

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS

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190

**HELEN TAMMERT**

Ecological role  
of physiological and phylogenetic diversity  
in aquatic bacterial communities



TARTU UNIVERSITY  
PRESS

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*This cell that had split, end to end  
Said, "I was quite upset and then,  
I didn't know who  
I should turn to.  
I was beside myself, my friend.  
A. Willis, 1994*



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## LIST OF ORIGINAL PUBLICATIONS

- I. **Tammert, H.**, Olli, K., Sturluson, M., Hodal, H. (2008). Bacterial biomass and activity in the marginal ice zone of the northern Barents Sea. *Deep-Sea Research II*, 55 (20–21), 2199–2209.
- II. **Tammert, H.**, Ott, I., Kisand, V. (2010). Ecophysiological status of bacteria in freshwater lakes during steep summer stratification. *Fundamental and Applied Limnology / Archiv für Hydrobiologie*, Volume 176 (1): 75–82.
- III. **Tammert, H.**, Olli, K., Lignell, R., Kisand, V. (manuscript, submitted). Labile carbon supplement induces growth of filamentous bacteria in the Baltic Sea.
- IV. **Tammert, H.**, Kisand, V., Noges, T. (2005). Bacterioplankton abundance and activity in a small hypertrophic stratified lake. *Hydrobiologia*, 547: 83–90.

My contribution to the articles is as follows:

- I. I provided data of all bacterial stainings, formalized general scientific ideas and concepts, and participated in writing of the manuscript
- II. I participated in samplings, lab work and in writing of the manuscript
- III. I participated in the mesocosm experiment, performed all analysis of bacterial abundances and wrote most of the manuscript.
- IV. I performed microbiological analysis and wrote most of the manuscript.

## LIST OF ABBREVIATIONS

ARC	actively respiring cells determined by CTC staining
BM	biomass of bacteria
BMcocc	biomass of nonfilamentous bacteria
BMfila	biomass of filamentous bacteria
BP	bacterial production
CA	correspondence analysis
CFA	carboxyfluorescein
CTC	5-cyano-2,3-ditolyl tetrazolium chloride
DAPI	4,6-diamidino-2-phenylindole
DGGE	denaturing gradient gel electrophoresis
EB	ethidium bromide
INT	bacteria with intact membranes by SYTO 9 staining
INT-dye	2,4-iodophenyl-3,4-nitrophenyl-5-phenyl tetrazolium chloride
Leu	leucine
MCB	bacteria with compromised cell membranes stained by SYTOX Green
OTU	operational taxonomic unit
PI	propidium iodide; marker of bacteria with compromised cell membrane
PCR	polymerase chain reaction
POC	particulate organic carbon
PON	particulate organic nitrogen
PP	primary production
Rh123	rhodamine 123
TNB	total number of heterotrophic bacteria by DAPI staining
Tdr	thymidine

# I. INTRODUCTION

Unlike eukaryotic plankton, which can often be taxonomically and metabolically categorized according to directly observable phenotypes, it has been more difficult to ascertain the core identities and physiological properties of planktonic Bacteria. The development of new technologies has highlighted the roles that bacteria are playing in biogeochemical cycling and ecosystem functioning. Increasing information regarding aquatic bacteria has shown that they exhibit high abundance, biomass, production and diverse community composition in lakes and oceans. Aquatic environments foster highly heterogeneous niches of chemical, physical and biological conditions which all have shown to affect bacterial community as a structuring force. A central goal of ecology is to understand how biodiversity is maintained. In marine and freshwater environment the complexity of factors that influence bacterial growth is difficult to follow. Therefore the threshold levels of bacterial community that respond to changing conditions in the environment are not well known. Optimizing growth rate at non-optimal nutrient levels must be an important aim for aquatic bacteria, given that marine and freshwater environments rarely offer growth-saturating concentration of nutrients. In the pelagic photic zone, nutrient limitation by e.g. phosphate, available nitrogen and degradable organic material, is believed to be a fundamental controlling factor for the community composition and activity of bacteria. Since eutrophication has become an increasing problem in marine coastal and estuarine areas, the question how heterotrophic bacteria respond to nutrient limitation is critical. In this study experimental approach was used to elucidate the effect of various combined additions of nitrogen, phosphorus and organic carbon to natural bacterial community.

The life strategy of species and an individual organism determines their competitive ability within the trophic level and to organisms in higher trophic levels. Responses to changing environmental conditions enable single bacterial cell to drive in various physiological states as a part of their life strategy. Among the continuum of physiological states active and alive categories have the highest ecologically relevance as actual mediators of bacteria related processes. Accurate assessment of physiological status and proper estimation of environmental bacterial abundance excluding usually abundant dead cells has remained a difficult methodological problem up to date. As a consequence the scaling of growth, metabolism and uptake of organic substrates to total number of cells yield numbers that underestimate the real activity of processes. In this thesis I discuss the ecological relevance to determine physiological state of aquatic bacteria and to use only the number of active or living bacteria in cell-specific calculations.

The current thesis shows the heterogeneity in aquatic bacterial world in different angles. The coastal Baltic Sea, Estonian stratified small lakes and the Arctic Barents Sea were used as model areas to describe bacterial heterogeneity and to find answers to several ecological questions listed as the aims of the study.

## 2. LITERATURE REVIEW

In the past few hundred years, our understanding of the role that microbes play in ecological and biogeochemical processes has grown at a phenomenal rate. Von Leeuwenhoek, who invented the microscope in the 17th century, was the first to observe microbes in lake water, but progress in microbiology came slowly. The field of microbial ecology did not come into existence until the middle of the 20th century. Early on, Vernadsky recognized the importance of microbes in global processes, and many scientists in the early 20th century acknowledged that bacteria and other microbes were an important but unquantifiable component of the ecosystem. For example, Lindeman (1942) put the microbial “ooze” at the centre of his diagram depicting trophic dynamics in lakes. Although Lindeman had little understanding of the influence of microbes in the carbon, energy, and nutrient fluxes that he studied in Cedar Bog Lake, USA. Riley (1951; by Cotner and Biddanda, 2002) recognized their importance in ocean geochemistry but lamented that appropriate methods for studying them were not available.

Just 40 years ago, the number of microorganisms in each millilitre of sea water was underestimated by a staggering three orders of magnitude (DeLong, 2009). Astronauts may have been exploring the Moon, but most of the microbial life on Earth remained largely undiscovered. The situation changed dramatically in the late 1970s and early 1980s, when method advancements like cell stains erythrosine for light microscopy and later acridine orange, DAPI etc. for epifluorescence microscopy corrected estimates of total cell numbers in natural aquatic environment. Over the next 25 years or so, local, regional and global estimates of microbial numbers, along with their bulk production and consumption rates in lakes and ocean surface waters were quantified and mapped. These data provided increasingly accurate estimates of the total biomass of planktonic microorganisms. Following studies gave estimates about their turnover, enlightening their perceived role and significance in the food webs but excluding the fact that not all bacteria are actively participating in biogeochemical cycles. As a consequence the cell-specific activities and turnover times were underestimated and we are spirally back in the beginning when the number of bacteria was underestimated by the culture based methods. Based on the development in microbiological methods the enthusiasm of using the total number of bacteria determined by DAPI should be history for now and replaced by the actual number of living bacteria.

Although the information about bacterial abundance is extremely useful, more specific data on the biology of planktonic Bacteria and Archaea have only recently become available, allowing us to address a new range of questions. Which taxa of Bacteria and Archaea are most dominant or biogeochemically important in particular lake or ocean provinces or depth strata? What are the most common microbial metabolic pathways, and how do they vary within and between communities and environments? How do the dynamic population shifts

and species interactions shape the ecology and biogeochemistry of the lakes and seas?

Recent advances in cultivation-independent metagenomics and transcriptomics, in which DNA/RNA from the microbial community is collected, sequenced and analysed en masse, as well as new cultivation technologies, have had a dramatic influence on our knowledge of microorganisms. The integrated perspective provided by a combination of cultivation-independent phylogenetic surveys, microbial metagenomics and culture-based studies has delivered a more detailed understanding of microbial life in the lakes and ocean.

## **2.1. Dead or alive – ecological point of view**

Assessment of cell viability is one of the main requirements in several areas of microbiology, from environmental research to industrial application. The need to determine bacterial viability is central to inferring the role of heterotrophic bacteria in aquatic food webs and their effect to biogeochemical cycles. The answer to the question whether cell is active, alive or dead is far from simple and remains challenging after years of research and continuing controversy.

Life is generally characterised by: (i) the presence of structures; (ii) changeable genetic information; (iii) metabolism or functional activity, and (iv) the ability to reproduce and grow (Nebe-von Caron and Badley, 1995). Living bacteria are generally characterized by their ability to reproduce. Probably because the capacity of a cell to multiply as determined by cultures has long been the single method available to microbiologists for bacterial viability assessment. Therefore, culturability and viability are often considered synonymous terms. Bacteria from natural environments, however, are often not easy to be grown in laboratory due to the lack of knowledge about the growth requirements. Increasing but only a very small fraction of aquatic bacteria have been successfully cultured. Therefore, other parameters of viability listed above need to be taken into account when the number or biomass of viable bacteria is of interest. The viability of aquatic bacteria has shown do depend on several factors leading to continuous shift between activation and limitation of cellular processes. As a result different levels of viability can be discriminated in a single cell level. In their paper Smith and del Giorgio (2003) questioned the validity of restricting the physiological state of bacterioplankton to such categories as active or inactive, instead proposing that they should be viewed as a continuum of physiological states. However, the discrete and over-simplistic notion of active versus inactive cells is useful until the development of methods to combine and validate the level of viability. I believe that the problem of describing bacterioplankton single-cell activity should be approached from the viewpoint of ecological relevance of these groups in aquatic environment. At least three ecologically functional groups can be distinguished. First, actively respiring bacteria, who are responsible for production and participating actively in chemical cycles as well as are main objects of selective grazing and viral

attacks. In this study, the active fraction of cells is determined by the ability of bacteria to utilize tetrazolium salts. Secondly, alive bacteria, which are determined by cell wall integrity. This is dynamic category which hosts cells with different activity levels, as well as dormant cells with intact cell walls. This group is like a gateway both to the heaven – to highly active state, or to hell – to cell death. Because of the inadequacy of methods it is difficult to establish whether some of these cells are truly dormant, inactive, or not growing. Precise definition of these physiological states is perhaps impractical in aquatic microbiology. Ecologically, the group of bacteria with intact cell walls maintains metabolism from low to high level participating in chemical cycles and are part of the food web. Third group, dead bacteria, is reported to form most abundant fraction among aquatic bacteria and should be considered only as detritus. Cell death is defined in this study as a lack of cell wall integrity.

A fundamental characteristic of cells is their ability to regulate growth in response to changing environmental conditions. Heterogeneity in an aquatic environment should lead bacteria to adapt physiologically as they attempt to maximize growth or survival, and should create a broad spatial-temporal diversity of niches in which different microbial physiological states may have a selective advantage. There is no question that at least some bacterial species are exquisitely well adapted for long-term survival (to centuries) in media with no energy sources (Ducklow, 2000). There is an enormous literature on the physiology and biochemistry on bacterial starvation-survival in cultures, and in various natural media and aquatic environments (Armada et al., 2003; Joux et al., 1997; Morita, 1997). Morita (1997) put forward the view that most of the biosphere is highly oligotrophic with respect to bacterial nutrition, and most bacteria in most habitats are in starvation-survival state. This view is supported by substantial evidence that sometimes a sizable fraction (<10 to >75%) of marine and limnetic bacterial assemblages are not active, as indicated by autoradiography (Karner and Fuhrman, 1997; Ullrich et al., 1996) or possess a vital respiratory chain (Freese et al., 2006; Rodriguez et al., 1992; Schumann et al., 2003). These observations lead to the understanding that bacterial assemblages in nature appear to be dominated by small, highly active subpopulations coexisting with large groups of less active, or perhaps temporarily inactive cells. In this thesis, I follow the concept of Smith and del Giorgio (2003) that there is a nested hierarchy of physiological states within natural bacterial communities and that there is no inherent problem with some methods yielding low proportions of “reactive” cells, because it is not unrealistic to think that a small fraction of the assemblage has much higher rates of activity than the rest. I believe that the actively respiring cells (ARC), having the highest metabolic activity, are responsible for the bulk of bacterial community metabolism in the aquatic environment.

The triggering factors of activation and inactivation are largely driven by growth environment. In general, physical factors (e.g. climatic conditions, oxygen, salinity, pH, pressure) are shaping the overall system favouring certain groups of bacteria. Chemical regimes are determining the food base where bacteria can grow up to exponential growth. Biological parameters are func-

tioning rather as abundance decreasing factors either through resource competition or by grazing and viral attacks. Given the diversity that coexists in natural assemblages, and the fact that not all bacterial populations are favoured by same conditions resulting in different growth rates, it should not be surprising that there is a full range of metabolic states in an assemblage.

The introduction of fluorescence microscopy lead to milestone discoveries in aquatic microbiology. Total bacterial abundance and biomass increased up to four orders of magnitudes compare to earlier studies in freshwater and ocean. This total number of bacteria has been the basic parameter estimated in numerous studies during the existence of aquatic microbial ecology. If we assume that at least half of the cells that contribute to the total number of bacteria are actually dead we have overestimated bacterial abundance by one or more orders of magnitude.

## **2.2. Cellular traits to determine physiological status of bacteria**

### **2.2.1. Bacteria with active respiratory system**

Tetrazolium salts, the artificial acceptors of electrons, are increasingly used as an indicator of the enzyme – dehydrogenase activity of an individual bacterial cell, also referred to as the activity of the electron transport system (ETS) and actively respiring bacteria (ARC; Fig. 1). Zimmermann et al. (1978) suggested using 2,4-iodophenyl-3,4-nitrophenyl-5-phenyl tetrazolium chloride (INT-dye) decomposed by the respiratory chain enzymes, for quantitative determination of active microorganisms in natural ecosystems. Rodriguez et al. (1992) developed the method by using CTC fluorogenic tetrazolium salt, i.e. triphenyltetrazolium chloride. Tetrazolium dyes are reduced from a colourless complex to a brightly coloured, intracellular, formazan precipitate by components of the electron transport system and/or a variety of dehydrogenase enzymes present in active bacterial cells. Since electron transport is directly related to cellular energy metabolism in respiring cells, the ability of cells to reduce tetrazolium compounds can be considered an indicator of bacterial activity. The fluorescent nature of CTC, despite to its similarity to INT-dye, allows faster and more convenient determination of intracellular formazan deposits. In addition, CTC is subject to easier and faster reduction as compared to INT-dye (the redox potential for CTC reaches 200 mV and  $\text{pH} \geq 4$ , whereas for INT-dye 90 mV and  $\text{pH} \geq 5$ ) and can be used in a wider range of pH (CTC 5–10, INT-dye 5.5–8.5; Smith and McFeters, 1997). The concentration of CTC applied in analyses of bacterioplankton and bacteriobenthos ranges between 1 and 10  $\text{mmol}^{-1}$  (Berman et al., 2001; Choi et al., 1999; Freese et al., 2006; Haglund et al., 2002; Rodriguez et al., 1992; Sondergaard and Danielsen, 2001).

The upper threshold levels may produce toxic effect on bacteria in samples (Lebaron et al., 2002; Servais et al., 2001; Ullrich et al., 1996). As the

incubation time for samples with CTC should be determined experimentally, it can differ from one experiment to another (Bartscht et al., 1999; Proctor and Souza, 2001). In general, the optimal time is in between 2–8 h for technique utilizing a fluorescence microscope, although some assays may involve an incubation time of up to 24 h (Berman et al., 2001; Choi et al., 1996; Sondergaard and Danielsen, 2001). Additional staining of all cells in an analyzed sample with fluorochromes, like DAPI, acridine orange or SYBR Green II, facilitates determination of active bacteria and a parallel count of the total bacterial number. Cells displaying dehydrogenase activity, during observation under an epifluorescence microscope with an excitation filter 546 and barrier filter 590, demonstrate the presence of brightly red formazan deposits (Choi et al., 1996; Howard-Jones et al., 2001).

Dehydrogenase activity of bacterial cell has correlated well with measures of either bacterial production (e.g. del Giorgio et al., 1997; Sherr et al., 1999), or respiration (Smith, 1998) in various ecosystems. The proportion of total cells scored as ARC tends to be low, generally less than 20% and sometimes just a few percent. These low proportions have motivated much of the criticism over CTC as a measure of activity in bacterial cells. While the use of this method has spread over the years (e.g. Haglund et al., 2002; Jugnia et al., 2000; Sherr et al., 1999), there is no lack of criticism over CTC as a means of distinguishing metabolically active cells (Karner and Fuhrman, 1997; Servais et al., 2001; Ullrich et al., 1999; Ullrich et al., 1996). The comparison with other methods of activity assessment have suggested that CTC staining underestimates the “true” abundance of active cells.

Microautoradiography (MAR), one of the earliest single-cell methods developed (Hoppe, 1976), is widely held as being the most sensitive technique for enumerating metabolically active bacteria (e.g. Karner and Fuhrman, 1997; Ullrich et al., 1999). This method detects bacteria that are actively engaged in substrate uptake (as measured by a radiolabeled tracer, typically thymidine, an amino acid, or a mixture of amino acids), and therefore presumably active in growth and metabolism. Critics of the CTC method points out that MAR detects a higher number of active bacteria than CTC does, but MAR results certainly do not support the notion that most bacterioplankton cells are uniformly active and growing. I suggest that it is not the method that should be criticised, but the interpretation of the results. CTC results should not be interpreted as MAR results. What the MAR results do suggest is that there is a large heterogeneity, even within a given system, in the proportion of cells that are apparently active in substrate uptake.

The second issue pointed out by Smith and del Giorgio (2003) I would like to address, is the intuition of many that reports of low fractions of active bacteria must, *a priori*, be wrong, simply because the numbers are low. The fact that a method may account for a high proportion of cells does not necessarily imply that it provides a more effective description of the distribution of single-cell activity that is “more correct”. Different methods have different thresholds and metabolic “targets” along the continuum of physiological states, and the

lack of agreement among them points to the large range of criteria possible for describing metabolic activity in bacterial communities (Smith and del Giorgio, 2003). It may be possible that the CTC method detects only this fraction of cells that have the highest metabolism (Smith and del Giorgio, 2003), the greatest role in transfer of energy and matter, also the highest RNA content (Oda et al., 2000), and consequently the highest fluorescence intensities as noted by detection with fluorescent in situ hybridization (del Giorgio and Bouvier, 2002). In this thesis, the concept that cells scored as ARC are having the highest metabolic activity is followed.

There are also other options to measure bacterial activity in single cell level (Fig. 1). Several observations have confirmed that different dyes can be loaded into bacteria and are subsequently actively removed by energised cells by pump activity. This is the case for Rh123 (Ueckert et al., 1995), carboxyfluorescein (Bunthof et al., 1999), and ethidium bromide (Nebe-von Caron et al., 1998). However, pump activity assays have only been applied to a few species in culture and are not universal enough to be applied to environmental samples.

### **2.2.2. Detection of cytoplasmatic membrane integrity**

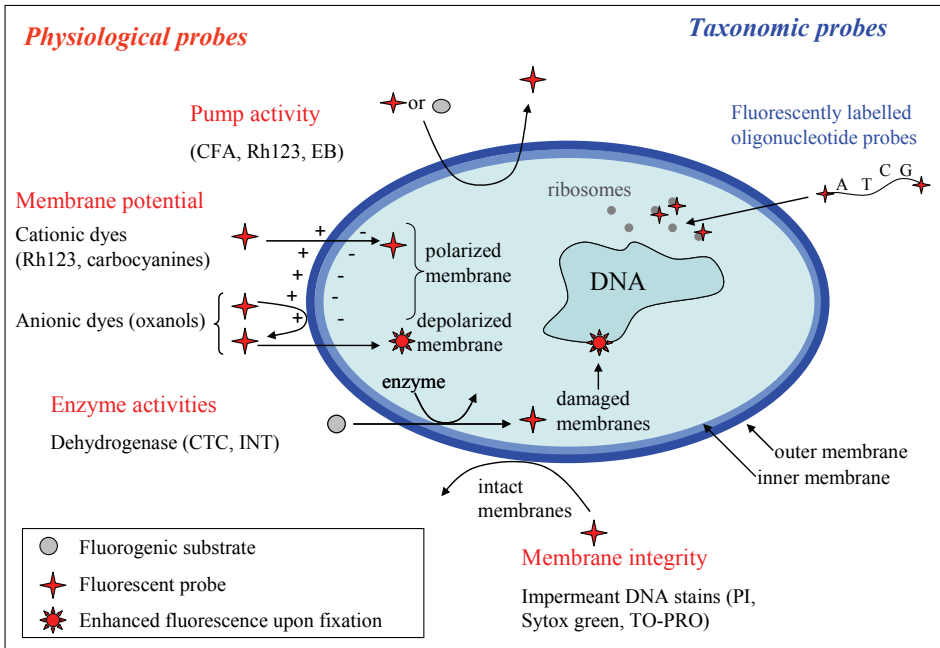
Membrane integrity as another aspect of the cell structure and physiology is one of the better-accepted criteria for distinguishing live cells from dead cells because of the assumption that cells with damaged membranes can not sustain any electrochemic gradient and are not able to resume growth. The loss of membrane integrity represents significant damage to the cells due to multiple functions linked to the plasma membrane (permeability barrier, transport, respiratory activity, etc.). Membrane integrity analysis is based on the capacity of the cells to exclude various chemical compounds, e.g., fluorescent dyes, which, when used at low concentrations do not normally pass through intact membranes. Most of the membrane integrity assays use nucleic acid stains, due to the high concentrations of nucleic acids within the cells and the large fluorescence enhancement exhibited by nucleic acid stains upon binding, leading to a clear separation between cells with intact and damaged cell membrane. The loss of membrane integrity as measured by uptake of membrane-impermeant dyes is generally considered irreversible and denotes cell death.

Different protocols and methodologies have been proposed to detect cell membrane damage in bacterioplankton cells (Fig. 1). Apart from direct inspection by transmission electron microscopy (Heissenberger et al., 1996), membrane polarity and integrity have been measured with probes such as ethidium bromide, calcofluor white, oxonols, carbocyanines, TO-PRO, Sytox Green, etc. (del Giorgio and Bouvier, 2002; Lebaron et al., 1998; Maranger et al., 2002; Mason et al., 1995; Schumann et al., 2003). The most widely used is probably propidium iodide (PI; e.g. Williams et al., 1998). Few researchers have used PI alone in studies of environmental samples (Lopez-Amoros et al., 1995), probably because of the methodological difficulties optimizing PI staining when it is used alone.

Most of these stains are nucleic acid labelling molecules that are either sized so that they do not pass through a healthy membrane but cross the membrane when it is damaged (Falcioni et al., 2008; Fig. 1). Lack of membrane permeability to large molecules (molecular weight 402–1355 Da of impermeant nucleic acid dyes supplied by Molecular Probes) is one of the properties of membrane integrity. These methods do not detect dead cells that have lost their cytoplasmic contents (Howard-Jones et al., 2001).

In order to simultaneously detect dead and intact cells double-staining with dyes which differ in their spectral characteristics and their ability to penetrate intact bacterial membranes is used. The principle of these protocols is based on energy transfer from an excited donor (the green stain) to an acceptor molecule (the red stain) according to the fluorescence resonance energy transfer phenomenon: the green stain is quenched in the presence of the red stain, and energy is transferred to the latter one if present (Falcioni et al., 2008). In several double-staining protocols PI with generic nucleic acid stain is used. The protocol devised by Barbesti et al. (2000) where PI is used together with Sybr Green, which is also applied to enumerate total bacterial abundance (Marie et al., 1997) is considered cheaper and more flexible than commercial kits. This methodology is applied both, in freshwater and marine bacterioplankton (Alonso-Saez et al., 2006). Another double-staining protocol Live/Dead BacLight viability kit (Molecular Probes) used in this study, has been applied in various areas of bacteriological research. Live/Dead BacLight viability kit was first applied by Lloyd and Hayes (1995) and to marine bacteria by Naganuma (1996) and Choi et al. (1996). One component of the kit, SYTO-9, penetrates inside cells with both intact or damaged membranes, staining the cells green, whereas PI only penetrates cells with damaged membranes, staining the cells red. When the dyes are used in combination, cells with intact membrane show a green fluorescence while cells with damaged membranes show a red fluorescence (SYTO-9 emission contributes to the excitation of PI by energy transfer). According to the manufacturer, SYTO-9 should penetrate intact membranes of a large number of Gram-negative and Gram-positive bacteria. However, Langsrud and Sundheim (1996) found that 30% of *Pseudomonas aeruginosa* strains tested ( $n = 18$ ) did not accumulate SYTO-9. To overcome this problem, other membrane permeable stains have been tested in combination with PI (e.g., SYTO-13; Davidson et al., 2004). Other authors have used other combinations of stains e.g., Hoechst and PI (Nebe von Caron and Badley, 1995); and Syto13 and Sytox Orange (Biggerstaff et al., 2006). Using flow cytometry, the interpretation of the green-versus-red-fluorescence cytograms could provide information not only on live (green) and dead (red) cells but also on “green-plus-red” particles, which have been identified as damaged cells (Gregori et al., 2003).

In addition to above described methods a new nanotechnological tool to count live or dead bacteria was introduced in 2009. This process measures the nanomechanical properties of cells walls by atomic force microscopy (Cerf et al., 2009).



**Figure 1.** Different cellular target sites for physiological and taxonomic fluorescent dyes. Modified after Joux and Lebaron (2000). CFA – carboxyfluorescein, Rh123 – rhodamine 123, EB – ethidium bromide, CTC – 5-cyano-2,3-ditolyl tetrazolium chloride, INT-dye – 2,4-iodophenyl-3,4-nitrophenyl-5-phenyl tetrazolium chloride, PI – propidium iodide.

### 2.3. Implication for bacterial growth and production

It is common practice in aquatic microbiology to scale growth, metabolism and uptake of organic substrates to the total bacterial abundance estimated using conventional epifluorescence techniques or flow cytometer. The resulting specific growth, production, respiration and uptake rates are extremely variable, both among and within the systems (del Giorgio et al., 1997), and much research in aquatic microbiology has focused on the mechanisms that regulate these rates. Based on the knowledge that only a small fraction of cells is active and productive in aquatic habitats, a smaller number than total bacterial abundance should be used in specific activity calculations. The bacterial production rates and fluxes measured by current methods are not affected by this consideration. However, specific rates of growth and activity must be higher if fewer cells are actively engaged in the process of interest. This argument opens up the possibility that bacteria in nature might be growing at rates substantially greater than estimated from bulk properties.

What is the growth rate at individual cell level in natural environments is not known. Already more than 40 years ago it was generally believed that aquatic

bacteria have variable generation times between 11 min to 11 h with mean about 4–5 h (Wood, 1967). Some historical records estimated mean generation times of bacteria 53 h with range from 20 to 200 h (Jannasch, 1969). Following decades has not changed the general paradigm – even the most famous slow growing bacteria from the SAR11 cluster (*Pelagibacter* spp.) demonstrated generation times of about 40 – 60 h (Rappe et al., 2002). Therefore, we have no direct evidence that individual bacteria normally grow more slowly than generation times above 100 h as often reported in environmental studies where production is scaled to total number of bacteria.

Unrealistically low bacterial growth rates ( $<0.02 \text{ day}^{-1}$ ) and long generation times (30–300 days) are reported for oligotrophic Arctic Waters (Anderson and Rivkin, 2001; Olli et al., 2007; Sherr and Sherr, 2003), whereas rates  $>1 \text{ day}^{-1}$  are commonly reported for more productive marine and freshwater communities (Riemann et al., 1984). However, if bacteria are isolated and their substrate turnover and growth is measured in the laboratory, they reach much higher rates than in nature (Kirchman et al., 1995).

In lakes, as well as in oceans, it is well accepted that bacterial assemblages are turning over very slowly in the most unproductive areas. This conclusion is drawn from observations that total bacterial abundance is relatively high but bacterial production is low in the most oligotrophic ecosystems (Ducklow and Carlson, 1992; Fuhrman et al., 1989; White et al., 1991). Along a gradient of increasing primary productivity, bacterial production varies greatly but the total bacterial abundance changes only slightly and is globally remarkably stable. In a study of 14 lakes del Giorgio et al. (1997) concluded, that growth rates scaled to active cells were, on average, similar in lakes and did not show any pattern along a gradient of increasing chlorophyll concentration, so there was no systematic change of bacterial growth rates with lake productivity. In contrast, growth rates scaled to the entire bacterial assemblage were positively correlated to chlorophyll, had a tenfold higher variability between lakes, compare to growth rates of active cells, and showed larger differences among lake strata. Scaling bacterial production to either the total number or the number of active cells thus results in very different patterns in bacterial growth rates among aquatic systems. More importantly, it appears that, for a given temperature, active cells in unproductive sites do not necessarily grow any more slowly than their counterparts in highly productive systems; they are simply less abundant (del Giorgio et al., 1997). This suggests that the differences in production must be related to changes in the number of intact or active bacteria. According to the logic of active bacterial detection, intact cells presumably have lazier electron transport system. However, the differences in activity levels are not known for us. If using the number of active bacteria in growth rate calculation we might slightly overestimate the overall growth rate. While growth parameters are calculated based on intact cell we are most likely underestimating specific rate of growth.

## 2.4. Resource limitation as structuring force of bacterial communities

The environment fosters an intricate combination of organisms, nutrients and physical matrices, harbouring almost immeasurable niche diversity. Within this context, microorganisms inhabit every conceivable location on earth, gaining energy by coupling most energetically favourable electron donors and receptors (Schmidt, 2006). In the optimum conditions biomass of bacteria can increase exponentially. It is frequently argued that bacteria growing at maximal rates are likely to be exception rather than the rule in many aquatic environments. Several factors affect bacterial growth resulting in constant balancing between favourable and unfavourable conditions for bacteria. Shortly, regulation of bacterial abundance by grazing and viruses; and gradients of physical and chemical parameters, which show considerable daily variation can affect bacteria. In the pelagic photic zone, nutrient limitation by, e.g. phosphate, available nitrogen, degradable organic material, iron and silica is believed to be a fundamental controlling factor for the community composition and activity of osmotroph microorganisms (organisms taking up dissolved nutrients *versus* phagotroph taking up particles). According to Liebig's law of the minimum, the yield of any organism is limited by the factor present in the lowest amount in relation to its requirements. Tilman's resource competition theory, tested on phytoplankton, states that under nutrient limitation in equilibrium conditions, those species which have either the lowest requirement for the limited resource or the highest ability to utilize it, will succeed in competition (Tilman, 1982).

Numerous studies have demonstrated inorganic N/P uptake by bacteria and/or growth limitation of these assemblages in a variety of marine and freshwater ecosystems (e.g. Carlsson and Caron, 2001; Kivi et al., 1993; Lignell et al., 2008; Thingstad et al., 1998; Zweifel et al., 1993). Collectively these studies indicate that bacteria utilize a significant fraction of organic matter and acquire N and/or P via the uptake of inorganic forms of these elements to their cells. Over 20 years ago, in the description of the microbial loop (Azam et al., 1983), bacterial carbon demand was recognised as potentially controlled by interactions between distinct factors such as bacterial physiology, predation, nutrient competition, and autochthonous release of dissolved organic matter from the food web. In many studies, planktonic bacteria have been found to be limited not only by inorganic nutrients, but also by a source of labile carbon (e.g. Carlsson and Caron, 2001; Kivi et al., 1993; Lignell et al., 2008; Thingstad et al., 1998; Zweifel et al., 1993).

The variations in the organic matter availability are typically accompanied by pronounced changes in bacterial abundance, productivity, ectohydrolase activities and colonization of particles (Smith et al., 1995). In principle, these changes may occur without major shifts in the phylogenetic composition of the bacterial community. Shifts in activity and surface attachment would represent plasticity in the bacterial phenotypes, with enzyme expression and growth being regulated in response to the available organic substrates (Riemann et al., 2000).

Alternatively, the observed changes could result from community succession, with bacteria with inherently different metabolic capabilities predominating at different times.

Beside the competition for limiting resources among species, competition between autotrophs and heterotrophs can shape bacterial activity and community composition in aquatic environments. Bacteria and phytoplankton are often thought to exist in a loose mutualism, where bacteria remineralize organically bound nutrients and phytoplankton supports bacterial growth with DOC (Daufresne and Loreau, 2001). Large fluctuations in primary production (PP) and algal biomass during the development of algal blooms cause distinct changes in the amount and quality of aquatic dissolved organic matter. However, under the conditions when bacterial production is limited by nutrients such as N and P instead of carbon, the relationship between algae and bacteria is considered to be rather competitive than mutualistic (Vadstein, 2000). Bacteria with higher growth potential, higher affinity for low nutrient concentrations and their large surface area to volume ratio should theoretically outpace phytoplankton in uptake of inorganic dissolved N and P. On the other hand, experiments and *in situ* observations show that slow growing and relatively uncompetitive phytoplankton can be successful in terms of biomass established in planktonic community (Lignell et al., 2003). Filamentous phytoplankton, without necessarily being superior in its physiological abilities for nutrient uptake, but subject to relaxed herbivorous pressure can grow side by side with heterotrophic bacteria in a nutrient-limited situation. Furthermore, the ability of nitrogen fixation of autotrophs, such as filamentous cyanobacteria *Aphanizomenon* sp, *Nodularia* sp and *Anabaena lemmermanni* typical in the Baltic Sea ecosystem, could be an extra advantage during N limitation.

Since eutrophication has become an increasing problem in marine coastal and estuarine areas, the question of nutrient limitation primarily of phytoplankton production and biomass has been central. It has been studied by a variety of methods such as nutrient contents of cells, nutrient uptake rates, physiological indicators and bioassays (Kuuppo et al., 2003 and reference herein). The overall conclusion of the bioassays carried out in coastal areas of the Baltic Sea is that the limiting nutrient varies not only spatially but also seasonally between phosphorus and nitrogen, while the open sea areas are largely nitrogen limited (Kivi et al., 1993; Seppälä et al., 1999). Bacterial productivity have shown to be controlled by the availability of both a carbon source and those nutrients that limit phytoplankton growth in the Baltic Sea (Lignell et al., 1992).

In case of permanently cold waters, bacteria have reported to live at temperatures well below their optima for growth, which implies a higher concentration threshold for utilization of dissolved substrates (Pomeroy and Wiebe, 2001). During polar winter, or under thick, snow-covered sea ice, substrate, originating mainly from autochthonous primary production might become limiting (Pomeroy et al., 1990; Rich et al., 1997). The lack of any unusual accumulation of dissolved organic matter in polar seawater shows that marine

heterotrophic bacteria do utilize labile DOC down to nanomolar concentrations at  $-1.8^{\circ}\text{C}$ . These results suggest that in polar region both free-living and attached bacteria are resource limited rather than directly affected by low temperatures. What is the fate of remineralization of organic matter in relation to vertical flux is not well studied.

In contrast to previous viewpoints, it has been suggested on theoretical grounds that substrate concentration normally should not be limiting to heterotrophic bacteria in the upper mixed layer (Thingstad and Lignell, 1997; Williams, 2000). Observations and experiments suggest that at least some heterotrophic bacteria have the ability to adjust their growth rate and body size according to the substrate concentration present (Thingstad et al., 2005; Wiebe and Pomeroy, 1972) and may be growing at near-maximum rates in both eutrophic and oligotrophic environments.

## **2.5. Bacterial community composition – who are they?**

Aquatic microbial ecology has long been challenged by the fact that only a minor fraction, i.e. much less than 1%, of the natural bacterioplankton community enumerated by light or epifluorescence microscopy can be cultivated, preferentially on solid media (Eguchi and Ishida, 1990; Kuznetsov, 1975; Razumov, 1932; Staley and Konopka, 1985). During the 1970s and 1980s, increasing evidence accumulated that the major fraction of the as yet uncultured bacteria actively grows and participates in the flux of energy and cycling of nutrients in aquatic ecosystems (Cole et al., 1988; van Es and Meyer-Reil, 1982). The next breakthrough occurred that same year with the development of polymerase chain reaction (PCR) technology (Mullis et al., 1986), which facilitated and invigorated cultivation-independent approaches. Employing purpose-designed oligonucleotide primers (Fig. 1), PCR methodology can be used to copy and amplify specific regions of DNA. PCR amplification, cloning, and sequencing of rRNA and DNA from environmental samples led to the discovery of numerous new taxa, and provided sound sequence information for the study of phylogenetic comparisons.

To improve efficacy, mainly from larger sample numbers, microbiologists have turned to DNA fingerprinting and to hybridization techniques. Fingerprinting methods take advantage of different properties of the amplified environmental sequences (e.g., sequence length, presence or absence of restriction sites, melting behaviour) to obtain a qualitative representation of the presence and abundance of different phylotypes in a sample. The application of molecular techniques based on PCR, such as the establishment of clone libraries and denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene sequences, revealed that the bacterioplankton community is constituted of many bacteria and phylotypes that had not been detected by culture based techniques (Giovannoni et al., 1990; Giovannoni and Rappe, 2000; Fuhrman et al., 1993;

Muyzer et al., 1993; Schmidt et al., 1991; Urbach et al., 2001; Zwart et al., 1998). By profiling the genetic diversity, composition, and structure of microbial communities, these techniques are valuable for tracking genotypic community changes over time, as well as for comparative analysis of microbial communities inhabiting different environments. Even though each of the methods has advantages and disadvantages, when they are used in combination, this “rRNA approach” for phylogenetic discovery and community study has been highly successful (Amann et al., 1995).

The difficulties facing researchers today relate less to the application of a specific method than to the need to understand what the methods tell us about the system and the ecological roles of the observed organisms (Amann et al., 1995). A considerable challenge is to understand bacterial interactions with each other, with autotrophs and predators in the relation to the environment. What heterogeneity in time and space do microbes really experience, and how do these effects shape microbial activity and microbial reactions at different scales?

It is reasonable to expect that changes in bacterial community composition are driven by the same environmental factors that affect bacterial abundance and activity (Riemann and Middelboe, 2002). There is also evidence for differences at taxonomic level in important ecological traits. For example Alphaproteobacteria and Betaproteobacteria have been observed to respond to predation differently (Jürgens et al., 1999; Pernthaler et al., 1997). Differences in competitive ability for resource acquisition have also been observed. For example, members of the Bacterioidetes group are distinctive in their ability to degrade high molecular weight polymers, although they can degrade low molecular weight compounds with reduced efficiency (Kirchman, 2002b). Various mesocosm studies have shown that distinct DOC components via direct supply or the experimental induction of phytoplankton blooms select for specific bacterial subcommunities or populations (e.g. Abell and Bowman, 2005; Lebaron et al., 1999; Pinhassi et al., 2004; Riemann et al., 2000; Schafer et al., 2001). The specific organic matter profile of various algae appears also to be an important selection factor for distinct bacterial communities and populations evolving in the phycosphere of algae (Rink et al., 2007; Schafer et al., 2002). In fact, Alphaproteobacteria, in particular the Roseobacter-clade, and the Sphingobacteria/Flavobacteria group appear to be most responsive to increased availability of phytoplankton-born DOC and organic sulfur compounds (Fandino et al., 2001; Grossart et al., 2005; Pinhassi et al., 2004; Riemann et al., 2000).

Long-term monitoring of aquatic bacterial communities with molecular fingerprinting methods have shown that community composition follow annually reoccurring patterns (Crump and Hobbie, 2005; Fuhrman et al., 2006; Shade et al., 2007). The recent efforts to model, formalize and explain temporal changes in microbial communities builds on an existing body of knowledge, wherein pronounced seasonal changes in certain components of bacterioplankton communities have been described in response to environmental change (Kan et al., 2006; Pernthaler et al., 1998; Pinhassi and Hagstrom, 2000; Schauer et al., 2003). Anderson et al. (2010) reported in study using massive deep

sequencing of ssuRNA gene fragment of the Baltic Sea bacterioplankton that community change was most highly correlated with change in phosphorus concentration and temperature leading to dramatic variation over time and repeatable high seasonal succession.

The highly dynamic nature of bacterial community composition suggests that the changes occurring in natural phytoplankton communities lead to significant effects on the structure and functioning of bacterial assemblages as well as on the nature and the rates of bacterially mediated organic matter cycling (Riemann et al., 2000). Accordingly, shifts in bacterial community structure may be important to carbon and nutrient flows in lakes and ocean (Fandino et al., 2001).

Another avenue of research has been to evaluate the view that “everything is everywhere”. It was first formulated by Beijerinck (Brock, 1961 by Pommier et al., 2005) and newly reintroduced by Fenchel and Finlay in a series of publications (e.g. Fenchel and Finlay, 2004). The recent analysis of 70 ssu rDNA sequences submitted to GenBank showed, that marine bacterioplankton components present unexpected global patterns of distributions including a high degree of endemism, where cosmopolitanism appears as an occasional trait (Pommier et al., 2005).

Although molecular methods have revolutionized microbial ecology, the field continues to profit from novel methodological developments occurring at breakneck speed (Logue et al., 2008). Techniques introduced in recent years, often derived from the medical field, are important new tools for studying microbial communities in greater detail. A key issue that many of these innovative techniques address concerns tighter linking between observed microbial diversity with ecological functions and biological strategies. The previous studies have emphasized the need to incorporate community composition into our conceptual thinking of the biogeochemical activities of marine and lake microbial assemblages.

## **2.6. Changes in bacterial morphotypes – ecological meaning**

Kevin D. Young (2006) wrote in his review about selective value of bacterial shape: “To be brutally honest, few people care that bacteria have different shapes. Which is a shame, because the bacteria seem to care very much”. The life strategy of species and an individual organism determines its competitive ability within the trophic level. Morphology (size and shape) as a part of a microbe’s life strategy is an important characteristic that affects cell physiology and trophic interactions. Bacteria actively modify their shapes. Some changes are temporary when moving from one growth phase to another or responding to nutritional alterations; or some accompany development of specialized cell structures like spores (Young, 2006). The variety of shape hints that organisms adapted a trait to cope with diverse environmental niches or conditions.

Uniformity, in the other hand, implies that there is a functional advantage to individual expression of trait (Young, 2006).

Some microbes possess a considerable degree of phenotypic plasticity with respect to cell size and morphology (Corno and Jürgens, 2006; Hahn and Hofle, 2001) due to natural selection. Grazing, as structuring force of bacterial standing stock, taxonomic structure and morphology, is well recognized. Since protists and metazooplankton are constantly seeking for food the success of bacteria to remain uneaten depends on the size and abundance of grazer as well as on the activity, abundance and morphology of bacteria. The selective grazers have shown to prefer active bacteria with suitable size for eating (del Giorgio et al., 1996). The measurements from 27 oceanic locations have shown that the percentage of phytoplankton (including bacteria) grazed per day range from 3 to 131% of standing stock (Sherr and Sherr, 2002). Cladocerans and rotifers were able to graze 4.1% of bacterial daily production in lakes (Agasild and Nøges, 2005) while ciliates have reported to be able to graze more than 100% of bacterial daily production (Kisand and Zingel, 2000). These examples show that grazing pressure can be very high most often either by heterotrophic flagellates or ciliates, depending to the food web. Grazing is size-selective with most protists feeding preferentially on medium-sized bacterial cells (Hahn and Hofle, 2001). As a result it leads to bi-directional shift in size distribution and domination of either by small bacteria (e.g. ultramicrobacteria) or by large morphotypes (e.g. filamentous forms, aggregates) under strong grazing pressure (Boenigk et al., 2004; Jürgens et al., 2000; Pernthaler et al., 2004). In addition to direct grazing effects to bacterial morphotypes the filament formation have shown to be stimulated by the grazer excretory products (Corno and Jürgens, 2006). In recent years small and filamentous bacteria have found not to be immune to grazing but having either size-independent species-specific vulnerability to grazing (Boenigk et al., 2004; Tarao et al., 2009) or are strongly reducing ingestion efficiency by bacterivorous flagellates (Wu et al., 2004).

It has been demonstrated that filamentous bacteria can belong to the phylogenetic groups of Alpha- and Beta-subclass of Proteobacteria as well as to the Bacteroidetes group (former name CFB – Cytophaga-Flavobacterium-Bacteroidetes group; Hahn et al., 1999; Jürgens et al., 1999; Simek et al., 1999). Among the numerous publications which have reported the presence of grazing-resistant morphotypes in bacterioplankton, only a few descriptive field studies have demonstrated a correlation of high protistan grazing pressure and occurrence of morphologically defined grazing-resistant bacteria (Güde et al., 1985; Jürgens and Stolpe, 1995; Pernthaler et al., 1996). Several laboratory studies, however, have demonstrated that filamentous and other complex morphologies provide efficient protection against protistan grazing (e.g. Hahn et al., 1999; Simek et al., 1997).

Filamentous morphology may provide other advantages. Elongation, supported by genotypic and phenotypic plasticity can be ecologically beneficial to bacteria by increasing a cell's uptake-proficient surface without changing its surface-to-volume ratio appreciably (Young, 2006).

Environmental microbiologists have long appreciated that bacterial morphology varies with growth rate and nutritional conditions. Cell size and shape plays a central role because they are related to diffusive nutrient transport towards the organism, and loss factors such as respiration and predation are size-dependent. Perhaps the most frequent shape change due to nutritional stress is filamentation, triggered by a limitation in the availability of one or more nutrients or carbon supply (Steinberger et al., 2002). In experimental study by Corno and Jürgens (2006) carbon starvation resulted in a rapid decrease in cell length and a shift towards small rods, which were highly vulnerable to grazing. Glucose addition, when unbalanced with respect to inorganic nutrients have found to lead to a bacterial community dominated by large “sausage”-formed bacteria (Havskum et al., 2003; Malits et al., 2004). Thingstad et al. (2005) have argued that this may be a specific strategy whereby some osmotroph microorganisms use a non-limiting substrate to simultaneously increase nutrient affinity and reduce predation.

As shown by Corno and Jürgens (2006) the distribution and frequencies of the different morphologies can be determined both by the substrate concentration (and resulting growth rate) as well as the actual grazing pressure. The direction of the response of bacteria to both selection factors is probably highly strain specific.

## 3. MATERIALS AND METHODS

### 3.1. Study areas and sampling

#### Paper I

Bacterial biomass and activity in the marginal ice zone of the northern Barents Sea was studied as a part of the international CABANERA project focusing on the pelagic benthic coupling processes in the northern Barents Sea. The field work was carried out during two expeditions (8–22 July 2003; 20 July–3 August 2004) onboard R/V Jan Mayen (University of Tromsø, Norway). The station locations selection was guided by compare–contrast approach based on our knowledge on Atlantic and Arctic water regimes and ultimately constrained by the ice conditions (Fig. 2). By large, the two expeditions took place during the same ecological season, but large variability from pre-bloom to post-bloom ecological settings was recovered at different locations, as well as a range of ice cover from 30% to 80%.

CTD profiles and water-column samples were taken at each station with a General Oceanic Rosette sampler equipped with 5 l Niskin bottles from fixed depths (1, 5, 10, 20, 30, 40, 50, 60, 90, 120, 150, 200 m, or as deep as the bottom topography allowed) and from the chlorophyll maximum (determined by the fluorescence profile of the water column). In station VII (Nansen Basin, off the Barents Sea shelf) additional samples were obtained from 300, 400 and 500 m.

To collect sinking aggregates, floating arrays of sediment traps (20, 30, 40, 50, 60, 90, 120, 150 and 200 m, or as deep as the bottom topography permitted) were moored to ice flows in each station and recovered ca. 24 h later. No bafflers were used in the cylinder opening and no poison was applied during the deployment. Upon retrieval, the contents of the sediment trap cylinders and Niskin bottles with water-column samples were drained into separate plastic containers and kept in dark at 0 °C until processing (<0.5 h).

#### Paper II

The sampling was carried out from July 29th to August 1st 1998 in three small stratified lakes in South Estonia (Fig. 2). The description of studied lakes is summarised in Table 1, **II**. All lakes studied were small moderately deep freshwater lakes, dimictic or biogenically meromictic with anoxic hypolimnetic waters. Physical and chemical parameters determined stable stratification during the study period. Relevant parameters for stratification definition were measured before sampling. Thereafter whole water column of the lakes was divided into three different layers: epi-, meta- and hypolimnion, according to temperature and oxygen content. Epilimnion was the uppermost layer with the highest and homogeneous temperature and oxygen concentration. Water layer fitted to the thermo- and oxycline was defined as metalimnion, this meant rapid decrease of temperature ( $>1.5^{\circ}\text{C m}^{-1}$ ) and oxygen concentration ( $>1.5\text{ mg O l}^{-1}\text{ m}^{-1}$ ) with small increase of depth. The deepest and also homogeneous hypolimnion did not have oxygen and the temperature was low and stable.

Samples were taken from 8 different depths by 2 l Ruttner sampler (length of the sampler 50 cm): two samples from epilimnion, 4 samples from metalimnion (interval from 0.5 m to 2 m) and 2 samples from hypolimnion (Table 1 in **II**). Sampling and field measurements were performed at noon (11:00–14:00 h).

### **Paper III**

The mesocosm experiment was carried out from 1 to 21 July 2003 in Tvärminne archipelago (Storfjärden station, depth 20–36 m) in the north-western part of the Gulf of Finland (Fig. 2), the Baltic Sea as a part of DANLIM project. This area is characterized by low salinity (ca. 6–7), absence of a permanent halocline and rapid changes in water mass with changing meteorological conditions (Niemi, 1975). A natural water column was enclosed in nine floating transparent plastic bags (double-layered polyethylene interspersed with reinforcing glass-fiber threads) each with a total volume of 51 m<sup>3</sup> (diameter 2.3 m; 12 m deep main cylindrical part and 2 m conical bottom part). The mesocosms were first lowered to about 15 m depth, then filled with water by lifting the collar up. All nine mesocosms were fastened to wooden rafts and connected separately to a central buoy (distance ca. 30 m from each mesocosm) to ensure even light conditions. Plastic covers were used to protect against contamination by birds, but exchange of gases between air and sea was not prevented.

During the first five days (hereafter referred to as the boosting period) eight mesocosms were treated daily with mineral nutrients (N and P; N = 1  $\mu\text{mol NH}_4\text{Cl-N l}^{-1} \text{d}^{-1}$ , P = 0.06  $\mu\text{mol KH}_2\text{PO}_4\text{-P l}^{-1} \text{d}^{-1}$ ) to induce a phytoplankton bloom. Thereafter, different combinations of N, P and labile dissolved organic carbon (13.3  $\mu\text{mol glucose-C l}^{-1} \text{d}^{-1}$ ) treatments were applied over 2 weeks (hereafter referred to as the experimental period) to evoke diverse nutrient limitation situations, controlling the carbon limitation of the bacteria and algal-bacterial competition for nutrients. Nutrient supplements five times greater than the N and P treatments used initially were supplied to create extreme nutrient limitation in the osmotrophic algal and bacterial communities. The ninth mesocosm received no nutrients during the whole experiment and served as a control (Table 1 in **III**).

Vertically integrated surface samples (0–7 m) of the mesocosms at 1 m intervals were taken at 06:00–08:00 h with a 7-l Limnos water sampler. Nutrients were added daily at 10:00–11:00 h (detailed technical description in Olli and Seppälä, 2001).

### **Paper IV**

Hypertrophic and stratified Lake Verevi, Estonia (Fig. 2) was sampled from 3 to 8 layers based on premeasured the oxygen and temperature at the deepest point of the lake. In 1991, 1993, 1994 and 1998 sampling was carried out by Ruttner or van Dorn sampler. In 2000 and 2001 a water pump (Masterflex N 7533–60) with “easy-load” pumphead (model 7518–12) connected to a tube (diameter 8 mm), designed for study of thin (20–25 cm) water layers, was used for sampling. In a diurnal study the samples were taken at 1 m intervals from the

layer of 0.5 to 7 m at 12:00 and 16:00 in August 2, and at 8:00 and 12:00 in August 3, 2001.



**Figure 2.** The map of studied areas.

## **3.2. Analytical methods**

### **3.2.1. Fluorescent markers to determine physiological states of bacteria**

The total number of bacteria (TNB) was determined by DAPI (4',6'-diamidino-2-phenylindole; PolySciences Inc) staining. Formaldehyde (2% final concentration; **III**, **IV**) or glutaraldehyde (2% final concentration **I**, **II**, **IV**) preserved sub-samples (2–5 ml) were incubated with DAPI (final concentration  $10 \mu\text{g ml}^{-1}$ ) for 5 min in the dark.

In paper **I** bacteria with compromised cell membrane (hereafter referred to as PI-stained cells) and intact cell membrane were distinguished by using LIVE/DEAD BacLight viability kit (MolecularProbes). One component of the kit,

green-fluorescing SYTO 9, serves as a viability marker. The second component, red-fluorescing propidium iodine (PI) stains cells with damaged cell membrane. Non-preserved samples (2–5 ml) were incubated for 15 min after simultaneous addition of both stains (1.5 mg ml<sup>-1</sup> final concentration).

In paper **II** membrane-compromised bacteria (MCB) were determined by 5 μM SYTOX Green (Molecular Probes Inc.) staining of 1 or 2 ml of fixed water sample for 5 minutes. The filters were double stained with DAPI for TNB as described above. Both the number of MCB and the total abundance of bacteria were counted on the same filter. DAPI staining was observed on the ultraviolet and SYTOX on the blue light. Intact bacteria (INT) were obtained subtracting membrane-compromised bacteria (MCB) from TNB (INT = TNB-MCB).

Actively respiring cells (ARC) were detected by staining with the fluorogenic tetrazolium dye CTC (5-cyano-2,3-ditoyl tetrazolium chloride, Polysciences Inc; Rodriguez et al., 1992). Non-preserved 1–2 ml subsamples were incubated with final concentration of 4 mmol l<sup>-1</sup> (**I**) or 0.5 mmol l<sup>-1</sup> (**II**) CTC (Polysciences Inc.) for 4 h based on previous calibration in the dark at in situ temperature. In paper **I** and the samples were counter stained with DAPI for 5 min (10 mg ml<sup>-1</sup> final concentration). ARC was determined by red fluorescence under green excitation light and DAPI-stained cells (hereafter referred to as total bacteria) were counted from the same filter under UV.

All staining procedures and incubations were done in darkness, at ambient pH, and near-ambient temperature. Incubation of live samples was terminated by adding glutaraldehyde (2% final concentration; **I**, **II**). After various staining procedures the samples filtered through a black 0.22 μm pore size polycarbonate filter (Osmonics Inc.) immediately after the end of incubation. Filters were air-dried, mounted into immersion oil and covered with a cover slip. The slides were kept frozen (-21°C) in dark until counting. Cell counts of bacteria were done as quickly as possible or within a 3 month period with Leica DMRB epifluorescence microscope with 100x oil immersion lens (PL Fluotar 100x/apertuur 1.30). Bacterial cells (usually 400 cells) on 15–30 fields were counted.

### 3.2.2. Bacterial biomass

Bacterial biomass in organic carbon units (BB) was calculated for each population of bacteria (TNB, ARC, INT) by using a conversion factor of 20 fg C cell<sup>-1</sup> (Lee and Fuhrman, 1987; **I**, **II**). Depth integrated bacterial biomass (mg C m<sup>-2</sup>) was calculated by trapezoidal integration over sampling depths (**II**). In paper **I** the vertical fluxes of bacterial biomass (mg C m<sup>-2</sup> day<sup>-1</sup>) were calculated from bacterial biomass in the sediment trap material and from the trap deployment time.

In paper **III** the biomass of nonfilamentous bacteria (hereafter referred to as BMcocc) was calculated using the empirically-determined indigenous summer bacterial C content of 18.5 fg C cell<sup>-1</sup> (unpublished). The biomass of filamentous bacteria (BMfila) was calculated using cell volume, obtained by mea-

suring the dimensions of the filaments stained with DAPI. Cells in each sample were measured on 40 microscopic fields with an ocular micrometer assuming a cylindrical geometry of the filaments. Filament volume was converted to cell carbon biomass by the factor  $0.22 \text{ pg C } \mu\text{m}^{-3}$  (Bratbak, 1985).

### 3.2.3. Bacterial production and growth rate

In paper **I** net bacterial production was determined with a dual labeling technique based on thymidine (Tdr) and leucine (Leu) incorporation as specified in Fuhrman and Azam (1982) and Kirchman et al. (1985), respectively. Three 10 ml replicates per sample were incubated for 2 h with  $10 \text{ nmol l}^{-1} \text{ }^3\text{H-Tdr}$  and  $50 \text{ nmol l}^{-1} \text{ }^{14}\text{C-Leu}$ . Thymidine incorporation was converted to cell production ( $\text{mg C m}^{-3} \text{ day}^{-1}$ ) by the factor  $1.1 \times 10^{18} \text{ cells mol}^{-1} \text{ }^3\text{H}$  incorporated (Riemann et al., 1987). Leucine incorporation was converted to protein production using the fractions 0.073 Leu/protein and 0.86 C/protein according to Simon and Azam (1989). Additional  $^{14}\text{C-Leu}$  incorporation to carbon production calculation was applied by a dilution factor of 2, as some isotope dilution is always present (Simon and Azam, 1989) and the correction factor of 1.27 because of the dual labelling approach with  $^3\text{H-Tdr}$  (Chin-Leo and Kirchman, 1988).

In paper **II** production of heterotrophic bacteria was measured in Lake Verevi and Lake Vellavere K ulaj rv by tritiated leucine incorporation (TLI) method (Kisand and N ges, 1998) in 3 replicates (+ 2 formalin killed blanks) in 5 ml water sample with  $^3\text{H-Leucine}$  addition ( $59.0 \text{ Ci mmol}^{-1}$ , Amersham Ltd., 15 nM). Leucine incorporation was converted into net carbon production (BP) using empirically determined conversion factor  $2.19 \text{ kg C mol}^{-1} \text{ Leu}$  (Kisand and N ges 1998).

In paper **III** net bacterial productivity (BP) was measured by a modified  $^3\text{H}$ -thymidine method (Fuhrman and Azam, 1982), the cells being separated from the liquid phase by centrifugation instead of filtration (Smith and Azam, 1992). Triplicate subsamples (each 1 ml) and one formalin-killed blank (2% final concentration) per mesocosm were dispensed in sterilised Eppendorf tubes after addition of  $^3\text{H}$ -thymidine (specific activity ca.  $80 \text{ Ci mmol}^{-1}$ ,  $10 \text{ nmol l}^{-1}$  final concentration; NEN Products) and incubated at in situ temperature for 1 h. Net bacterial productivity was estimated as in paper **I**, applying the  $^3\text{H}$ -thymidine conversion factor of  $1.1 \times 10^{18} \text{ cells mol}^{-1} \text{ Tdr}$  (Riemann et al., 1987).

In paper **IV** bacterial production was estimated by the tritiated thymidine incorporation method (Riemann et al., 1987). Triplicate 10 ml subsamples of each sample (+3 formaldehyde killed blanks) were treated with  $10 \text{ nM } ^3\text{H}$ -thymidine (Amersham; specific activity  $26 \text{ Ci mmol}^{-1}$ ). The subsamples were incubated 30 min at room temperature. Cold base–acid–ethanol extraction was used for purification of DNA as described by (Bell et al., 1983). The uptake of thymidine was converted to the number of produced cells by using conversion factor of  $2 \times 10^{18} \text{ cells per mole of incorporated thymidine}$ .

The bacterial growth rates (BGR;  $\mu$ ) of different physiological groups were calculated as bacterial production divided by bacterial carbon biomass according to Kirchman (2002a; **I, II, III**). Generation times were calculated as  $G = 1/\mu$ .

### **3.2.4. Bacterial community composition by PCR-DGGE (III)**

Water samples (100 ml) were filtered through 0.22  $\mu\text{m}$  pore filters (Poretics, 47 mm diameter). Nucleic acids were extracted from these filters using an SDS-polyphosphate buffer for lysis, zirconia beads for bead-beating and phenol/chloroform for extraction as described previously (Stevens et al., 2005). The primers GM5F (341F, with GC-clamp) and DS907RM, specific for *Bacteria*, were used to amplify fragments of about 550 bp by the polymerase chain reaction (PCR) using an Eppendorf Mastercycler with RedTaq™ (Sigma). Denaturing gradient gel electrophoresis (DGGE) was performed with a D-Code System (Bio-Rad) or DGGEK-1001–220 (CBS Scientific) according to Brinkhoff and Muyzer (1997), using a 20–70% denaturing urea/formamide gradient on a polyacrylamide gel for 20 h at 100 V constant voltage. Gels were stained with SYBR Gold (Molecular Probes) and visualized by a BioDoc Analyse Transilluminator (Biometra) or Typhoon Trio (Amersham).

Each band (DNA fragment travelling to a particular position) was assumed to represent a single operational taxonomic unit (OTU). Banding patterns were compared using band positions, and binary coding (1 – band existing, 0 – no band) was used to create a data matrix (Jaccard similarity matrix), which was analyzed by correspondence analysis (CA) to create a low-dimensional space of significant variation in the data matrix (ordination). Sample scores were used to analyze the statistical similarity or difference between samples in respect of community diversity. These differences (samples grouped by nutrient treatments as described above) were subjected to multivariate discriminant analysis and statistical significance was tested by the multivariate randomization test (1000 permutations).

For sequencing of PCR-DGGE bands DNA was excised from the DGGE gels with sterile pipette tips and small pieces of acrylamide gel were placed in 20  $\mu\text{l}$  sterile water and allowed to release solutes by diffusion overnight at 4°C. Parallel DNA bands from different samples (lanes) travelling visually to the same position were analyzed and used as a template for reamplification using the primers GM5F and DS907RM. The PCR products were purified with Quantum Prep PCR Kleen spin columns (Bio-Rad) and cloned into the pGEM Easy-T vector system (Promega). Clone identity was confirmed by a re-run of the DGGE gel to determine that their positions accorded with the bands that had been excised. The PCR products were then purified with Quantum Prep PCR Kleen spin columns (Bio-Rad) and directly sequenced using the Applied Biosystem 3730XL according to the manufacturer's directions.

The accession numbers of all the DGGE bands sequenced (EU878136 to EU878168) can be found at the GenBank website. The phylogeny of the sequences obtained was determined using nucleotide search at NCBI. Detailed phylogenetic analysis was performed with ARB software (Ludwig et al., 2004) using the 16S rRNA database version of ssu\_jan04\_corr\_opt.

## 4. RESULTS AND DISCUSSION

### 4.1. Objectives of the study

The present dissertation is based on the studies of ecological relevance of aquatic bacteria in contrasting environments like Estonian stratified freshwater lakes, the coastal Baltic Sea and the marginal ice zone of the Barents Sea.

The following questions motivated this thesis:

1. What are bacterial growth rates in various aquatic environments?
2. Does the number of active bacteria respond to increasing substrate availability?
3. Do the sinking organic aggregates produced in the euphotic zone host higher biomass of active bacteria than free-living in the watercolumn?
4. Does the availability or limitation of nutrients alter the bacterial community composition?
5. Are heterotrophic bacteria successful competitors for mineral nutrients compared to autotrophs in the presence of additional labile carbon?
6. What is the ecological meaning of the development of filamentous bacteria?

### 4.1. Low abundance of actively respiring cells

Overall, the actively respiring bacteria comprised ca. 10% of the total cell count both in the Barents Sea (**I**) and in Estonian small lakes (**II**). Low abundance of active aquatic bacteria compared to the total number of cells has been reported several times in the literature both in temperate and polar areas (Freese et al., 2006; Huston and Deming, 2002; Jugnia et al., 2000; Schumann et al., 2003; Sherr et al., 1999). It is acceptable that only a small fraction of cells is favoured by the conditions in the growth environment and are responsible for the production of new cells. However, there might be other reasons contributing to the low number of active cells. First, the low proportions of ARC might be partly due to the high electron transport activity required for bacteria to become stained with CTC (Sherr et al., 1999). In addition to methodical problems discussed earlier, low proportion of ARC could be the result of naturally occurring processes. The cells with compromised membrane, which prevailed in the total number of bacteria correlated well in the Barents Sea with the proxies of substrate availability like Chl, POC, PON, but also with bacterial production estimates (**I**). Also, active bacteria had a positive correlation with POC, PON and somewhat weaker correlation with Chl, bacterial and primary production (Table 4 in **I**). This indicates that the principal source for the PI-stained cells in the water column can only be the most actively growing bacterial fraction (e.g., the ARC). However, the mechanisms that contribute to the accumulation of dead bacteria are most probably resource limitation, and loss factors such as viral lysis and grazing.

Similarly to the Antarctic Ocean as reported by Davidson et al. (2004), the active bacteria in our Barents Sea study comprised approximately half of the intact bacterial population (**I**). In lakes, active bacteria formed mostly smaller fraction of INT (up to 31%; **II**). Choi et al. (1999) argued that the percentage of ARC increases with increasing substrate availability. This is intuitively understandable, but contrasts our results in the Barents Sea where ARC did not reveal particular concentration increase e.g. compared to the total count, in sites of elevated algal and bacterial activity, like the sub-surface chlorophyll and bacterial production maxima (**I**). Contrary to the Barents Sea, within Estonian small lakes the share of active bacteria on total number of cells depended on the availability of organic matter (**II**). In more productive Lake Verevi with higher BP:PP ratio increased abundance of ARC as well as higher percentage on TNB and intact bacteria compare to deeper Lake Vellavere K ulaj rv was observed (**II**). However, in the Barents Sea the cells with intact membranes had higher probability to score as actively respiring bacteria than in lakes (**I**).

In sites of high bacterial density, like the euphotic zone of lakes or the Barents Sea, as well as the sinking aggregates, resource competition might account in formation of microheterogeneity of nutrients. Enhanced activity in ‘hotspots’ could lead to rapid micro-scale substrate limitation, starvation and slow-down of the metabolism, switching the cells into an inactive state or death. If low-metabolic-activity cells are permeable to PI, as suggested by previous studies (Gasol et al., 1995; Hahn and Hofle, 2001; Monger and Landry, 1992), it could explain the accumulation of dead cells in our study (**I**, **II**), as well as in earlier works.

Another mechanism accounting for the formation of low abundance of active bacteria could be the selective ingestion of actively growing and dividing bacteria by nano-heterotrophs. If bacterivores are able to discriminate against dormant and dead bacteria, as have been demonstrated in numerous studies (e.g. Gasol and Del Giorgio, 2000; Hahn and Hofle, 2001), the latter may accumulate relative to the active ones in environments with elevated bacterial production and grazing rates like the sinking aggregates or chlorophyll and primary production maxima. Grazing selectivity can reportedly result in active bacteria being consumed at rates around four times that of inactive cells (del Giorgio et al., 1996; Gasol and del Giorgio, 2000). Preferential grazing on actively growing and dividing cells could partly explain the reduced abundance of active bacteria in sites of high microbial activity and concomitant accumulation of cells with compromised membrane (**I**, **II**). In addition to the above discussed mechanisms, viruses could play a role by attacking bacteria preferentially in higher density regions as shown by various reports (Steward et al., 1992; Thingstad, 2000; Thingstad and Lignell, 1997).

## 4.2. Sinking particles hosted similar number of active bacteria than water column (I)

On average, the contribution of total bacterial biomass ( $7.7 \text{ mg C m}^{-2} \text{ day}^{-1}$ , range  $2.9\text{--}38.2 \text{ mg C m}^{-2} \text{ day}^{-1}$ ) was a small, but measurable fraction (median 3.3%, range 0.9–22.1%) of the POC vertical flux in the Barents Sea. The fraction of ARC from the total count in the sediment traps was on average 0.09 (range 0.02–0.20). This fraction did not change much with depth, but differences between stations were notable with lowest numbers in the off-self station. Contrary to our hypothesis, sinking particles did not host higher numbers of active cells compare to free-living bacteria. However, intact cells were more likely to score as active in sinking particles. Why do our results contradict to previously observed higher abundances of active cells as a part of vertical flux in the Arctic Ocean? Two reasons are likely to contribute – time and space. At first the time of sampling is important due to the strict seasonality and inter-annual variability in the spring bloom connected with the melting and retreat of the ice-edge in association with lengthening daylight (Sakshaug, 2004). In our study, the sea-ice algal communities were not well-developed and POC vertical flux below the ice was relatively low (Reigstad et al., 2008; Tame-lander et al., 2008). Neither was the ice-water interface considered as a significant source of labile DOC (Gasparovic et al., 2007). Secondly, every locations has specific factors affecting the biological compartment of the ocean. Four times higher abundances of ARC in sediment traps compare to water column were observed in a very productive North Water polynia where the aggregates originated largely from ice-algae (Huston and Deming, 2002). Rich et al. (1997) reported a very high bacterial production (occasionally exceeding planktonic primary production) in the central Arctic Ocean, supported by a conspicuous ice-associated algal production. In contrast, Sherr and Sherr (2003) and Olli et al. (2007) reported relatively low bacterial production (compared to the primary production) in the central Arctic Ocean in combination with a poorly developed ice-algal community. According to Wassmann et al. (2006) the annual integrated primary production is higher and less variable in Atlantic ( $130 \text{ g C m}^{-2} \text{ year}^{-1}$ ) compared to Arctic water-influenced areas of the Barents Sea ( $70 \text{ g C m}^{-2} \text{ year}^{-1}$ ) with an overall average of  $93 \text{ g C m}^{-2} \text{ year}^{-1}$  for the Barents Sea. These examples are showing the regional variability of Arctic Ocean and point to the need to be careful in interpretation and extrapolation of data obtained from one location to another in polar areas.

Arctic region has received increasing attention in recent years due to the decline in the sea ice extent. One of the most serious challenges in this century will be to understand how climate change – past, present and future – influences life in the oceans. One of the issues to address is the importance of bacteria in the attenuation of vertical flux. The models have predicted warmer climate in the Arctic region with pronounced changes in the sea ice regime. The consequences are higher primary productivity and increased vertical flux of POC and PON. Are heterotrophic bacteria able to sequester the new production or

will it sink to the bottom? This question needs further attention because we lack the adequate baseline data with which to compare contemporary observations and to determine whether climate variability alters microbial metabolism and marine ecosystem services. Effectively we are conducting a global-scale experiment, but without a control.

### **4.3. High growth rates of bacteria both in eutrophic and oligotrophic areas**

In our studies (I, II) the bacterial production was normalized to different physiological groups assuming that the dormant and dead bacteria do not support growth (the exceptions are growth parameters calculated for TNB). Three categories of cells, active bacteria, intact bacteria and total number of cells, were under the interest. The main aim was to show the principal difference of scaling bacterial production to these categories of cells to answer ecological questions.

At first, the results of bacterial growth rates and generation times when normalized to total number of bacteria (Fig. 3). In the Barents Sea the bulk bacterial growth rates in the surface layer (0–50 m), based on Tdr incorporation and total cell counts, ranged from 0.04 to 0.16 day<sup>-1</sup> (0.27 in station XIII; **I**), average generation time ~16 days (**I**). These data are within the typically reported growth rate values in the Arctic surface waters ranging from 0.05 to 0.5 day<sup>-1</sup>, with an average of about 0.1 day<sup>-1</sup> (Rich et al., 1997). Surprisingly, the growth rates of bacteria in hypertrophic lakes (**II**) based on Leu incorporation were even lower than in the Arctic if calculated for the total cell number, which is a routine procedure in environmental microbiology. Growth rate ranged from an unrealistically low 0.02 to 0.06 day<sup>-1</sup> with extremely low value (0.002 day<sup>-1</sup>) in the hypolimnion of Vellavere K ulaj arv (Table 3 in **II**). Accordingly, generation time up to ~2 years was calculated (**II**). Such a long time to form new generation of bacteria is not realistic with respect to maintaining bacteria-based processes in any aquatic environment.

When bacterial production was normalized to active bacteria generation time ranged in lakes from a few hours (epilimnion of Lake Vellavere K ulaj arv) to one month (hypolimnion of Lake Verevi; **II**; Fig. 3). Similarly, the use of active bacteria in calculations scaled up the growth parameters in the Barents Sea (**I**). In the upper layer (0–50 m) of the ocean growth rate as high as 0.63 up to 2.65 day<sup>-1</sup> was calculated. Respective generation time 38 up to 9 hours, shortest in station XIII. Below the euphotic zone bacterial growth rates decreased and were generally <0.5 day<sup>-1</sup> for actively respiring cells (Fig. 2). Thus, the generation times based on ARC are comparable to the usual range of pure culture generation times, a few days or less than a day.

When considering the third category of cells, living bacteria according to cell wall integrity, growth rates increased on average four times in lakes (**II**) and five times in the Barents Sea (**I**) compare to the TNB-based growth rate. In

Lake Verevi, generation times calculated from the INT population were ca. 4.2 days range, and in the upper layers of Lake Vellavere Kälajärv between 10–20 days (II). In the euphotic zone of the Barents Sea the generation time ranged from 9 hour to 4 days. Below the euphotic layer bacterial growth rates decreased and generation times were generally longer than 5 days for intact bacteria (I). Again, the growth rates and generation times were similar to as measured for pure isolates.

In case of the Barents Sea, bacterial production was also measured with leucine incorporation, which shows protein production rate and should be an indicator of cell growth (I). Bacterial growth rates based on Leu incorporation were clearly higher compare to Tdr incorporation. When normalized to intact or ARC bacteria, growth rates exceeded  $5 \text{ day}^{-1}$  in many occasions.

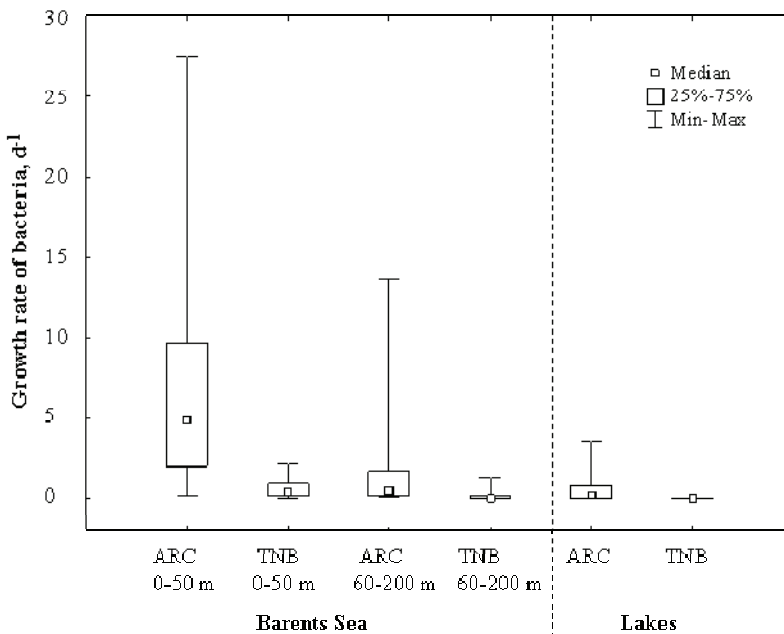
The specific growth rates of filamentous bacteria are not well known. In the nutrient addition experiment (III) the input of 5N and NPG treated mesocosms yielded higher TNB based growth rates ( $1.01$  and  $0.74 \text{ d}^{-1}$ , respectively) compare to other manipulations ( $0.58 \pm 0.05 \text{ d}^{-1}$ , in average). Glucose and nitrogen additions had also a statistically significant effect on bacterial growth rates ( $p < 0.05$ ). These growth rates are calculated based on the total C biomass (III) and are comparable to that of lakes when only active bacteria are used (II).

In general population dynamics, the birth, growth and death are clearly defined events of various types of life cycles. The mortality of bacteria is a known process in microbial ecology but traditionally rather ignored. The results shown in this study are good examples how TNB based growth rates are unrealistically low. While the measurements of processes like bacterial production or fluxes are not influenced by the considerations above, when scaled to population level the difference is obvious. The impact to our evaluation of rate measurements using different physiological groups as mediators of actual processes is remarkable. Focusing on the intact and active fraction of the bacterial population gives substantially higher growth rates than the bulk properties (I, II).

Some models have been introduced to overcome the problem of nongrowing cells without measuring the actual size of active bacteria (Kirchman et al., 1982; Torrétón and Dufour, 1996). However, these models require time-series long enough to extrapolate from the latter stages of growth in the total counts back toward time zero and assume, that nongrowing cells remain nongrowing. The application of these models to predict active cell number is only seldomly used and has questionable value given the fluctuation in active compartments of environmental bacterial communities.

Another possibility to establish ecophysiological status of bacterioplankton is an analytical evaluation of the relationship between the abundance or biomass and production. Large-scale comparisons of bacterial abundances and production rates among systems have shown to correlate and bacterial production increases with total bacterial density with slopes between 1.12 and 1.22 on a log-log scale (Cole et al., 1988; White et al., 1991). In our study of lakes the slopes of BP and TNB were 0.91 in Lake Verevi and 0.82 in deeper Lake

Vellavere K ulaj rv (II). The relationship of BP and active bacteria were different: it was steep in Lake Vellavere K ulaj rv (2.84) but not in shallower Lake Verevi (0.49) (Fig. 4 in II). Because of their steep log-slopes (except in Lake Verevi), these empirical models imply, that bacterial abundance is considerably less variable than bacterial production, and that cell-specific bacterial production increases with system productivity (Fuhrman et al., 1989). In detail, a steep slope in such a relationship means higher growth efficiency and stronger dependence on carbon supply (Kisand et al., 1998). Thus, in deeper lake, the community included highly active bacteria that had stronger dependence on the carbon supply (II).



**Figure 3.** Box-and-whisker plots of bacterial growth rates ( $\mu$ ,  $\text{day}^{-1}$ ) scaled to the entire assemblage (TNB) and to the active fraction of cells (ARC) in the layers of 0–50 m and 60–200 m of the Barents Sea and in Estonian lakes. All the samples for study area were pooled.

We have shown in this study that the Barents Sea tends to have lower numbers and proportion of active cells and lower rates of bacterial production compared to more productive Estonian lakes, so that these variables tend to co-vary. The results of the present study also demonstrate that, at least in the Barents Sea, bacterial production is directly proportional to the number of active bacteria (I). Growth rates, however, are higher in the Barents Sea compared to Estonian lakes. More importantly, it appears that, for a given temperature, active cells in

unproductive sites do not necessarily grow any more slowly than their counterparts in highly productive systems; they are simply less abundant (I).

In the light of above discussed results I suggest to include only active and living bacteria to the conceptual thinking of processes in the aquatic environment. At this point the quantitative information about the fraction of active bacteria vs. total bacteria in lake and seawater is almost lacking.

#### 4.4. Shift of bacterial morphotypes in carbon addition

In the study of resource limitation of the Baltic Sea bacterioplankton the most striking result was the change in bacterial community morphology. The triggering factor of this change was the availability of labile carbon added to experimental enclosures as glucose. All nine mesocosms had initially natural Baltic Sea bacterial community dominated by cocci and rod-shaped cells. In the presence of glucose the abundance of small cell forms decreased towards the end of the experiment and was replaced by filamentous bacteria. Glucose addition favoured both the proportion and biomass of filamentous bacteria ( $p < 0.05$ ), up to  $199 \mu\text{g C l}^{-1}$ . They contributed 84% of the total bacterial biomass in the mesocosm which received daily addition of 5 times nitrogen and carbon (Fig. 2B in III). Filamentous bacteria constituted an average of 56.9% ( $\text{SD} \pm 16.9$ ) of the total bacterial carbon biomass in mesocosms supplemented with glucose, in contrast to 2.5% ( $\text{SD} \pm 3.2$ ) of the carbon BM in mesocosms without additional glucose (Fig. 2B in III). Simultaneous enrichment with nitrogen and phosphorus in addition to labile carbon was sufficient for balanced growth without nutrient limitation. The absence of either nitrogen or phosphorus in other two mesocosms, however, did not retard the growth of filaments. It was surprising that such conditions, limitation of either nitrogen or phosphorus, supported the growth of elongated bacteria.

Using the traditional concept that efficient nutrient competitors are small with high surface to volume ratio, one should predict a selection for small-sized heterotrophic bacteria. Contrary to expectation, the addition of glucose to mesocosms was found to induce the growth of large bacteria. Our finding is consistent with both freshwater and marine experiments, which show that filamentous bacteria can develop preferentially when a sufficient organic carbon supply is available (Havskum et al., 2003; Jansson et al., 2006).

The life strategy of filaments was to use a competitive advantage to increase cell size by using non-limiting substrate. This idea was first proposed by (Øvreas et al., 2003) and theoretically evaluated by Thingstad et al. (2005). As a result the theory for nutrient diffusion was formulated by Thingstad (2005) and it states that it is the *surface : cell requirement of limiting element* ratio, rather than the *surface : volume* ratio, that is important. The distinction is crucial, since it opens up the possibility for life strategies that use a non-limiting element to increase cell size and explains the empirical evidence in our mesocosm experiment.

The domination of filamentous bacteria was surprising but the presence of an organism is, in itself, a proof of the success of the life strategy (Thingstad et al., 2005). It thus seems fundamental to understand the factors determining the success of a particular strategy. In case of our mesocosm study the elongation was a successful strategy, because the abundance of filaments accumulated during the addition of glucose. These cells either outcompeted smaller bacteria or had phenotypic plasticity to increase from coccoid to filament. This question is discussed in the next chapter.

The next subject I would like to address is the ecological consequence of the dominance of filamentous cells to the aquatic environment. In one aspect bacteria have potentially higher total biomass compare to the usual situation when coccoid or rod-shaped bacteria dominate in the community. In second aspect they are growing slower than smaller bacteria leading to lower BP:PP ratio. Third aspect is reduced bacterivory on large bacteria meaning low transfer rate of organic matter to higher trophic levels through the microbial food-web. The filamentous bacteria are functioning as remineralises and the contribution to microbial loop is of minor importance. As a consequence the organic matter produced by autotrophs and incorporated into the filamentous cells is settling out. Fourth aspect is nutritional requirement. The filamentous bacteria, which have high affinity to mineral nutrients will probably succeeded in competition with smaller cells. The availability of suitable carbon form, however, can be the bottleneck because filament formation requires labile organic carbon while smaller cells can cope in the presence of more complicated organic carbon forms. According to our study, the labile organic carbon produced by cyanobacterial bloom was not sufficient to boost the development of filamentous heterotrophic bacteria. The scenario of filamentous bacterial domination, according to results of our mesocosm experiment in the coastal area of the Baltic Sea, can become realistic only if C is introduced to the system by land run-off from anthropogenic sources or by natural processes in the watershed (e.g. leaching from soil).

Can we be certain, that changes in bacterial morphology were not triggered by grazing pressure in our experiment? The answer is yes, because the species composition of protist as well as the wet weight of ciliates and heterotrophic flagellates was similar in all nine mesocosms including the control (III, Fig. 5). Potential grazing by meta-zooplankton was also uniform in all the mesocosms as the community structure and biomass of zooplankton was similar throughout (Kangro et al., 2007). We do not have any grazing experiments to support our perception. However, the fact that in the mesocosms where carbon was not added the biomass of filamentous cells remained minor should be convincing.

In marine systems, the occurrence of filaments has seldom been reported (Havskum and Hansen, 1997) referring to usual dominance of other morphotypes and exclusion of possible short-time periods with higher abundance of heterotrophic filamentous bacteria. For marine environments only a few experimental data about filament formation is available (Nebe-von Caron and Badley, 1995; Havskum et al., 2003; Lebaron et al., 1999). The development of

grazing-resistant filamentous bacteria has frequently been observed in freshwater lakes, where they can temporarily attain significant biomass (Pernthaler et al., 2004; Schauer and Hahn, 2005). To our knowledge this study was first to reveal filamentation of bacterioplankton triggered by excess carbon in the Baltic Sea.

#### **4.5. Nutrient limitation of bacteria and competition with algae**

Optimizing growth rate at non-optimal nutrient levels must be an important aim for aquatic bacteria, given that marine and freshwater environments rarely offer growth-saturating concentration of nutrients. The experimental set-up described in paper **III** allowed to make the nutrient limitation observable and controllable. In addition to more frequently tested N and P limitation, the glucose was added to the mesocosms as readily available carbon source (**III**). The most remarkable changes in bacterioplankton diversity, morphology and production occurred as a result of labile carbon addition. However, the addition of nitrogen elevated significantly bacterial production relative to other mesocosms (SAS, MIXED procedure,  $p < 0.001$ ; Table 3 in **III**). The need for nitrogen suggest that bacteria, similar to phytoplankton, were initially nitrogen limited. In the line of theoretical model by Thingstad et al. (2005), filamentous bacteria, which developed in carbon added mesocosms were able to increase in biomass without increasing cellular requirements for N and P. However, the deficiency of N was obvious in case of bacterial production in mesocosms which received only P addition and P together with carbon. Single nitrogen addition drove bacteria to the deficiency of carbon and P. Bacterial community responded quickly to various nutrient regimes and those who had higher affinity to available nutrients and mechanism to compensate missing resources were successful. Constant change in community diversity profiles indicates niche separation and specialization of bacteria.

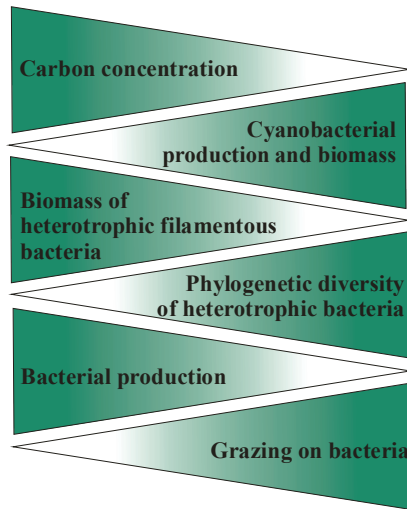
Simultaneous addition of N and P evoke phytoplankton bloom composed mainly of filamentous cyanobacteria typical to the Baltic Sea (Kangro et al., 2007). This enabled us to investigate the resource-competition between autotrophs and heterotrophic bacteria. The increase in the biomass of cyanobacteria remained lowest in the bag with 5NG addition, while 5N addition lead to 2-fold higher cyanobacterial biomass by the end of the experiment. In conclusion, labile carbon addition had significant negative effect to primary production (GLM,  $p < 0.005$ ) while N (GLM,  $p < 0.05$ ) and combined N and P additions had strong positive effect (GLM,  $p < 0.003$ ). These results strongly suggest that in the presence of glucose, filamentous heterotrophic bacteria, who increased steadily their biomass, were more efficient by taking up the added nitrogen. The competition become even more unbalanced towards heterotrophic bacteria when the nitrogen fixed by autotrophs become available to heterotrophs in the last week of experiment.

It has been shown that a large fraction of the uptake of mineral nutrients can be in the bacterial size fraction (Suttle et al., 1990). The idea that bacteria can compete with phytoplankton for mineral nutrients, rather than supply them as remineralizers, has been discussed for many years (Bratbak and Thingstad, 1985; Currie and Kalff, 1984; Pengerud et al., 1987; Thingstad et al., 1993). However, the bacterial success in competition through increasing the biomass without cellular requirements for N and P was first shown in our study in the Baltic Sea.

It has been suggested, that filamentous phytoplankton, without necessarily being superior in their physiological abilities for nutrient uptake, but subject to relatively low grazing pressure from rapidly responding microzooplankton, could theoretically succeed in a nutrient-limited situation (Havskum et al., 2003). Similar principle can be applied to filamentous bacteria and the success of either heterotrophic or autotrophic filaments depends on the environmental conditions and competitive abilities. In addition, the competitors might become controlled by viruses, as shown in case of filamentous bacteria by Øverås (2003).

In the epilimnion of small stratified Lake Verevi the high primary production should satisfy bacterial needs of nutrients and carbon. However, TNB and BP did not correlate neither with algal biomass nor primary production but instead TNB was negatively correlated with zooplankton biomass (**IV**). No relationship between abundance and activity of bacteria was found because the number of bacteria was controlled by predators. Therefore more organic carbon was produced during the productive season than utilized by heterotrophic bacteria in the epilimnion. The results also indicated that the excess of primary production was partly consumed during the clear water phase in June after phytoplankton bloom in May and in late autumn when phytoplankton activity had collapsed but bacteria still remained highly productive afterwards. Also the diurnal dynamics of PP and BP showed uncoupled variations of algal and bacterial activity: BP was the highest in the afternoon when PP decreased (Fig. 4 in **IV**).

Bacteria in the thermocline could depend more on abiotic environment at the same time remineralizing organic matter and releasing inorganic nutrients, as correlation of bacterial abundance and BP with inorganic nutrients and temperature was strong ( $r > 0.4$ ,  $p < 0.05$ ; **IV**). Hypolimnetic bacteria were not highly active (or measurements of BP failed in anoxic waters) and their high numbers were supported rather by specific conditions (lack of most eukaryotic organisms, therefore no grazing, undisturbed environment etc.) than high productivity. However, the bacterial activity in binding of nutrients in hypolimnion is important as the concentrations of total phosphorus and nitrogen were strongly correlated with TNB.



**Figure 4.** The response of cyanobacteria and heterotrophic bacteria to the amendment of labile organic carbon in the Baltic Sea mesocosms.

#### **4.6. Carbon amendment changed bacterial community composition**

One of the central goals in ecology is to understand how biodiversity is generated and maintained. Our mesocosm study to investigate the response of different bacterioplankton parameters to combined amendments of N, P and C added valuable information about the succession in bacterial community composition. The DGGE results showed that the addition of excess carbon lead to statistically different phylothetic patterns (CA and permutations test,  $p < 0.05$ ) and decreased diversity in bacterial community.

The initial phylotype richness in the enclosures, filled with Baltic Sea coastal water, was around 10 phylotypes. During the first part of the experiment when only N and P was added, the diversity increased up to 19 bands per mesocosm and remind close to that level for the rest of the experiment. The overall detected phylotype number in the mesocosms was 73. Surprisingly, only two phylotypes were common; they were observed in 20 and 21 out of the 35 samples. Several phylotypes were very rare; almost half the bands (33) were observed in only 1–3 samples. Temporal community changes were more pronounced compared to the variation between nutrient treatments within one day; the samples clearly clustered day by day. These results reflect high plasticity and potential of bacterial community to respond to conditions present in the environment. The results clearly point that bacterial diversity is more sensitive to changing conditions than the bulk abundance. And that the production of

bacteria is maintained by fluctuating consortium of cells having different phylogenetic affiliation.

Similar changes what we observed during the experiment are likely to happen in the water column in case of changes in DOC or nutrient regime as a response to physically or biologically mediated processes. Conclusions based on mesocosm experiment should be extrapolated to the natural aquatic environment with extreme care. However, experimental studies are important and the only possibility for elucidating single factors affecting the composition of bacterial communities. Field studies have been carried out in various ecosystems have shown that the composition of bacterial communities undergoes temporal changes, for example during the course of a phytoplankton blooms (Fandino et al., 2001; Larsen et al., 2004; Yager et al., 2001). These changes often reflect the changing environmental conditions and DOM supply and also indicate which bacteria are mainly involved in the biogeochemical cycling of elements and flux of energy.

The community composition of the bacterioplankton in the mesocosms was typical to the Baltic Sea coastal waters, being dominated by various Bacteroidetes phylotypes and accompanied by Actinobacteria, Alpha- and Beta-proteobacteria. It changed significantly over the course of the experiment (Figs. 3 and 4 in **III**). Mesocosms supplemented with glucose became statistically significantly different from other mesocosms at day 17. By that time domination of Bacteroidetes phylotypes in carbon amended mesocosm become obvious (Fig. 3 in **III**). A similar trend was observed in the control mesocosm. However, the bacterial community in the control became statistically different from the other mesocosms from day 5 onwards.

We observed a decrease in bacterial richness in the mesocosms supplemented with glucose. Presumably the affinity of some of these species to available nutrients as well as the ability to increase in size probably lead to out-competition of other phylotypes. The phylogenetic affiliation of filamentous bacteria which dominated both in the abundance and biomass in carbon amended mesocosms is not known. Since Bacteroidetes bacteria were prevailing group (6 phylotypes out of 9) they could be the source of filaments. Our data suggest that they belonged to species that were not abundant in the original water mass.

The present study shows the highly dynamic nature of bacterial community composition and strongly suggests that nutrient-induced changes in natural phytoplankton communities lead to significant effects on the structure and functioning of bacterial assemblages as well as on the nature and the rates of bacterially mediated organic matter cycling. Hence, this study emphasizes the need to incorporate community composition into our conceptual thinking of the biogeochemical activities of aquatic microbial assemblages.

## CONCLUSIONS

The present study reveals that bacterial behaviour in aquatic environment is like in the Wall Street. What we can see about it depends on what we are looking at. For example if we focus only on the total number of bacteria we can see just up and downs in figures similar to Wall Street indices and we hardly understand what is behind those numbers. When we deal to look what makes these fluctuations we see very busy and calculated cost effective activities of bacteria similar what is happening in the stock exchange markets. Bacteria are constantly changing the uptake of nutrients to optimize growth rate. Low affinity to labile organic carbon has shown to enable increase in size without increasing the need for mineral nitrogen and phosphorus. The heterogeneity in efficiency to take up available resources is reflected in constant changes in the community composition. Beneath the cover of total abundance small number of active bacteria is responsible for most of the bacteria mediated processes. It is the bargaining for life. As researchers we should be curious enough to find out what is really going on in the bacteria's life and smart enough to see the ecological relevance of these traits. And the road ahead will be tremendously exiting.

The investigations of this study have shown the highly diverse world of bacteria and the ecological conclusions based on the results are as follows:

1. Bacterial growth rates both in polar and temperate region were similar to what has measured in optimal growth conditions when consider that only the active bacteria are responsible for the production and turnover of organic material. This finding opens up the new concept of high bacterial activities in polar areas which has included dead and inactive bacteria to growth parameter calculations.
2. Free-living active bacteria did not reveal particular concentration increase in sites of elevated algal and bacterial activity, like the sub-surface chlorophyll and bacterial production maxima in the Barents Sea. In Estonian lakes, the number of active bacteria was positively related with the organic matter concentration.
3. The sinking organic matter which was produced in the euphotic zone did not host higher biomass of active bacteria compare to the free-living cells in the marginal ice zone of the Barents Sea. These results contradict with previous investigations in more productive regions with well-developed sea-ice algal communities in the Arctic Ocean. The spatial and regional variability of primary and secondary production in the Arctic Ocean point to the need to be careful in interpretation and extrapolation of data obtained from one location to another in polar areas.
4. Resource manipulation led to changes in bacterial community composition with most pronounced differences with carbon addition.
5. Heterotrophic bacteria were better competitors for mineral nutrients than planktonic autotrophs with the presence of additional labile carbon in the Baltic Sea.

6. The filamentous bacteria represented effective means to overcome nutrient limitation and predation by using carbon to increase size without elevated cellular requirements for nitrogen and phosphorus.

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## SUMMARY IN ESTONIAN

### Veebakterite füsioloogilise ja fülogeneetilise mitmekesisuse ökoloogiline roll

Looduslik veekeskkond sisaldab väga erinevaid keemiliste, füüsikaliste ja bioloogiliste parameetrite koosmõjusid, mis kujundavad bakterite koosluse füsioloogilise ja liigilise struktuuri. Keskkonnatingimustest sõltuvalt muutub bakterirakkude aktiivsus pidevalt. Seetõttu sisaldab bakterikooslus puhkavaid kuni kõrge aktiivsusega rakke. Meie arusaam veekogus toimuvatest bakteriaalsetest protsessidest sõltub paljuski sellest, millist parameetrit me uurime. Keskendudes ainult bakterite üldarvu väärtustele, mis kõiguvad üles-alla nagu börsiindeksid, ei tea me varieerumise põhjuseid. Veebakterite üldarvu muutusi lähemalt uurides näeme bakterite aktiivset ja kalkuleeritud kasumlikku käitumist sarnaselt aktsiaturgudega. Pidevalt muutuva süsiniku- ja energiaallikate kontsentratsiooni tingimustes toimuva konkurentsi hinnaks on elu. Kasvukiiruse optimeerimiseks on bakterid võimelised muutma toiteainete omastamise efektiivsust ning vajadusel säilitades madalat ainevahetuse taset jääma ootama paremaid elutingimusi. Bakteritele vajalike toiteainete ja süsiniku omastamise efektiivsuse muutused peegelduvad ka bakterite koosluse mitmekesisuses. Käesolev doktoritöö näitab, et väga laialdaselt kasutatud veebakterite arvukuse hindamise meetoditega arvestame bakterite üldarvu hulka ka paljud surnud bakterid, mida peaks käsitlema kui detriiti. Tulemusena ülehindame aktiivsete rakkude hulka vees ning alahindame bakterite kasvukiiruseid.

Käesolev doktoritöö näitab veebakterite mitmekesisust ja ökoloogilised järeldused on järgnevad:

1. Bakterite kasvukiirused on polaar ja parasvöötme aladel sarnased optimaalsetel tingimustel mõõdetud kasvukiirustega juhul, kui arvestatakse ainult aktiivsete bakterite hulka, kes vastutavad produktsiooni ja orgaanilise aine lagundamise eest. Järelikult on polaaralade bakterid palju aktiivsemad, kui on arvatud varasemas uurimustes. Traditsiooniliselt on veemikrobioloogias kasvukiiruste arvutamisel aluseks bakterite üldarv, mis sisaldab ligikaudu 50% ulatuses surnud baktereid.
2. Veesambas vabalt elavate aktiivsete bakterite arvukus ei olnud Barentsi meres suurem kõrgema bakterite produktsiooni ning klorofüllü kontsentratsiooniga veekihtides. Eesti järvedes sõltus aktiivsete bakterite hulk orgaanilise aine sisaldusest.
3. Settivatele orgaanilise aine osakestele kinnitunud aktiivsete bakterite arvukus ei ole Barentsi meres suurem võrreldes vees vabalt elavate bakteritega. Saadud tulemused erinevad Põhjajäämere produktiivsemates piirkondades tehtud uurimustest, kus jääga seotud vetikad olid väga arvukad. Vetikate ja bakterite produktsioon on ajaliselt ja ruumiliselt varieeruv ning uurimistulemuste interpreteerimisel ja laiendamisel kogu Põhjajäämerele tuleb olla ettevaatlik.

4. Bakterite koosluse liigiline struktuur muutus sõltuvalt toiteainete kättesaadavusest. Suurimaid muutusi põhjustas orgaanilise süsiniku lisamine.
5. Heterotroofsed bakterid olid mineraalse lämmastiku ja fosfori omastamisel vetikatest edukamad juhul, kui keskkonnas oli piisavalt orgaanilist süsinikku.
6. Läänemeres tehtud toitumiskatses kasutasid filamentsed heterotroofsed bakterid orgaanilist süsinikku kehamassi suurendamiseks vajamata suuremaid lämmastiku ja fosfori kontsentratsioone. Filamentse morfoloogiaga rakud olid väiksematest bakteritest edukamad toiteainete limitatsiooni ja ärasöömissurve tingimustes.

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## **PUBLICATIONS**

# CURRICULUM VITAE

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Physiological and phylogenetic diversity as well as population dynamics of bacteria in their natural aquatic environments.

## Publications

1. Tammert, H., Ott, I., Kisand, V. (2010). Ecophysiological status of bacteria in freshwater lakes during steep summer stratification. *Fundamental and Applied Limnology (Archiv für Hydrobiologie)*, Volume 176 (1): 75–82.
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3. Tammert, H., Olli, K., Sturluson, M., Hodal, H. (2008). Bacterial biomass and activity in the marginal ice zone of the northern Barents Sea. *Deep-Sea Research II*, 55 (20–21), 2199–2209.

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6. Tammert, H., Kisand, V., Nõges, T. (2005). Bacterioplankton abundance and activity in a small hypertrophic stratified lake. *Hydrobiologia*, 547, 83–90.
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9. Nõges, P., Haberman, J., Kangur, K., Kolk, R., Künnap, H., Nõges, T., Tammert, H., Timm, H., Timm, T., Zingel, P. (2002). *Võrtsjärve seisund*. Eesti keskkonnaseire Tartu: Tartu Ülikooli Kirjastus, 69–73.
10. Kisand, V., Tammert, H. (2000). Bacterioplankton strategies for leucine and glucose uptake after a cyanobacterial bloom in an eutrophic shallow lake. *Soil Biology & Biochemistry*, 32(13), 1965–1972.

#### **International courses attended:**

- Workshop “Water management – monitoring of water quality”, Uppsala University, Sweden, August 3–9, 1998.
- Workshop “Planning and management for sustainability in the Baltic Sea”, Kalmar University, Sweden, August 1–26, 1999.
- NorFa course “Particle analysis and aquatic microbial communities”, Bergen, Norway, June 5–12, 2001.
- NARP International Symposium/workshop “C flux and climate change: The Nordic contribution to a panarctic perspective” Sigulda, Latvia, November 1–7, 2002.
- NorFa student workshop “Integrated modelling”, Trondheim, Norway, September 12–15, 2002.
- NorFa workshop “Integrated eutrophication management”, Tallinn, Estonia, December 6–10, 2002.
- NorFa workshop “Integrated approaches to drainage basin nutrient inputs and coastal eutrophication” Tvärminne, Finland, November 26–30, 2003.
- NordForsk symposium/workshop “Climate and changing pathways of marine Arctic pollution” Tromsø, Norway, November 28–December 3, 2005.

**International conferences:**

- “Enzymes in the Environment: Activity, Ecology and Applications” Granada, Spain, July 12–16, 1999. Poster presentation: Tammert, H. & Kisand, V., “Leucine and glyucose “metabolism” in eutrophic shallow lake after a cyanobacterial bloom”.
- “7th European Marine Microbiology Symposium”, Noordwijkerhout, Netherlands, September 17–22, 2000.
- “Shallow Lakes 2002, International Conference on Limnology of Shallow Lakes”, Balatonfüred, Hungary, May 25–30, 2002. Poster presentation: H. Tammert & Kisand V., “Dead or alive aquatic bacteria? Comparison of the different staining methods”.
- “9th Symposium on Aquatic Microbial Ecology”, Helsinki, Finland, August 21–26, 2005. Oral presentation: Tammert, H., Olli, K., Lignell, R. & Kisand, V., “Diversity and activity of bacterioplankton during a cyanobacterial bloom in a large-scale mesocosm experiment”.
- “Arctic Frontiers Science Conference: Food web dynamics and biogeochemical fluxes in the Arctic Ocean”, Tromsø, Norway, January 23–26, 2007. Oral presentation: H. Tammert, K. Olli & M. Sturluson, “Bacterial activity and physiological groups in the marginal ice zone of the northern Barents Sea”
- The 5<sup>th</sup> Shallow Lakes Conference, Punta del Este, Uruguay, November 23–28, 2008. Oral presentation: Tammert, H., Kisand, V., Nõges, T., „Inter-annual dynamics of bacterial community composition in a large shallow lake”.

**Membership of societies, fellowships:**

Since 1999	Estonian Society of Microbiology
Since 1999	Federation of European Microbiological Societies
Since 2005	Estonian Naturalists Society
1999	FEMS young scientist travel grant
2000	FEMS young scientist travel grant
2010	FEMS research fellowship award

# ELULOOKIRJELDUS

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## Haridus

1991–1994 Tartu 5. Keskkool  
1994–1999 BSc hüdrobioloogia erialal, Tartu Ülikool  
1999–2002 MSc hüdrobioloogia erialal, Tartu Ülikool  
Alates 2002 PhD õpe, Tartu Ülikool. 2007–2009 lapsepuhkusel

## Teenistuskäik

1996–2005 Eesti Põllumajandusülikool, zooloogia ja botaanika instituut, Võrtsjärve limnoloogijaam; vanemlaborant  
Alates 2005 Eesti Maaülikool, põllumajandus- ja keskkonnainstituut, limnoloogiakeskus; vanemlaborant  
Alates 2006 Tartu Ülikool, Loodus- ja tehnoloogiateaduskond, Tartu Ülikooli Tehnoloogiainstituut; teadur

## Teaduslik ja arendustegevus

### Peamised uurimisvaldkonnad:

Veebakterite füsioloogiline ja fülogeneetiline mitmekesisus ning populatsiooni dünaamika looduslikus keskkonnas.

## Publikatsioonide loetelu

1. Tammert, H., Ott, I., Kisand, V. (2010). Ecophysiological status of bacteria in freshwater lakes during steep summer stratification. *Fundamental and Applied Limnology (Archiv für Hydrobiologie)*, Volume 176 (1): 75–82
2. Tšertova, N., Kisand, A., Tammert, H., Kisand, V. (2010). Low seasonal variability in community composition of sediment bacteria in large and shallow lake. *Environmental Microbiology Reports* (in press).
3. Tammert, H., Olli, K., Sturluson, M., Hodal, H. (2008). Bacterial biomass and activity in the marginal ice zone of the northern Barents Sea. *Deep-Sea Research II*, 55 (20–21), 2199–2209.

4. Zingel, P., Nõges, P., Tuvikene, L., Feldmann, T., Järvalt, A., Tõnno, I., Agasild, H., Tammert, H., Luup, H., Salujõe, J., Nõges, T. (2006). Ecological processes in macrophyte- and phytoplankton-dominated shallow lakes. *Proceedings of the Estonian Academy of Sciences. Biology, Ecology*, 55, 280–307.
5. Ott, I., Timm, H., Rakko, A., Mäemets, H., Kübar, K., Tammert, H., Laas, A., Tambets, M., Järvet, A., Kübar, R. (2006). Viljandi järv – kas looduslik või oluliselt muudetud? Sammul, M. (Toim.). *Eesti Looduseuurijate Seltsi aastaraamat (76–93)*. Tartu: Eesti Looduseuurijate Selts.
6. Tammert, H., Kisand, V., Nõges, T. (2005). Bacterioplankton abundance and activity in a small hypertrophic stratified lake. *Hydrobiologia*, 547, 83–90.
7. Tammert, H., Kisand, V. (2004). Bacterioplankton. Haberman J., Pihu, E., Raukas, A. (Toim.). *Lake Võrtsjärv*. Estonian Encyclopaedia Publishers Ltd, 207–215.
8. Tammert, H., Kisand, V. (2003). Bakteriplankton. Haberman, J., Pihu, E., Raukas, A. (Toim.). *Võrtsjärv*. Tallinn: Eesti Entsüklopeediakirjastus, 249–258.
9. Nõges, P., Haberman, J., Kangur, K., Kolk, R., Künnap, H., Nõges, T., Tammert, H., Timm, H., Timm, T., Zingel, P. (2002). Võrtsjärve seisund. *Eesti keskkonnaseire Tartu: Tartu Ülikooli Kirjastus*, 69–73.
10. Kisand, V., Tammert, H. (2000). Bacterioplankton strategies for leucine and glucose uptake after a cyanobacterial bloom in an eutrophic shallow lake. *Soil Biology & Biochemistry*, 32(13), 1965–1972.

**Erialane enesetäiendus:**

- Kursus “Water management – monitoring of water quality”, Uppsala Ülikool, Rootsi, 3–9 august, 1998.
- Kursus “Planning and management for sustainability in the Baltic Sea”, Kalmar University, Rootsi, 1–26 august, 1999.
- NorFa kursus “Particle analysis and aquatic microbial communities”, Bergen, Norra, 5–12 juuni, 2001.
- NARP rahvusvaheline kursus “C flux and climate change: The Nordic contribution to a panarctic perspective” Sigulda, Läti, 1–7 november, 2002.
- NorFa kursus “Integrated modelling”, Trondheim, Norra, 12–15 september, 2002.
- Elektronmikroskoopia alane enesetäiendus Bergeni Ülikoolis merebioloogia uurimiserühma juures Norras, 16–30 september, 2002.
- NorFa kursus “Integrated eutrophication management”, Tallinn, Eesti, 6–10 detsember, 2002.
- NorFa kursus “Integrated approaches to drainage basin nutrient inputs and coastal eutrophication” Tvärminne, Soome, 26–30 november, 2002.
- NordForsk kursus “Climate and changing pathways of marine Arctic pollution” Tromsø, Norra, 28. november – 3. detsember, 2005.

**Osalemine konverentsidel:**

- “Enzymes in the Environment: Activity, Ecology and Applications” Granada, Hispaania, 12–16 juuli, 1999. Poster ettekanne: Tammert, H. & V. Kisand, “Leucine and glyucose “metabolism” in eutrophic shallow lake after a cyanobacterial bloom”.
- “7th European Marine Microbiology Symposium”, Noordwijkerhout, Holland, 17–22 september, 2000.
- “Shallow Lakes 2002, International Conference on Limnology of Shallow Lakes”, Balatonfüred, Ungari, 25–30 mai, 2002. Poster ettekanne: H. Tammert & V. Kisand, “Dead or alive aquatic bacteria? Comparison of the different staining methods”.
- “9th Symposium on Aquatic Microbial Ecology”, Helsinki, Soome, 21–26 august, 2005. Suuline ettekanne: Tammert, H., Olli, K., Lignell, R. & V. Kisand, “Diversity and activity of bacterioplankton during a cyanobacterial bloom in a large-scale mesocosm experiment”.
- “Arctic Frontiers Science Conference: Food web dynamics and biogeochemical fluxes in the Arctic Ocean”, Tromsø, Norra, 23–26 jaanuar, 2007. Suuline ettekanne: H. Tammert, K. Olli & M. Sturluson, “Bacterial activity and physiological groups in the marginal ice zone of the northern Barents Sea”
- The 5<sup>th</sup> Shallow Lakes Conference, Punta del Este, Uruguai, november 23–28, 2008. suuline ettekanne: Tammert, H., Kisand, V., Nõges, T., „Inter-annual dynamics of bacterial community composition in a large shallow lake”.

**Teadusorganisatsiooniline tegevus, stipendiumid:**

Alates 1999	Eesti Mikrobioloogide Ühenduse liige
Alates 1999	Federation of European Microbiological Societies liige
Alates 2005	ELUS liige
1999	FEMS noore teadlase stipendium.
2000	FEMS noore teadlase stipendium.
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