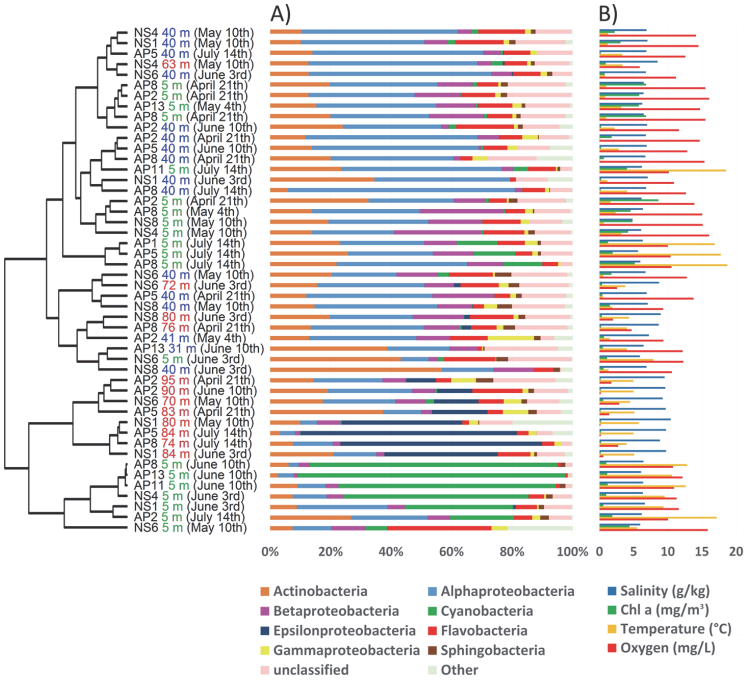

Fig 5. The relative abundance of the eight most abundant bacterial classes and a pooled group of unclassified ribotypes (A) that are accompanied with environmental parameters (B). The dendrogram is adopted from Fig 3.

doi:10.1371/journal.pone.0122304.g005

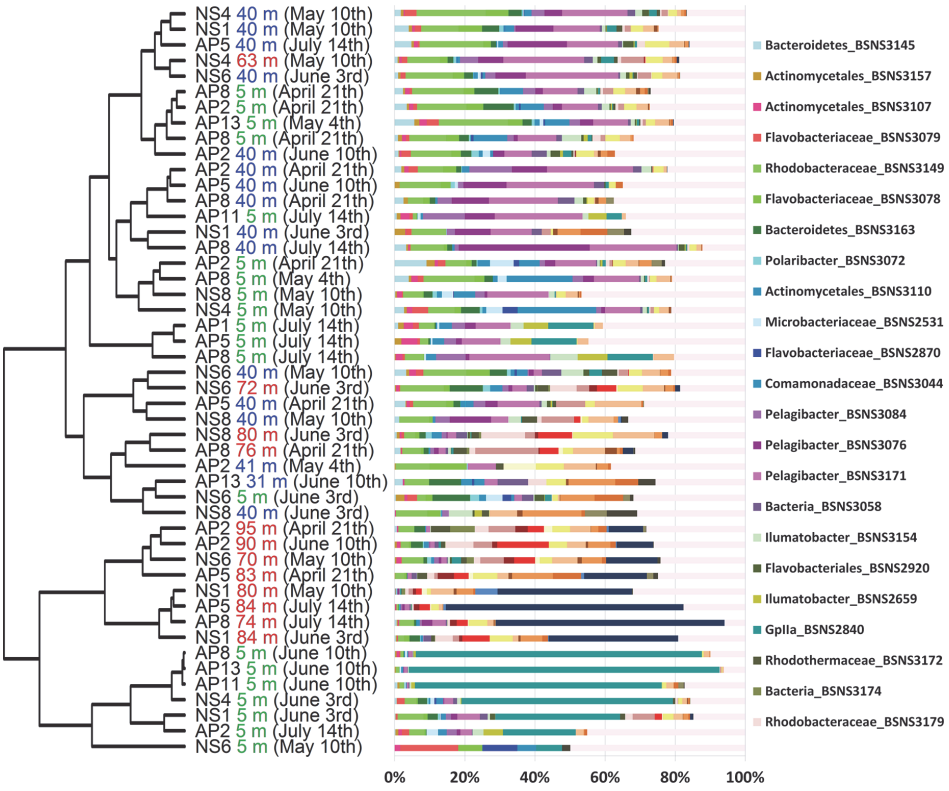

Fig 6. The relative abundance of dominant OTUs (>300 sequences in the entire dataset). The dendrogram is adopted from Fig 3.

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independent methods, although an underrepresented fraction of *Gammaproteobacteria* and *Verrucomicrobia* was noted [34,39,42,44,60].

Moreover, many relatively abundant OTUs are closely affiliated with sequences previously isolated from the Baltic Sea (Table 4), as expected due to the long residence time of the Baltic Sea and annually reoccurring patterns of bacterioplankton succession [44]. Two OTUs that managed in some cases to contribute to over 50% of the BCC (Fig 6) can be considered permanent and well-adapted local populations. One of these OTUs, BSNS2840, had an identical match to a sequence isolated from laminae of the Gulf of Finland sediments dating back to the Late Litorina Sea [61]. Hence, this population may have been present in the area for over 3000 years. It was classified as a member of the cyanobacterial clade GpIIa (*Synechococcus* by database affiliation) and it contributed up to 88.7% of the BCC in the surface layer in June, making it the most abundant picocyanobacterium of the dataset (Fig 6). OTU BSNS2840 was also numerous in the winter picoplankton community in the same sampling area [42].

The prevalence of *Cyanobacteria* during summer is typical of the seasonal succession of phytoplankton in the area and is caused by multiple environmental conditions, most notably the increase in temperature and the depletion of nitrates in the surface layer after the spring bloom (Table 2), which gives a distinct advantage to diazotrophic cyanobacteria [62]. The

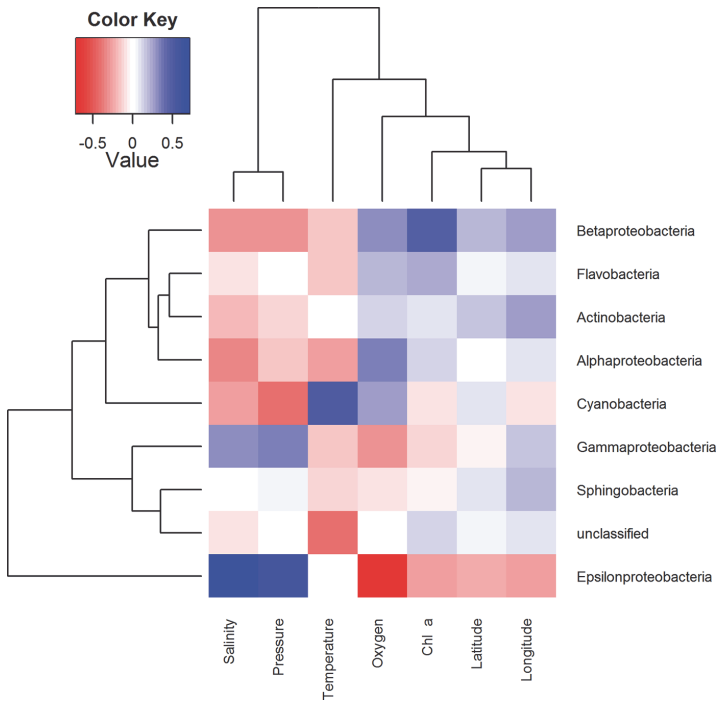

Fig 7. Pearson correlations between environmental parameters (columns) and classes of bacteria (rows). Colors indicate r-values. The dendrograms represent complete linkage clustering of the samples based on the similarities in r-values.

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variability of the fraction contributed by *Cyanobacteria* within samples that were collected during same cruise indicates patchy nature of the bloom. In addition, it is important to point out that *Synechococcus* has been shown to occupy the anoxic and dark zone in the central Baltic Sea [38,63] and Chesapeake Bay [25]. The underlying mechanisms behind this phenomenon remain unexplored to our knowledge. The most abundant OTU in the hypoxic/suboxic layer, BSNS3177, was classified as *Sulfurimonas* (genus of *Epsilonproteobacteria*). One characteristic feature of chemolithoautotrophic bacteria in the Baltic Sea is that the majority of cells belong to the Sulfurimonas GD17 group [64], which oxidizes H_2S with NO^{3-}. The prevalence of single domant strain of epsilonproteobacterium is also reflected in our results. Ribotype BSNS3177 contributed to a large fraction of the BCC in the hypoxic/suboxic near-bottom layer at the westernmost stations and at AP2 and AP5 in mid-summer (Fig 6). This suggests dispersion from the Baltic Proper to the Gulf of Finland, as proposed by our previous study [42]. Moreover, the *Sulfurimonas* strain co-occurred with OTU BSNS3126 (Fig 9), classified as *Bacteroidetes*, and both were affiliated with sequences isolated earlier from the anoxic zone of central Baltic Sea (Table 4) [65]. Linkage to the same isolation source was also demonstrated by the co-occurring pair of ribotypes BSNS3168 and BSNS3178, classified as representatives of *Gammaproteobacteria* (affiliated with the SUP05 clade) and *Flavobacteriaceae*, respectively. These correlations probably represent cooperation activities.

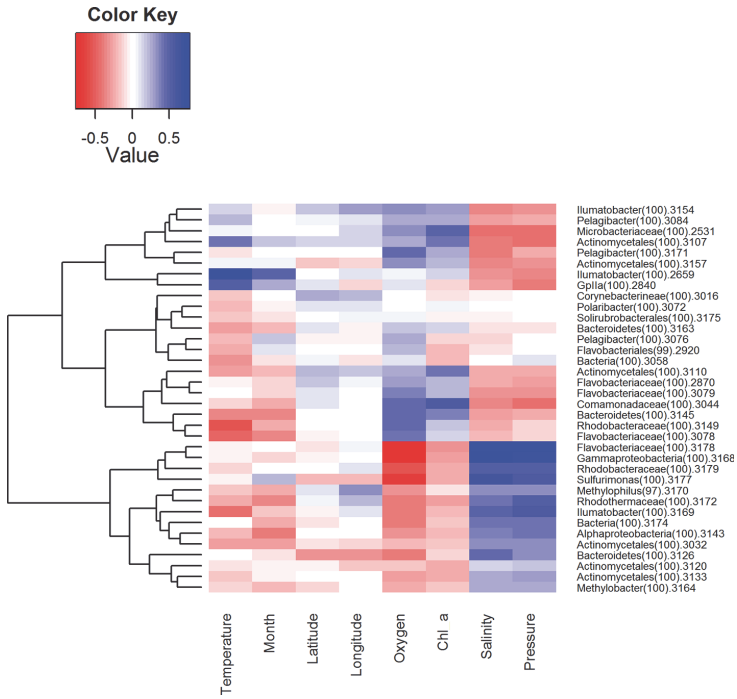

Fig 8. Correlations between environmental parameters and OTUs (>300 sequences in whole dataset). Colors indicate the r-values of Pearson correlations. The dendrograms represent complete linkage clustering of the samples based on the similarities in r-values.

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The *Epsilon-* and *Gammaproteobacteria* have been identified as major chemolithoautotrophic groups in the central Baltic Sea [40,41,64–67] and also globally in other marine OMZs [31,68]. The GD17 group is capable of a chemotactic response to nitrate [69]. The chemotactic swimming strategies of marine bacteria are especially significant in patchy nutrient seascapes [70], and in OMZs, the corresponding genes have been shown to be over-represented [39,71].

Heterotrophic *Alphaproteobacteria* dominated in the upper oxygenated layer of water. In addition, *Candidatus* Pelagibacter (ribotypes BSNS3076, BSNS3084 and BSNS3171 accounted for 15.9%) was the most abundant genus in the whole dataset. *Candidatus* Pelagibacter belongs to the SAR11 clade, which is the most abundant type of organism in the world's oceans [12,72,73] and has been found to be numerous in the Baltic Sea as well [35,42]. Another prevalent alphaproteobacterial OTU in oxygenated waters, BSNS3149, was classified as *Rhodobacteraceae* (order *Rhodobacterales*). It had an identical match with a sequence isolated from a coastal North Sea diatom bloom [74]. Both groups, *Rhodobacterales* and SAR11, contribute to the conversion of dimethylsulfoniopropionate to dimethylsulfide [75]. Interestingly, both groups have been found to be numerous in OMZs in both oxic and anoxic layers, but their metabolic adaptations for anaerobic growth remain to be uncharacterized [31].

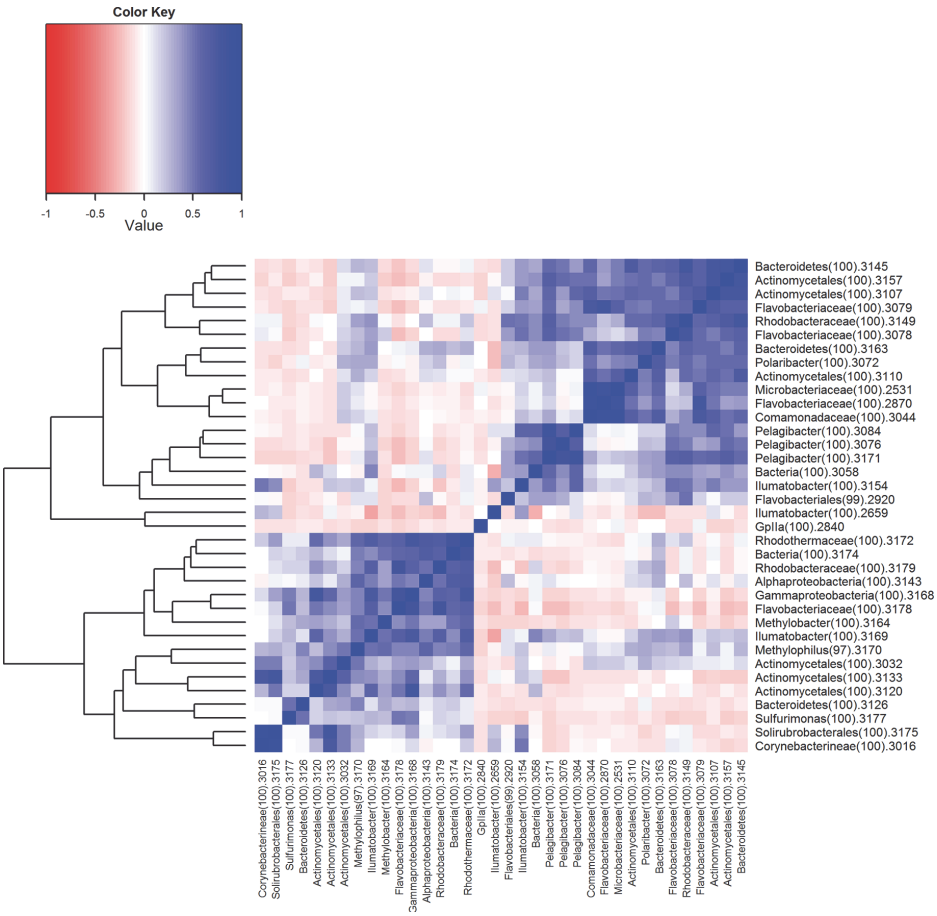

Fig 9. Co-localization analysis between the most abundant ribotypes, which are accompanied by classifications. Colors indicate the r-values of Pearson correlations. The dendrogram represents complete linkage clustering of the samples based on the similarities in r-values.

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Importance of methane and trace metal gradients

Methane is mainly generated in the sediment from which it transfers into the water column. A methane gradient with decreasing concentrations toward the water surface is usually observed in the Baltic Sea [76]. Microbial oxidation of methane in the water column represents an important sink of methane. Different electron acceptors can be used for methane oxidation (sulfate, nitrate, nitrite, iron and manganese), but using oxygen produces a considerably larger energy yield [77].

As a consequence, the hotspot for microbial methane oxidation activity in central Baltic Sea is at the upper part of the redoxcline [78]. Interestingly, the corresponding group consists of only type I methanotrophic bacteria [79,80]. Our results suggest that the same group is also represented in the Gulf of Finland with gammaproteobacterial OTU BSNS3164

(*Methylobacter*) as the most abundant representative. It is important to consider that our samples were collected above and under the redoxcline. In addition, OTU BSNS3143 corresponded via databases to *Methylosinus trichosporium*, a type II methane-oxidizing alphaproteobacterium previously found in surface sediments of the Gotland Deep [81]. Considerably more relevant was betaproteobacterial OTU BSNS3170, classified as *Methylophilus*, an obligate methylotroph common to the Baltic Sea [38,42,82]. All these three groups were more abundant at the 40 m depth (Fig 6).

Redox-sensitive trace metals (e.g. iron and manganese) provide another energy source for microbes and can serve as electron donors above the redoxcline. When oxygenated, insoluble compounds form aggregates that will sink below the redoxcline and can be used as electron acceptors [83–85]. Our results suggest that members of *Actinomycetales* (order of *Actinobacteria*) may inhabit this niche, because several relatively abundant OTUs (BSNS2659, BSNS3154 and BSNS3169) were classified as *Ilumatobacter*. All known and described members of *Acidimicrobiaceae* are capable of iron oxidation [86]. The found OTUs exhibited different patterns of distribution indicating separate strategies. For example, OTU BSNS2659 was more abundant at 5 m in July. However, two other *Ilumatobacteria* and three unclassified *Actinobacteria* (OTUs BSNS3032, BSNS3120, BSNS3133) occurred mainly at 40 m and in the near-bottom layers, supporting the iron-oxidation hypothesis. Interestingly, almost all of these OTUs (except OTU BSNS3169) had nearly identical matches to brackish and temporarily anoxic estuaries (Table 4).

Spring bloom associated community influenced by freshwater clades

The Gulf of Finland has a relatively large input of freshwater compared to the Baltic Proper and southern basins. This impacts the BCC through the salinity distribution of the gulf and the influx of populations originating from freshwater sources [33–35,44]. Consequently, some relatively abundant OTUs were highly similar to sequences obtained from rivers and lakes (Table 4). Overall, the prevalence of phylogenetic groups considered to be characteristic of freshwater ecosystems, like *Actinobacteria* and *Betaproteobacteria*, exhibited a positive correlation with longitude (Fig 7), i.e. increasing in abundance towards less saline parts of the gulf. This concurs with previous reports of BCC dynamics along the salinity gradient of the Baltic Sea [35].

Annually recurring phytoplankton spring blooms in the Gulf of Finland are co-dominated by diatoms and dinoflagellates and exhibit high spatiotemporal variability [87]. The alternating dominance of bottom-up and top-down interactions result in a succession of heterotrophic bacterial groups with different growth strategies throughout the phytoplankton bloom [74,88]. Although our sampling period covered only part of the spring bloom, groups representing different strategies could be distinguished.

Bacterial lineages considered fast growing "opportunistic" types that utilize dissolved organic matter (DOM) like *Betaproteobacteria* and *Flavobacteria* [88–90] were accompanied by more slow-growing and grazing-resistant members of the AcI lineage of *Actinobacteria* [88,91,92]. Most of the OTUs connected to the spring bloom were clustered together by the correlation analysis (upper 12 OTUs in Fig 7), including the discussed unclassified *Rhodobacteraceae* that were affiliated with the diatom bloom.

Members of *Flavobacteria* are often overrepresented in the spring BCC of the Baltic Sea and are major contributors to the degradation of high molecular weight carbon [39,82,93]. OTUs BSNS2870, BSNS3078 (both classified as *Flavobacteriaceae*) and BSNS3163 (unclassified *Bacteroidetes*) were closely related to sequences previously isolated from the Baltic Sea (Table 4). We identified several OTUs classified as *Polaribacter* (member of *Flavobacteria*), with OTU

BSNS3072 as the only relatively abundant representative. This group contributes to the degradation of phytoplankton-derived organic matter via high expression of sulfatases [74].

Overall, *Betaproteobacteria* had the strongest correlation with Chl *a* concentrations, especially unclassified *Comamonadaceae* (BSNS3044) which stood out on the OTU level (Figs 6 and 7, respectively). Members of *Comamonadaceae* have been previously identified in spring bacterioplankton communities on several occasions [39,82]. Moreover, mesocosm experiments have shown a negative impact of higher temperature on this psychrotolerant lineage (Lindh *et al*., 2012).

We identified three relatively abundant members of *Actinobacteria* (OTUs BSNS3107, BSNS3110 and BSNS3157) that were closely related to the freshwater AcI lineage characterized by small cell sizes. The presence of small bacteria can be considered a clear indication of grazing pressure by bacterivorous nanoflagellates. Metagenomic profiling of pelagic and benthic bacteria during the spring (Landsort Deep) reveled that genes for the degradation of polyaromatic hydrocarbons (like cellulose and chitin) belong mainly to *Actinobacteria* and more specifically to *Mycobacterium* [39]. These genes were mainly found in the sediments, and because they are affiliated with aerobic groups, the authors considered sedimentation most likely. In our dataset, OTU BSNS3016, classified as *Corynebacterineae* and closely affiliated with *Mycobacterium*, comprised 8.7% of the total sequences isolated at a depth of 40 m in the beginning of June; therefore, our results support the sedimentation hypothesis.

Conclusions

The availability of electron acceptors is a critical determinant of the marine ecosystem structure, and we conclude that oxygen concentration is a major environmental factor impacting the BCC in the near-bottom layer of the Gulf of Finland. We conclude that chemolithotrophic groups dispersing from the central Baltic Sea become dominant members of the BCC in the suboxic/anoxic layer when it is formed. Some members of *Actinobacteria* inhabit the layer above the redoxcline and most probably contributing to the oxidation of ferrous iron. Our results also led to the conclusion that the Gulf of Finland has likely a more diverse composition of methanotrophic bacteria than the central Baltic Sea. The BCC at the surface layer was strongly impacted by phytoplankton seasonal succession, as many abundant OTUs in April and May could be associated with the spring phytoplankton bloom. In addition, in the beginning of summer, inorganic nutrient depletion and rising temperatures led to the proliferation of picocyanobacteria. We determined one dominant lineage of *Synechococcus*, which due affiliation contributes to the conclusion that some well-established phylogenetic lineages have persisted in the area for over 3000 years. Furthermore, our results support the emerging pattern of related microbes occupying the OMZs throughout the world and suggests that core of the bacterioplankton community of the Gulf of Finland is part of that a redox-specialized bacterial network, which by definition can be considered a metacommunity.

Supporting Information

S1 Fig. Rarefaction curves outlined from each sample representing the relation between the number of sequences and the number of operational taxonomic units (OTUs) identified with 97% similarity threshold. Different depths are marked with letters: 5 m (a), 40 m (b) and near-bottom layer (c).
(PNG)

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Author Contributions

Conceived and designed the experiments: PL IL MM. Performed the experiments: PL IL. Analyzed the data: PL JS IL VK MM. Contributed reagents/materials/analysis tools: IL UL MM. Wrote the paper: PL IL UL VK MM.

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Paper III

Laas, P, Šatova, E., Lips, I., Lips, U., Simm, J., Kisand, V., Metsis, M. (2015). Near-bottom hypoxia impacts dynamics of bacterioplankton assemblage throughout water column of the Gulf of Finland (Baltic Sea). *PLoS ONE,* 11(5): e0156147.

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RESEARCH ARTICLE

Near-Bottom Hypoxia Impacts Dynamics of Bacterioplankton Assemblage throughout Water Column of the Gulf of Finland (Baltic Sea)

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Abstract

Over the past century the spread of hypoxia in the Baltic Sea has been drastic, reaching its 'arm' into the easternmost sub-basin, the Gulf of Finland. The hydrographic and climatological properties of the gulf offer a broad suite of discrete niches for microbial communities. The current study explores spatiotemporal dynamics of bacterioplankton community in the Gulf of Finland using massively parallel sequencing of 16S rRNA fragments obtained by amplifying community DNA from spring to autumn period. The presence of redoxcline and drastic seasonal changes make spatiotemporal dynamics of bacterioplankton community composition (BCC) and abundances in such estuary remarkably complex. To the best of our knowledge, this is the first study that analyses spatiotemporal dynamics of BCC in relation to phytoplankton bloom throughout the water column (and redoxcline), not only at the surface layer. We conclude that capability to survive (or benefit from) shifts between oxic and hypoxic conditions is vital adaptation for bacteria to thrive in such environments. Our results contribute to the understanding of emerging patterns in BCCs that occupy hydrographically similar estuaries dispersed all over the world, and we suggest the presence of a global redox- and salinity-driven metacommunity. These results have important implications for understanding long-term ecological and biogeochemical impacts of hypoxia expansion in the Baltic Sea (and similar ecosystems), as well as global biogeography of bacteria specialized inhabiting similar ecosystems.

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Introduction

Two of the most significant issues facing coastal zone management are habitat loss and alteration due to anthropogenic pollution fuelled by eutrophication, which in the case of the Baltic Sea is amended with vicious positive feedback loop caused by oxygen depletion leading to increased phosphate release from sediments [1]. Oxygen minimum zones have been expanding on a global scale during the geologically recent deglaciation [2]. There has been about 10-fold increase of hypoxia in the Baltic Sea during past 115 years [3]. Depletion of oxygen alters ecosystem energy flow from macrobenthic organisms to microbes [4]. It is estimated that due to hypoxia about 30% of total secondary production is lost in the area [5], and these losses are expected to increase with the expansion of the oxygen-depleted area.

Estuaries represent the interface between marine and freshwater habitats hosting unique and mixed communities of microorganisms. Spatiotemporal studies of variability of bacterioplankton community composition (BCC) in coastal margins have shown that the spatial variability can extend over seasonal changes, given a sharp gradient of environmental factor (e.g. salinity, oxygen) [6, 7]. A recent study of spatiotemporal dynamics of artificially oxygenated fjord (Byfjord, Sweden) that has comparable trophic conditions with the Baltic Sea demonstrated that the amount of oxygen available shaped the bacterial communities, regardless of the depths or the season they were collected in [7].

The presence of redox zone, and thereby different chemical gradients of electron donors and acceptors in variable limiting conditions results in redox-driven niche partitioning. Such geographically distant oxygen minimum zones can be inhabited by related bacterial lineages [8, 9]. A previous spatiotemporal investigation in the Gulf of Finland showed that the main factor shaping the BCC is not only the redox-driven niche partitioning, but 'key species' are indeed closely affiliated with bacterial sequences isolated from other similar oxygen depleted ecosystems.

Vertical and/or horizontal surveys of the Baltic Sea have demonstrated that various environmental gradients have a profound impact on BCC [10–19] and cause zonation of functional capabilities [20, 21]. However in temperate climates dramatic seasonal shifts and annually repeating patterns in the BCC should not be overlooked [22–27]. Though, datasets restricted to single dimensions, such as time series from single station survey, can be hard to analyse in order to distinguish between arbitrary shifts in water masses and actual biological succession in bacterioplankton communities driven by changes in environmental condition [24]. The present investigation was undertaken to breach this limitation.

Here we present a study that compares BCC in multiple dimensions: two spatial scales (vertical and horizontal variation) and time. For this purpose massively parallel sequencing of amplified 16S rRNA gene fragment libraries was performed. Samples were collected from two transects in the Gulf of Finland from spring to autumn in 2012. The goal of the present investigation is to characterize spatiotemporal patterns of microbial community composition in hydrographically complex Gulf of Finland that is also the sub-basin to where the oxygen depleted zone is expanding from the Baltic Proper.

Materials and Methods

Ethics statement

No specific permits were required for the described field studies. The location is not privately-owned or protected in any way. The field studies did not involve endangered or protected species.

Study area and sample collection

Water sampling aboard the R/V Salme was performed on two transects in the central part of the Gulf of Finland from April to October of 2012 (Fig 1). The cross-gulf AP-transect was sampled to characterize south-north salinity gradient maintained by a cyclonic circulation pattern in the gulf. This residual circulation consists of an outflow of fresher waters (originating from the main river discharge at the eastern end of the gulf) in the northern part and an inflow of saltier waters from the Northern Baltic Proper along the southern coast [28]. Vertical stratification of the water column and water exchange with the Northern Baltic Proper are characterized by high variability both in long-term [29] and in monthly scales [30]. The measurements along the Keri-transect were conducted to sample the deepest site in the central gulf where anoxic bottoms could occur with relatively high probability. Samples were collected from three to five depths, depending on the transect (S1 Table). A rosette sampler (M1018, General Oceanics, Inc.) equipped with Niskin water samplers (volume 1.7 l) was used for sampling. For background information the vertical profiles of temperature and dissolved oxygen were obtained with an OS320plus CTD probe (conductivity, temperature, depth probe with oxygen sensor, Idronaut s.r.l) while chlorophyll *a* fluorescence was registered with a Seapoint chlorophyll *a* fluorometer.

Sample Processing

Water samples for microbial community DNA extraction were collected into sterile bottles (Nalgene, Thermo Scientific Inc.) and immediately filtered on 0.2 μm filters (Puradisc FP 30; Whatman, Inc.) after preliminary filtration through 5.0 μm prefilters (Puradisc FP 30; Whatman, Inc.). The design of filtration system is presented in [17]. The sample volume varied between 0.5 and 1.0 liters. Filters were kept frozen at—20°C until community DNA was extracted with a PowerSoil® DNA Isolation Kit (MO BIO Laboratories, Inc.). Few modifications were made to the protocol: syringe filters were incubated with the lysis buffer in the casing at 60°C for 30 minutes and then eluate was removed. Water samples for flow cytometry were fixed with paraformaldehyde (PFA, 1% final concentration). Cell counts were obtained with Accuri C6 flow cytometer (Becton Dickinson) after being stained with SYBR Green I DNA dye for 15 minutes at room temperature [31].

Amplification of bacterial 16S rRNA gene sequences

The bacterial 16S rRNA gene V1-V2 hypervariable regions were amplified in polymerase chain reactions (PCR) using universal bacterial primers BSF8 and BSR357 complemented with 8 nt index and Illumina adapter sequences [32, 33]. PCR was performed with Smart-Taq Hot Red 2X PCR Mix (Naxo, Estonia), 1 μl of extracted DNA, 0.2 μM of each primer, using the following cycling parameters: 15 minutes denaturation at 95°C followed by 3 cycles (30 sec at 95°C, 30 sec at 50°C, 60 sec at 72°C), 28 cycles (30 sec at 95°C, 30 sec at 65°C, 60 sec at 72°C) and a final extension at 72°C for 7 minutes. PCR reactions were run in a Thermal cycler 2720 (Applied Biosystems). Oligonucleotides were removed from pooled PCR product library using the QIAquick® PCR Purification Kit (Qiagen, Inc.). Single end sequencing of V2 hypervariable region was performed on Illumina MiSeq next generation sequencing platform using the v3 kit (a service provided by The Estonian Genome Center Core Facility).

Bioinformatics

Reads with low quality ($Q<30$) and shorter than 225 bp (basepairs) were removed from the dataset. Operational taxonomic unit (OTU) was defined using the average neighbor-clustering algorithm of MOTHUR 1.34.4 [34] with 97% similarity threshold. Reference sequences were

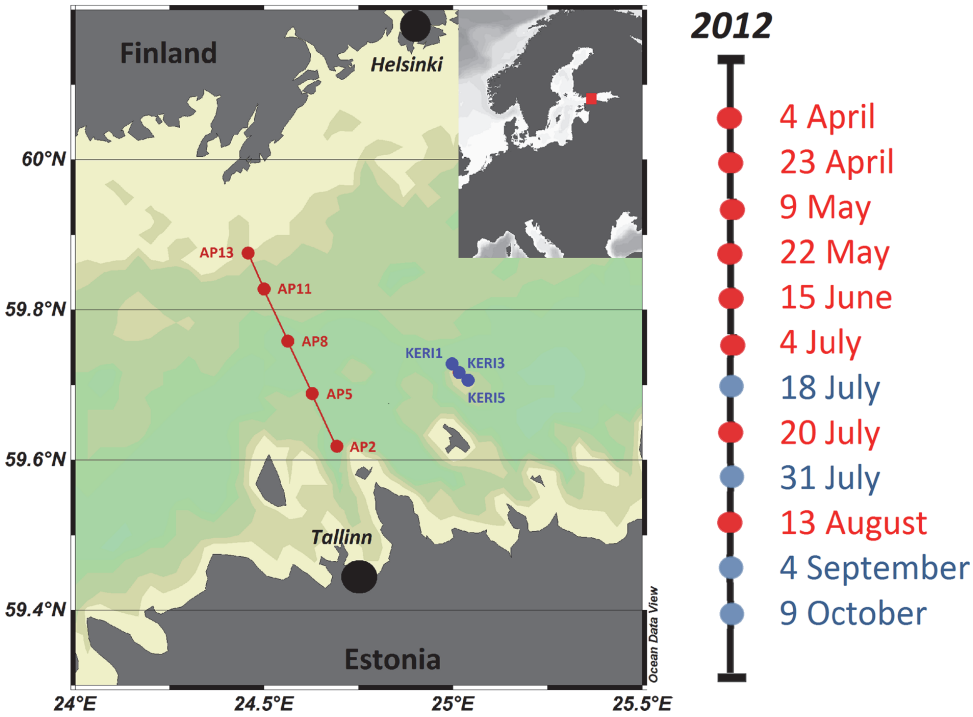

Fig 1. Map of central Gulf of Finland supplemented with AP- and KERI-transects, stations are color-coded red and blue, respectively. The timeline of the 2012 sampling cruises is provided with corresponding color-codes. Four samples were also collected on 8th November of 2011. The tan to bright green colours give an indication of the topography of the study area.

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selected from the SILVA ribosomal RNA database release 102 [35]. The UCHIME algorithm was used to remove chimeric DNA sequences caused by PCR errors [36]. Taxonomic assignments were processed by the Ribosomal Database Project (RDP) naïve Bayesian Classifier [37] and BLAST [38] searched against a NCBI (National Center for Biotechnology Information) nucleotide database (release 206, http://www.ncbi.nlm.nih.gov/). Sequences matching eukaryotic DNA were discarded from the dataset. Statistical analyses were carried out with program R version 3.1.2 (http://www.r-project.org), ACE (Abundance-based Coverage Estimation) [39] and Chao1 [40] richness estimates were calculated using VEGAN package [41]. The same package was used to carry out the non-metric multidimensional scaling (NMDS) analyses. Week number was used as an independent variable for time. For data visualizations ggplot2 package was used [42]. All sequences are uploaded to the GenBank and are available under accession numbers KT855220–KT860044.

Results

Physicochemical factors

The study covered the central region of the Gulf of Finland and was carried out on two transects (Fig 1). These transects provided both vertical and horizontal spatial coverage of the area.

Dramatic shifts in environmental parameters took place over the sampling period (Fig 2). The halocline at 60–80 m depth is quasi-permanent in the gulf because of the freshwater inflow from rivers in the surface layer and water exchange with Baltic Proper in the near-bottom layer. Samples collected from 5 m horizon had an average salinity of 5.58 g kg^{-1}(n = 63, SD = 0.25) and on the other hand, > 60 m layer had an average salinity of 8.70 g kg^{-1} (n = 70, SD = 0.68). The dissolved O_2 concentration had negative co-variation with salinity and corresponding average values for O_2 were 11.3 (SD = 2.2) and 1.8 mg L^{-1} (SD = 1.4, MIN = 0.0). The temperature remained quite stable in the near-bottom layer, with on average 4.6°C (SD = 0.45), but the surface layer temperatures varied in a large extent ranging from just under 1°C in the beginning of April and reaching up to 18°C in July. The thermocline became more pronounced in June. Three phytoplankton blooms typically occur in the Baltic Sea: spring, summer and autumn blooms; in decreasing order by biomass [43]. Consequently, the highest chlorophyll *a* concentrations in the surface layer were recorded during spring (almost up to 15 mg m^{-3}), followed by summer and autumn values.

Picoplankton cell counts

Highest cell count numbers were recorded at the surface layer (< 25m, n = 34; S1 Fig) reaching up to 3.6 x 10^6 cells ml^{-1} (mean = 1.9 x 10^6, SD = 0.7 x 10^6), and as expected the cell abundance had significant correlation with temperature (r = 0.60, $p < 0.001$; Pearson). At the intermediate layer (25–65 m, n = 68) usually lowest cell counts were observed (mean = 3.93 x 10^5 cells ml^{-1}, SD = 2.6 x 10^5, n = 37) and at the hypoxic/suboxic near-bottom layer (>65 m, n = 35) the picoplankton abundance increased compared to overlaying water, with on average 0.79 x 10^6 cells ml^{-1} (SD = 4.12 x 10^5). The double-stranded DNA staining technique used does not provide discrimination between *Bacteria* and *Archaea*, therefore, obtained results reflect overall prokaryotic picoplankton abundances.

Observed and estimated bacterioplankton diversity

After quality-filtering and removal of chimeric and eukaryotic sequences, a total of 955,023 16S rRNA gene hypervariable region V2 reads were used in this analyses. These sequences were divided between 181 different samples, with on average 5,310 (SD = 3,070, MIN = 1,840, MAX = 23,720) sequences per sample. A total of 4,692 OTUs (97% similarity cut-off) were obtained, out of which 2,840 were singletons (single-read OTUs). Chao1 and ACE species richness estimates for each sample are listed in S2 Table. There were only small differences between the estimates. Chao1 species richness estimates were recorded highest at the near-bottom layer, with on average 394 OTUs (SD = 168, n = 61). At the intermediate layer the estimates were slightly lower, with on average 344 OTUs (SD = 125, n = 62); and at the surface layer the average estimated diversity was lowest (mean = 299, n = 58), however the variability was highest (SD = 329). The Chao1 species richness estimates were significantly higher at the near-bottom layer than at the surface and intermediate layers (t-test, $p<0.1$ in both cases). There was no statistical difference between the two upper layers (t-test, p = 0.33).

Dynamics of BCC

Eleven bacterial classes accounted for 86.4% of overall BCC and spatiotemporal patterns of these classes help to decrypt the dynamics of the community structure on a broader scale (Fig 3). *Alphaproteobacteria* contributed the largest fraction of the dataset, mainly occupying the upper oxygenated water (on average 51.1% of BCC above 65 m; Fig 3). Their dominance at the surface layer was only challenged by *Cyanobacteria* during summer bloom (reaching 53.7% of BCC; Fig 3). On the other hand, there were also bacterial classes that occurred mainly in

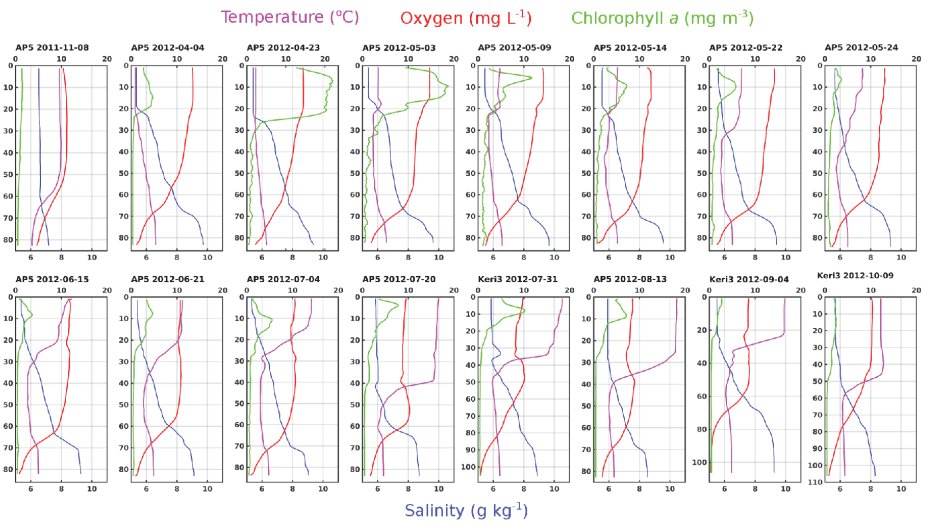

Fig 2. Water column profiles of stations AP5 and Keri3. Temperature (°C, magenta), oxygen (mg/L, red) and chlorophyll *a* (mg/m^3, green) are on upper x-axis. Salinity (g/kg, blue) is on bottom x-axis. The sampling date is marked as following: YYYY-MM-DD.

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hypoxic/suboxic near bottom layer, constituting of mostly *Epsilonproteobacteria*, but also of *Delta-*, *Gammaproteobacteria*, and one class level group that could be classified only on phylum level (unclassified *Bacteriodetes*). Below 65 m these groups contributed on average 31.7%, 19.4%, 6.9% and 0.6% of BCC; respectively. The relative abundance of *Gammaproteobacteria* peaked in April (21.7% of BCC; Fig 3).

Actinobacteria, *Betaproteobacteria*, and *Flavobacteria* were found throughout the water column (Fig 3). These bacterial classes contributed on average 12.9%, 6.6% and 5.8 of BCC; respectively. However, their occurrence revealed distinct spatiotemporal dynamics. *Betaproteobacteria* and *Flavobacteria* contributed to a larger fraction in spring, increasing in relative abundance in the intermediate as well hypoxic near-bottom layer (Fig 3). The fraction of *Actinobacteria* became more predominant in late summer and peaked in autumn (up to 36.4% of BCC), although its relative abundance also peaked at the end of May at intermediate and near-bottom layers (Fig 3). TM7 genera *incertae sedis* appeared only in autumn samples, with a maximum relative abundance of 3.7% (Fig 3).

At the species level, the bacterioplankton community structure was much more complex, despite the fact that sequence distribution between OTUs was uneven. Sorted by their relative abundance, the top 17 OTUs accounted for more than 1% of the total dataset each, adding up to 76% of the total dataset and are henceforth referred to as 'relatively abundant OTUs' (Table 1, Fig 4). The following 56 OTUs in relative abundance were in the range of 0.1–1% (totalling up 16%; S2 Fig) and can be considered as 'common' [24]. Last 4619 OTUs contributed less than 0.1% (i.e. 'relatively rare'). Moreover, when abundance cut-off for relatively rare taxa was set to 0.01%, then still 4440 OTUs met the criteria, leaving 179 OTUs in the range 0.01–0.1%. However, considering occurrence in relation to the whole dataset discriminates against opportunistic 'pulse' populations that can be relatively abundant in limited time-space and therefore ecologically relevant. There were 101 OTUs that contributed a minimum of 1%

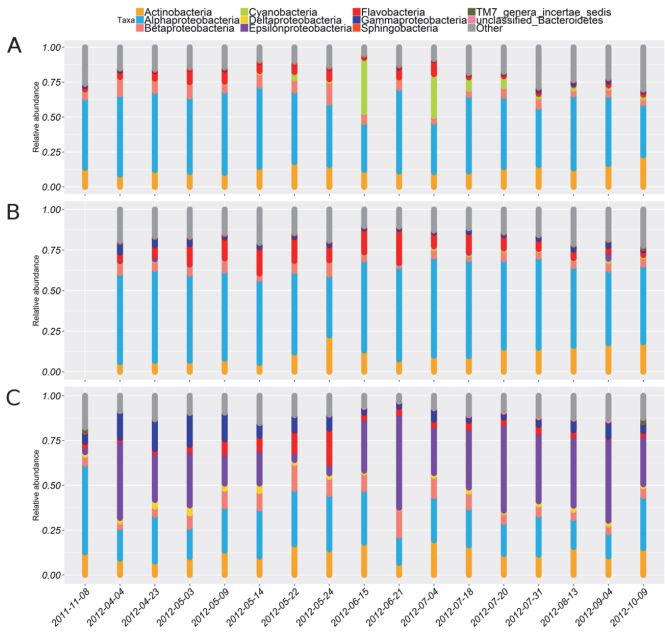

Fig 3. The relative abundance of dominant bacterial classes given in three depth ranges: 5–25 m (A), 25–65 m (B) and >65 m (C).

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Table 1. Relatively abundant OTUs, their taxonomic affiliation according to the RDP classifier, their closest neighbours in the GenBank database with isolation source.

OTU	Classification (classification confidence—%)	Genbank accession	Similarity score	Isolation source
OTU1	'*Candidatus* Pelagibacter' (100)	EU801724	0.987	Chesapeake Bay, MD
OTU2	*Sulfurimonas* (100)	KC492833	0.987	Baltic Sea redoxcline, 119 m depth
OTU4	*Rhodobacteraceae* (100)	JX015552	0.987	marine bulk water (the North Sea)
OTU5	GpIIa (100)	AY151238	0.991	isolate
OTU6	'*Candidatus* Pelagibacter' (100)	EU799240	1.000	Newport Harbour, RI
OTU7	*Ilumatobacter* (100)	HM446118	0.987	West Thumb 98 (Yellowstone Park)
OTU12	*Micrococcineae* (75)	DQ316352	0.992	freshwater
OTU14	*Bacteria* (100)	EU801283	0.992	Chesapeake Bay, MD
OTU15	*Ilumatobacter* (100)	EU800762	0.996	Delaware Bay, NJ
OTU16	*Comamonadaceae* (100)	FR685984	0.995	marine biome, fjord, coastal water
OTU17	*Rhodobacteraceae* (100)	HQ153846	0.983	shallow hydrothermal vent (depth of 189.1 m)
OTU18	*Flavobacteriaceae* (100)	AM279175	0.983	marine water (*Gymnodimium catenatum*)
OTU19	*Bacteroidetes* (100)	JX015752	0.995	marine bulk water (the North Sea)
OTU20	*Methylobacter* (100)	AF152597	0.991	isolate
OTU21	*Methylophilus* (82)	AJ400352	0.987	North Sea
OTU26	'*Candidatus* Pelagibacter' (100)	EU800094	0.996	"Delaware Bay, NJ
OTU29	*Bacteroidetes* (100)	GQ259245	0.942	surface water (the Arctic ocean)

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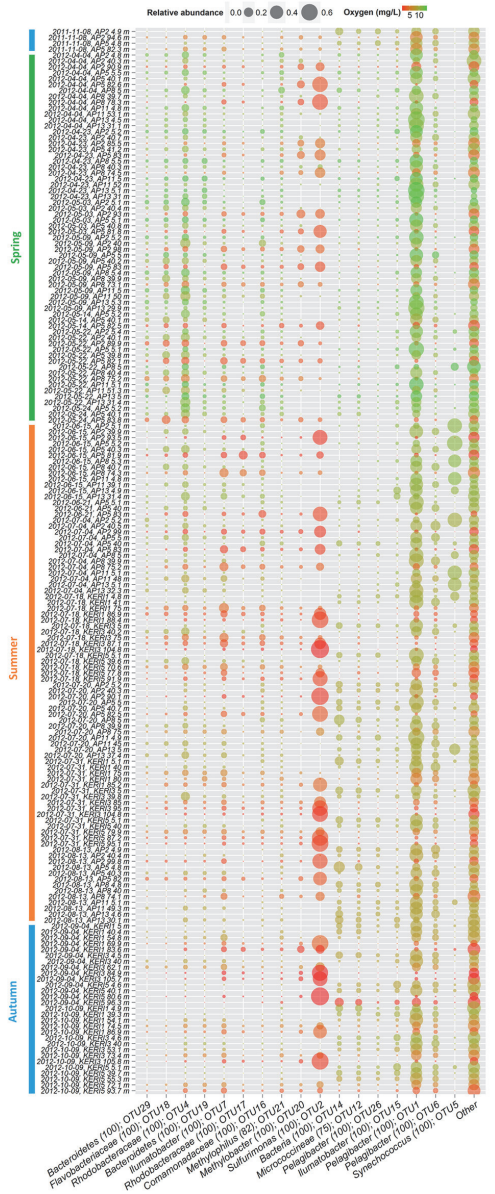

Fig 4. Occurrence patterns of relatively abundant OTUs (top 17). OTUs are ordered by their co-localization.

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of BCC in at least one of the samples. Lowering the threshold to 0.1%, this number quadrupled and resulted in 413 OTUs.

Variability of BCC in relation to environmental conditions was analysed using detrended correspondence analysis (DCA) with added linear fitting of environmental parameters onto the ordination (S3 Fig). The DCA was chosen for all of samples and OTUs because it helps to overcome the 'arch effect' that accompanies correspondence analyses. The relevance of salinity, depth, and oxygen were statistically highest, yielding r^2 values of 0.773, 0.742 and 0.630 ($P<0.001$ in each case), respectively. These three parameters were followed by temperature ($r^2 = 0.289$, $P<0.001$).

Non-metric multidimensional scaling (NMDS) analysis of relatively abundant and common OTUs was carried out (based on Bray-Curtis dissimilarity matrix) and supplemented with the linear fitting of environmental parameters onto the ordination (Fig 5). As a result, three general

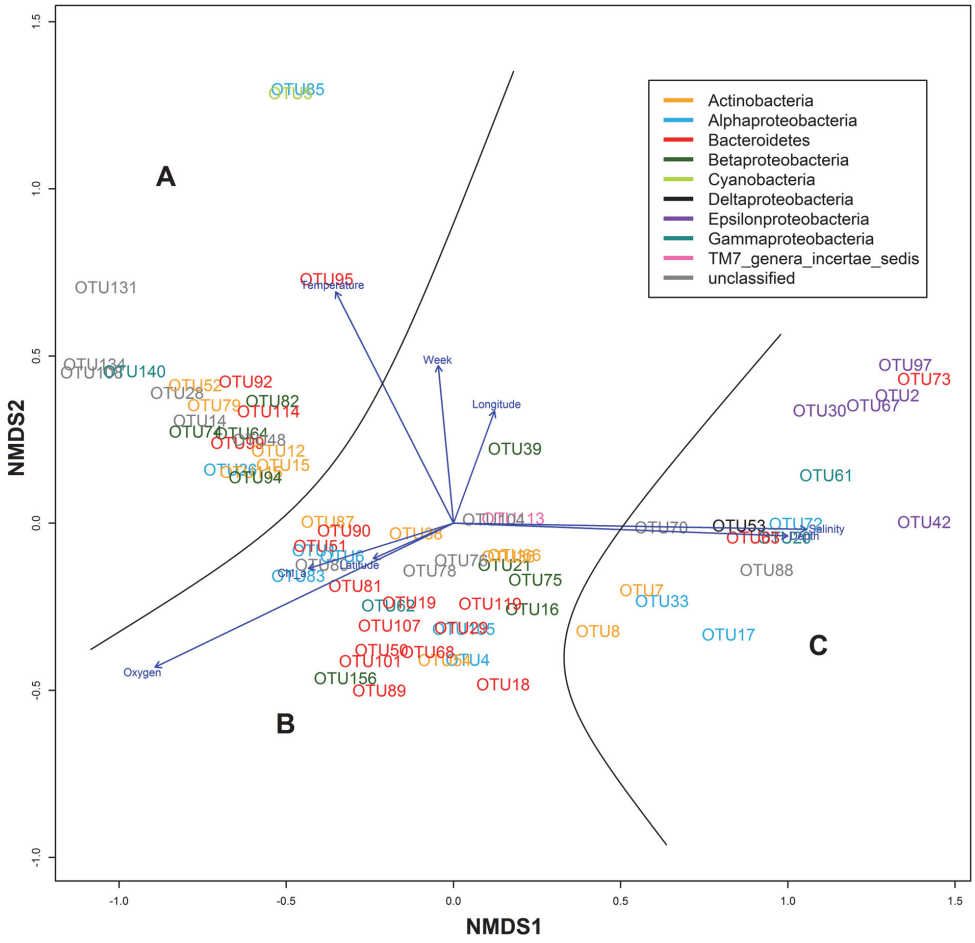

Fig 5. NMDS plot of abundant and common OTUs fitted with environmental parameters (stress score: 0.125). The added black lines divide OTUs into three major groups, which are marked with letters for discussion.

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groups can be distinguished: OTUs that were mainly found in surface layer in summer and autumn (group A); spring phytoplankton bloom-associated OTUs, including populations that became more prevalent in deeper layers after the bloom had ended (group B); and OTUs that were mostly found in hypoxic waters (group C in Fig 5). Therefore, seasonal succession (with important impact of changing temperature); and a covarying trio of salinity, pressure and oxygen could be established as main factors influencing the occurrence of these OTUs.

Most dominant populations in the 'hypoxic group' were members of *Epsilonproteobacteria*: OTU2, OTU30, OTU67, OTU97 (classified as *Sulfurimonas*) and OTU42 (classified as *Arcobacter*). OTU2 contributed up to 71% of BCC and had 99% sequence similarity to the representative of *Sulfurimonas* GD1/GD17 subgroup, which has been shown to be key chemolithoautotrophic taxa in the Gotland Deep [44, 45].

The major bulk of *Alphaproteobacteria* was contributed by OTUs classified as "*Candidatus* Pelagibacter" (OTU1, OTU6, OTU26, and OTU83). OTU1 and OTU6 remained transiently abundant with minor variations in time (Fig 4, S2 Fig). OTU26 was more prominent at surface layer towards the autumn (Fig 4) and clustered together with relatively abundant actinobacterial OTUs: OTU12 (*Micrococcineae*), OTU15 (*Ilumatobacter*) and OTU14 (closest database affiliation to *Micrococcineae*; Table 1). Some of these OTUs exhibited similar presence in samples collected autumn in 2011 and 2012 (Fig 4, S2 Fig).

Unicellular cyanobacterium *Synechococcus* (OTU5) and co-occurring OTU85 (*Rhodobacteraceae*) formed a separate cluster (Fig 5), and a significant and strong relationship between OTUs and temperature suggests that temperature had a strong impact on their occurrence. OTU95 (*Flavobacteriaceae*) and OTU39 (classified as *Alcaligenaceae*) appeared during the peak of *Synechococcus* relative abundance, only in deeper layers (S2 Fig). During summer bloom *Synechococcus* displayed a patchy distribution at the surface layer above the thermocline as its relative abundance varied along AP-transect; members of "*Candidatus* Pelagibacter" remained dominant in the intermediate layer, and representatives of *Sulfurimonas* contributed the largest fraction at the hypoxic near-bottom layer (Fig 6).

Relatively abundant and common OTUs associated with the spring phytoplankton bloom could also be distinguished as a separate co-localizing cluster in the middle bottom of NMDS plot (Fig 5). This positioning is explained by negative correlations with time and their spread throughout the water column, which resulted in weak correlations with dissolved oxygen concentrations. These OTUs contributed a large fraction of BCC above the halocline during the

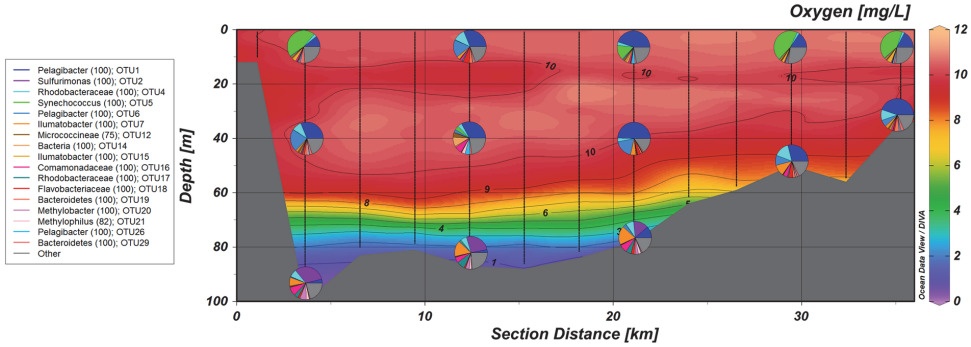

Fig 6. Cross-section of AP-transect on 2012-07-04 supplemented with relative abundances of top 17 OTUs.

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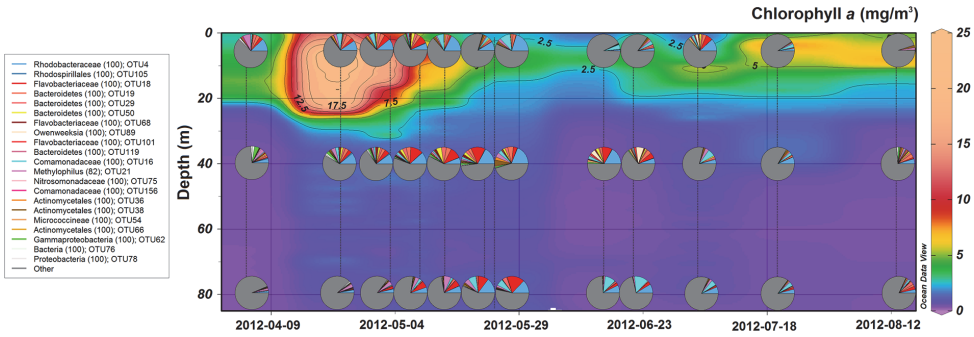

Fig 7. Time series of Chlorophyll *a* at station AP5. Pie charts indicate the relative abundance of OTUs that were associated with the spring phytoplankton bloom. Note that the second surface community (2012-04-23, 5 m depth) was recorded at station AP2 not AP5.

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spring bloom and remained abundant at intermediate layer till mid-summer (Fig 7). The most relevant bacterial populations by their relative abundance were classified as *Rhodobacteraceae* (OTU4), *Comamonadaceae* (OTU16) and *Bacteroidetes* (OTU19 and OTU29). The fraction of these OTUs also increased in the near-bottom hypoxic layer as the spring bloom progressed (Fig 7). Interestingly, betaproteobacterial OTU16 (*Comamonadaceae*) reached its maximum abundance in hypoxic conditions in the beginning of summer (Fig 7). During that period also OTU7 (*Ilumatobacter*) and OTU17 (*Rhodobacteraceae*) became more predominant in the hypoxic layer (reaching 10.3% and 17.8% of BCC, respectively), but the opposite trend was displayed by OTU2 (Fig 4). OTU21 (*Methylophilus*) was present at deeper layers throughout the sampling period; however, it contributed a larger fraction during the spring bloom and had its maximum abundance occurred at 5 m depth at the beginning of April (Fig 7).

Discussion

High-throughput marker gene sequencing has opened a new gateway to investigate aquatic microbial communities. The present study explores the abundances of picoplankton and BCC of the Gulf of Finland in three dimensions: horizontally from south to north, vertically from the surface to near-bottom layer, and temporally from spring to autumn. Similar investigations are quite rare as most temporal studies lack spatial dimension and *vice versa*. The focus of this study is on spatiotemporal patterns and phylogenetic relationships of OTUs that are relatively abundant or common. However, our results and previous investigations of BCC of the Baltic Sea [12, 15–17, 22, 24] also reflect the presence of the 'rare biosphere', which is represented by the long tail pattern found in rank-abundance curves of bacterial species [46–48]. That long tail is composed mostly of dormant cells and can produce a pulse of ecosystem activity by resuscitation [49], acting as a reservoir of genetic and functional diversity [50]. The presence of the 'rare biosphere' is also reflected from our present results, as well as from previous investigations of BCC of the Baltic Sea using next-generation sequencing platforms [12, 15–17, 22, 24]. However, the focus of this study is on spatiotemporal patterns and phylogenetic relationships of OTUs that are relatively abundant or common. The switch point between the 'tail' and the common or abundant bacterial populations is, of course, subjective, and 0.1% of the total dataset was selected for this analysis [51].

Spatiotemporal niche partitioning of the BCC

The approach used, massively parallel sequencing of the amplified 16S rRNA gene fragment libraries, enabled culture-independent and detailed view in the dynamics of bacterioplankton community. Spatiotemporal surveys of BCC in ecosystems with sharp environmental gradients (like river mouths, hypoxic estuaries or coastal regions) have demonstrated that spatial variation overpowers seasonal succession [6, 7, 16]. Our results reflect the same trend, but also demonstrate in fine detail the seasonal succession throughout the water column. There were 17 relatively abundant OTUs. However four dominant OTUs (OTU1—"*Candidatus* Pelagibacter", OTU2—*Sulfurimonas*, OTU4 –*Rhodobacteraceae* and OTU5—*Synechococcus*) could be considered as core populations of bacterioplankton in the Gulf of Finland.

There is no sill between the Gulf of Finland and Baltic Proper, hence the deep inflow to the gulf takes place just below the co-occurring oxy- and halocline [28]. Consequently, taxa characteristic of the sulfidic zone of the central Baltic Sea are dispersed into the Gulf of Finland [15–17]. Our results of agree well with findings from these studies. Nitrate-reducing and sulphide-oxidizing *Sulfurimonas gotlandica* (98.7% similarity to OTU2) is capable of motility and chemotaxis; hence, it is typically more abundant near the oxycline [52, 53]. In contrast, sulfate-reducing *Deltaproteobacteria* are more prominent in deeper anoxic layers (below 140 m) [54]. As a consequence, *Deltaproteobacteria* contribute small and *Sulfurimonas* make up large fraction of the bacterial community in the near-bottom layer of the Gulf of Finland (Fig 3). The sulphur reducers in the gulf are mainly found in the sediments [55].

Alphaproteobacterial SAR11 clade (candidate order "*Pelagibacterales*") and also Roseobacter group constitute high proportions of the marine bacterioplankton globally and in the geographically close North Sea [55–58]. Both of these groups were made up OTUs that can be considered core populations for the area. The Baltic Sea is known to harbour several phylogenetic lineages of the "*Pelagibacterales*" that exhibit niche partitioning along the salinity gradient [12, 54]. Results from the present and previous studies indicate that this group is a transiently abundant heterotrophic group in the surface layer (S2 Fig, Fig 6) [16, 17]. Members of SAR11 clade and order *Rhodobacterales* are mostly involved in the transport and metabolism of low-molecular-weight organic compounds [58–60]. The latter includes degradation of phytoplankton-produced dimethylsulfoniopropionate (DMSP) to dimethylsulfide (DMS) [61]. It is highly probable that these groups facilitate these processes also in the Gulf of Finland, especially given that OTU4 had its highest occurrence during the spring phytoplankton bloom.

The coordinated and cooperative activities of bloom-associated 'specialists' are critical in the biogeochemical cycles of the ecosystem for determining the fate of the organic biomass. Various members of *Actinobacteria*, *Betaproteobacteria*, *Bacteroidetes* and *Verrucomicrobia* have been demonstrated to be responsible for the degradation of high-molecular-weight organic compounds in the Baltic Sea [21, 62, 63] and in the North Sea [58, 64]. In general, temporal dynamics of these groups at the surface layer are in line with previous investigations [22, 24]; for example, representatives of *Bacteroidetes* were more abundant during spring and early summer, and members of *Actinobacteria* were prevalent in late summer and autumn (Fig 4). However, to the best of our knowledge, the present study is the first that provides simultaneous insight into dynamics of BCC below surface layer in this region (Fig 7, S2 Fig).

Annually recurring spring phytoplankton blooms in the Gulf of Finland consist mainly of diatoms and dinoflagellates [65]. During spring bloom, a large fraction of annual primary production is carried out [43] and therefore these bloom-associated bacterial populations hold great ecological significance. Our results demonstrate shifts in BCC during the bloom (Fig 7) and likely sedimentation of some OTUs to the deeper layers. The fraction of bloom-associated populations steadily increased at near-bottom hypoxic layer probably due to attachment to

decaying phytoplankton cells or other settling particles (Fig 7). It is important to keep in mind that bacteria attached to particles or aggregates larger than 5.0 μm were excluded from the dataset due to prefiltration. However, certain bacterial lineages are known to switch between free-living and particle-attached lifestyle and also some of the particle-associated groups can get detached during the filtration process. These possibilities have to be considered, especially because there is a clear influx of populations into the deeper layers that in previous data points were prevalent in the surface layer.

At the beginning of the spring bloom, OTU4 (Roseobacter group) betaproteobacterial OTU21 (*Methylophilus*) and three members of *Bacteroidetes* (OTU18, OTU19, and OTU29) constituted a considerable fraction of BCC. The presence of OTU21 shifted rapidly from the surface to deeper layers (Fig 7). Representatives of the *Methylophilaceae* family (*Betaproteobacteria*) have recently been identified as dominant DMS-degrading populations in Tocil Lake sediment and soil collected from *Brassica oleraceae* field [66]. This finding suggests that two relatively abundant and common OTUs that were classified as *Methylophilus* could also be metabolizing DMS, especially considering that OTU21 had its closest affiliation to sequence isolated from samples from the North Sea after an algal bloom (Fig 7).

The spatiotemporal patterns of bloom-associated bacterial populations provide valuable insight into mechanisms of community assembly in relation to the phytoplankton community and the fate of organic matter produced by phytoplankton. One important aspect of the current study is to shed light on phytoplankton bloom related shifts in BCC that take place in deeper hypoxic layers. To start off, the relative abundance of representatives of *Bacteroidetes* decreased at the surface layer as the bloom progressed, but at 40 m depth, some OTUs remained present until the beginning of summer (Fig 7). However, by the end of the spring bloom OTU18 (*Flavobacteraceae*) contributed a large fraction of BCC at the near-bottom hypoxic layer at the end of May (Fig 7). This could occur due detachment from lysing phytoplankton cells. However, the occurrence of unclassified *Flavobacteraceae* in oxygen depleted water was also observed during the spring phytoplankton bloom in Byfjord; in the following year, when oxygen conditions improved, the abundance of that group was drastically reduced [7]. This gives an indication that degradation of organic matter in hypoxic conditions provides a specific niche, especially considering that some of the members of *Bacteroidetes* were mostly found in the oxygen depleted near-bottom layer (OTU63 and OTU73; Fig 5).

Similarly, betaproteobacterial OTU16 (*Comamonadaceae*) appeared at surface layer in the end of the spring bloom and peaked in abundance after sedimentation at hypoxic layer in the beginning of summer. Members of *Comamonadaceae* have shown to be involved in denitrification and to be active in the hypoxic zone [67, 68]. In addition, OTU7 (*Ilumatobacter*) became more abundant in the hypoxic layer during the spring bloom; the same group was associated with diatom degradation in the near-bottom layer of Lake Baikal [69].

The spatiotemporal patterns of these populations provide valuable insight into mechanisms of community assembly. The presence of certain bloom-associated populations is affected not only by phytoplankton community but also by the presence of a hypoxic zone. The fact that the abundance of some of the OTUs increased in the hypoxic zone suggests that they are probably capable of switching the terminal electron acceptor, and therefore the oxygen depleted zone provides a particular niche for them. These results point toward combined effects of substrate- and redox-driven niche partitioning on the BCC.

In addition to substrates provided by phytoplankton, there are compounds produced by bacteria in the sediments, like methane [70], which as powerful greenhouse gas bears global significance. The abundance and activity of methane-oxidizing bacteria in the water column are crucial regulators of methane emission to the atmosphere. Our results demonstrate a vertical distribution of different potentially methanotrophic bacteria. The most dominant of which

were classified as *Methylobacter*, like OTU20, which was mainly found in the hypoxic near-bottom layer, where the methane concentrations are potentially highest [70, 71]. Also, OTU21 and OTU94 were classified as *Methylophilus*, a non-methane-oxidizing bacterial group which usually utilizes other C1 substrates [72]. However, the representatives of *Methylobacter* and *Methylophilus* have been demonstrated to cooperate in methane utilization [73, 74] and experiments with such methane-consuming communities suggested that, in low oxygen concentrations, members of *Methylobacter* dominate over representatives of *Methylophilus* and *vice versa* [74]. Our *in situ* results confirm these previous findings. Overall, our results reflect the importance of C1 substrate utilizing bacterial populations in the gulf as they contribute a significant fraction of BCC throughout the water column (Fig 4).

Emerging pattern of cosmopolitan specialists

It is well established that salinity has a major impact on bacterioplankton community assemblage, gene expression and metabolic activity in aquatic ecosystems, including the Baltic Sea [12, 20, 75]. Over the past decade, there has been accumulating evidence that certain phylogenetic lineages (for example members of SAR11, *Rhodobacterales*, *Methylophilales*, *Synechococcus*, SUP05) contribute a significant fraction of BCC in geographically distant, but hydrographically close (brackish and periodically/partially hypoxic) estuaries [7, 16, 76–78]. Moreover, recent genome level comparisons using metagenome-assembled genomes have demonstrated that the Baltic Sea and the Chesapeake Bay harbour not only phylogenetically close members, but exactly the same species [59]. The similarity of communities in distant locations fits the oft-repeated 'everything is everywhere, but the environment selects' concept in ecology [79, 80]. This concept agrees well with the species-sorting paradigm of metacommunity theory [81], by which the geological barriers are rendered irrelevant and local environmental conditions play a central role in the community assembly process. From our previous studies, we have concluded that these communities can be considered as a metacommunity, and now this statement is backed with more evidence [16].

Hugerth *et al.* [59] put the selective emphasis on the salinity range, coining it a 'brackish microbiome', but their analyses included reads obtained only from the sea surface communities. However, the similarities in BCC were observed with estuaries also suffering from oxygen depletion. The results of the present study clearly demonstrate the structuring effect of dissolved oxygen, because some bacterial populations achieve an advantage in the hypoxic zone through very likely using alternative electron acceptors (e.g. denitrification). The notable presence of usually oxic surface bacterial populations also in the hypoxic zones has raised questions of their anoxia-tolerance and capability to use alternative electron acceptors [16, 82]. Functional capability to use alternative electron acceptors gives a distinct advantage to bacteria in such oxygen depleted ecosystems and can provide specific niches as demonstrated by bloom-associated OTUs (Fig 7).

Thereby, rather than putting salinity or redox conditions in the central role in bacterioplankton community assemblage in the Baltic Sea, it should be looked as a combination of the two. Hence, these communities can be considered as metacommunity driven by gradients of redox conditions and salinity. An analogy would be a symphony orchestra on a world tour: it plays the same melodies (ecosystem services), and although some musicians (bacterial populations) may vary between concerts, they play the same instrument (functional niche). In the case of the Baltic Sea, one of these 'unique musicians' is *Sulfurimonas gotlandica*, as sulfur-oxidation in most of these other communities is carried out by SUP05 clade members [7, 76–78, 82].

Conclusions

The present study provided a detailed insight into spatiotemporal patterns of BCC in the Gulf of Finland, a stratified estuary suffering from oxygen depletion in the near bottom layer. Thousands of OTUs were identified in the framework of this study, but most of them belong to the rare biosphere, which can be accessed by 'deep' sequencing. Only around one hundred OTUs could be considered relatively common or abundant. Oxygen availability explained most of the variability of BCC, however, seasonal dynamics were observed both in oxygenated and hypoxic layers. Especially intriguing were the spatiotemporal occurrence patterns of heterotrophs responsible for degradation of the spring phytoplankton bloom-derived organic matter, as a combination of substrate- and redox-driven niche partitioning could be observed. These spatiotemporal niche specializations among bacterioplankton communities are crucial for predicting ecosystem functioning and understanding multilevel effect of oxygen depletion to the Baltic Sea, and in addition, help explain biogeography of certain cosmopolitan species.

Supporting Information

S1 Fig. Picoplankton total cell count numbers supplemented with oxygen concentration (mg/L).
(TIF)

S2 Fig. Occurrence patterns of abundant and common OTUs (top 73). OTUs are ordered by their co-localization.
(TIF)

S3 Fig. Detrended correspondence analysis of the bacterioplankton community composition fitted with environmental parameters. Red crosses represent individual OTUs (n = 4692) and circles represent different samples (n = 181).
(TIF)

S1 Table. Sample collection metadata and physicochemical background data.
(DOCX)

S2 Table. Species richness estimates.
(DOCX)

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Author Contributions

Conceived and designed the experiments: PL IL MM. Performed the experiments: PL EŠ. Analyzed the data: PL JS UL EŠ. Contributed reagents/materials/analysis tools: IL UL MM. Wrote the paper: PL IL VK UL MM EŠ.

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ABSTRACT

Spatiotemporal niche-partitioning of bacterioplankton community across environmental gradients in the Baltic Sea

Estuaries and other coastal waterbodies account for most anthropogenic influenced ecosystems in the world. Such ecosystems also encounter similar problems; most of which are mediated or directly caused by micro-organism: eutrophication fuelled oxygen depletion, harmful algal blooms, production of hazardous compounds (e.g. H_2S and CH_4), and infections by faecal-derived pathogens. Hence, understanding the mechanisms of microbial community assemblage and functional roles in these habitats regarding ecosystem management and sustainable development of these areas becomes crucial. The objective of this Ph.D. thesis was to further the progress of the theory of microbial ecology through investigating dynamics of bacterioplankton community composition (BCC) in one of such ecosystems – the easternmost sub-basin of the Baltic Sea, the Gulf of Finland.

Massively parallel tag sequencing of 16S rDNA fragments was used to determine bacterioplankton community structure throughout all seasons, in variable locations and at different depths. The substantial spatiotemporal physicochemical heterogeneity was reflected on the contrasting dynamics of the microbial community. Combined redox-, salinity- and substrate-driven niche partitioning were identified as major factors shaping the BCC. The Gulf of Finland is vertically stratified: the permanent halocline is co-localized with oxycline, the seasonal thermocline is preventing mixing with the nutrient rich intermediate layer. The inflow of hypoxic and more saline Baltic Proper waters in the near bottom layer increase the vertical stratification of the water column and strength of different clines. The most contrasting shifts in the BCC occurred vertically across the oxycline. Bacterial populations (mainly chemolithoautotrophic *Epsilonproteobacteria*) capable of using alternative terminal electron acceptors to oxygen becoming predominant in the hypoxic layer.

Some of the relatively abundant taxa had their closest database affiliations isolated from geographically distant estuaries, but with similar physicochemical structuring. The similarities expanded to the overall phylogenetic structuring of BCC in these estuaries. These results contribute to the understanding of emerging pattern in BCCs that occupy hydrographically similar estuaries dispersed all over the world and suggest the presence of a global redox- and salinity-driven metacommunity. We conclude that capability to survive (or benefit from) shifts between oxic and hypoxic conditions is vital adaptation for bacteria to thrive in such environments. The results of the present study have important implications for understanding long-term ecological and biogeochemical impacts of hypoxia expansion in the Baltic Sea (and similar ecosystems), as well global biogeography of bacteria specialized inhabiting similar ecosystems.

RESÜMEE

Bakterplanktoni koosluse ajalis-ruumiline nišijaotus läbi keskkonnagradientide Läänemeres

Estuaarid ja teised rannikumere elupaigad on ühed enim inimtegevuse poolt mõjutatud ökosüsteemid. Neis ökosüsteemides esinevad sarnased, kaudselt või otseselt vee mikroorganismide poolt põhjustatud probleemid, nagu eutrofeerumisest tingitud hapnikuvaegus, kahjulikud vetikaõitsengud, mürgiste või kahjulike ainete (näiteks H_2S and CH_4) ja fekaalse reostusega kaasnevate patogeenide esinemine. Seetõttu on bakterplanktoni koosluse koosseisu kujunemise mehhanismide ja funktsionaalsete rollide mõistmine nimetatud ökosüsteemides oluline, seda eriti ökosüsteemi jätkusuutliku majandamise kontekstis. Käesoleva doktoritöö eesmärgiks oli edendada mikroobi ökoloogia alaseid teadmisi ning selgitada bakterplanktoni koosluse nišijaotuse mõjutegureid ühes inimtegevusest tugevalt mõjutatud ökosüsteemis – Soome lahes.

Doktoritöö raames viidi läbi bakterplanktoni koosluse koosseisu ajalis-ruumilise muutlikkuse analüüs kasutades kogu koosluse DNA-st PCR-amplifitseeritud 16S rRNA geeni fragmentide mass-sekveneerimist. Analüüsi teostamiseks koguti proove kõikidel aastaaegadel erinevatest Soome lahe piirkondadest ja sügavustest. Soome lahe füüsikalis-keemiline heterogeensus läbi aja ja ruumi peegeldus ka bakterplanktoni koosluse koosseisu dünaamikas. Redoks-, soolsus ja substraadi-põhised nišijaotused osutusid peamisteks teguriteks bakterplanktoni struktuuri mõjutamisel. Soome laht on vertikaalselt kihistunud ning suurimad muutused bakterplanktoni koosluses toimusid läbi koosesineva hapniku ja soolsuse hüppekihi. Põhjalähedases hüpoksilises kihis domineerisid populatsioonid, mis on võimelised hapniku asemel kasutama alternatiivseid terminaalseid elektronide aktseptoreid, peamiselt kemolitoautotroofsed epsilonproteobakterid.

Mitmed suhteliselt arvukad bakterite populatsioonid omasid lähimaid andmebaasi vasteid annotatsioonidega, mis olid isoleeritud sarnase füüsikalis-keemilise taustaga estuaaridest mujal maailmas. Soome lahe bakterplanktoni koosluse laiem võrdlus teiste sarnaste ökosüteemidega tõi esile veelgi enam sarnasusi koosluste fülogeneetilises struktuuris. Saadud tulemused võimaldavad näha teatud ökotüüpide globaalset esinemismustrit, mis omakorda viitab kosmopoliitse soolsuse ja redokstingimuste poolt kujundatud metakoosluste esinemisele. Vertikaalselt kihistunud ökosüsteemis saavad eelise bakterite populatsioonid, kes on võimelised taluma ajutist hapniku puudust või suudavad kasutada alternatiivseid terminaalseid elektronide aktseptoreid. Doktoritöö tulemused aitavad paremini mõista pikaajalisi ökoloogilisi ja biogeokeemilisi mõjusid, mis kaasnevad hüpoksia levikuga Läänemeres (ja sarnastes ökosüsteemides).

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Inglise keel	Kõrgtase
Vene keel	Kesktase
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5. Teadustegevus

Laas, P., Simm, J., Lips, I., Metsis, M. (2014). Spatial variability of winter bacterioplankton community composition in the Gulf of Finland (the Baltic Sea). *Journal of Marine Systems*, 129, 127 - 134.
Laas, P., Simm, J., Lips, I., Lips, U., Kisand, V., Metsis, M. (2015). Redox-specialized bacterioplankton metacommunity in a temperate estuary. *PLoS ONE*, 10(4), e0122304

Laas, P., Šatova, E., Lips, I., Lips, U., Simm, J., Kisand, V., Metsis, M. (2015). Near-bottom hypoxia impacts dynamics of bacterioplankton assemblage throughout water column of the Gulf of Finland (Baltic Sea). *PLoS ONE,* 11(5): e0156147.

6. Juhendatud väitekirjad

Elina Šatova, magistritöö „Bakterplanktoni koosluse ajalis-ruumiline muutlikkus läbi vertikaalsete hüppekihtide Soome lahes“. Tallinna Tehnikaülikool, Geenitehnoloogia instituut, 2015.

7. Kursused

17.09-15.10.2010 – praktikant MESOAQUA eksperimendil “*Dynamics of microbial food webs and mixotrophy in a light gradient*” (Kreeta, Kreeka)

28.05.2013 – BD Accuri C6 koolitus (Lund, Rootsi)

CURRICULUM VITAE

1. Personal data

Name	Peeter Laas
Date and place of birth	14 September 1985, Estonia
E-mail address	peeter.laas@msi.ttu.ee

2. Education

Educational institution	Graduation year	Education (field of study/degree)
Tartu Secondary School of Business	2001	Secondary school
Hugo Treffner Gymnasium	2004	High school
University of Tartu	2008	Gene technology, BSc
University of Tartu	2010	Gene technology, MSc

3. Language competence/skills (fluent, average, basic skills)

Language	Level
Estonian	Native language
English	Fluent
Russian	Average
German	Basic Skills

4. Professional employment

Period	Organisation	Position
2009-2010	Institute of Ecology and Earth Sciences at UT	Lab assistant
2010-2012	Marine Systems Institute at TUT	Engineer
2013-present	Marine Systems Institute at TUT	Junior Researcher

5. Scientific work

Laas, P., Simm, J., Lips, I., Metsis, M. (2014). Spatial variability of winter bacterioplankton community composition in the Gulf of Finland (the Baltic Sea). *Journal of Marine Systems*, 129, 127 - 134.

Laas, P., Simm, J., Lips, I., Lips, U., Kisand, V., Metsis, M. (2015). Redox-specialized bacterioplankton metacommunity in a temperate estuary. *PLoS ONE*, 10(4), e0122304

Laas, P., Šatova, E., Lips, I., Lips, U., Simm, J., Kisand, V., Metsis, M. (2015). Near-bottom hypoxia impacts dynamics of bacterioplankton assemblage throughout water column of the Gulf of Finland (Baltic Sea). *PLoS ONE,* 11(5): e0156147.

6. Theses supervised

Elina Šatova, Master's Thesis „Spatio-temporal variation of bacterioplankton community composition across vertical clines in the Gulf of Finland“. Tallinn University of Technology, Department of Gene Technology, 2015.

7. Special courses

17.09-15.10.2010 – participated as a trainee in MESOAQUA experiment “Dynamics of microbial food webs and mixotrophy in a light gradient” (Crete, Greece)

28.05.2013 – BD Accuri C6 meeting/training (Lund, Rootsi)

DISSERTATIONS DEFENDED AT
TALLINN UNIVERSITY OF TECHNOLOGY ON
NATURAL AND EXACT SCIENCES

1. **Olav Kongas**. Nonlinear Dynamics in Modeling Cardiac Arrhytmias. 1998.
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