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CARINA BUNSE

BACTERIOPLANKTON IN THE LIGHT OF  
SEASONALITY AND ENVIRONMENTAL DRIVERS



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of seasonality and environmental drivers**



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**Bacterioplankton in the light of seasonality and environmental drivers**  
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## Abstract

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Bacterioplankton are keystone organisms in marine ecosystems. They are important for element cycles, by transforming dissolved organic carbon and other nutrients. Bacterioplankton community composition and productivity rates change in surface waters over spatial and temporal scales. Yet, many underlying biological processes determining when, why and how bacterioplankton react to changes in environmental conditions are poorly understood. Here, I used experiments with model bacteria and natural assemblages as well as field studies to determine molecular, physiological and ecological responses allowing marine bacteria to adapt to their environment.

Experiments with the flavobacterium *Dokdonia* sp. MED134 aimed to determine how the metabolism of bacteria is influenced by light and different organic matter. Under light exposure, *Dokdonia* sp. MED134 expressed proteorhodopsin and adjusted its metabolism to use resources more efficiently when growing with lower-quality organic matter. Similar expression patterns were found in oceanic datasets, implying a global importance of photoheterotrophic metabolisms for the ecology of bacterioplankton.

Further, I investigated how the composition and physiology of bacterial assemblages are affected by elevated CO<sub>2</sub> concentrations and inorganic nutrients. In a large-scale experiment, bacterioplankton could keep productivity and community structure unaltered by adapting the gene expression under CO<sub>2</sub> stress. To maintain pH homeostasis, bacteria induced higher expression of genes related to respiration, membrane transport and light acquisition under low-nutrient conditions. Under high-nutrient conditions with phytoplankton blooms, such regulatory mechanisms were not necessary. These findings indicate that open ocean systems are more vulnerable to ocean acidification than coastal waters.

Lastly, I used field studies to resolve how bacterioplankton is influenced by environmental changes, and how this leads to seasonal succession of marine bacteria. Using high frequency sampling over three years, we uncovered notable variability both between and within years in several biological features that rapidly changed over short time scales. These included potential phytoplankton-bacteria linkages, substrate uptake rates, and shifts in bacterial community structure. Thus, high resolution time series can provide important insights into the mechanisms controlling microbial communities.

Overall, this thesis highlights the advantages of combining molecular and traditional oceanographic methodological approaches to study ecosystems at high resolution for improving our understanding of the physiology and ecology of microbial communities and, ultimately, how they influence biogeochemical processes.

## Keywords

marine bacteria, marine microbiology, seasonal succession, ocean acidification, proteorhodopsin, photoheterotrophy, microbial time series

## Svensk sammanfattning

Bakterier är nyckelorganismer i marina ekosystem. De är viktiga för näringsämnenas kretslopp tack vare sin förmåga att transformera löst organiskt kol och andra näringsämnen. Bakteriesamhällets artsammansättning och produktivitet i ytvatten förändras ständigt i både tid och rum. Många underliggande biologiska processer såsom när, varför och hur bakterieplankton reagerar på årstider och andra miljöförändringar är fortfarande dåligt förstådda. I denna avhandling har jag gjort experiment med modellorganismer och naturliga bakteriesamhällen samt fältstudier för att studera molekylära, fysiologiska och ekologiska mekanismer som gör det möjligt för marina bakterier att anpassa sig till sin miljö.

Experiment med flavobakterien *Dokdonia* sp. MED134, syftade till att bestämma hur bakteriers metabolism påverkas av ljus och olika organiska substrat. Vid ljusexponering uttryckte *Dokdonia* sp. MED134 det ljuskänsliga proteinet proteorodopsin och andra gener. Särskilt vid tillväxt med substrat av lägre kvalitet så ledde detta till en tydlig anpassning av ämnesomsättningen. Liknande genuttrycksmönster återfanns även i havet, vilket tyder på att bakteriellt nyttjande av solljus kan vara globalt viktig för bakteriers ekologi.

Vidare undersökte jag hur naturliga bakteriesamhällen och bakteriers ämnesomsättning påverkas av förhöjda CO<sub>2</sub>-halter och tillgång på oorganiska näringsämnen. I ett storskaligt experiment visade vi att bakteriernas produktivitet och artsammansättning förblev stabil tack vare justeringar av genuttrycket som respons på CO<sub>2</sub> stress. För att upprätthålla pH-homeostasen under förhöjda CO<sub>2</sub> nivåer och låga näringsförhållanden, uttryckte bakterierna flera gener relaterade till respiration, membrantransport och fotosyntes. Vid förhöjda näringsförhållanden och växtplanktonblomningar, var sådana regleringsmekanismer inte nödvändiga. Detta visar att den näringsfattiga miljön i det öppna havet är känsligare för havsförsurning än kustnära områden.

Slutligen utförde jag fältstudier i Östersjön för att bestämma hur marina bakterier påverkas av miljöförändringar och hur det leder till säsongsmässiga successionsmönster. Med hjälp av högfrekvent provtagning hittade vi flera biologiska fenomen som varierade påtagligt och snabbt både mellan och inom år, såsom kopplingar mellan växtplankton och bakterier, näringsupptag och bakteriesamhällets sammansättning.

Denna avhandling visar de stora fördelarna med att kombinera metoder i molekylärbiologi och mikrobiell oceanografi för att nå kunskap om marina bakteriers fysiologi och ekologi och hur de påverkar näringsämnenas kretslopp i havet.

*“The face of the sea is always changing. Crossed by colors, lights, and moving shadows, sparkling in the sun, mysterious in the twilight, its aspects and its moods vary hour by hour. The surface waters move with the tides, stir to the breath of the winds, and rise and fall to the endless, hurrying forms of the waves. Most of all, they change with the advance of the seasons.”*

*Rachel Carson, The sea around us*



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## List of Papers

### Paper I

Joakim Palovaara, Neelam Akram, Federico Baltar, **Carina Bunse**, Jeremy Forsberg, Carlos Pedrós-Alió, José M. González, and Jarone Pinhassi. 2014. Stimulation of growth by proteorhodopsin phototrophy involves regulation of central metabolic pathways in marine planktonic bacteria. *Proceedings of the National Academy of Sciences USA* 111: E3650-E3658.

### Paper II

**Carina Bunse**, Daniel Lundin, Christofer MG Karlsson, Neelam Akram, Maria Vila-Costa, Joakim Palovaara, Lovisa Svensson, Karin Holmfeldt, José M. González, Eva Calvo, Carles Pelejero, Cèlia Marrasé, Mark Dopson, Josep M. Gasol, Jarone Pinhassi. 2016. Response of marine bacterioplankton pH homeostasis gene expression to elevated CO<sub>2</sub>. *Nature Climate Change* 6: 483-487.

### Paper III

**Carina Bunse**, Daniel Lundin, Markus V. Lindh, Johanna Sjöstedt, Stina Israelsson, Sandra Martinez-Garcia, Federico Baltar, Saraladevi Muthusamy, Benjamin Pontiller, Christofer M.G. Karlsson, Catherine Legrand and Jarone Pinhassi. Seasonality and co-occurrences of free-living Baltic Sea bacterioplankton. *Manuscript*

### Paper IV

Stina Israelsson\*, **Carina Bunse\***, Federico Baltar, Mireia Bertos-Fortis, Emil Fridolfsson, Catherine Legrand, Elin Lindehoff, Markus V. Lindh, Sandra Martinez-Garcia and Jarone Pinhassi. Seasonal dynamics of Baltic Sea plankton activities: heterotrophic bacterial function under different biological and environmental change. *Manuscript*

\* *These authors contributed equally to this work.*

### Paper V

**Carina Bunse** and Jarone Pinhassi. 2017. Marine bacterioplankton seasonal succession dynamics. *Trends in Microbiology* 25: 494-505

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## Additional published papers not included in this thesis

Anna Godhe, Conny Sjöqvist, Silke Sildever, Josefin Sefbom, Sarah Harðardóttir, Mireia Bertos-Fortis, **Carina Bunse**, Susanna Gross, Emma Johansson, Per R. Jonsson, Saghar Khandan, Catherine Legrand, Inga Lips, Nina Lundholm, Karin E. Rengefors, Ingrid Sassenhagen, Sanna Suikkanen, Lisa Sundqvist and Anke Kremp. 2016. Physical barriers and environmental gradients cause spatial and temporal genetic differentiation of an extensive algal bloom. *Journal of Biogeography* 1;43(6):1130-42.

**Carina Bunse**, Mireia Bertos-Fortis, Ingrid Sassenhagen, Silke Sildever, Conny Sjöqvist, Anna Godhe, Susanna Gross, Anke Kremp, Inga Lips, Nina Lundholm, Karin Rengefors, Josefin Sefbom, Jarone Pinhassi and Catherine Legrand. 2016. Spatio-temporal interdependence of bacteria and phytoplankton during a Baltic Sea spring bloom. *Frontiers in Microbiology* 7:517.

Luisa Hugerth, Markus V. Lindh, Conny Sjöqvist, **Carina Bunse**, Catherine Legrand, Jarone Pinhassi, Anders Andersson. 2016. Seasonal dynamics and interactions among Baltic Sea prokaryotic and eukaryotic plankton assemblages. *Diva preprint* urn:nbn:se:kth:diva-186160

## List of Abbreviations

autochthonous DOM: phytoplankton-derived dissolved organic matter  
allochthonous DOM: dissolved organic matter derived from terrestrial runoff  
DOM: dissolved organic matter  
DOC: dissolved organic carbon  
chl *a*: chlorophyll *a*  
LMO: Linnaeus Microbial Observatory, time-series station in the Baltic Sea  
POM: particulate organic matter  
RuBisCo: Ribulose-1,5-bisphosphate carboxylase/ oxygenase  
TCA cycle: tricarboxylic acid (TCA) cycle, also known as citric acid cycle  
OTU: operational taxonomic unit, used to group “species” based on gene similarity

# Introduction

*“If you don’t like bacteria, you’re on the wrong planet.”*

*Stewart Brand*

## Role of bacterioplankton in marine ecosystems

Marine microbes rule the oceans. Prokaryotes, including marine bacteria, owe their success to several properties: For example, they have populated the planet for a long time (~3.7 billion years (Nutman, et al. 2016)), and they are uniquely adapted to their specific habitat or niche. Marine prokaryotes can grow fast, are ubiquitously distributed and they display high cell numbers in the water column (Kirchman 2016). Marine prokaryotic cells can thrive in all oceanic habitats; from the deep sea to surface waters and from the polar seas to equatorial regions (Karl 2007, Kirchman 2008).

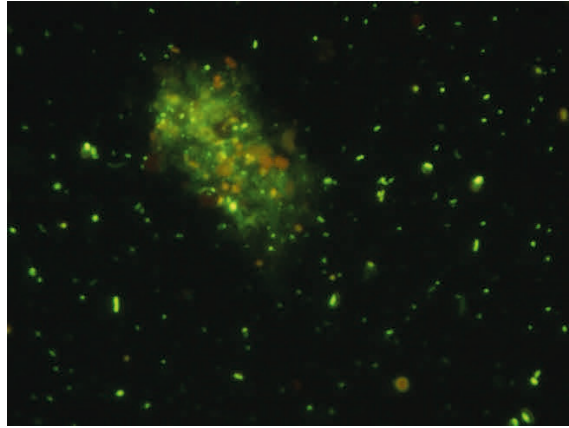
Traditionally, marine microbes are classified according to two main trophic states; photoautotrophs (organisms producing organic compounds via photosynthesis for carbon and energy) and heterotrophs (organisms gaining carbon and energy via degradation of organic compounds). During photosynthesis, carbon dioxide and water are transformed into glucose and oxygen and the synthesized organic material is subsequently used for biomass and growth of the primary producer. In the oceans, phytoplankton fix approximately 48.5 Pg carbon per year via primary production processes, which is equivalent to the carbon fixed by all terrestrial plants combined (Field, et al. 1998). Major portions of the organic material are released into the water continuously (as particulate organic matter or dissolved organic matter, POM or DOM respectively), through processes such as cell lysis, during ‘sloppy feeding’ by grazers or viral lysis (see for example (Fuhrman 1992, Wilhelm, et al. 1999)). Heterotrophic organisms in turn rely on these organic molecules for cellular carbon and energy production (Karl 2014) (Figure 3), and the bacterioplankton sequentially degrade organic material in the seawater (Arrigo 2005). They therefore play a major role in total nutrient turnover. Furthermore, these bacterioplankton also mineralize organic compounds from

land runoff (so called allochthonous organic matter), influencing mainly coastal carbon budgets (Tranvik 1992). Bacterioplankton, therefore, control the turnover rates and biogeochemical reactions of carbon, and other nutrients like phosphate and nitrogen (Kirchman 2008). Consequently, the total biomass and activities of marine microbes have massive impacts on global water nutrient cycling, marine food webs and productivity.

Marine microbes (organisms smaller than 100  $\mu\text{m}$  and only seen with the help of a microscope) include bacteria, archaea, viruses, protists and fungi (Fuhrman 2009, Kirchman 2008). In this thesis, I refer to components of the marine microbial community: bacterioplankton broadly describing marine prokaryotes, i.e. bacteria and archaea, and phytoplankton including photosynthetic eukaryotic algae, such as diatoms and dinoflagellates, as well as cyanobacteria.

## Functional diversity

Bacterioplankton are functionally very diverse. Biogeochemical functions conducted by marine microbes include  $\text{CO}_2$  fixation and production of organic material, degradation of organic material, mineralizing and oxidizing dissolved organic matter for biomass production, control of prey, reducing  $\text{N}_2$  to ammonium (nitrogen fixation), oxidizing ammonium to nitrate (nitrification) or reducing nitrate to  $\text{N}_2$  (denitrification) (Kirchman 2008). The 'functional groups' conducting these tasks are divided into compartments like primary producers, heterotrophs, grazers and viruses,  $\text{N}_2$ - fixers, nitrifiers and denitrifiers (Kirchman 2008). Bacterioplankton groups can be named based on their source of energy and carbon compounds used as building blocks, e.g. photoheterotroph or chemoautotroph. However, a strict classification of the functional groups is often not straightforward, as many organisms are mixotrophic, that is a combination of the above functions (Kirchman 2008). Further 'minor' functions can have massive impacts on microbial communities and food webs. For example, extracellular enzymes hydrolyze large molecules into smaller ones that can be transported into cells (Hoppe 1993). While several studies are currently redefining our knowledge of substrate exchanges and production of marine microbial communities (Amin, et al. 2012, Durham, et al. 2015), many underlying concepts, gene functions and traits are still to be explored; an exciting task for future studies. Functional diversity, and to some extent bacterioplankton phenotypes (that is biochemical or physiological properties), can be estimated from their gene repertoire and gene expression patterns. Gene expression patterns of different metabolic pathways or functional enzymes thus provide information of which biochemical reactions



*Figure 1. A marine microbial community visualized under an epifluorescent microscope. The picture is taken from Baltic Sea seawater filtered onto 0.2  $\mu\text{m}$  polycarbonate filter and stained with SYBR Gold, sampled at Linnaeus Microbial Observatory 14/10/2014 and magnified under an Olympus BX50 microscope (1000x magnification).*

an organism conducts. This may further help us understand how organisms are adapted to the environment and how they can react to changes in growth conditions (such as substrate concentrations and resource quality, light exposure,  $\text{CO}_2$  concentrations or temperature).

### **Photoautotrophy**

Oxygenic photosynthesis is best described in cyanobacteria, algae and plants (Hanada 2016) of which single celled cyanobacteria have a central role in bacterioplankton communities. Photoautotrophs gain their energy and reducing power from light and carbon from  $\text{CO}_2$  (Figure 3, Table 1). Organisms conducting oxygenic photosynthesis use the proton gradient for energy, gain their carbon from  $\text{CO}_2$  and receive electrons from  $\text{H}_2\text{O}$  (via  $\text{CO}_2$  fixation in the photosystems) (Karl 2014). Interestingly, it has been argued that obligate photoautotrophic organisms may not exist in nature (Karl 2014), as they also contain the metabolic means take up and consume organic molecules to obtain carbon and nutrients (such as in the glycolysis or TCA cycle). For example *Prochlorococcus*, a single celled cyanobacterium, frequently consumes organic substrates despite its expected photoautotrophic lifestyle (Björkman, et al. 2015, Church, et al. 2006, Gómez-Pereira, et al. 2013, Mary, et al. 2007) and diatoms can take up dimethylsulfoniopropionate (DMSP) and vitamins (see for example (Droop 1957, Vila-Costa, et al. 2006)).

Genes for oxygenic photosynthesis are often among the most expressed genes in surface water communities (see for example Figure 3) and gene expression of several autotrophic organisms (such as cyanobacteria) oscillate

on a daily basis (Aylward, et al. 2015). Genetically, photoautotrophs can be recognized by the photosystem machinery and RuBisCo (Pichard, et al. 1996).

Table 1. Light capture and energetic sources of photoautotrophic, heterotrophic and photoheterotrophic organisms (adapted from (Karl 2014)).

	Photoautotrophy	Heterotrophy	Photoheterotrophy
Energy source	H <sup>+</sup> gradient	DOC oxidation	H <sup>+</sup> gradient and/or DOC oxidation
Electron source	H <sub>2</sub> O	DOC	DOC
Carbon source	CO <sub>2</sub>	DOC	DOC
Light capture	photosynthesis	Not available	AAP photosystem, proteorhodopsin

## Heterotrophy

Heterotrophic organisms rely on organic molecules for cellular carbon and energy production (Karl 2014) (Figure 2). Heterotrophic bacteria occupy different ecological and physical niches, which characteristics are encoded in their genomes. In particular, they contain a very diverse set of genes to degrade polymers and monomers to gain energy by oxidizing DOM to C<sub>3</sub>-compounds that are ultimately further reduced in glycolysis and TCA pathways. TCA cycle pathways are not unique to heterotrophs as also photoautotrophic organisms contain them, despite some modifications in cyanobacteria (Allen, et al. 2011, Zhang, et al. 2011), therefore these pathways are not distinctively suitable as identifier genes or pathways for heterotrophy. It is difficult to pinpoint one or a few heterotrophic genes that are shared among all heterotrophic bacteria, as many enzymes degrading polymers are very specific. Possibly, heterotrophy is best described as the absence of photosynthesis and RuBisCo. Despite the large amount of ecological and physical niches of heterotrophic bacteria, some biogeochemically relevant genes, mainly enzymes degrading polymers can describe heterotrophic processes in a given community. For example, oxidative genes can be indicative for carbon processing (e.g. chitinase, protocatechuate (aromatic ring cleavage) or acetylpolymine aminohydrolase (Moran 2008)). But, not all heterotrophic marine bacteria contain all or some of the mentioned functional genes (Moran 2008) resulting in an overall lower expression of such gene families compared to phototrophic genes, such as photosynthetic reaction center protein (Figure 2).

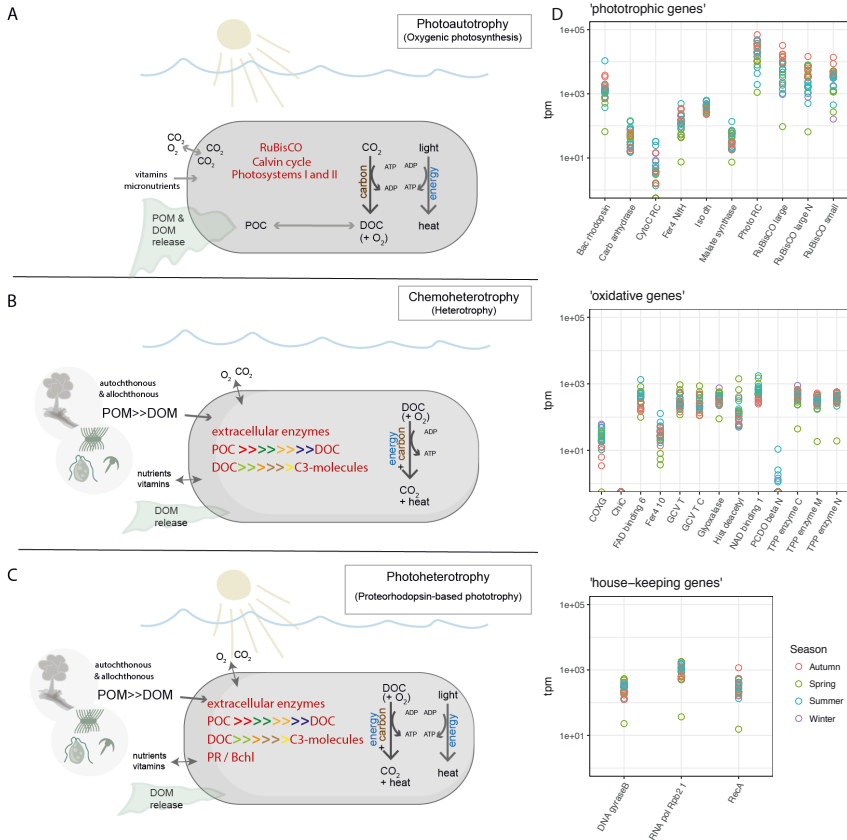


Figure 2. Conceptual figure displaying marine bacterioplankton and trophic characteristics; photoautotrophy (A), chemoheterotrophy (B) and proteorhodopsin-based photoheterotrophy (C). The flow of energy and carbon is adapted from (Karl 2014). Important biochemical enzymes or pathways are highlighted in red (e.g. RuBisCO), the different colors in the POC to DOC transformation indicate a variety of different enzymes and functions. To the right, relative transcript abundance for phototrophic, oxidative and house-keeping genes are presented, oxidative genes were selected from a gene list described by (Moran 2008) (D). The metatranscriptomic data originate from a Baltic Sea transcriptome dataset (unpublished: at LMO, RNA samples were taken roughly monthly during 2012-2014, prefiltered through 3  $\mu\text{m}$  and collected on 0.2  $\mu\text{m}$  filters). Extracted, cleaned and linearly amplified RNA was sequenced using an Illumina Hiseq instrument, quality controlled and mRNA was extracted from all RNA sequences using sortmeRNA (Kopylova, et al. 2012), annotated against the Baltic Sea Reference Metagenome assembly (BARM, (Alneberg, et al. manuscript)) and grouped into Pfam-protein families. Transcripts were standardized by dividing the counts per gene by the total amount of annotated counts per sample; tpm denotes transcripts per million. Transcripts are color-coded according to season.

## Photoheterotrophy – the light effect

Photoheterotrophic bacterioplankton can utilize light energy in several ways; aerobic anoxygenic photosynthesis, anaerobic anoxygenic photosynthesis or through proteorhodopsins-based energy capture from light. Despite that these organisms use different light capture systems, most of them use DOC as ATP, electron and carbon source, and light generated  $H^+$  gradient to obtain additional energy (Table 1), but see anaerobic phototrophic bacteria, that do not use DOC for carbon and energy (Yurkov, et al. 1998). Aerobic anoxygenic phototrophs (AAP) belong to many different phyla (Hanada 2016) and AAP bacteria have photosynthetic reaction centers with bacteriochlorophyll but not the capacity to oxidize water into oxygen (Hanada 2016, Yurkov, et al. 1998). Thus, light supplements the energetic needs for AAP bacteria, for example the increased proton gradient supports ATP production and substrate transport (Yurkov, et al. 1998).

Proteorhodopsins (PR) are light activated proton pumps that are embedded in the cell membrane of mainly prokaryotic cells and pump protons across the membrane when activated by light (Pinhassi, et al. 2016). Yet also some eukaryotic phytoplankton, fungi and viruses were described to contain proteorhodopsins (Brown, et al. 2006, Nagel, et al. 2002, Yutin, et al. 2012). Recently, several studies have investigated phylogeny, gene expression patterns and potential energetic advantages for PR-containing organisms. Proteorhodopsins are expressed by various bacterioplankton community members (Ottensen, et al. 2013). Generally, when light strikes the seven-helix protein, a conformational change in the inner chromophore pumps one ion through the channel (Sharma, et al. 2006). The most common proteorhodopsin type pumps protons out of the cytosol while other rhodopsin types can pump sodium ions out or even chloride ions in (Yoshizawa, et al. 2014). Upon light induced proton pumping, the resulting proton motive force across the membrane can in turn fuel the ATP synthase to build ATP (Béjà, et al. 2001). While for some described photoheterotrophic bacteria no growth stimulation was detected upon the growth in light, some bacteria showed responses when grown in light compared to darkness (Del Giorgio, et al. 2011, Gómez-Consarnau, et al. 2007, Gómez-Consarnau, et al. 2016, Kimura, et al. 2011). Some bacteria have been shown to have enhanced proteorhodopsin gene expression during daylight compared to night or have a prolonged survival in light compared to darkness (Akram, et al. 2013, Aylward, et al. 2015). Other bacteria, such as *Psychroflexus torquis* (*Flavobacteria*) show enhanced proteorhodopsin gene expression under salt stress (Feng, et al. 2013). In the *photobacterium angustum* S14 (*Gammaproteobacterium*) light effects are enhanced under higher cell biomass rather than low substrate availability (Courties, et al. 2015). It is now known that proteorhodopsin-containing bacteria are widespread in marine surface environments and comprise up to more than half of the bacterioplankton (Béjà, et al. 2001, Campbell, et al.

2008, de la Torr , et al. 2003, Pinhassi, et al. 2016). Therefore, their impact on biogeochemical nutrient cycling is potentially tremendous and requires further investigations.

## Microbial species concept

The classical biological species (CBS) concept was developed for interbreeding eukaryotic organisms; applying these CBS-rules and classification for asexually reproducing bacteria is difficult, if not impossible, and therefore a number of other species concepts have been proposed. I will not discuss how a ‘true bacterial species’ can, or should be, defined, but rather explain the practical taxonomic approach that is currently widely used in microbial oceanography to group bacterial diversity into “operational taxonomic units” (OTUs) or populations.

Traditionally, marine bacteria were classified using characteristics identified in monocultures, such as when grown on agar plates or in seawater cultures (Hagstr m, et al. 2017). With advances in DNA extraction and bioinformatics, DNA-DNA hybridization techniques were initially used to study relatedness between bacterial isolates (Rossell -M ra, et al. 2015). Specifically, a degree of >70% cross-hybridization between genomic material of two isolates was classified as the same species (Rossell -M ra, et al. 2015). Later, the DNA-DNA hybridization degree was translated to approximate nucleotide identity levels of the highly conserved small subunit of the ribosomal gene (16S rRNA gene) of bacteria (Pedros-Alio, et al. 2015, Rossell -M ra, et al. 2015, Woese 1987). Whilst this method was first used for bacterial isolates and clones, it rapidly became a common tool for microbiologists working with environmental genetic material using amplicon-sequencing methodologies (e.g. (Sunagawa, et al. 2015)). Using this method, OTUs are clustered at a certain threshold (e.g. 97% sequence identity) of the 16S rRNA gene to classify them as the “same”. When applied to environmental samples, this led to the realization that only about 1% of marine bacteria are ‘culturable’ on standard media, that is, are able to be described and identified by traditional methods (Hagstr m, et al. 2017). This was later known as the great plate count anomaly (Hugenholtz 2002, Staley, et al. 1985). Some ecotypes (a sequence cluster coupled with distinct ecological attributes (Cohan 2006)) or populations can be classified at higher thresholds (Pedros-Alio, et al. 2015). For example, *Prochlorococcus* and *Synechococcus* 16S rRNA genes are within 96% identical, however they belong to different species (Rocarp, et al. 2002). Moreover, within the *Prochlorococcus* clade, many different ecotypes are described, which inhabit different ecological and spatial niches, typically exhibiting more than 97% 16S rRNA gene sequence identity (Kashtan, et al. 2014, Malmstrom, et al. 2010). To increase resolution, more conservative clustering thresholds (>98.7%) have been used to describe

heterotrophic bacteria populations (Rosselló-Móra, et al. 2015), using programs such as *dada2* or *Deblur* (Amir, et al. 2017, Callahan, et al. 2016). These techniques use all “true” amplicon-sequences in a dataset, correcting sequencing errors using a statistical approach. Therefore, they are able to disentangle closely related populations (Amir, et al. 2017, Callahan, et al. 2016). In recent years, ‘high throughput sequencing’ instruments and techniques were developed that are faster, more efficient and cheaper compared to older methods (Caporaso, et al. 2012). Methods such as 16S rRNA gene amplicon techniques, are therefore now frequently used to annotate microbial datasets taxonomically and even metagenomic and metatranscriptomic analyses generate datasets of functionally annotated genes.

In summary, genetic techniques such as 16S rRNA gene amplicon techniques are a widely used approach to study bacterial taxonomic diversities, due to their economic and technical practicality. These detailed taxonomic techniques are currently painting a new picture of the vast microbial diversity existing on our planet.

## **Taxonomic diversity in the Baltic Sea**

Bacterioplankton communities are taxonomically diverse. Laboratory studies of model organisms provided insights about growth characteristics, genotypic and phenotypic traits of some marine bacteria classes, especially *Alphaproteobacteria* (such as the SAR11 clade and roseobacters) and *Flavobacteria*. For example, *Alphaproteobacteria*, can have distinct strategies: SAR11 clade organisms (such as *Candidatus Pelagibacter ubique*) have a streamlined genome (Giovannoni, et al. 2005). They mainly have transporters with a broad substrate range additional to a number of specific substrate targets, such as amino acids, osmolytes or N-compounds (Giovannoni, et al. 2005). They also contain many ABC transporters exhibiting high substrate affinities (Giovannoni, et al. 2005). Yet, despite the advances in deciphering *Candidatus P. ubique* behavior in cultures, other SAR11 strains remain to be cultivated to disentangle their growth characteristics. Other *Alphaproteobacteria*, such as roseobacters (e.g. *Silicibacter pomeroyi*) contain an overall large gene repertoire and can be cultivated (Brinkhoff, et al. 2008, Buchan, et al. 2005). Members of this lineage can degrade aromatic compounds, sulfidic compounds, DMSP or oxidize carbon monoxide (Buchan, et al. 2005). Yet, while members of this family (and possibly other bacterial phyla) are able to conduct all these functions, not all members contain the entire gene repertoire (Buchan, et al. 2005), making extrapolation from taxonomy (that is taxonomic annotation of organisms) to function (such as phenotypic traits) somewhat complicated. Thus, while some roseobacters are highly specialized for certain substrates, others can use a wide variety of DOM (Brinkhoff, et al. 2008). These different traits lead to an overall high

abundance of roseobacter and SAR11- clade bacteria in marine systems, yet occupying different niches (Brinkhoff, et al. 2008, Buchan, et al. 2005, Giebel, et al. 2011). Furthermore, other copiotrophic bacteria such as *Flavobacteria* are often found attached to particles, especially during and after phytoplankton blooms (Buchan, et al. 2014), which is encoded in gliding motility genes (González, et al. 2011). For the *Dokdonia* sp. MED134, for example, many lipoproteins and secretion proteins, carbohydrateester hydrolysis and many predicted peptidases are reported (González, et al. 2011).

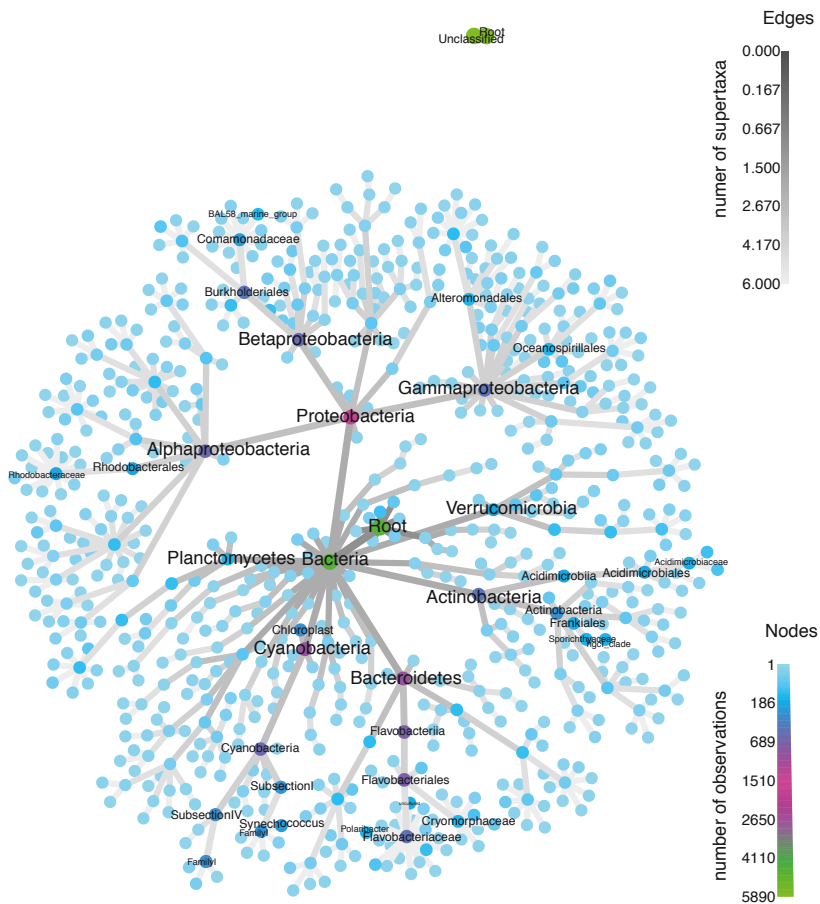


Figure 3. All bacterial genera identified at Linnaeus Microbial Observatory (LMO) in the western Baltic Proper during 2011-2017. The LMO dataset is based on 16S rRNA amplicon sequences, analyzed with dada2 and drawn using the metacoder and ape packages in R (n=660) (Callahan, et al. 2016, Foster, et al. 2017, Paradis, et al. 2004). Each leaf node displays one genus, node color gradient shows the number of genera (observations) for each taxon, edges display the number of supertaxa.

*Dokdonia* sp. MED134 thus has a preference for peptides over polysaccharides (González, et al. 2011), explaining its growth in peptone medium. These three bacterial classes are abundant in seawater samples, and also in the Baltic Proper they display major roles, together with other marine bacteria (Lindh, et al. 2015) (Figure 3).

The Baltic Sea is a semi enclosed sea in northern Europe that displays a salinity gradient from freshwater-like waters in the Bothnian Bay, salinities around 7 PSU in the Baltic Proper to marine salinities (~33 PSU) in the Skagerrak. In the Baltic Sea, two extensive phytoplankton blooms appear yearly, a spring bloom that is composed of dinoflagellates and diatoms, and a summer bloom of filamentous cyanobacteria (Karjalainen, et al. 2007, Klais, et al. 2011, Suikkanen, et al. 2007, Wasmund, et al. 1998, Wasmund, et al. 2011, Wasmund, et al. 2003). Sometimes, a smaller autumn bloom consisting of diatoms can be observed (Wasmund, et al. 2011). The intensity of spring blooms exhibits inter-annual variability and diatoms and dinoflagellates shift in dominance (roughly oscillating over decades) (Klais, et al. 2011, Wasmund, et al. 2011). During summer, filamentous cyanobacteria thrive in warm, stratified surface water conditions (Bertos-Fortis, et al. 2016, Karjalainen, et al. 2007, Legrand, et al. 2015). Because of the multiple phytoplankton bloom occurrences per year, the Baltic Sea is a suitable environment to study the response of heterotrophic bacteria to phytoplankton blooms and to investigate their impact on biogeochemical cycles (via for example heterotrophic production estimates) and consequences for the ecosystem.

In bacterioplankton community composition datasets in the Baltic Sea, the freshwater influence is particularly obvious given the abundance of *Actinobacteria*, which are associated with low saline and brackish waters (Dupont, et al. 2014, Herlemann, et al. 2011, Lindh, et al. 2015). Towards more saline waters in the southern Baltic Sea, *Alphaproteobacteria* and *Gammaproteobacteria* increase in relative abundance during summer (Dupont, et al. 2014). In the Western Baltic Proper, at Linnaeus University Observatory (LMO), *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria* are the most abundant members of the proteobacterial clades (Figure 3 and (Dupont, et al. 2014, Herlemann, et al. 2011, Lindh, et al. 2015)). Class *Flavobacteriia*, *Roseobacter* clade species (*Alphaproteobacteria*) and *Gammaproteobacteria*, that are commonly following eukaryotic phytoplankton blooms (Buchan, et al. 2014, Bunse, et al. 2016, Pinhassi, et al. 2004, Riemann, et al. 2000, Teeling, et al. 2012, Teeling, et al. 2016), are also frequently observed in the Baltic Sea (Laas, et al. 2016, Lindh, et al. 2015). Not to be neglected are members of the phylum *Planctomycetes* and *Verrucomicrobia*, which were detected in elevated abundances during summer (Andersson, et al. 2010, Lindh, et al. 2015).

## **Bacterioplankton community structure**

Life and microbial communities are highly dynamic. The community structure of bacterioplankton (that is, species composition and the corresponding abundances of populations) changes with time, space, depth, interactions and nutrient availability (Fuhrman 2009, Fuhrman, et al. 2008). Photosynthetic bacteria have growth rates of about  $0.75 \text{ d}^{-1}$ , as for the single celled cyanobacterium *Synechococcus* (Kirchman 2016). Marine heterotrophic bacteria *in situ* often reach growth rates between  $0.1\text{-}0.3 \text{ d}^{-1}$ , with abundances of about half a million to millions of cells per millilitre (Kirchman 2016, Lindh, et al. 2015, Whitman, et al. 1998). Growth rates and cell abundances depend on the lifestyle of the bacterioplankton population, they can be oligotrophic (for example SAR11, that is adapted to low nutrient concentrations) or copiotrophic (e.g. members of the class *Flavobacteria*). In the low-nutrient (oligotrophic) ocean, oligotrophic bacteria can compete for resources, for example by using high-affinity and low capacity transport systems to exploit substrates (Hagström, et al. 2017). Copiotrophic bacteria (sometimes also called opportunistic bacteria) on the other hand can quickly respond to organic nutrient input, grow and build biofilms (Hagström, et al. 2017, Pinhassi, et al. 1997). Under nutrient-rich conditions in monocultures and under optimal temperatures, copiotrophic marine bacteria exhibit growth rates between  $0.2 \text{ h}^{-1}$  to  $1 \text{ h}^{-1}$  while oligotrophic marine bacteria show growth rates up to  $0.02 \text{ h}^{-1}$  in seawater cultures (Hagström, et al. 2017). Therefore, the potential for fast changes in microbial community structure are vast and become especially evident during times of nutrient input, such as during coastal phytoplankton blooms, where community structure can change within days (Lindh, et al. 2015, Needham, et al. 2016, Pinhassi, et al. 2004, Riemann, et al. 2000, Teeling, et al. 2012). Community compositions change depending on which bacteria grow actively and which die, but also functional capacities of communities can change. For example, in polar regions, shifts in community composition and functional capacities of microbial communities changed from a photosynthesis-fueled community during summer to a more chemotrophic community during dark winters (Ghiglione, et al. 2012, Grzyski, et al. 2012). Remarkably, such changes can appear at a single sampling location (Grzyski, et al. 2012), revealing a vast functional diversity of microbial communities throughout the year.

### **Temporal patterns**

Microbial communities change over time. Applying microbial ecology and molecular biology tool boxes on samples from marine microbial time-series, it is now possible to disentangle which populations in the microbial community are doing what, when and how across seasons. Included in this quest, and a main objective for microbial oceanographers and environmental biochemists, is to measure how bacterial communities, use their genetic repertoire and gene

expression patterns to regulate the turnover of DOM. This can be studied at different scales; from a broad scale such as ocean wide basins and overall biomass fluxes for a modelling perspective, to narrower scale location at high temporal resolution to understand the biological processes typical to different environments. One way to infer the appropriate scale is to consider water masses, time frames and distances from a bacterial viewpoint. Given a common non-motile bacterial cell is about  $\sim 0.4 \mu\text{m}$  in diameter, distances to other bacteria or aggregates which display potential food sources, are long. Brownian diffusion moves cells through the surrounding seawater so that they reach about 80 nl per day (Stocker 2012). Nutrients can then be taken up in the diffusion boundary layer, covering a few cell diameters (Stocker 2012). Swimming microbes on the other hand can reach a volume of about 0.8 ml a day, that is about 10 000 times the volume reached by non-motile bacteria (Stocker 2012). Furthermore, if bacteria can turn over half of their metabolite pool in one second, and express genes within minutes, this leads to growth rates of close to  $1 \text{ h}^{-1}$  in enriched medium (Hagström, et al. 2017, Shamir, et al. 2016). Notably, bacterial processing of DOM at the microscale ultimately influences ocean basin-scale carbon fluxes for example through the microbial loop, carbon storage, and carbon fixation itself (Azam 1998). Logically short timescales are important for microbes at the physiological level, such shifts can be semidiurnal (tides), diurnal (biological phototrophic production), weekly, monthly and seasonal (Gunderson, et al. 2016). Thus, also yearly timeframes remain of interest to oceanographers due to broad level factors such as seasons, and even beyond that, climate. Overall, marine seasons are driven by differences in light intensity and resulting temperatures that in turn impact on water chemistry and physical traits of seawater.

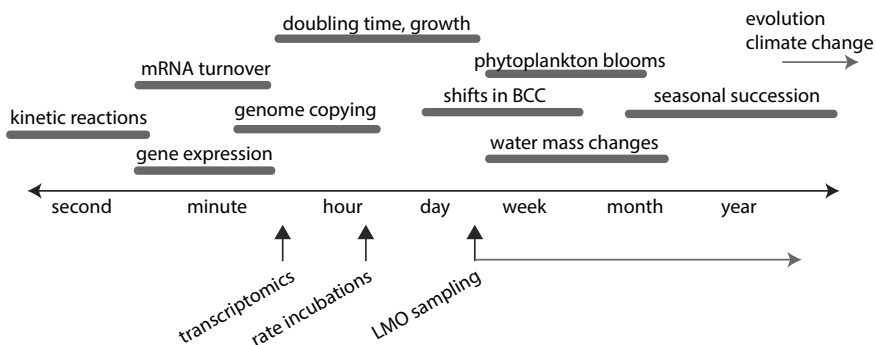


Figure 4. Conceptual summary of relevant timescales for bacterioplankton. Cellular and metabolic processes are very fast ( $< \text{hour}$ ) while environmental changes (such as phytoplankton blooms, temperatures or seasons) occur at successively longer time frames. In the Baltic Proper at LMO, samples for this thesis were sampled approximately twice a week and bioassays were incubated for one to two hours, while transcriptomic samples were processed within half an hour.

## **Multiple climate change drivers (*do bacteria care?*)**

Anthropogenic activities, such as combustion of fossil fuels, have increased CO<sub>2</sub> concentration in the atmosphere and resulted in increased  $p\text{CO}_2$  values in seawater, particularly intensive in the last century (Doney, et al. 2009, Dore, et al. 2009, Pelejero, et al. 2010). Due to carbon chemistry in seawater, elevated CO<sub>2</sub> absorption leads to a shift in the equilibrium from CO<sub>2</sub> towards bicarbonate ions, resulting in increased proton concentration (reduced pH) (Doney, et al. 2009). A reduction of 0.3-0.4 pH, as projected for the end of this century by models of Haugan et al. (1996), could thus lead to up to 150% increase in H<sup>+</sup> concentrations and 50% decrease of CO<sub>3</sub><sup>2-</sup> concentrations ((Doney, et al. 2009, Haugan, et al. 1996, Orr, et al. 2005) and references within). Marine microbial cells are separated to the seawater only through thin membranes, cell walls or calcified capsules. Higher proton concentrations in the surrounding seawater will consequently increase the proton pressure on the membrane. Ocean acidification directly affects calcification of several calcareous plankton (such as coccolithophores, reviewed in (Doney, et al. 2009) and (Hutchins, et al. 2017)). Phytoplankton photosynthesis might be expected to increase under predicted ocean acidification conditions, aided by potential decreasing needs for energetically costly CO<sub>2</sub> concentrating mechanisms (Hutchins, et al. 2017). Yet, how ocean acidification will affect overall phytoplankton community composition and productivity are often hard to predict, also as they further depend on temperature and nutrients (Hutchins, et al. 2017).

Additional predicted climate changes include for example increased land runoff, changes in trace metal concentrations, and rising seawater temperatures (Boyd, et al. 2012). While these are global trends, it needs to be highlighted that many stressors differ locally, regionally and globally (Boyd, et al. 2012). For example, models for the Baltic Sea area indicate that warming and increased precipitation may have profound effects on ice cover and salinity in the brackish water system, while in the Mediterranean Sea decreased precipitation and warming are anticipated (Andersson, et al. 2015, Giorgi, et al. 2008). Future climate change and ocean acidification research is therefore urgent to determine the effect of multiple stressors on communities and ecosystems, rather than single driver and single species studies (Riebesell, et al. 2015).

In a climate change perspective, it is important to consider that natural bacterial communities are continuously exposed to changes in water chemistry, temperatures, as well as nutrient, substrate, O<sub>2</sub> and CO<sub>2</sub> concentrations. For example, during phytoplankton blooms, CO<sub>2</sub> fixation leads to increases in pH (Joint, et al. 2011). Seasonal changes may increase surface water temperatures by up to 20°C during summer compared to winter (Ward,

et al. 2017). Some studies conclude direct as well as indirect effects of elevated CO<sub>2</sub> on bacterial community composition, respiration or productivity (Arnosti, et al. 2011, Grossart, et al. 2006, James, et al. 2017, Krause, et al. 2012, Piontek, et al. 2010), while others have found no significant effects on the bacterial communities (Allgaier, et al. 2008, Hartmann, et al. 2015, Oliver, et al. 2014, Roy, et al. 2008). As there is no consensus on how microbes will respond to ocean acidification or combined multiple stressors (Hutchins, et al. 2017, Pachauri, et al. 2014), one prominent hypothesis is that bacterioplankton and carbon export, via the microbial loop, will not be significantly altered in the future (Joint, et al. 2011). Yet, the IPCC report states that for “microbes, a conceptual foundation suitable to support an integrated understanding of climate impacts on individual species and communities is lacking” (Pachauri, et al. 2014). If marine bacteria can withstand the proton pressure of elevated CO<sub>2</sub>, for example with the help of proton pumps that are involved in bioenergetics (Joint, et al. 2011), it would be desirable to understand the underlying biochemical processes and enzymatic reactions. If they cannot household internal proton concentrations under predicted future ocean scenarios, this could lead to potential shifts in bacterial production and respiration rates, resulting differences in growth efficiencies, competition, abundances and community composition.

## Aims

*“Indeed, why not an issue on marine microbes? This field is fascinating, not only because it is the study of that which cannot be seen with the naked eye but also because it is the study of organisms that are fundamental to the functioning and health of the ocean.”*

*L.M. Proctor and D.M. Karl, A Sea of Microbes*

This thesis addresses how important environmental drivers influence bacterial responses using experiments with model species and natural assemblages as well as field studies. Further, this thesis aims to identify bacterioplankton succession patterns and activities as response to seasonal changes in the Baltic Proper.

Using laboratory approaches and a high-frequency time series study, I specifically aimed to:

- determine how the metabolism of proteorhodopsin-containing, flavobacterium *Dokdonia* sp. MED134 is influenced by light and different organic matter (using gene expression analysis) (Paper I)
- determine how the community composition and physiological processes of marine bacteria are affected by elevated CO<sub>2</sub> concentrations and inorganic nutrient loads (Paper II)
- determine how microbial activities and community composition are influenced by temporal changes and growth conditions and how this leads to seasonality in microbial functioning in temperate marine systems (Paper III, IV, V)

## **A brief overview of methodology**

*“In one drop of water are found all the secrets of all the oceans.”*

*Kahlil Gibran*

*“When we’re 1,000 km from shore, and our \$32,000-a-day cruise depends on getting one last profile to 4,000 m depth, we’ll find the duct tape, silicon sealant, and dry nitrogen to make things work.”*

*Richard Spinrad, A Sea of Microbes*

### **Sampling sites**

Time-series data in this thesis derive from the Linnaeus Microbial Observatory (LMO), situated in the Western Baltic Proper, approximately 11 km off the coast of Kårehamn, Öland (N 56° 55.8540', E 17° 3.6420'). At LMO, surface water samples collected at a depth of 2 m were sampled approximately twice a week (during spring to autumn 2011-2013), once a month (2014), and bi-weekly (2015-2016). Water was transported to the laboratory in Kalmar in 10 l carboys where it was subsequently processed further for community composition analysis and bacterial activities (Paper III & IV). Water for the mesocosm experiment in paper II derived from the Mediterranean Sea in Blanes Bay (Figure 5).

### **Cell enumeration and productivity estimates**

Flow cytometry and microscopy were used for bacterial enumeration. For Flow cytometry analysis, water samples were fixed with formaldehyde (~3% final concentration) and preserved frozen until analysis. After staining the bulk bacterioplankton using SYBR Green or Syto13, instruments like FACSCalibur (Becton Dickinson) or Cube8 (Partec) were used to count the stained cells. For microscopic analysis, microbial cells were similarly fixed with formaldehyde, stained with SYBR Gold and filtered on black polycarbonate filters, mounted on a glass slide and counted under the epifluorescence microscope (Olympus BX50, 1000x magnification).

Phytoplankton biomass was counted under the light microscope and estimated measuring chl *a* fluorescence. Secondary/ heterotrophic production was measured using  $^3\text{H}$ -labelled leucine uptake into cells over time (Smith, et al. 1992). Further substrate uptake rate constants were measured via labelled substrate ( $^{14}\text{C}$  or  $^3\text{H}$ ) uptake into biomass over a set incubation time (Paper IV). Additionally, the activity of extracellular enzymes (such as beta-glucosidases or aminopeptidases), indicative for heterotrophic polymer degradation, was measured at the sampling site, LMO, as described in (Baltar, et al. 2016).

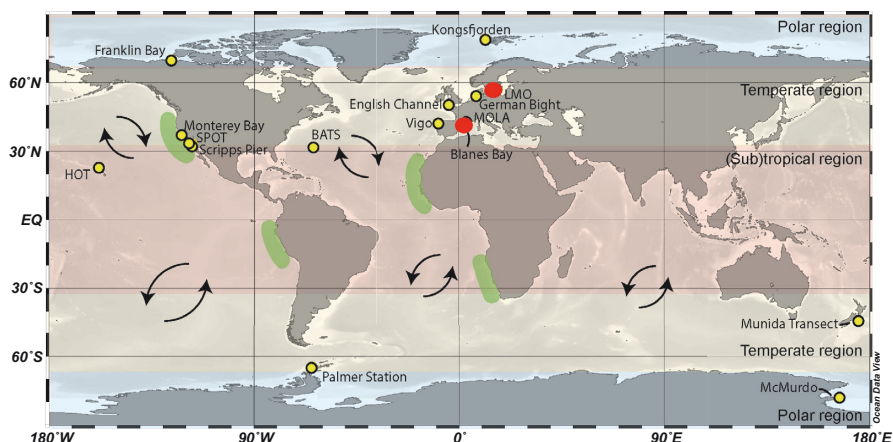


Figure 5. World map depicting selected microbial time-series (in yellow points) and visualizing polar, temperate and tropical regions. The figure is redrawn from Paper V (Bunse, et al. 2017). Station LMO and Blanes Bay, where water samples for this thesis derived from, are highlighted in red. Green shadows indicate upwelling zones.

### Model organism work: flavobacterium *Dokdonia* sp. MED134

In paper I, growth and gene expression of the photoheterotrophic flavobacterium *Dokdonia* sp. MED134 was investigated. *Dokdonia* sp. MED134 was isolated on Zobell agar plates (ZoBell 1946) in the Northwest Mediterranean Sea and is one of the marine bacteria whose genome was sequenced during the Gordon and Betty Moore Foundation marine microbiology initiative (González, et al. 2011). It was previously described to use proteorhodopsin-generated energy (Gómez-Consarnau, et al. 2007, Gómez-Consarnau, et al. 2016, Kimura, et al. 2011). Building on this, *Dokdonia* sp. MED134 was grown in artificial seawater medium enriched with complex DOM (peptone and yeast extract) or alanine (alanine and trace concentrations of yeast extract, for supply of essential vitamins and amino acids) to assess its photoheterotrophic traits (Paper I). Upon growth, gene

expression was monitored by harvesting cells, RNA extraction and RT-qPCR amplification of gene products using gene-specific primers (Paper I). Particulate organic carbon of harvested biomass was measured by harvesting cells of grown cultures on precombusted filters, drying and subsequent analysis for carbon content (Costech ECS 4010 Elemental Analyzer; Costech International). Bacterial production and bicarbonate uptake were measured using the above productivity estimates

## **16S rRNA gene amplicon data**

For bacterial community composition analysis, bacterioplankton cells were collected on 0.2  $\mu\text{m}$  filters (Sterivex filters, after prefiltration through 3.0  $\mu\text{m}$  polycarbonate filters) and DNA was extracted using protocols adapted from (Boström, et al. 2004, Lindh, et al. manuscript). The V3V4 region of the 16S rRNA gene was amplified using primers 341f-805r as described in (Hugerth, et al. 2014). Subsequently, Illumina adapters were attached to the amplicon product through a second PCR, and adapter-ligated DNA was pooled and cleaned according to Lindh et al. (unpublished manuscript). The resulting DNA libraries were sequenced on Illumina Miseq instruments at the SciLifeLab in Stockholm (using 2x300 bp).

For the LMO amplicon dataset described in this thesis, we wanted to be able to identify populations by distinct sequences of the 16S rRNA gene, and further to have a continuously running time-series dataset, to be able to add data of future sampling campaigns. Therefore, we decided to use curated sequences, instead of traditional clustering methods obtaining OTUs. Thus, after quality control, the resulting raw sequences were processed using the *dada2* pipeline (Paper III) (Callahan, et al. 2016). Resulting sequences (populations from here) were further analyzed in Rstudio using a variety of packages, mainly *dplyr*, *vegan*, *ggplot2*, or *Mfuzz* (Kumar, et al. 2007, Oksanen, et al. 2007, Wickham 2009, Wickham, et al. 2015).

## **Metatranscriptomics**

For metatranscriptomic analysis of bacterioplankton communities in paper II, seawater from Blanes Bay (41°40'13.5"N, 2°48'00.6"E) was incubated under different nutrient and CO<sub>2</sub> conditions for 9 days in 200 l tanks. Bacterioplankton biomass from day 9 was pre-filtered (3.0  $\mu\text{m}$ ) and harvested on 0.2  $\mu\text{m}$  filters. RNA was extracted and processed using a protocol adapted from (Poretsky, et al. 2009). Briefly, after RNA extraction and remaining genomic DNA removal (RNEasy kit (Quiagen) and TURBO DNA-free kit (Ambion)), rRNA was depleted (mRNA-ONLY prokaryotic mRNA isolation kit (Epicenter Biotechnologies), MICROBExpress (Ambion) and MICROBEnrich (Ambion)). Then, the MessageAmp II-Bacteria kit (Ambion) was used to synthesise cDNA and linearly amplify the RNA. The resulting RNA was shotgun sequenced on an Illumina HiSeq2000 sequencing instrument.

Briefly, the bioinformatics pipeline involved quality control, trimming, ribosomal RNA filtering, assembly of the sequences, open reading frames (ORFs) were called on contigs (Paper II). These ORFs were annotated using M5NR SEED (Overbeek, et al. 2005), KEGG (Kanehisa, et al. 2007), and RefSeq (Overbeek, et al. 2005) databases using BLAST (Altschul, et al. 1997). For quantitative annotations, the original sequences were mapped back against annotated ORFs using *Bowtie2* (Langmead, et al. 2012) and relative transcript abundances were calculated as counts per million (CPM). SEED categories were subsequently used for grouping functional genes into broader metabolic categories.

### **Method limitations in the field of microbial ecology**

All methods have limitations and uncertainties. The currently used methods, which have been employed here, have been evaluated and are in use by other groups or research fields, or might be traditional methods in oceanography. Regardless, interpreting the results should be conducted in consideration of the possible methodological drawbacks and biases. For example, in the field of microbial oceanography, filtration is central in collecting planktonic biomass, sterilize medium or to size-fractionate microbial communities. However, it remains a challenge to separate phytoplankton cells from bacterioplankton, and autotrophic cells from heterotrophic cells prior to incubation experiments. Fragile cells can be disrupted on filters, resulting in unintended cell lysis. Cells which are larger than the filter pore-size can be pushed through pores due to pressure, or conversely, cells can be caught on the filter due to organic material or particles, although their cell size should allow them to pass through pores. Further, the chosen filter pore size (in this thesis mostly 0.2  $\mu\text{m}$ ) dictates a threshold of which bacteria are included in the analysis, missing smaller cells (Brown, et al. 2015). Some of these limitations can be bypassed with cell-sorting flow cytometers or specific probes for fluorescent *in situ* hybridization (FISH) methods, yet these methods are mostly applied after incubation experiments, thus not entirely disentangling direct or indirect effects of e.g. multiple stressors in community experiments.

Several of the projects discussed in this thesis rely on ‘high throughput sequencing’ techniques, such as 16S rRNA gene amplicon techniques, metagenomic or metatranscriptomic analysis. These sequencing methods have revolutionized our understanding of microbial communities and are relatively straightforward to use, efficient and cheap, and allow comparing datasets from distantly related locations or over different time intervals. These methods are, however, biased with limitations, for example, nucleic acid extraction efficiencies and biases, time-dependent degradation of biological material, primer bias, PCR and sequencing efficiencies (see for example (Parada, et al. 2016)). Furthermore, the suitability of the 16S rRNA gene for relative abundance assessment of prokaryotic communities is problematic in itself as many bacterial taxa are polyploid (Soppa 2017). Consequently, shotgun-

sequencing projects (which do not amplify specific genes) often differ from amplicon datasets in community composition (Tessler, et al. 2017). Moreover, functional and taxonomic annotations are only as good as the databases to which they are annotated. Often, a high number of unknown genes may be present in samples, potentially limiting our interpretations. Furthermore, combined taxonomic and functional annotations are difficult to obtain and often not sufficiently detailed for non-model organisms or *in situ* assemblages. Despite their limitations, high throughput sequencing techniques have opened a “black box” of opportunities and advances that were hidden from scientists before the development of efficient extraction and deciphering of nucleic acid material. Supplemented with other methods, such as qPCR, FISH, activity measurements or bacterial abundances, they are good indicators and generate solid hypotheses that can be further investigated and tested. Thus, the supremacy and advantages of the mentioned methods (or the lack of alternatives) overpower their possible limitations.

## Results and Discussion

### Light effects on model organism *Dokdonia* sp. MED134

Physiological traits and gene expressions of metabolic pathways of photoheterotrophic marine bacteria are not much studied, thus hampering our understanding of how photoheterotrophic organisms impact on biogeochemical nutrient cycling. In paper I, we investigated light enhanced growth and bicarbonate uptake rates as an indication for anaplerotic carbon fixation of proteorhodopsin-containing *Dokdonia* sp. MED134. Estimates of the contribution of anaplerotic carbon fixation to bacterial biomass in lab cultures range from negligible 1.4% to substantial 15% (Roslev, et al. 2004, Tang, et al. 2009). In paper I, bicarbonate contributed between 5% and 31% of cell carbon and was elevated during growth in light for *Dokdonia* sp. MED134. In a previous study, also an aerobic anoxygenic phototrophic bacterium (*Acidiphilium rubrum*) was reported to have enhanced CO<sub>2</sub> fixation rates in light (Kishimoto, et al. 1995). *In situ* estimations of anaplerotic carbon fixation rates of heterotrophic or photoheterotrophic bacterioplankton in surface waters are to my knowledge not existent. It is difficult to measure rates to precisely estimate the contribution of anaplerotic carbon fixation of bacterioplankton among phytoplankton communities, hampering our understanding of how abundant these metabolisms are in nature. This in turn leaves us with an incomplete picture of the carbon cycle in marine ecosystems, with special respect to the microbial loop. It could thus be that anaplerotic carbon dioxide fixation is so far overlooked, not significant or that it is already included in <sup>14</sup>C primary production estimates (depending on methodology and e.g. filter sizes as discussed in the method section) but that we attribute it to phytoplankton primary production.

Interestingly, proteorhodopsin gene expression was low during exponential growth and increased during late exponential and stationary phase (Paper I). The glyoxylate shunt is a pathway that permits the use of C<sub>2</sub>-compounds to fill up cellular carbon supplies when glucose is limiting and bypasses

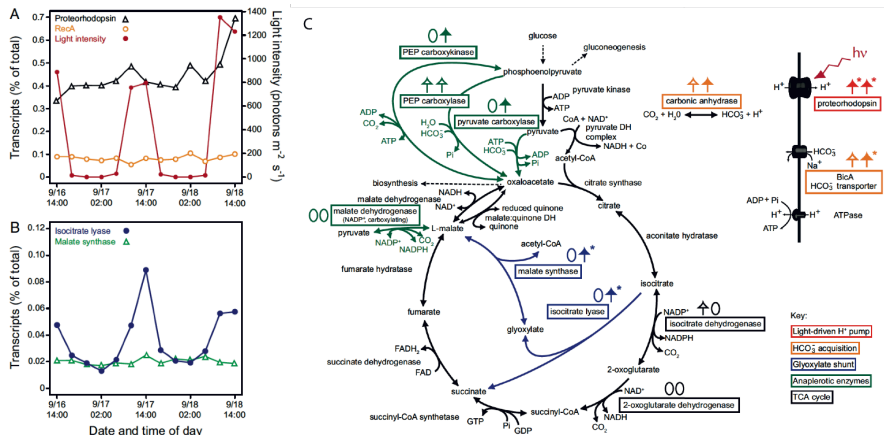


Figure 6. Relative transcript abundance and light intensity of the Monterey Bay dataset (A and B) and schematic TCA cycle (C) indicating the regulation of the studied genes, the figure is redrawn from Paper I (Palovaara, et al. 2014). Arrows indicate if genes were upregulated and 0s indicate no different expression upon growth in light.

decarboxylation steps of the traditional TCA cycle (Lorenz, et al. 2002) (Figure 6) and has been described for AAP bacteria (e.g. roseobacters (Moran, et al. 2007)). During growth in light in alanine medium, the gene expression of proteorhodopsin and the two glyoxylate shunt genes (isocitrate lyase and malate synthase) was enhanced in *Dokdonia sp.* MED134 (Paper I, Figure 6). In complex medium on the other hand, the glyoxylate shunt genes were only minimally expressed. The enhanced expression of the glyoxylate shunt genes isocitrate lyase and malate synthase in light in alanine medium suggests a relocation of the metabolic pathways from a more heterotrophic metabolism (TCA cycle centered) towards a photoheterotrophic and anaplerotic bicarbonate-fixing metabolism (Paper I). Interestingly, diel patterns of isocitrate lyase gene expression could be observed in the bacterioplankton community off Monterey Bay (Paper I, Figure 6). Subsequently, high glyoxylate shunt gene expression of several photoheterotrophic bacterioplankton clades (such as SAR11, SAR86 or SAR116) has been observed, with higher gene expression during day in the California coast and the North Pacific Subtropical Gyre (Aylward, et al. 2015). The glyoxylate shunt therefore seems to play an important role for proteorhodopsin-containing marine bacteria. Together, these results imply that photoheterotrophic bacterioplankton have the capacity for anaplerotic carbon fixation and glyoxylate shunt gene expression, that might aid them when nutrient quality and quantity is limiting in the environment.

# **Bacterioplankton responses to elevated CO<sub>2</sub>**

*“The cell never acts; it reacts” Ernst Haeckel*

## **Elevated CO<sub>2</sub> in high chlorophyll environments**

Responses of bacterioplankton to climate stressors appears to differ between study systems (Hutchins, et al. 2017, Pachauri, et al. 2014). This has led to the proposal that marine bacteria and the functioning of the microbial loop might not be significantly different in the future ocean (Joint, et al. 2011). Yet, the underlying biochemical and physiological processes and stimuli with which marine bacteria respond to projected environmental drivers remain largely unknown. Ocean acidification experiments frequently study the combined effects of bacterioplankton and phytoplankton, for example in mesocosm experiments. In order to be able to study phytoplankton in sufficient cell numbers, growth is often stimulated by addition of inorganic nutrients. Consequently, DOM concentrations increase, that in turn can support heterotrophic bacterioplankton growth. As during phytoplankton growth, CO<sub>2</sub> uptake and respiration lead to vast changes in pH over a short time (Joint, et al. 2011), phytoplankton-coupled bacteria may have a natural resilience to pH shifts. Given that the effects of ocean acidification on marine biota may be more severe under oligotrophic, rather than coastal areas (Duarte, et al. 2013), nutrient loads in experiments should be carefully assessed.

In paper II we tested if marine bacteria would respond to elevated CO<sub>2</sub> in mesocosm experiments with Mediterranean seawater under high and low nutrient loads (High-chl, and Low-chl) for 9 days. Under elevated nutrient concentrations, phytoplankton bloomed, leading to high chl *a* values (~28 µg l<sup>-1</sup> (High-chl), (Paper II)). Furthermore, pH values increased due to carbon dioxide fixation (~8.3 in High-chl-control and 8.1 in High-chl-acidified compared to 7.8 at day 3) and bacteria responded to the phytoplankton biomass with enhanced production and growth (Paper II). The bacterial community composition showed only minor differences in the elevated CO<sub>2</sub> treatment compared to the control treatment (Baltar, et al. 2015). The transcriptomic response of the community resulted in <50 significantly differentially abundant transcripts upon acidification under High-chl (0.3% of total genes, EdgeR  $p < 0.01$ ), and among the significant genes, we did not detect obvious stress-related patterns. These results imply that bacteria in high nutrient conditions, such as in coastal waters or during phytoplankton blooms, might not be significantly affected by elevated CO<sub>2</sub> concentrations. Yet, the gene expression patterns could be camouflaged by a high gene expression response to the declining phytoplankton bloom (Duarte, et al. 2013, Spilling 2007). Alternatively, it could reflect the overall elevated pH range upon phytoplankton growth, instead of a pH below a critical threshold that requires a transcriptomic regulation (Paper II).

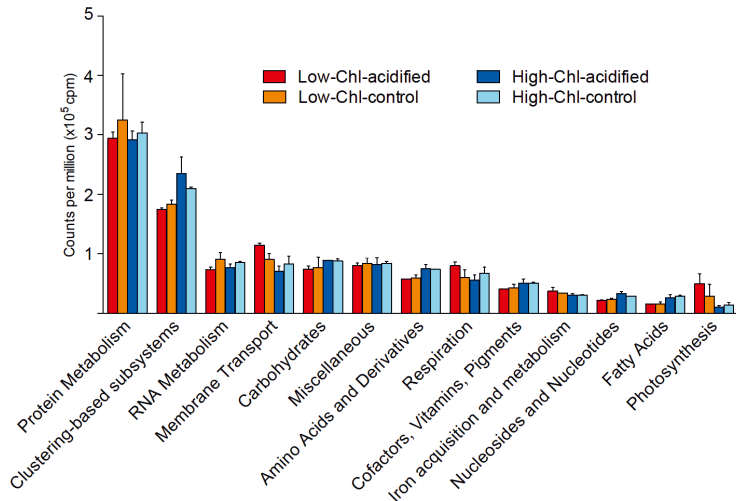


Figure 7. Transcripts from paper II, grouped into major SEED categories under low- and high- chlorophyll conditions, figure is redrawn from (Bunse, et al. 2016). Presented are mean relative RNA transcript abundances, normalized to counts per million (CPM), of duplicate samples. The colors represent control mesocosms (orange), compared to CO<sub>2</sub> treated mesocosms (red), nutrient enriched control mesocosms (turquoise) and nutrient enriched and CO<sub>2</sub> treated mesocosms (blue).

## Elevated CO<sub>2</sub> in low chlorophyll environments

In our mesocosm experiment under low nutrient conditions (Low-chl), chl *a* values remained below 2.3 μg l<sup>-1</sup> and pH decreased from 8.1 to ~7.8 (acidified Low-chl) and ~7.9 (control Low-chl) respectively (Paper II). Bacterioplankton community composition did not significantly differ between acidified and control mesocosms under Low-chl conditions (Baltar, et al. 2015). However, we detected more pronounced significant differences in relative transcript abundance compared to the High-chl treatment (>300 genes, 1.9% of total genes, EdgeR  $p < 0.01$ ). Transcripts of gene functions grouping into respiration, light harvesting, membrane transport, RNA and protein metabolism had a higher relative abundance in acidified Low-chl mesocosms compared to control mesocosms (Paper II). Many abundant genes that increased in acidified conditions could be associated with pH homeostasis mechanisms such as proton pumps (~20% of differentially abundant transcripts) (Paper II). Given similar bacterial abundance and productivity between acidified and control mesocosms (Paper II), these gene expression patterns would suggest that bacterioplankton possess the potential to physiologically adapt to elevated CO<sub>2</sub> concentrations.

Furthermore, different bacterial families expressed distinct genes in response to acidification. For example, SAR11-clade bacteria mainly expressed a pyrophosphate-energized proton pump used to expel excess protons (Paper II). A study in the offshore Atlantic found no effects of short-term 2.5-fold acidification exposure on the productivity of SAR11, suggesting physiological resilience to acidification (Hartmann, et al. 2015). Our study could thus provide an explanation of how SAR11, and other bacteria, regulate their transcriptome to respond to, and potentially overcome challenges in pH. Interestingly, proteorhodopsin was highly abundant and significantly differently abundant upon acidification in Low-Chl, hinting to an important role in pH homeostasis regulation. Proteorhodopsin has been previously hypothesized, but not shown, to modulate responses of bacterioplankton to ocean acidification because of its mode of action: light-driven proton pumping (Fuhrman, et al. 2008).

Considering that the observed mechanisms to expel protons across the cell membrane against the proton gradient are energetically costly, bacteria would potentially have to assign more energy to these processes under acidification. Under long-term exposure to elevated CO<sub>2</sub> concentrations, this could leave less energy for other processes, such as growth. Thus, despite unchanged bacterial production estimates in our nine-day experiment, increased proton expulsion and respiration rates could, under extended periods of elevated CO<sub>2</sub>, impact on bacterial growth efficiencies. In fact, a recent study showed that even short-term elevation of CO<sub>2</sub> can increase respiration of microbial communities and subsequently increase the cell-specific DOC removal and loss of organic carbon for the ecosystem (James, et al. 2017). Yet, predictions of how bacteria will respond to ocean acidification remain difficult, particularly due to the tremendous functional diversities of heterotrophic bacterioplankton (Hutchins, et al. 2017). Future studies are recommended to further incorporate respiration measurements (in addition to productivity estimates) to explore the energetic cost of pH homeostasis under low-nutrient, open-ocean scenarios. Multiple stressors as well as a differentiation between direct and indirect effects of ocean change (Boyd, et al. 2016, Hutchins, et al. 2017, Riebesell, et al. 2015), have the potential to uncover underlying fundamental physiological traits and functions of microbes to ocean climate changes, and overall gradients in marine systems.

## **Proteorhodopsin photoheterotrophy in marine systems – future outlooks**

The role of proteorhodopsin for marine bacteria could be more diverse than previously hypothesized. Figure 8 summarizes the role of proteorhodopsin under the environmental conditions studied in this thesis. Depending on nutrient quality and quantity, bacterial growth stage or pH stress, the

proteorhodopsin-resulting proton motive force could aid marine bacteria in different ways (Paper I&II). Given its occurrence across diverse phyla, high abundance in surface bacterioplankton genomes and its high gene expression levels, it is likely that even more proteorhodopsin-based functions exist in nature. Moreover, the latent role of photoheterotrophic bacteria for carbon fixation and energy turnover remains to be quantified *in situ*, seasonally and in different oceanic environments (for example using high-frequency time series and metatranscriptomics). Further recent instrumental advances, such as cell-sorting flow cytometry, single cell DNA and mRNA sequencing or FISH of functional genes – coupled to traditional oceanographic and microbiological techniques and experiments, promise great advances to uncover the functions and specific role of photoheterotrophic microbes.

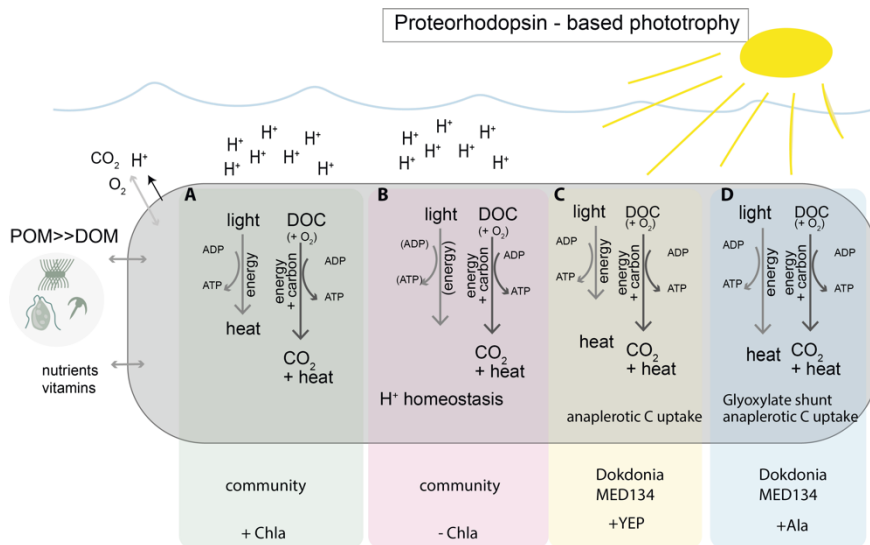


Figure 8. Conceptual figure summarizing photoheterotrophic, metabolic traits of proteorhodopsin, analyzed in this thesis (Paper I & II). In paper II, under elevated  $\text{CO}_2$  in high chl a conditions (green, A), proteorhodopsin was similarly abundant in comparison to control conditions. Relative gene expression patterns of the bacterial community showed enhanced proteorhodopsin relative abundances under elevated  $\text{CO}_2$  and low chl a conditions (red, B), possibly due to enhanced pH homeostasis regulation (paper II). In paper I, *Dokdonia* sp. MED134 expressed TCA cycle enzymes, proteorhodopsin and anaplerotic reaction enzymes under light exposure in YEP medium (yellow C), and switched the TCA metabolism towards glyoxylate pathway expression in alanine medium (blue, D).

## Seasonal bacterioplankton trends in Baltic Sea

Traditional time series have revealed persistent seasonal fluctuations and overturn of physical, chemical and some biological parameters in temperate marine areas (see for example Paper V, (Tiselius, et al. 2015, Wasmund, et al. 2011). Microbial time series have shown that, generally, seasonal succession of marine bacteria in temperate areas depends on environmental drivers such as light, day length, nutrient availability and especially temperature (Paper V) (Gilbert, et al. 2009, Gilbert, et al. 2012, Johnson, et al. 2006, Pinhassi, et al. 2000, Yung, et al. 2015). In addition to abiotic factors, biotic drivers have been identified including phytoplankton biomass, organic matter concentrations and quality, as well as biological interactions (Needham, et al. 2016, Pinhassi, et al. 2000, Teeling, et al. 2012, Teeling, et al. 2016).

In the LMO high-frequency dataset, we found overall predictable temperature and nutrient patterns and phytoplankton and bacterioplankton biomass dynamics. Seasonally, many environmental factors showed autocorrelation patterns; such as light and temperature, during spring when light and temperatures increased, phytoplankton grew and inorganic nutrients became depleted (Paper IV). During summer on the other hand, inorganic nutrient concentrations were low whereas temperatures and summer phytoplankton biomass were elevated (Paper IV). Dominating phyla of the eukaryotic phytoplankton spring bloom (diatoms and dinoflagellates) varied between 2011 and 2014; however, timing and succession of the spring bloom (in April) and summer bloom (early July) remained stable (Paper IV). In the Baltic Sea, decadal shifts in spring bloom dominance have been described with a recent tendency for dinoflagellate dominance over diatoms in the Western Baltic Proper (Legrand, et al. 2015, Suikkanen, et al. 2007, Wasmund, et al. 2011). We show that bacterioplankton displayed recurring seasonal trends at coarse taxonomic resolution, such as at class level (Figure 9, Paper III). On average, *Flavobacteria* had strongly elevated relative abundances during spring while *Actinobacteria* were relatively more abundant in late summer/autumn, and *Verrucomicrobia* peaked during summer but exhibited very low relative abundances during other times of the year (Figure 9, Paper III).

Repeating seasonal bacterioplankton community composition patterns would suggest that the community is predictable based upon seawater conditions (Fuhrman, et al. 2006). It would also imply that environmental parameters are more important than trophic interactions (Gilbert, et al. 2012). But do these deterministic effects explain all the fine-scale microbial population dynamics and productivities? Stochastic effects such as colonization, death, dispersal or migration could result in more variable patterns (Zhou, et al. 2017). For example, if, by chance, one bacterial cell is

closer to a particle compared to another, it might have advantages to colonize it and grow, which would subsequently result in an earlier progression.

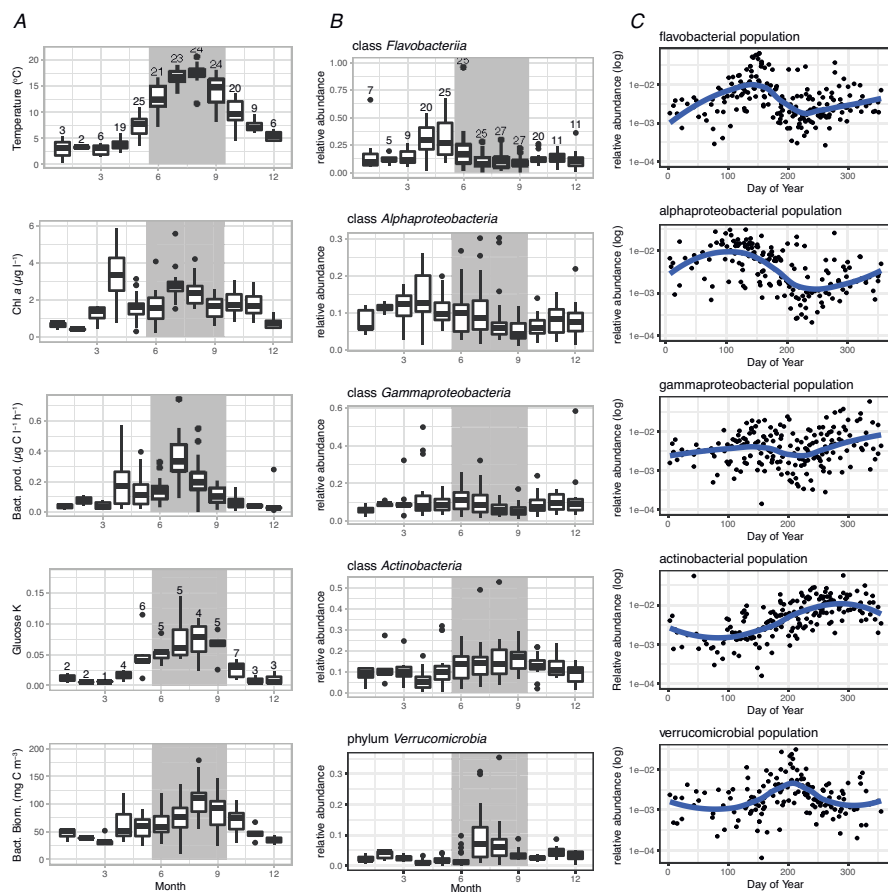


Figure 9. Boxplots grouped per month of environmental parameters (panel A) and bacterioplankton patterns (panel B), as well as dotplots of the most abundant population per class (one per class) (panel C). The data originate from the LMO time series (panel A, 2011-2014, Paper IV) and 3-0.2  $\mu\text{m}$  filter fraction in 2011-2016 (panel B and C, Paper III). Note that the Chl a graph is cut at y-axis for visualization purposes and omits one data point (April 2011; 13.45  $\mu\text{g l}^{-1}$ ). In panel B, bacterial relative abundances are summed by class (or phylum respectively) for each sampling date. The relative abundances of the most abundant populations are plotted against the day of year, blue lines indicate smoothed conditional means (method loess from ggplot2 package (Wickham 2009)). Abbreviations denote bacterial production (Bact. prod.), Glucose uptake rate constant K (Glucose K), bacterial biomass (Bact. Biom.). Numbers above temperature boxes indicate number of samples for each month of environmental samples (2011-2014), but see glucose K, while numbers above Flavobacteriia boxes indicate number of samples for 16S rRNA gene data (panel B and C, 2011-2016). Grey background panels indicate summer months.

In fact, in our study, bacterial abundances and activities (that is, heterotrophic productivity estimates, substrate uptake rate constants and enzyme activities) varied greatly throughout the year and exhibited higher values during summer compared to winter (Paper IV). Accordingly, bacterial production correlated with several environmental factors such as temperature, Chl *a*, bacterioplankton biomass, phytoplankton biomass such as cyanobacteria and flagellates and negatively to nutrients such as nitrate and phosphate (Spearman's  $\rho$ ,  $p$ -value  $<0.001$ , Paper IV). Similarly, bacterial biomass correlated to multiple abiotic and biotic factors (temperature, salinity, phosphate, total N, DOC, Chl *a*, bacterial production, summer phytoplankton biomass (cyanobacteria and flagellates)) (Spearman's  $\rho$ ,  $p$ -value  $<0.001$ , Paper IV). We did not detect noteworthy bacterioplankton growth prior to the spring bloom, indicating a reliance on phytoplankton-derived substrates for the heterotrophic bacterioplankton community. Furthermore, uptake rate constants of several substrates (such as glucose, amino acids and organic acids), correlated with temperature, bacterial production and cyanobacterial biomass (Spearman's  $\rho$ ,  $p$ -value  $<0.001$ , Paper IV). This implies that a combination of abiotic and biotic parameters could represent important stimuli of microbial activities.

## **Dynamics in Bacterioplankton community structure**

Whereas seasonally recurring patterns in bacterioplankton community composition have been observed in several marine areas (Paper V, (Fuhrman, et al. 2006, Gilbert, et al. 2012)), also inter-annual variability has been detected when studying bacterial communities at higher temporal and taxonomic resolution (Gilbert, et al. 2012, Linz, et al. 2017, Teeling, et al. 2016). Accordingly, several underlying factors influencing ecotype dynamics, bacterioplankton evolution and niche expansion are still unidentified or possibly stochastic (Vergin, et al. 2013, Zhou, et al. 2017).

In paper IV, we observed a substantial variability over short time scales in many bacterial parameters. For example, microbial substrate uptake rate constants displayed the highest variability during times of water mass stratification during summer, i.e. presumably homogeneous surface water conditions (Paper IV). Among these variable patterns, stochastic effects could play a role for which populations bloom: for example, the history of the community, i.e. which populations were already growing, which were in a resting stage, or which populations were decreasing in cell numbers, just prior to the sampling time points. In Paper V, we hypothesized that preceding increasing abundances of populations could fuel further growth of populations in comparison to competitor populations as response to 'disturbances' such as nutrient input or changes in stratification. Further, assuming that closely

related bacterial populations can have a slightly different gene repertoire (as for example detected for actinobacterial acIV-MAGS (metagenomics amplified genomes) at LMO (Hugerth, et al. 2015)), a considerable part of the measured variability in substrate preference and bacterial activities in this dataset, could be related to shifting bacterial populations or ‘ecotypes’.

Table 2. Variability of relative abundances of dominant classes (and phylum *Verrucomicrobia*) in the free-living fraction at LMO over the time period 2011-2016, table is redrawn from Paper III.

Taxa	Min	Max	Mean	Variation
<i>Flavobacteriia</i>	0.0089	0.949	0.1707	107-fold
<i>Gammaproteobacteria</i>	0.0105	0.5845	0.0935	56-fold
<i>Alphaproteobacteria</i>	0.0100	0.3021	0.0909	30-fold
<i>Actinobacteria</i>	0.0035	0.5273	0.1254	150-fold
<i>Verrucomicrobia</i>	0.0006	0.3538	0.0395	570-fold
<i>Cyanobacteria</i>	0.0022	0.6226	0.1969	280-fold

A high frequency time-series study recently suggested that winter and summer assemblages in temperate bacterioplankton communities resulted from switching between closely related strains with different temperature preferences (Ward, et al. 2017). Such variability and shifts in community structure might only to a lesser extent be detected in a community analysis at class level or at coarse time resolution such as monthly intervals (as presented in Figure 9B). Yet, even at class level, variability in community composition at LMO became evident; among summed relative abundances per sampling time point, *Flavobacteriia* varied 107-fold during the sampling period, *Actinobacteria* ranged 150-fold, *Cyanobacteria* varied 280-fold and verrucomicrobial abundances varied up to 570-fold (Table 2). To further unravel if bacterioplankton community structure could explain intra-annual variability in metabolic rates, we assessed whether distinct abundant bacterioplankton populations displayed seasonal preferences. Using the LMO 16S rRNA gene amplicon dataset of the free-living bacterioplankton community we further tested if bacterioplankton populations co-occurred with other populations of similar or dissimilar taxa.

Among the most abundant bacterioplankton populations, many populations were not stable over time (Paper III). For example, many *Actinobacteria* populations showed relatively elevated abundances during late summer, while some *Flavobacteriia* displayed relatively elevated abundances during spring or late summer, respectively (Figure 9, Paper III). Soft clustering analysis based on standardized relative abundances per population of high frequency sampling over three years (2011-2014) grouped populations of similar taxa together and clusters mostly consisted of populations from the same family (Paper III). Yet, populations displayed inter-annual variation in timing, amplitude and width of abundance peaks (Paper III).

To investigate whether bacterioplankton populations and their abundance patterns could be associated to distinct phytoplankton classes or seasons, cluster mean abundances of the 100 most abundant populations were correlated against abiotic and biotic environmental data. The most abundant bacteria populations (cluster means) correlated to spring or summer related environmental conditions (Paper III).

Among the spring clusters, mainly *Flavobacteriaceae*, *Rhodobacteriaceae* and *Acidimicrobiaceae* correlated to elevated nutrients and spring phytoplankton biomass (the sum of diatom and dinoflagellate biomass, Spearman correlations,  $p > 0.01$ , Paper III). Furthermore, some cluster means (including populations of the bacterial families *Cyclobacteriaceae*, *Sporichthyaceae*, *Acidimicrobiaceae* and LD29 *Verrucomicrobia*) significantly correlated to summer parameters (elevated temperatures, cyanobacterial biomass and DOC concentrations, Spearman correlations,  $p > 0.01$ , Paper III). In conclusion, bacterioplankton populations displayed seasonal preferences, potentially coupled to phytoplankton-derived substrates and further influenced by temperature. Moreover, dominating populations changed over short timescales as observed in many short-term peaks.

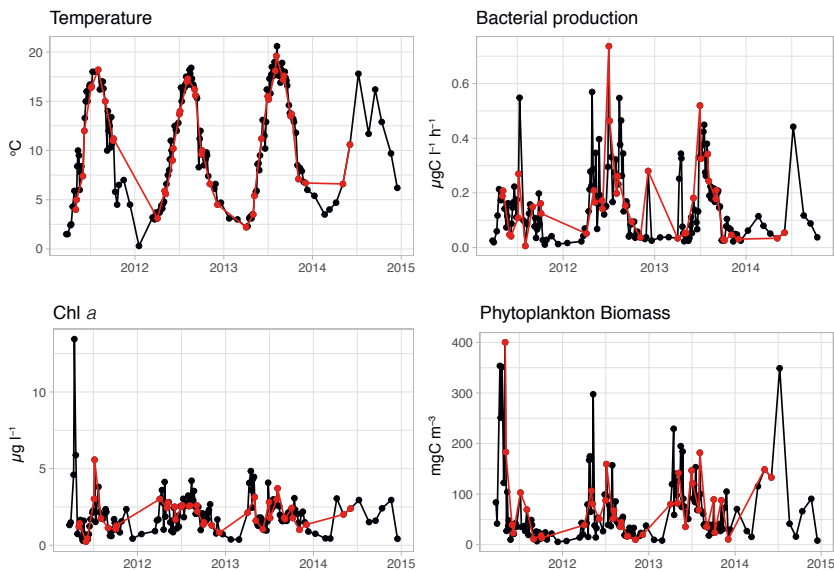


Figure 10 Subsampled data of the LMO high-frequency time series during 2011-2014, redrawn from paper IV. Data points that were sampled during the first week of the month (day 1-7), were labeled in red, underlying black points indicate all measured data for temperature (°C), bacterial production ( $\mu\text{g C l}^{-1} \text{h}^{-1}$ ), chl a ( $\mu\text{g l}^{-1}$ ) and phytoplankton biomass ( $\text{mg C m}^{-3}$ ).

Assuming that different bacterial lineages have distinct genetic properties, it is possible that a substantial portion of the variability observed in heterotrophic rates in the Baltic Sea, is influenced by changes in bacterioplankton community composition.

## **Microbial food web dynamics: insights and future outlooks**

Going back to the original questions of who in the microbial community is doing what, when, and how; so far, as also shown in this thesis, we have begun to understand which bacterial classes and populations are abundant throughout the year in the Baltic Proper (Paper III & V). This thesis also investigated ‘what’ is done by the bacterioplankton community, such as bacterial biomass and activity estimations (heterotrophic production, substrate preferences and enzymatic activities) (Paper IV). Underlying metabolic and physiological processes (which population is doing what, and which functional genes do they possess to do it) are though more slowly disentangled, although ongoing analyses are addressing this issue (e.g. Figure 3, Figure 9, Paper V). It is possible that similar processes as discussed in Papers I and II are involved in driving community composition and heterotrophic activities, among other ‘yet-to-be-found’ and regulatory mechanisms. Further, co-occurrences between bacterial populations and correlation analysis to environmental parameters might provide further clues about possible biological interactions.

Summarizing, with the help of the high frequency sampling scheme at LMO, we identified several important characteristics of the phytoplankton and bacterioplankton assemblages (Paper III & IV):

- high inter-annual variation in spring phytoplankton bloom dynamics and species dominance (i.e. shifts between diatoms and dinoflagellates)
- a twin peak in filamentous cyanobacterial biomass during summer, possibly consisting of different cyanobacterial species (paper IV)
- distinct bacterioplankton seasonal patterns at coarse taxonomic rank (class level)
- different bacterial taxa respond to spring phytoplankton bloom compared to summer phytoplankton bloom (e.g. *Flavobacteria* showed relatively higher abundances in spring, whereas *Verrucomicrobia* responded to summer bloom) (paper III)

- different bacterial biomass and production dynamics following the spring diatom- and dinoflagellate bloom, compared to after the summer cyanobacterial bloom
- bacterial activities (heterotrophic production, substrate uptakes and extracellular enzyme activities) displayed high variance during stratified surface water conditions in summer
- the high variance in biological parameters, could lead to an over- or underestimation by monthly sampling (Figure 10, Paper IV)

Future research challenges and research directions could include understanding underlying physiological processes shaping microbial communities. It would be desirable to further understand biological interactions between organisms, for example between phytoplankton and bacterioplankton populations, such as how succession is regulated by metabolic interdependences among planktonic communities (Paper V). Additionally, studies addressing the impact and specificity of grazers on seasonal bacterioplankton succession patterns, and the proportion of picocyanobacteria to bulk bacterioplankton abundances and productivities, have the potential to disentangle missing links in our understanding of Baltic Sea microbial communities. Also, outstanding research questions as defined in Paper V, such as which thresholds of physical, chemical, and biological factors are dependent on the transition between seasons and how marine bacteria communities will react to projected climate change scenarios. A paramount future line of research would be to study how specific seasonal transcription patterns of functional genes of different bacterioplankton populations relate to bacterioplankton activities and biogeochemical rates, for example photoheterotrophic traits. Here, high resolution sampling schemes focused across particular time periods could provide important insights into the regulation of marine ecosystems.

## Conclusions

This thesis has contributed to a deepened understanding of when and how marine bacteria respond to environmental changes and gradients, and how such responses may partly explain the distribution and success of particular bacterial taxa in marine ecosystems. Overall, using laboratory approaches and a high-frequency time series study, I discovered that bacterioplankton communities reacted to changes in the environment at a transcriptional level, by altering productivity rates or by changes in community structure.

Under light exposure and different organic nutrient qualities, model organism *Dokdonia* sp. MED134 upregulated the gene expression of proteorhodopsin and other genes to change its metabolism towards a more resourceful state. Similar metabolic functional traits of proteorhodopsin-containing bacterioplankton were found in several marine ecosystems and could further play important roles for marine bacteria to thrive and to encounter changing DOM gradients in natural ecosystems.

Under elevated CO<sub>2</sub>, bacterioplankton could keep their productivity and community structure unaltered by adapting the gene expression. To maintain pH homeostasis, bacteria induced higher expression of genes related to respiration, membrane transport and light acquisition, such as proteorhodopsin, under low-nutrient conditions. Under high-nutrient conditions with phytoplankton blooms, such regulatory mechanisms were not necessary, indicating that pelagic microbes are potentially more vulnerable to ocean acidification compared to populations in coastal habitats.

Using the LMO time series study in the Baltic Sea, this thesis discovered pronounced temporal dynamics in many investigated microbial variables including latent phytoplankton-bacteria linkages, substrate uptake rates, and shifts in bacterial community structure. Environmental conditions, and especially seasonal physicochemical and biotic changes, highly impacted on microbial productivities and abundances. Also, bacterioplankton community

composition changed seasonally at class level, despite detected inter-annual variability among genera and populations. Many abundant bacterioplankton populations displayed spring or summer preferences and accordingly correlated to seasonally changing environmental parameters, such as biomass of different phytoplankton phyla. Thus, high resolution time series can provide important insights into the interplay and scaling between factors ultimately determining the success and seasonal succession of different bacterial populations.

In this thesis, I highlight the advantages of combining methodological approaches to study bacterioplankton at high resolution, for improving our understanding of mechanisms controlling microbial communities and biogeochemical processes. Such advances in knowledge of fundamental metabolic and physiological processes underlying plankton succession could further aid to obtain a four-dimensional picture of how microbial communities relate to the turnover of DOM in the oceans.

## **My contribution to the individual papers**

Paper I	Laboratory work and data analysis.
Paper II	Data analysis and writing of the manuscript.
Paper III	Time series sampling and laboratory work (2012-2015), study design, data analysis and interpretation, writing of the manuscript.
Paper IV	Time series sampling and laboratory work (2012-2014), data analysis and interpretation, writing of the manuscript.
Paper V	Literature research, data analysis, interpretation and writing of the manuscript.

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*“An oceanographer is not (a) solitary sailor.” L.M. Proctor*

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