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Cycling of dissolved and particulate organic matter in the pelagic marine environment: Impact of phytoplankton community mortality and microbial degradation

SAMU ELOVAARA

Division of Ecosystem and Environment Research Program
Faculty of Biological and Environmental Sciences
Doctoral Programme in Wildlife Biology Research (LUOVA)
University of Helsinki
and
Marine Research Centre
Finnish Environment Institute

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Contribution of the authors

	I	II	III
Original idea	TT	TT	SE, TT
Study design	TT, DF	TT, SE	SE, HK
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EA = Eero Asmala, EER = Eeva Eronen-Rasimus, DF = Daniel Franklin, HK = Hermanni Kaartokallio, KS = Kristian Spilling, MD = Maria Degerlund, MV = Mari Vanharanta, SE = Samu Elovaara, TT = Tobias Tamelander

I: SE processed and analyzed the data together with MV. SE conducted all statistical analyses. SE wrote the manuscript together with MV.
II: SE collected the data together with MD and TT. SE processed and analyzed the data together with MD and TT. SE conducted all statistical analyses. SE designed and wrote the first draft of the manuscript.
III: SE designed the study together with HK. SE conducted the experiment and collected all data. SE analyzed all data with the exception of bacterial community composition and optical properties of dissolved organic matter. SE conducted all the statistical analyses with the exception of those related to the bacterial community composition. SE designed and wrote the first draft of the manuscript.

Supervised by	Dr. Tobias Tamelander, University of Helsinki, Finland
	Dr. Hermanni Kaartokallio, Finnish Environment Institute, Finland
Advisory committee members	Dr. Jaanika Blomster, University of Helsinki, Finland
	Prof. Bo Gustafsson, Stockholm University, Sweden
Reviewed by	Prof. John Berges, University of Wisconsin Milwaukee, USA
	Prof. Daniel Thornton, Texas A&M University, USA
Examined by	Prof. Paul del Giorgio, Université du Québec à Montréal, Canada
Custos	Prof. Sakari Kuikka, University of Helsinki, Finland

CYCLING OF DISSOLVED AND PARTICULATE ORGANIC MATTER IN THE PELAGIC MARINE ENVIRONMENT: IMPACT OF PHYTOPLANKTON COMMUNITY MORTALITY AND MICROBIAL DEGRADATION

SAMU ELOVAARA

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Cell lysis, as a consequence of adverse conditions, has been recognized as an important loss process among phytoplankton, in addition to the well-known loss processes of grazing and sinking. Cell lysis has been connected to increased release of carbon fixed by phytoplankton as dissolved organic carbon (DOC), the primary carbon source for pelagic heterotrophic bacteria. This has the potential to enhance pelagic remineralization at the cost of reduced sedimentation of organic carbon. Cell lysis may, therefore, have global consequences as the ratio of pelagic remineralization to sedimentation widely determines whether oceans function as a source or a sink of atmospheric carbon. However, the subject has been studied predominantly in oceans and oligotrophic marine regions. The Baltic Sea is different from these environments and the causes and consequences of phytoplankton cell lysis may, therefore, be expected to differ.

The studies included in this thesis are the first attempt to study phytoplankton cell lysis and its effect on carbon cycling in the Baltic Sea. The focus of the thesis is mainly on elucidating the abiotic and biological controls of cell lysis and its relationship with pelagic DOC concentration. These were studied on a spatial scale during a spring bloom on an area covering the Gulf of Finland, the Åland Sea and the Baltic Proper, and on a temporal scale during a two-year long monitoring campaign in an estuary in the northern Gulf of Finland. In both studies the proportion of cells undergoing lysis was measured using a membrane impermeable nucleic acid stain to indicate cells with compromised membrane integrity.

The spatial monitoring study revealed considerable variation in the proportion of cells undergoing lysis with generally higher proportion of dying cells in deep water (1-10 m: average 84%, range 67-91%; 30 m: average 77%, range 62-90%; 60 m: average 71%, range 58-86%) and among nanophytoplankton (surface water average: 64%), as compared to smaller eukaryotic picophytoplankton (surface water average: 88%) and picocyanobacteria (surface water average: 82%). No clear correlations between cell lysis and nutrient concentrations were found, although there was a weak correlation between the proportion of intact eukaryotic picophytoplankton and phosphate concentration (R^2 = 0.13, p = 0.029). No connection between cell lysis and DOC concentration was found. Also during the temporal monitoring campaign variation of cells undergoing lysis was high (surface water average: 62%, range 18-97%). Again,

no correlation between nutrient concentrations and cell lysis was found, although this time there was a weak negative relationship between the proportion of cells undergoing lysis and DOC concentration ($R^2 = 0.15$, $p = 0.0185$). In both studies some indication was found that phytoplankton lysis is less prevalent in conditions where interspecific phytoplankton competition is low.

Details of the flow of carbon from phytoplankton to pelagic heterotrophic bacteria was studied experimentally using two phytoplankton species (a dinoflagellate *Apocalathium malmogiense* and a cryptophyte *Rhodomonas marina*). Contrasting species specific differences were found in their ability to transfer carbon from the inorganic pool via DOC to bacterial biomass and in the composition of the emerging bacterial community. The smaller *R. marina* released more bioavailable DOC and attracted a bacterial community mainly consisting of copiotrophs (bacteria thriving when DOC is abundant and highly bioavailable), which likely directs more carbon towards microbial loop. The DOC released by the larger *A. malmogiense* was less bioavailable. If these results can be generalized to other taxa of similar size, the fast consumption of DOC released by R. marina may partially explain why no relationship between the lysis of small phytoplankton and DOC concentration was found.

The overarching conclusion from the two field studies is that the environmental conditions, such as nutrient limitation, that have been shown to promote cell lysis in oligotrophic marine regions are not the main determinants of cell lysis in the Baltic Sea. Also, the high ambient DOC concentration and terrestrial runoff in the Baltic Sea seem to mask the effect of cell lysis on DOC concentration. The group and species specific differences in both cell lysis and carbon cycling indicate that investigating cell lysis on lower taxonomic levels will help to connect cell lysis to carbon cycling.

Samu Elovaara, Division of Ecosystem and Environmental Research Program, Faculty of Biological and Environmental Science, University of Helsinki P.O. Box 4 (Yliopistonkatu 3), 00014, University of Helsinki, Finland; Tvärminne Zoological Station, University of Helsinki, J. A. Palménin tie 260, 10900, Hanko, Finland; Finnish Environment Institute, Marine Research Centre, Latokartanonkaari 11, 00790, Helsinki, Finland

LIST OF ABBREVIATIONS

%LC	Percentage of living phytoplankton cells
$a_{CDOM}(254)$	Absorption coefficient at 254 nm
BA	Bacterial abundance
BGE	Bacterial growth efficiency
BP	Bacterial production
BR	Bacterial respiration
Chl *a*	Chlorophyll *a*
CDOM	Colored dissolved organic matter
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DON	Dissolved organic nitrogen
FDOM	Fluorescent dissolved organic matter
F_v/F_m	Photochemical efficiency (the ratio between variable and maximum Chlorophyll *a* fluorescence)
H	Shannon diversity index
HIX	Humification index
KPI	Key point incubation
PA	Phytoplankton abundance
PCD	Programmed cell death
Peak C	Fluorescence peak C
Peak T	Fluorescence peak T
POC	Particulate organic carbon
POM	Particulate organic matter
PON	Particulate organic nitrogen
PP	Primary production
PUA	Polyunsaturated aldehyde
$S_{275\text{-}295}$	Spectral slope between 275 and 295 nm
TDN	Total dissolved nitrogen
TZS	Tvärminne Zoological Station
VLP	Virus-like particle

CONTENTS

1. INTRODUCTION 9
1.1. Pelagic microbial carbon cycling 9
1.1.1. Primary production by phytoplankton 9
1.1.2. Grazing and sedimentation as pathways of particulate organic carbon 9
1.1.3. Dissolved organic carbon pool 10
1.2. DOC release from phytoplankton 11
1.3. Cell lysis as a phytoplankton loss pathway 13
1.4. Consequences of phytoplankton cell lysis for carbon cycling 14
2. OBJECTIVE OF THE THESIS 16
3. MATERIALS AND METHODS 17
3.1. Study area 17
3.2. Overview of data collection 18
3.2.1. Spatial data (I) 19
3.2.2. Temporal data (II) 20
3.2.3. Experimental data (III) 21
3.3. Statistical analyses 23
3.4. Summary of the methods 24
4. RESULTS 27
4.1. Trends and variability of phytoplankton cell lysis (I, II) 27
4.1.1. Spatial variation during spring bloom (I) 27
4.1.2. Temporal variation (II) 27
4.1.3. Reliability of %LC determination 29
4.2. Controls of phytoplankton cell lysis (I, II) 30
4.2.1. Nutrients and environmental conditions (I, II) 31
4.2.2. Phytoplankton community composition and other organisms (I, II) 32
4.3. Effects of phytoplankton cell lysis on carbon cycling (I, II) 33
4.3.1. DOC release and POC:DOC partitioning (I, II) 34
4.3.2. Sedimentation of organic matter (I, II) 34
4.4. Carbon cycling dynamics between phytoplankton and bacteria (III) 35
4.4.1. Phytoplankton growth and primary production 35
4.4.2. Bacterial production and ^{14}C transport 36
4.4.3. Bacterial community 39
4.4.4. DOM transformations 40
5. DISCUSSION 44
5.1. Causes for phytoplankton cell lysis in the Baltic Sea (I, II) 44

5.1.1. Comparison of trends in cell lysis in the Baltic Sea to other systems 44
5.1.2. Relationship between cell lysis and abundance 44
5.1.3. Effect of removal from the photic layer on phytoplankton cell lysis 45
5.1.4. Effect of grazers and viruses on phytoplankton cell lysis 46
5.1.5. Effect of nutrients and salinity on phytoplankton cell lysis 48
5.1.6. Effect of species interactions on phytoplankton cell lysis 50
5.2. Consequences of phytoplankton cell lysis in the Baltic Sea (I, II) 52
5.2.1. Phytoplankton cell lysis and photosynthetic efficiency 52
5.2.2. Effect of cell lysis on DOC release 53
5.2.3. Effect of cell lysis on the sedimentation of particulate matter 55
5.3. Carbon cycling between phytoplankton, DOC and heterotrophic bacteria (III) 56
5.3.1. DOC production, transformation and consumption 57
5.3.2. Response of bacteria to DOC 60
5.3.3. Ecological implications of species specific DOC dynamics 63
6. CONCLUSIONS 66
7. ACKNOWLEDGEMENTS 69
8. REFERENCES 71

1. INTRODUCTION

1.1. Pelagic microbial carbon cycling

1.1.1. Primary production by phytoplankton

The biomass of phytoplankton in the ocean is estimated to be 0.25-0.65 Pg C (Falkowski & Raven 2007), whereas the biomass of terrestrial plants is approximately 450-1000 Pg C (Falkowski & Raven 2007, Bar-On et al. 2018). Still, phytoplankton fix about 50 Pg carbon annually and are considered to be responsible of half of the global primary production (PP) (Field et al. 1998). This carbon is initially fixed as particulate organic matter (POM) within phytoplankton biomass but, due to rapid turnover of phytoplankton cells and the dynamic nature of microbial food web, is quickly transferred to other trophic levels of the aquatic food web.

Carbon fixed by the phytoplankton is directly channeled into the pelagic food webs when phytoplankton are grazed. Some organic matter is released from phytoplankton as dissolved organic matter (DOM), either by cellular mechanisms or due to external stressors (Thornton 2014). When phytoplankton or organisms feeding on phytoplankton-derived organic matter sink, carbon is, at least temporarily, removed from the short term pelagic trophic cycling (Honjo et al. 2014). Part of the pelagic organic carbon is returned back to inorganic carbon by respiration (Robinson 2019). The fate of the carbon fixed by phytoplankton depends on the trophic state of the system and the relative importance of these phytoplankton loss pathways.

The combined mass of all organic carbon pools (DOM and living and dead POM) is estimated to be around 1000 Pg (Falkowski & Raven 2007). This is a significant carbon pool on planetary scale, higher than the atmospheric CO_2 reservoir (~780 Pg C, (Emerson & Hedges 2008)). To understand carbon cycling in the ocean, and the global role of oceanic carbon cycling, it is, therefore, crucial to understand where the carbon fixed by phytoplankton ends up and which mechanisms control the relative prevalence of the different trophic pathways.

1.1.2. Grazing and sedimentation as pathways of particulate organic carbon

Grazing of phytoplankton by protistan and zooplankton grazers channels carbon to higher trophic levels and larger animals. The living biomass in the ocean (autotrophs and heterotrophs combined) is estimated to be 1-2 Pg C (Falkowski et al. 2000). Larger animals retain carbon in the pelagic system but contribute to POM sedimentation through fecal matter and, eventually, by dying and sinking (Turner 2015). Grazers of phytoplankton may also release organic carbon in the environment through sloppy feeding and dissolution from fecal pellets (Lignell et al. 1993, Saba et al. 2011). Grazers also convert organic carbon back to CO_2 through respiration and release remineralized nutrients. The effectiveness of grazing depends on the phenology of phytoplankton and their grazers. If phytoplankton grow in

absence of grazers, they will develop a high biomass bloom and possibly deplete the system of nutrients. When such a bloom collapses sedimentation is the most prevalent loss pathway (Turner 2015).

Some of the sinking POM reaches the deep water layers or the seafloor, fueling the biological pump (Honjo et al. 2014, Boyd et al. 2019). Biological pump is a natural carbon sequestration mechanisms and an important aspect of the climatic control by the world ocean. All POM is susceptible to sinking, although larger particles sink considerably faster. The sinking rate of phytoplankton cells depend on their cell structure and ability for locomotion (Padisák et al. 2003). Fast sinking phytoplankton, such as diatoms, contribute effectively to the biological pump (Agustí et al. 2015). Slow sinking buoyant or motile phytoplankton, such as dinoflagellates, are expected to mainly be consumed in the euphotic zone, contributing to pelagic POM and DOM pools (Tamelander & Heiskanen 2004).

1.1.3. Dissolved organic carbon pool

Aquatic environment contains a vast array of extracellular organic molecules and particles suspended in the water column. As aquatic organisms exudate, excrete, and cells are damaged and broken, all kinds of biological molecules (carbohydrates, amino acids and proteins, lipids, nucleic acids and metabolic intermediates and end products) can be found freely in the water column but, due to different resistance to abiotic and biological degradation and different buoyancy, their relative share in the water column is different compared to living cells and tissues.

Dissolved organic carbon (DOC) is operationally defined as the fraction of organic carbon that passes through a filter of certain pore size. Various cutoffs have been used but GF/F (Whatman) filters have become common (nominal pore size 0.7 μm). Effective pore size of these filters can be reduced by combustion, after which their retentive capacity is comparable to 0.2 μm membrane filters (Nayar & Chou 2003). Due to the limitations of the operational definition, DOC pool practically consists not only of organic molecules of all sizes (low molecular weight to high molecular weight molecules), but also colloids, cell fragments and even viruses and small cells. DOC is the largest reservoir of organic carbon in the ocean, approximately 660 Pg C (Hansell et al. 2009). Autochthonous DOC may originate directly from living phytoplankton (Thornton 2014) or from heterotrophs (Steinberg & Landry 2017). Bacterial hydrolyzation may also produce DOC from larger particles (Azam & Malfatti 2007). Especially in coastal seas the DOC pool may partly consist of allochthonous organic matter of terrestrial origin but the flux of allochthonous DOC into the sea depends greatly on the processes at the catchment area and it is, therefore, difficult to give global estimates of the contribution of allochthonous DOC (Mitrovic & Baldwin, (2016) and references therein).

As the primary energy source for heterotrophic bacteria (Ducklow & Carlson 1992) DOC is considered one of the main components of aquatic food

webs. DOC released from phytoplankton is often highly bioavailable and, therefore, rapidly consumed by pelagic heterotrophic bacteria (Larsson & Hagström 1979, Jiao et al. 2010, Sarmento & Gasol 2012, Pedler et al. 2014). Especially amino acids and good energy sources, such as monosaccharides and labile polysaccharides, are quickly removed from the DOC pool (Meon & Kirchman 2001). As the bacteria remineralize DOC and produce different organic molecules, they return necessary nutrients (Amin et al. 2009, Christie-Oleza et al. 2017) and other substances such as vitamins (Croft et al. 2005) to be used by the phytoplankton. Bacterial remineralization of sinking organic matter weakens the efficiency of the biological pump and shortens the carbon sequestration time in the system (Ploug et al. 1999, Kwon et al. 2009). Some of the carbon in DOC goes through microbial loop to higher trophic levels mediated by the bacterivorous organisms (Azam et al. 1983). As a result of preferential use of labile DOC by heterotrophic bacteria, the pelagic DOC pool consists mostly of less bioavailable DOC. DOC that has undergone considerable bacterial degradation and many DOC species of terrestrial origins have very long residence times. Based on the biological reactivity and lifetime of the different components of the DOC pool, DOC can be divided into fractions on the continuum from labile to ultra-recalcitrant DOC with decreasing biological relevance (Hansell 2013) and generally with decreasing molecular size (Benner & Amon 2015). Bacterial processing of labile DOC towards more recalcitrant DOC effectively removes carbon from the short term biological carbon cycle and is known as the microbial carbon pump (Jiao et al. 2010).

Depending on the composition of DOC and surrounding conditions, DOC may accumulate in the water column (Hedges 1992, Mari et al. 2017), aggregate and sink (Engel et al. 2004), or be consumed by DOC feeding organisms, especially pelagic heterotrophic bacteria (Kujawinski 2011). The rates of these processes determine the prevalent fate of DOC and thus greatly determine total carbon cycling pathways.

1.2. DOC release from phytoplankton

Phytoplankton release DOC into the pelagic DOC pool through different mechanisms, some of which can be considered passive or non-controlled while others are actively controlled by the cell (Thornton 2014). Much of the DOC release has been attributed to the 'overflow' release of excess photosynthate in conditions where e.g. nutrient acquisition cannot keep up with photosynthesis (Fogg 1983, Zlotnik & Dubinsky 1989). Later it has become evident that phytoplankton also release molecules with specific purposes, such as signaling molecules (Vardi et al. 2006, Amin et al. 2015).

The quantity and composition of organic matter released from phytoplankton depend on the phytoplankton species (Romera-Castillo et al. 2010, Sarmento et al. 2013, Becker et al. 2014, Mühlenbruch et al. 2018) and growth phase (Chen & Wangersky 1996,

Biddanda & Benner 1997, Urbani et al. 2005) with a general, although species dependent, trend towards higher cell specific release of DOC and towards the production of higher molecular weight molecules after the exponential growth phase. These mechanisms are not fully understood, however, and the proportion of fixed organic matter released as DOM may also be independent of growth phase (López-Sandoval et al. 2013). The release of organic matter is also affected by environmental conditions. For example, the C:nutrient ratios of released DOM may increase under nutrient limitation (Saad et al. 2016) and the phytoplankton may release carbohydrates with no N and P (Myklestad 1995). DOM produced by nutrient limited phytoplankton may be less bioavailable to bacteria (Obernosterer & Herndl 1995).

Phytoplankton may lose some small hydrophobic organic molecules passively through their cell membranes (Bjørnsen 1988, Thornton 2014). Large or hydrophilic organic molecules require active transport and, therefore, phytoplankton may be better able to regulate their release (Baines & Pace 1991). Therefore, it can be assumed that whenever these molecules are found in the DOM pool, they have either been released for specific purposes, or they have been released when the cell membranes have been destroyed.

Different types of DOM produced by phytoplankton are used with different preferences by heterotrophic bacteria (Sarmento & Gasol 2012, Teeling et al. 2012, Sarmento et al. 2013) and the phytoplankton community composition may, therefore, affect the composition of the emerging bacterial community (Romera-Castillo et al. 2011). Labile DOM attracts copiotrophic bacteria, which are bacteria specialized for conditions where bioavailable organic matter is abundant. In the Baltic Sea DOM is always abundant and, therefore, bacteria which use highly bioavailable DOM are commonly called copiotrophs. Copiotrophs are capable of quickly draining the DOM pool of its most bioavailable labile components (Pedler et al. 2014). Consequently, it can be assumed that a phytoplankton community releasing less labile DOM will support bacteria specialized in utilizing semi-labile and less labile DOM. Because the phytoplankton benefit from pelagic remineralization conducted by bacteria, different phytoplankton species can be assumed to benefit from bacterial communities which efficiently remineralize the characteristic DOM produced by individual phytoplankton species.

In addition, a variety of interactions mediated by chemical signaling between phytoplankton and bacteria shape the way phytoplankton and bacteria are associated (Seymour et al. 2017). Some of these interactions may be necessary for the optimal growth of certain phytoplankton species (Bolch et al. 2011) or their associated bacteria (Thompson et al. 2012). Interactions among phytoplankton and bacteria may affect the composition and properties of DOM released by phytoplankton (Mühlenbruch et al. 2018). Some interactions affect the fate of carbon on large scale by e.g. enhancing

phytoplankton production through growth hormones (Amin et al. 2015) or by alleviating iron limitation (Amin et al. 2009), or by enhancing sedimentation (Gärdes et al. 2011). These interactions are not necessarily uniform in different environments and may be affected by e.g. nutrient availability (Gärdes et al. 2012).

1.3. Cell lysis as a phytoplankton loss pathway

Studies in the past two decades have indicated that a considerable proportion of phytoplankton cells may not be healthy and viable (e.g. Brussaard et al. 1995, Agustí et al. 1998, Veldhuis et al. 2001, Agustí 2004, Berman-Frank et al. 2004, Hayakawa et al. 2008, Rychtecky et al. 2014, Kozik et al. 2019). These cells are often detected by diverse membrane probes which identify cells with compromised membrane integrity (Veldhuis et al. 2001, Agustí & Sánchez 2002), and which are, therefore, considered to be moribund (Kroemer et al. 2009) although defining the actual point of no-return for microbial death is difficult (Davey 2011).

Phytoplankton cell lysis and loss of membrane integrity has been connected to adverse environmental conditions such as nutrient stress (Berges & Falkowski 1998, Agustí & Sánchez 2002, Lasternas et al. 2010), suboptimal temperature (Agustí & Duarte 2000), high UV radiation (Berges & Falkowski 1998, Llabrés & Agustí 2006), viral lysis (Brussaard et al. 2001) and to various chemicals often used as algicides (Fan et al. 2013).

Biochemical studies have identified key metabolites related to autocatalytic cell death pathways, highlighting the importance of programmed cell death (PCD) as a cause of cell lysis among phytoplankton (Bidle 2015). PCD can be initiated by diverse environmental stressors, such as nutrient limitation (Berman-Frank et al. 2004), high temperature (Bouchard & Yamasaki 2009) suboptimal salinity (Ross et al. 2019), viral infection (Schatz et al. 2014), pathogenic bacteria (Bramucci & Case 2019) and senescence (Franklin & Berges 2004). Sophisticated molecular controls connect cell death to environmental stressors (Vardi et al. 2006). PCD is a conserved cellular mechanism among phytoplankton and has been evolutionarily associated with community structuring and increased fitness among related phytoplankton (Durand et al. 2016).

Cell death can also be induced by allelochemicals produced by other phytoplankton (Poulin et al. 2018). For example polyunsaturated aldehydes (PUA) produced by some phytoplankton reduce growth and viability of other phytoplankton species (Casotti et al. 2005, Ribalet et al. 2007, Ribalet et al. 2014). PUA production is common among diatoms (Wichard et al. 2005) but not limited to them (Hansen et al. 2004, Vidoudez, Nejstgaard, et al. 2011).

The effects of varying abiotic stressors on phytoplankton depend on the taxa; some phytoplankton have a wider tolerance range than other coexisting taxa (Alonso-Laita & Agustí 2006, Rychtecky et al. 2014). Such taxa could survive better in rapidly changing

environments, whereas more sensitive taxa succumb to external stressors and show higher mortality and lysis rates.

In this thesis cells undergoing lysis were identified using a membrane probe. In papers I and II the proportion of phytoplankton with intact membranes to total phytoplankton abundance (PA) is referred to as viability of the phytoplankton community. This terminology has been widely used in similar sense in literature dealing with phytoplankton cell death and lysis (Brussaard et al. 2001, Agustí & Sánchez 2002) and should not be directly interpreted according to the definition in classical microbiology where viability refers to the ability to divide. Cells which are non-viable according to the classical definition may still have intact membranes and, therefore, they are not sensitive to membrane probes. Consequently, membrane probes are not viability tests in a classical sense. To avoid confusion, in paper III and in the synthesis of this thesis the term percentage of living cells (%LC) is used instead for the proportion of phytoplankton cells which exclude the membrane probe.

1.4. Consequences of phytoplankton cell lysis for carbon cycling

Biochemical consequences of phytoplankton cell death can be diverse. The photosynthetic efficiency of phytoplankton decreases when they progress along the cell death pathway (Veldhuis et al. 2001). Therefore, PP capacity of some cells within the phytoplankton community may be limited even though the cells still seem intact to visual inspection.

Phytoplankton cell death can result in cell lysis, thereby providing DOM to the pelagic microbial food web (Franklin et al. 2006, Thornton 2014). In the final stages of the cell death pathway the cell membrane becomes increasingly permeable until the cell finally disintegrates (Veldhuis et al. 2001). This causes the release of cellular contents not expected to be released through normal exudation mechanisms. As the cells release more of their biomass as DOM, cell death can be expected to direct the flow of carbon toward the pelagic DOC pool and the microbial loop (Orellana et al. 2013) instead of sinking of particulate organic carbon (POC) and the biological pump (Kwon et al. 2009). Therefore, phytoplankton cell death may reduce carbon sequestration. The ability of viruses to release organic matter from phytoplankton to DOC and POC pools (viral shunt) is well known (Suttle 2007), but the quantitative contribution of the different causes of phytoplankton cell lysis on carbon cycling is unclear. For example, there is evidence that upon cell death some phytoplankton, e.g. diatoms, release compounds which stimulate the remineralization of POC, enhancing pelagic carbon cycling at the cost of organic matter sedimentation (Edwards et al. 2015).

The consequence of cell death on carbon cycling is not always simply the release of DOC since synchronized PCD induced cell death may initiate the collapse of phytoplankton blooms and therefore, contribute to sinking of carbon, as shown for *Trichodesmium* by

Bar-Zeev et al. (2013). Some phytoplankton undergoing cell death produce transparent exopolymer particles (Berman-Frank et al. 2007, Kahl et al. 2008, Thornton & Chen 2017). These compounds can produce sustenance for pelagic heterotrophic bacteria (Carrias et al. 2002) and potentially stay in the pelagic system due to having lower density than water (Mari et al. 2017), but they may also promote particle aggregation and increase carbon export (Kahl et al. 2008, Turner 2015).

Biogeochemical effects of phytoplankton lysis have mainly been studied in oligotrophic marine environments where it has been shown to directly contribute to pelagic DOM pool (Agustí & Duarte 2013). There have been fewer investigations of phytoplankton lysis in the coastal seas. Estimates of the effect of phytoplankton lysis on carbon cycling in the coastal regions are important since they have been estimated to be responsible of ~12% of the marine PP and ~86% of the total carbon burial in the ocean (Dunne et al. 2007). The coastal seas are often more influenced by terrestrial and anthropogenic DOM and nutrient inputs. The higher nutrient levels point to other prevalent causes for phytoplankton mortality than nutrient deficiency. The higher DOM concentration might mitigate the importance of DOM released from dying phytoplankton, as opposed to open ocean. On the other hand, the shallower depth of coastal seas means that the sedimentation times are shorter, and any effects of phytoplankton lysis on the efficiency of the biological pump could be more pronounced.

2. OBJECTIVE OF THE THESIS

The extent of seasonal and spatial variation in phytoplankton mortality, its environmental controls and subsequent effects on carbon cycling in the Baltic Sea are not known. Due to the very different nature of the Baltic Sea compared to open oceans (see 3.1.) the environmental conditions causing mortality among phytoplankton are also expected to be different in the Baltic Sea. An explorative approach with open-ended objectives was chosen due to the limited data and knowledge on the topic in a Baltic Sea context.

The main objective of the thesis was to improve the knowledge of mortality in the phytoplankton community in the northern Baltic Sea, its impact on the concentrations of POC and DOC, and their bioavailability to pelagic heterotrophic bacteria. A secondary objective was to elucidate the importance of phytoplankton lysis under different trophic conditions in order to improve the understanding of how environmental changes affect organic matter cycling. These objectives were approached through three distinct sub-objectives.

The first sub-objective was to determine spatial (I) and seasonal (II) variations in phytoplankton cell lysis in the total phytoplankton community and in different trophic conditions, and how it relates to DOC and POC concentrations. This task was approached on field monitoring campaigns (I, II), which aimed to cover the natural seasonal dynamics (II) and different regions (I) in the Baltic Sea.

The second sub-objective was to determine POC:DOC partitioning by phytoplankton taxa characterized by different functional traits, and their effects on the composition of organic matter regarding the most relevant organic molecule types. The effect of phytoplankton community composition on POC:DOC partitioning was investigated during the spatial sampling campaign (II). POC:DOC partitioning and more detailed organic matter dynamics were investigated experimentally using phytoplankton cultures (III).

The third sub-objective was to determine the bioavailability of organic matter produced by phytoplankton to pelagic heterotrophic bacteria. The response of heterotrophic bacteria on DOM produced by phytoplankton was investigated experimentally using phytoplankton cultures (III).

3. MATERIALS AND METHODS

3.1. Study area

All three studies were conducted in the Baltic Sea or using phytoplankton cultures isolated from the Baltic Sea. Spatial data (I) was collected from open water sites on the Baltic Proper, the Åland Sea and the Gulf of Finland (Figure 1). Temporal data (II) was collected at Storfjärden (59°86'N, 23°26'W), an intensely studied site at the mouth of Karjaanjoki estuary in the archipelago of northern Gulf of Finland (Figure 1). Experiments (III) were conducted at the Tvärminne Zoological Station (TZS, 59°84'N, 23°25'W, Figure 1). Phytoplankton cultures used in the experiments had originally been isolated from Storfjärden and the bacterial inoculations used in the experiments were collected from the close vicinity of TZS.

The Baltic Sea is a semi-enclosed shallow sea area with a limited connection to the North Sea via Danish Straits. Due to limited salt water inflow and small water volume compared to the large heavily populated catchment area, Baltic Sea is brackish with decreasing northward salinity gradient (approximately 6 at Storfjärden), and heavily eutrophied due to anthropogenic activities (Fleming-Lehtinen et al. 2008).

In coastal environments, such as the Baltic Sea, DOC can have several origins, with riverine runoff often being a substantial source (Alling et al. 2008, Kulinski & Pempkowiak 2008, Hoikkala et al. 2015). Terrestrial DOC is mostly retained in river estuaries of the Baltic Sea and has its greatest influence on the coastal Bothnian Sea while the open-sea area of the western Gulf of Finland and the Baltic Proper show primarily autochthonous origin of DOC (Hoikkala et al. 2015). Terrestrial DOC contributes to the DOC pool especially in coastal zones (Hoikkala et al. 2015) which might reduce the importance of DOC released from dying phytoplankton, a process expected to be less significant in the open Baltic Sea.

The northern Baltic Sea freezes over in winter but the extent of ice cover varies. In spring when the ice melts the increase in light and temperature, and the increase in inorganic nitrogen concentrations due to melt water, initiate a spring bloom of phytoplankton. The spring bloom has previously been mainly diatom populated, but there is evidence of an increase in dinoflagellate dominated spring blooms in later years (Klais et al. 2011). Termination of the spring bloom is mainly caused by N depletion (Graneli et al. 1990). In the late summer there is typically a second phytoplankton bloom (Hällfors et al. 2013), often dominated by diazotrophic cyanobacteria benefiting from warm temperature and low inorganic N:P ratio supported by internal P loading (Vahtera et al. 2007). During productive season the euphotic zone is very shallow, especially at coastal zones (Kratzer et al. 2003). At the same time, climate change is causing structural and functional shifts in the communities of aquatic ecosystems (Li et al. 2009, Kahru et al. 2016), with potential implications for sedimentation (Tamelander et al. 2017)

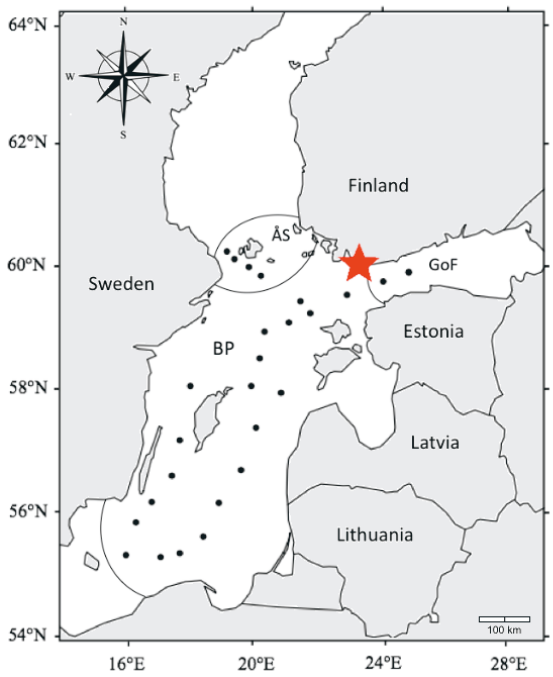

Figure 1. Study area and sampling sites. Black points mark the sampling sites for spatial data (I). The red star marks the location of TZS and Storfjärden, the sampling site of temporal data (II) and the point of origin for phytoplankton cultures used in the experiments (III). BP = Baltic Proper, GoF = Gulf of Finland, ÅS = Åland Sea

and biogeochemical cycles (Spilling et al. 2018) in the Baltic Sea.

3.2. Overview of data collection

Because %LC in phytoplankton communities had not been previously investigated in the Baltic Sea one of the most important goals of the study was to investigate the extent of spatial (I) and temporal (II) variation of %LC in natural phytoplankton communities. In addition to environmental monitoring, an experiment was conducted to investigate the species specific differences in carbon cycling from phytoplankton to bacteria, with the focus on DOM production and consumption (III). The principal purpose of the experiment was to clarify the mechanisms responsible for the observed absence of clear relationship between %LC among phytoplankton and DOC pool (I, II).

A membrane integrity probe (Sytox Green nucleic acid stain (Invitrogen)) was used to detect cells with compromised membranes in all studies. The green fluorescence (excitation: 488 nm, emission: 523 nm) of Sytox Green stained cells (i.e. cells with damaged membrane) was detected using flow cytometry (I, III) or epifluorescence microscopy (II). When using microscopy, stained cells were distinguished from non-stained cells by discretion of the microscopist. This was considered reliable, because Sytox Green fluorescence was generally very bright and easy to observe (Figure 2). When using flow cytometry the distinction between stained and non-stained cells was not so clear. This may be due to different staining response (Peperzak & Brussaard 2011) or different green autofluorescence (Tang & Dobbs 2007) of different species. Therefore, an arbitrary limit of five times the background green fluorescence was used to discern stained cells from non-stained cells (Veldhuis et al. 2001, Timmermans et al. 2007).

%LC was calculated by dividing the abundance of cells that are not stained by Sytox Green (i.e. cells with intact membranes) by total cell abundance.

Membrane permeability indicator was chosen as the test to identify healthy and intact cells because it detects a crucial aspect of cell death related to organic matter concentration, namely the compromised membrane integrity allowing for spontaneous release of cellular contents. It is important to consider, that while Sytox Green staining identifies membrane damaged cells, i.e. cells which currently or later upon disintegration release cell contents into the environment, the method does not quantify the rate of release of cell contents. Therefore, the differences in the DOM release from e.g. large vs. small cells or from recently vs. earlier ruptured cells cannot be compared using this method. The analysis, therefore, only aimed to answer the very broad question; whether the presence of membrane damaged cells correlates with higher DOC concentrations.

A suite of biological and biogeochemical variables was also monitored to investigate if phytoplankton cell lysis can be directly linked to generally measured environmental variables (I, II). Especially nutrient concentrations were focused on, because nutrient limitation has previously been connected to phytoplankton cell lysis (see 1.3.), and because phytoplankton growth in the Baltic Sea is often limited by nitrogen (Tamminen & Andersen 2007). Another priority was DOC concentration as high DOC concentrations during periods of low %LC might be an indicator of cell contents released from dying phytoplankton (see 1.4.).

3.2.1. Spatial data (I)

Water samples were collected during a research cruise (CFLUX16) onboard the R/V Aranda, Finnish Environment Institute, from 4th to 15th April 2016. Seawater samples were collected from several depths using Niskin bottles on a Rosette sampler. Cell lysis was investigated on the pico- and nanophytoplankton because of their importance to overall productivity, and

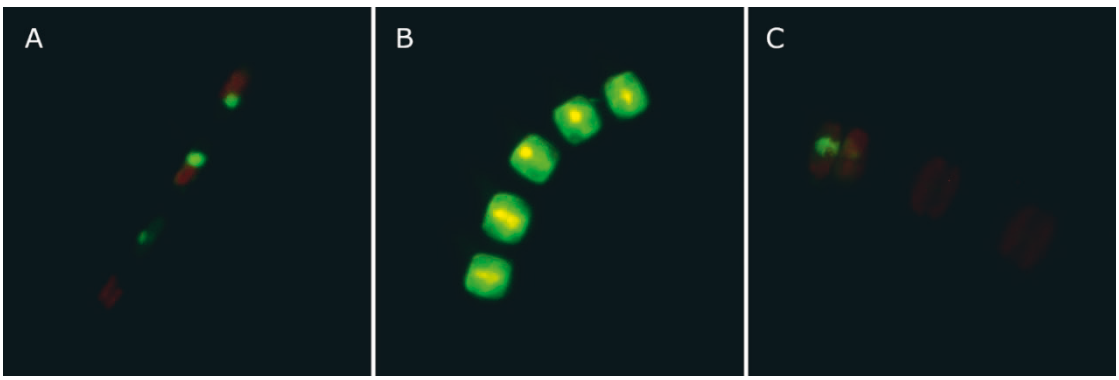

Figure 2. Epifluorescence microscopy images (not to scale) of Sytox Green stained phytoplankton cells demonstrating Chl *a* autofluorescence (red) and Sytox Green fluorescence (green). Partially stained *Skeletonema marinoi* (A), fully stained *Thalassiosira* sp. (B) and partially stained and unstained *Thalassiosira* sp. (C).

because cells mostly corresponding to these size classes could be easily determined using flow cytometry using forward scatter and orange fluorescence (excitation: 488 nm, emission: 610/30 nm) allowing for fast and easy assessment of abundance and %LC. Samples were analyzed without pre-filtration in order to avoid artefactual damage on the cells. No clogging of the flow cytometer was evident despite the lack of pre-filtration (maximum particle size capacity of the instrument, as reported by the manufacturer: 100 µm). Gating was used to exclude larger cells (by high frontal scatter) and other particles such as cell fragments and heterotrophic bacteria (by low red fluorescence). Because the study focused on individual cells, an attempt was made to exclude chains and colonies of cells. Area of scatter and fluorescence signals was used instead of height with the purpose of giving chains of cells an artificially large FSC value which would cause them to be excluded by the gating. Because flow cytometry does not allow the identification of these phytoplankton to species level, they were pooled into broad groups according to the optical properties of the cells. The first group (G1) included cells with low forward scatter and high orange fluorescence (phycoerythrin) and was assumed to contain mostly picocyanobacteria. The second group (G2) included cells with comparable forward scatter but lower orange fluorescence and was considered to contain most of the picoeukaryotes. Cells in the third group (G3) expressed intermediate to high orange fluorescence and higher forward scatter than the two previous groups and were assumed to contain larger cells mostly consisting of nanophytoplankton. Sytox Green fluorescence was detected from the total community and from each group individually using 536/40 nm bandpass filter.

Nutrient concentrations and the presence of larger phytoplankton species were analyzed as possible controls for cell lysis. The photochemical efficiency, the ratio between variable and maximum Chlorophyll *a* (Chl *a*) fluorescence (F_v/F_m), was analyzed as an indicator of health of the total phytoplankton community. DOC concentration was analyzed to detect significant DOC release from membrane damaged phytoplankton. Samples were analyzed onboard R/V Aranda (abundance, community composition and %LC of small phytoplankton, Chl *a*, inorganic nutrients) or at the Marine Research Center of Finnish Environment Institute (abundance and community composition of large phytoplankton, DOC).

3.2.2. Temporal data (II)

Sampling started in March 2015 at the beginning of the spring bloom and continued until the December 2016 (21 months). The investigation focused on the variation in the proportion of dying cells within the phytoplankton community and the possible biological and biogeochemical controls (inorganic nutrients, abundance of virus-like particles (VLP)) and consequences (POC:DOC partitioning, particulate matter sedimentation, abundance of free-living heterotrophic bacteria (BA)).

Samples were collected from surface water and from 20 m (2015 only) using a Limnos sampler and from 24 h sediment traps deployed at 20 m. The sediment traps were acrylic cylinders with inner diameter of 7.2 cm and height–diameter ratio of 6:1, filled with filtered seawater with salinity adjusted to 10. Concentrations of Chl *a*, POC, particulate organic nitrogen (PON), DOC, total dissolved nitrogen (TDN) and inorganic nutrients were measured from surface samples. DON concentration was calculated by subtracting nitrate, nitrite and ammonium concentrations from TDN. Also phytoplankton abundance, community composition and %LC, and BA was measured. In sediment traps the sedimentation rates of POC, PON and TDN were measured. In 2015 phytoplankton %LC was also measured in sediment traps and in the 20 m water sample. All the laboratory analyses were conducted at the TZS.

As a part of investigating the POC:DOC partitioning the %LC of sinking phytoplankton cells was considered. It has been shown that dead or senescent cells of certain species sink faster than living members of the same species even if there are no visible differences in the cells, and this may be due to loss of flotation aids (Padisák et al. 2003). Therefore, it was investigated if Sytox Green stained cells differed in sinking rate compared to healthy phytoplankton.

3.2.3. Experimental data (III)

In the experimental study the ecophysiology of two different phytoplankton species and its effect on microbial carbon cycling from DIC uptake to bacterial DOC processing was investigated. A larger (3391-12764 μm^3, (Olenina et al. 2006)) dinoflagellate *Apocalathium malmogiense* (G.Sjöstedt) Craveiro, Daugbjerg, Moestrup & Calado 2016 was compared to a smaller (217 μm^3, (Olenina et al. 2006)), fast growing, cryptophyte *Rhodomonas marina* (P.A.Dangeard) Lemmermann 1899. These phytoplankton species were acquired from the FINMARI culture collection/SYKE Marine Research Centre (*A. malmogiense* (syn. *Scrippsiella hangoei*), culture id: SHTV-2, isolated in Storfjärden, Tvärminne by Anke Kremp in 2002; *R. marina*, culture id: Crypto08-A2, isolated in Storfjärden, Tvärminne by Anke Kremp in 2008). Both species are common in the Baltic Sea during spring. These phytoplankton cultures were inoculated with natural bacteria from the Baltic Sea and then investigated experimentally for the effect of species-specific differences in PP and DOM production on carbon flow from phytoplankton to bacteria, and bacterial DOM consumption, production and community composition. The phytoplankton cultures were grown in artificial sea water to minimize the effect of growth medium on optical DOM properties.

The experiment was conducted at TZS during the winter 2017-2018 in two parts. Triplicate non-axenic unialgal batch cultures were grown in F/2 growth

medium in 5 L Erlenmeyer flasks in 4 °C in approximately 60 µmol photons s^{-1} m^{-2} under light-dark regime of 14 h and 10 h. Different triplicate batches were grown for both parts of the experiment with identical setup and growth conditions.

In the first part, hereafter called *DOM release experiment*, the phytoplankton and bacteria present in the cultures were grown together for over 4 months and the carbon flow from phytoplankton to bacteria, DOM alterations and bacterial activity were monitored at the start of the exponential growth phase and at two later stages. During these three monitoring occasions (hereafter referred to as 1st, 2nd and 3rd KPI after key point incubation) phytoplankton cultures were incubated with natural bacteria for 24 h and sampled at 0, 4, 8 and 12 h (+ extra sampling at 24 h for net PP). This experiment addressed the combined effect of the phytoplankton species and their adjacent bacteria on carbon cycling. In the second part, hereafter called *DOM consumption experiment*, the phytoplankton were grown to high density after which the phytoplankton and most of the bacteria were filtered out. The filtrate, inoculated with natural bacteria, was incubated for 7 days to compare the isolated effect of the bacterial communities on DOM processing. For the DOM release experiment temperature was increased to 10 °C to enhance the bacterial processes for easier detection. Both experiments were conducted partially overlapping but always so that for each species DOM release experiment was followed by DOM consumption experiment. A control unit containing only F/2 medium and natural bacterial inoculum was used to investigate how the natural bacterial community develops and how their DOC processing differs in the growth medium in the absence of DOM derived from the cultured phytoplankton and competition from cultured bacteria.

In the DOM release experiment concentrations of Chl *a*, POC, PON and inorganic nutrients were measured and bacterial community composition was determined before each KPI. Two different sample sets were incubated simultaneously at each KPI. In the first set, hereafter referred to as *production line*, phytoplankton cultures and bacteria (associated bacteria and the natural bacterial inoculum) were incubated in light and PP, bacterial production (BP) and ^{14}C transfer from ^{14}C-$NaHCO_3$ via phytoplankton to DOC pool and bacterial biomass were measured. Transfer of ^{14}C to DOC was investigated by filtering PP samples through 0.45 µm GD/X (Whatman) syringe filters and by measuring the radioactivity in the filtrate. Transfer of ^{14}C from DOC to bacterial biomass was investigated by incubating the previously mentioned filtrate for 4 h in dark after which the incubation was stopped by addition of 50% trichloroacetic acid and the particulate biomass in the samples was centrifuged for analysis of radioactivity. PP was used to calculate community respiration according to Spilling et al. (2019).

In the second set, hereafter referred to as DOM line, phytoplankton were removed by 0.8 µm filtration and bacteria (associated bacteria and the natural bacterial inoculum) were

incubated in dark and DOC concentration, optical properties of DOM, and BA were measured. Because the complex collection of molecules that comprise the aquatic DOC pool is near impossible to comprehensively describe with reasonable effort, optical properties of DOM were used to assess alterations in the DOM pool. Optical properties of colored and fluorescent DOM (CDOM and FDOM respectively) are easy to measure and can be used as proxies of DOM source and bioavailability (Coble 1996). CDOM produced by phytoplankton differ in composition depending on phytoplankton species (Romera-Castillo et al. 2010) and is further altered by bacterial DOM utilization (Romera-Castillo et al. 2011).In this study special emphasis was put on the following fluorescence variables: absorbance coefficient at 254 and 440 nm ($a_{CDOM}(254)$ and $a_{CDOM}(440)$, respectively) as general indicators of optically active molecules and light attenuation, DOC-normalized absorbance at 254 nm as an indication of DOC aromaticity ($SUVA_{254}$, (Weishaar et al. 2003)), absorption spectral slope between 275 and 295 nm as a proxy of molecular size ($S_{275-295}$, (Helms et al. 2008)), fluorescence peaks T and C (Coble 1996) as proxies of protein-like and humic-like DOM, respectively, humification index (HIX, (Zsolnay et al. 1999)) as an indicator of relative humification of DOM and biological index (BIX, (Huguet et al. 2009)) as an indicator of autochthonous DOM.

During the 7-day incubation of the DOM consumption experiment DOC concentration, optical properties of DOM and BA, BP and bacterial community composition were measured daily. Bacterial respiration (BR) was measured continuously using oxygen optodes. BP and BR were used to calculate bacterial growth efficiency (BGE).

3.3. Statistical analyses

Statistical analyses of data in papers I-III were performed following similar logic. Groupwise comparisons were performed with Welch-ANOVA, which allows for more difference in variance among treatments than regular ANOVA. This was done because the small sample sizes often resulted in unequal variance. Games-Howell post-hoc test was used to compare combinations of groups of significant Welch-ANOVA results. Correlations were tested using regression analyses. Linear regression was used when the dependent variable was continuous, generalized linear model with negative binomial distribution was used when the dependent variable was counts, and generalized linear model with beta distribution was used when the dependent variable was proportions.

In papers I and II the data sets were pooled and analyzed without considering spatial (I) and temporal (I and II) elements of the data set in the statistical models. In paper I this was done because the progression of the spring bloom was at different stages at different sampling stations making it difficult to relate the progression of the bloom to the order of samplings. In paper II temporal aspect of the monitoring campaign was not considered relevant because the

sampling interval was long from the perspective of the microbial processes at work at the sampling site and, therefore, the samplings could be considered independent without significant lagging influence from the previous sampling. Autocorrelation was investigated and considered low enough to be safely ignored in the regression analyses. The pooled data set was further divided into seasons, to investigate if the processes governing the relationships among the measured variables changed among the seasons.

Because multiple comparisons were performed for each data set and because the data sets were so small that individual observations often had effect on the tests of significance, *p*-values higher than 0.001 were considered suggestive only. No post-hoc tests for multiple comparison were made as the *p*-value limit of 0.001 was considered a sufficient protection against type I error. The results of regression analyses yielding *p*-values up to 0.05 were reported but caution was used when conclusion were drawn from such results. All statistical analyses were done using R (R Core Team 2019). The statistical analysis of the bacterial community data is presented in paper III.

3.4. Summary of the methods

Measurements and analyses were conducted according to previously published methods (Table 1) and described in detail in papers I-III.

Table 1. Summary of methods: Analyses were conducted by 1 = authors with notable contribution by the author of the thesis, 2 = other authors, 3 = Tvärminne Zoological Station, University of Helsinki, 4 = Marine Research Centre, Finnish Environment Institute, 5 = Institute of Biotechnology, University of Helsinki, - = analysis not conducted

Category	Variable	Method	Reference	I	II	III
Phytoplankton	Abundance and community composition, large species	Microscopy of Lugol-solution fixed samples	(HELCOM 2015)	4	2	-
	Abundance and community composition, small species	Flow cytometry	(Olson et al. 1993, Smith 2009, Tarran & Bruun 2015)	2	-	-
	Abundance	Flow cytometry		-	-	1
	C content	Automatic analysis with EnvPhyto phytoplankton counting program	(Menden-deuer & Lessard 2000, Olenina et al. 2006)	4	-	-
		Calculation	(Menden-deuer & Lessard 2000)	-	2	-
	F_v/F_m	Fluorescence induction (OJIP) curve (AquaPen fluorometer, Photon Systems Instruments) with 450 nm excitation light after dark acclimation		2	-	-
	%LC	Flow cytometry of Sytox Green stained samples	(Veldhuis et al. 2001, Timmermans et al. 2007)	2	-	1
		Epifluorescence microscopy of Sytox Green stained samples	(Veldhuis et al. 2001)	-	2	-
	Primary production	^{14}C-method	(Steemann Nielsen 1952, Gargas 1975)	-	-	1
Bacteria	Abundance of bacteria and virus like particles	Flow cytometry	(Gasol et al. 1999)	-	1	1
	Respiration	OXY-4 micro oxygen meter (PreSens)		-	-	1
	Production	^{3}H-thymidine and ^{14}C-leucine incorporation method	(Smith & Azam 1992)	-	-	1
	Community composition	DNA extraction (DNeasy Power Soil kit (Qiagen))	Kit specific instructions	-	-	1
		Sequencing (PCR, Illumina MiSeq)		-	-	5
		Bioinformatics (primer removal, read merging and processing, taxonomic classification	(Martin 2011, Quast et al. 2013, Callahan et al. 2016)	-	-	2

Physical and chemical variables	Temperature and salinity	Conductivity-temperature-depth sensor (CTD)		4	1	-
	NH_4^+	Colorimetric method	(Grasshoff et al. 1983)	4	3	-
	NO_3^-, NO_2^- and PO_4^{3-}	Colorimetric method	(Grasshoff et al. 1983)	4	3	3
	DSi	Colorimetric method	(Grasshoff et al. 1983)	-	3	-
	DOC, TDN	High-temperature catalytic oxidation (HTCO, Shimadzu TOC-V CPH carbon and nitrogen analyzer)	(Sharp et al. 1993)	4	3	3
	POC, PON	Automated nitrogen and carbon analysis-mass spectrometry (Europa Scientific ANCA-MS 20-20 15N/13C mass spectrometer)	(Salonen 1979)	-	3	3
	Chl *a*	Spectrofluorometric detection (Varian Cary Eclipse fluorometer, Agilent)	(Jespersen & Christoffersen 1987)	2	1	1
	DOM optical properties	Absorbance measurement (CDOM, Shimadzu 2401PC spectrophotometer), fluorescence measurement (FDOM, Varian Cary Eclipse fluorometer, Agilent)		-	-	1
		EEM processing	(Coble 1996, Murphy et al. 2010, Massicotte 2016)	-	-	2
Miscellaneous	Photic zone depth	Calculation from Secchi depth	(Luhtala & Tolvanen 2013)	1,4	-	-
Statistical analyses	ANOVA and regression analyses	R (betareg, MASS, userfriendlyscience)	(Venables & Ripley 2002, Cribari-Neto & Zeileis 2010, Peters 2018)	1	1	1
	PCoA, dbRDA, PERMANOVA, ANOVA (bacterial community)	R (vegan)	(Anderson 2001, Oksanen et al. 2019)	-	-	2

4. RESULTS

4.1. Trends and variability of phytoplankton cell lysis (I, II)

4.1.1. Spatial variation during spring bloom (I)

The total and relative abundance of the measured nano- and picophytoplankton groups (i.e. groups G1, G2 and G3) varied throughout the sampling area (Table 2 in paper I). All three groups were most abundant in the Åland Sea and the Gulf of Finland and least abundant in the southernmost stations of the Baltic Proper. In general, abundance was highest in the 0-10 m photic layer and lower at 30 m and 60 m (Figures 3 and 4 in paper I). Because of the gating protocol which was planned to exclude larger cells and chains, and because the contribution of filamentous cyanobacteria to the bloom was minimal (Lipsewers et al. 2020), these data most likely successfully encompass only the target groups. At many stations the abundance of individual groups was so low that %LC could not be measured at all depths or at all.

Total %LC of the phytoplankton community varied little, but variation was high among the different phytoplankton groups. Average %LC remained almost unchanged in the photic zone, decreased slightly at 30 m and was significantly lower at 60 m (Figure 3A). The %LC of G3 was lower than the %LC of G2 at most sampling depths and showed greater variation, but average %LC did not differ significantly among the sampling depths (Figure 3B). G2 %LC at 60 m depth was significantly lower than at 1-10 m. At some stations, the 60 m sample was taken below the halocline (data not shown). The %LC of G2 at 60 m at those stations was slightly lower than at stations where the halocline was deeper than 60 m.

%LC among phytoplankton groups differed among sampling stations (3 m measurements, Figure 3C). The %LC of G1 and G2 were similar across the study area but the %LC of G3 was significantly lower compared to the two picophytoplankton groups. At some stations, e.g. in the Baltic Proper, dying cells accounted for more than half of the G3 population.

4.1.2. Temporal variation (II)

Phytoplankton %LC varied from 18 to 97% (average of the whole sampling period: 62%) during the 21 month monitoring period (Figure 4). Phytoplankton community %LC was always less than 100% meaning that at any given time a fraction of the community consisted of dying cells. On average, %LC was lowest in summer. In general, %LC was high when total PA was high, such as during spring blooms and fall, but abundance explained only a small part of the variation in %LC. Variation in the %LC was considerable throughout the sampling period suggesting that the ecological consequences of phytoplankton cell lysis are not strictly limited to certain seasons or events, such as the demise of the spring bloom.

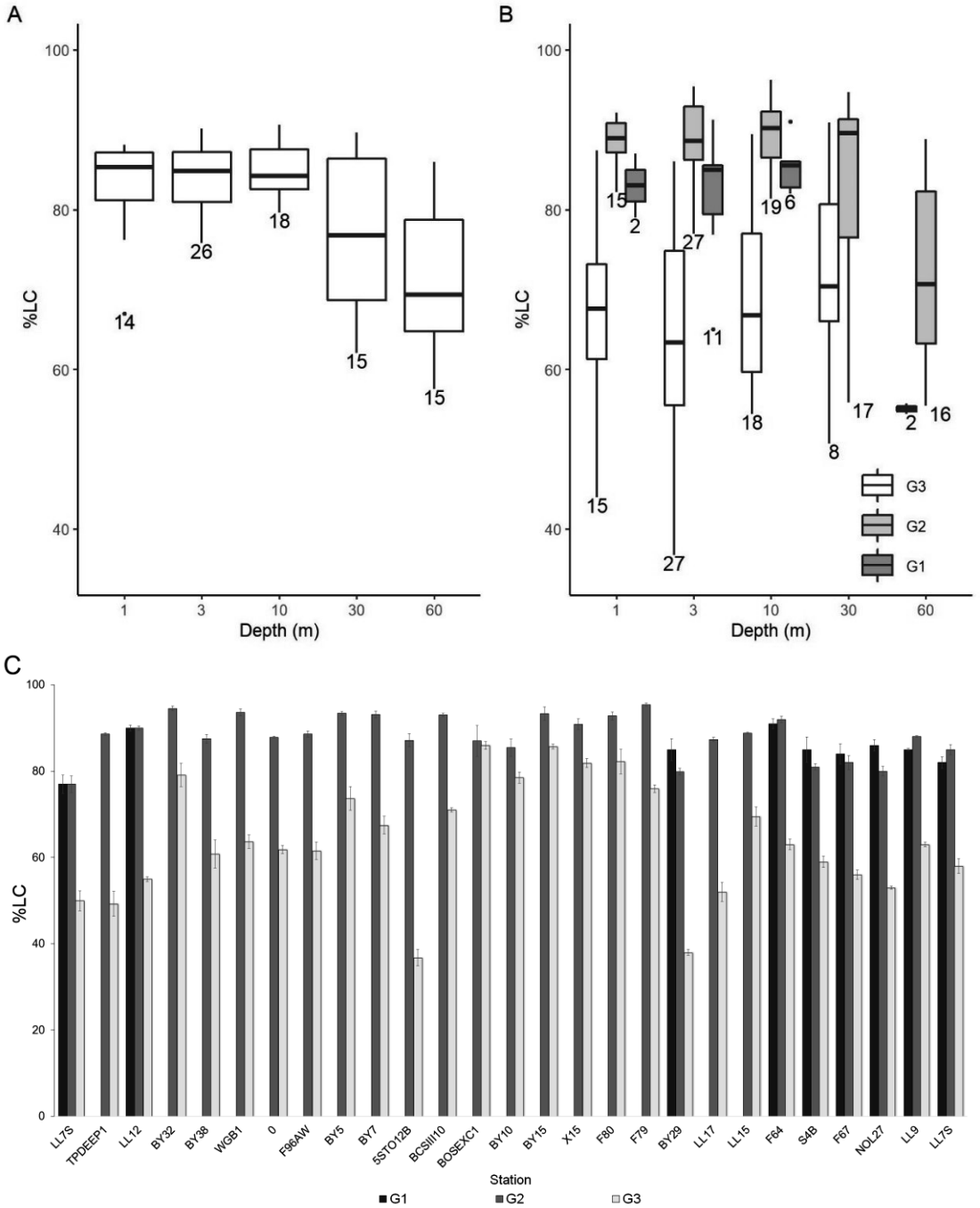

Figure 3. %LC of all nano- and pico-sized phytoplankton (A) and groups G1, G2 and G3 (B) at different depths, when all the stations are pooled by depth. Lower and upper hinges of boxes mark the first and third quartiles, respectively, while the whiskers extend to the lowest and the highest value within 1.5 times the interquartile range. C: %LC of groups G1, G2 and G3 at 3 m depth. The station order on the x-axis follows the cruise route, which started and ended at the Gulf of Finland (station LL7S).

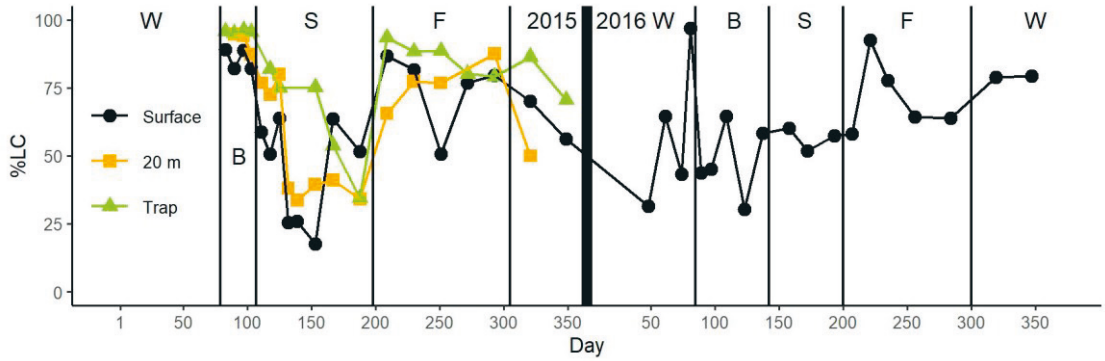

Figure 4. %LC of phytoplankton through the total sampling period in surface water (circles) in 20 m water column (squares) and in 20 m sediment trap (triangles) Seasons were defined according to typical seasonal progression in the Baltic Sea using trends in temperature, Chl *a* concentration and nitrate concentration (see II) and are divided by vertical lines. W, B, S and F stand for winter (low Chl *a* and temperature, high nitrate), spring bloom (increase and subsequent decrease in Chl *a*, decreasing nitrate), summer (low Chl *a*, increasing temperature) and fall (increase and subsequent decrease in Chl *a*, decreasing temperature), respectively.

4.1.3. Reliability of %LC determination

Because %LC determination using flow cytometry (I) relies on fluorescence thresholds, there is inherently more uncertainty compared to visual inspection using epifluorescence microscopy (II). Much of this uncertainty is caused by the potentially different staining sensitivities of different phytoplankton to Sytox Green (Peperzak & Brussaard 2011). For example the higher %LC of G1 and G2 than G3 could be a true observation or caused by different sensitivity to Sytox Green staining between the groups. In order to control this uncertainty, heat-killed samples were used to assess the fluorescence intensity of the community when all the cells are dead (see paper I for details).

An average of 12% (minimum: 3%, maximum: 28%) of the heat-killed phytoplankton did not express Sytox Green fluorescence. There was no relationship between the %LC in the heat-treated controls and the abundance ratios of any of the flow cytometry-based phytoplankton groups. This indicates that some dead cells do not stain with Sytox Green and that this insensitivity to staining does not correlate, at least clearly, with high proportions of any of the groups. However, it does not tell anything about tendency of living cells to erroneously stain with Sytox Green. Thus, there is an unknown probability of false positives (living cells staining with Sytox Green) and a somewhat known probability range of false negatives (dead cells not staining with Sytox Green). If the average number of cells which respond poorly to Sytox Green staining is the same between heat-killed and natural samples (that is, it applies also to cells which have died through other means than heat), then the analysis of paper I slightly overestimates the %LC. Regardless, this overestimation would generally be low, and because there are

no clear trends between staining of heat-killed cells and the abundance ratios of the phytoplankton groups, it can be cautiously assumed that the staining sensitivity of dead cells within all the groups is equal, albeit not complete. There was also no significant difference in flow cytometry total event counts between unstained and stained samples, which suggests that Sytox Green staining does not disturb the analysis by e.g. aggregating the cells.

However, there was a significant positive relationship between Chl *a* concentration and %LC in the heat-treated controls. This suggests that the Sytox Green concentration that was used was not sufficient to stain all the phytoplankton in dead controls when their density was high, which might lead to lower fluorescence intensity and thus to overestimation of %LC. This possible overestimation cannot be proven nor removed retrospectively and this conclusion, therefore, emphasizes the need to adapt the concentration of Sytox Green to Chl *a* concentration, phytoplankton biomass, or POC in the future, preferably using saturation tests. Because of this, dead controls were not used to mathematically correct for the %LC measurements, but only to report an uncertainty: a possible overestimation of %LC.

Determining %LC using epifluorescence microscopy is less prone to interpretation errors related to Sytox Green staining. Possible binding of Sytox Green to surface of living cells can be ignored, as it can be clearly seen if nucleus is stained (Figure 2A, C), which was perceived as a clear indicator that Sytox Green had passed inside the cell. All such partially stained cells were counted as dead when determining %LC. Because of this, it is safe to say that minor staining differences due to differential staining of dead cells of different phytoplankton species should not affect the analysis, as long as some green fluorescence could be observed.

Regardless of its limitations, Sytox Green is a commonly used membrane probe, which functioned well with most of the species tested by Peperzak and Brussaard (2011) including small local species such as *Rhodomonas baltica*. Therefore, the results of this thesis are assumed to be comparable with other studies investigating phytoplankton lysis at the community level.

4.2. Controls of phytoplankton cell lysis (I, II)

A summary of relationships between %LC and measured abiotic and biological variables which were hypothesized to affect %LC are presented in Table 2.

Table 2. Summary of all the measured variables hypothesized to affect %LC and the interactions between the variables and %LC. + = positive relationship, - = negative relationship, 0 = no detected relationship, ? = unclear relationship, NA = not measured

Variable		Paper I	Paper II
Nutrients			
	NH_4^+	0	0
	$NO_3^- + NO_2^-$	0	0
	PO_4^{3-}	G1 & G3: 0; G2: +	0
	Si	NA	0
Other phytoplankton			
	Chl *a*	?	0
	Total PA	G1 & G3: 0; G2: -	+
	Diatom abundance	G1 & G3: 0; G2: -	NA
	Diversity of phytoplankton community	NA	-
Other			
	Removal from surface layer	-	0
	VLP abundance	NA	0

4.2.1. Nutrients and environmental conditions (I, II)

The pooled analysis of all the samples of the spatial monitoring revealed that G3 abundance at 3 m depth correlated positively, albeit only slightly, with ammonium concentration (Figure 6 in paper I). G2 abundance correlated negatively with temperature and phosphate concentration (Figure 6 in paper I).

At many stations in the Baltic Proper, the nitrate concentration was not detectable, indicating that the phytoplankton community had already consumed most of the available nitrate. The only correlation between abiotic factors and %LC during the spatial monitoring cruise was the weak correlation between phosphate concentration and G2 %LC. G3 %LC did not correlate with any of the measured environmental variables.

Relationships between nutrients and %LC during the temporal monitoring were likewise unclear. Immediately following the 2015 spring bloom, when most of the nutrients had been consumed, %LC dropped quickly to its minimum value. Later in the summer phytoplankton biomass and %LC increased again despite the low nutrient concentrations. Nitrate concentration was low (often below the accurate determination limit of 0.25 µmol L^{-1}) during the productive periods of both years. Phosphate was occasionally depleted during the productive periods but sometimes showed values as high as 0.85 µmol L^{-1}. Nutrient concentrations had no direct correlation with %LC. Depleted nitrate concentration and occasionally depleted phosphate

concentration during the productive period suggest that the phytoplankton growth was mostly nitrogen limited, as is typical at the region (Tamminen & Andersen 2007), but periods of simultaneous nitrogen and phosphorus limitation might have occurred. There was no correlation between POC:PON ratio and %LC. POC:PON varied between 7 and 28 with highest values during winter, suggesting that low nitrate concentration did not clearly affect seston C:N stoichiometry, nor could it be used to predict events of low %LC, during the productive season. Si concentration remained > 3.5 µmol L^{-1} even during periods of high diatom abundance suggesting that Si was never a limiting nutrient for diatoms.

Abundance and %LC had different predictors; G2 abundance correlated negatively with phosphate concentration even though G2 %LC correlated positively with phosphate concentration. Concurrently, G3 abundance correlated positively with ammonium concentration, but G3 %LC did not. It seems, therefore, that the environmental variables controlling PA cannot directly be used to predict phytoplankton %LC.

4.2.2. Phytoplankton community composition and other organisms (I, II)

%LC of G3 and G2 seemed to be higher whenever the total phytoplankton density was low (as indicated by direct flow cytometry counts and Chl *a* concentration). This was especially pronounced in the negative relationship between G2 %LC and total flow cytometry-based PA (Figure 5A) and between G2 %LC and diatom biomass (Figure 5B). Diatoms dominated the biomass of large phytoplankton, as defined on class level by light microscopy (data not shown).

There was a weak correlation between the total phytoplankton cell abundance and %LC during the total temporal sampling period. Out of a total of 38 %LC measurements only 5 showed a %LC less than 40% and these events of low %LC invariably coincided with low PA and low Chl *a* concentration. The lowest %LC, therefore, occurred when conditions were overall unfavorable for phytoplankton growth. High PA coincided with high %LC especially during 2015 spring bloom and during the peaks of the late summer blooms. During most other samplings there did not seem to be any relationship between phytoplankton %LC and abundance.

The phytoplankton communities at Storfjärden differed in species composition during the spring blooms of 2015 and 2016 (Figure 3 in paper II). In 2015 the spring bloom phytoplankton community consisted mainly of diatoms and it was dominated by a single diatom species *Skeletonema marinoi* and the duration of the bloom was about 20 days shorter than in 2016. In 2016 the spring bloom community was mixed, consisting mainly of diatoms and dinoflagellates, and had two biomass peaks. The bloom period in the fall of 2015 was mixed with a succession from dinoflagellates to cyanobacteria. Periods of dominance by a single phytoplankton group, as identified by low group level (diatoms, dinoflagellates, other flagellates, cyanobacteria and other phytoplankton)

Shannon index values (H, see paper II for details about calculation), always co-occurred with high %LC (> 80%, Figure 5C). During the S. *marinoi* dominated 2015 spring bloom %LC remained high (> 80%) throughout the bloom. On the contrary, during spring 2016 both %LC and phytoplankton community composition were more variable than in 2015. During this mixed 2016 bloom %LC was much lower than during 2015 spring bloom, varying between 30 and 65%. The rest of the dataset is less conclusive but on day 209 at the onset of the 2015 late summer bloom there was one more occasion when the phytoplankton community was dominated by only one group (dinoflagellates, mainly *Heterocapsa triquetra*) and the %LC was high. Toward the next sampling on day 230 the phytoplankton biomass had almost doubled, the community became more mixed (H = 1.0), and %LC dropped, although only from 87 to 81%. The change in %LC is nevertheless negative as opposed to the general trend of increased %LC with higher PA. Two out of three observations of low H and high %LC were from the 2015 diatom dominated spring bloom and one from the dinoflagellate dominated day 209 of 2015. Even though there was no direct correlation between the two variables, there were no cases where both %LC and diversity would have been low. No relationship between %LC and abundance of VLPs was detected.

4.3. Effects of phytoplankton cell lysis on carbon cycling (I, II)

A summary of relationships between phytoplankton %LC and potential consequences in the organic matter cycling are presented in Table 3.

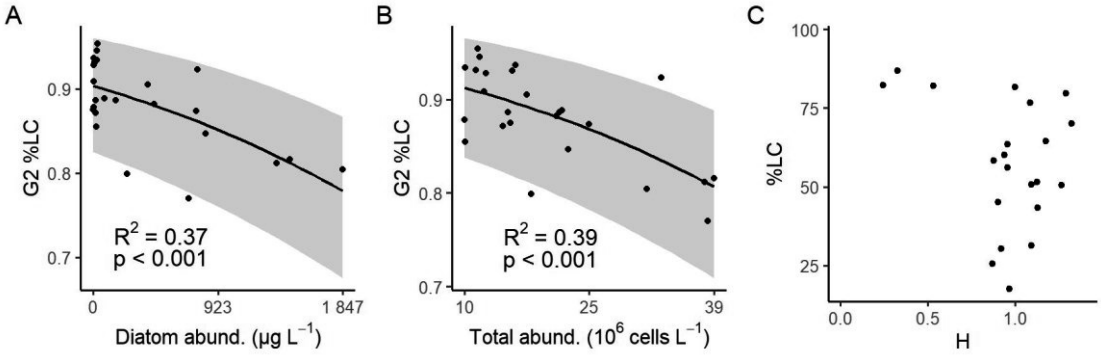

Figure 5. Fitted values (line) and 95% quantiles (shaded area) of beta regression models presenting significant predictors of %LC in paper I (A, B). C: Phytoplankton community %LC presented against Shannon index (H) calculated based on the biomass of phytoplankton pooled into diatoms, dinoflagellates, other flagellates, cyanobacteria and other phytoplankton.

Table 3. Summary of all the measured variables hypothesized to be influenced by %LC and the interactions between the variables and %LC. + = positive relationship, - = negative relationship, 0 = no detected relationship, ? = unclear relationship, NA = not measured

Variable		Paper I	Paper II
Organic matter			
	DOC	0	-
	POC	NA	0
	POC:DOC	NA	0
	Sedimentation of organic matter	?	0
Other			
	F_v/F_m	+	NA
	BA	NA	?

4.3.1. DOC release and POC:DOC partitioning (I, II)

During the spatial monitoring cruise no relationship between %LC and DOC could be found. The F_v/F_m correlated with G2 %LC indicating that low %LC is generally related to lower productivity (Figure 6A).

During the temporal monitoring campaign DOC correlated negatively with %LC, although this correlation was very weak, when the whole sampling period was considered (Figure 6B). However, when the data were divided into seasons, this correlation was only significant during spring bloom. POC:DOC ratio was not significantly related to %LC or changes in DOC concentration. There was no correlation between %LC and DON or PON. The riverine inflows appeared to supply organic matter to the study site because there was a clear negative relationship between salinity and DOC (Figure 6C). DOC concentration was best explained by salinity and %LC together.

There was no noticeable relationship between phytoplankton %LC and the abundance of bacteria (total, HNA or LNA) during the total monitoring period. Occasionally, BA increased during or immediately after a drop in %LC, for example after spring and late summer blooms in 2015. In summer there was a significant inverse relationship between BA and %LC indicating possible DOC release from dying cells to bacteria.

4.3.2. Sedimentation of organic matter (I, II)

Neither suspended nor sinking POC, PON or Chl *a* fractions were significantly related to %LC during the temporal monitoring campaign. The %LC in the sediment traps was in general slightly higher than in the surface or 20 m (Figure 4) but the average %LC among the different depths did not differ significantly. During the 2015 spring bloom %LC of sinking phytoplankton, which consisted mainly of *S. marinoi*, was ~ 95%. High %LC of sinking cells was not restricted to diatoms only, since the same phenomenon was observed during the autumn bloom when

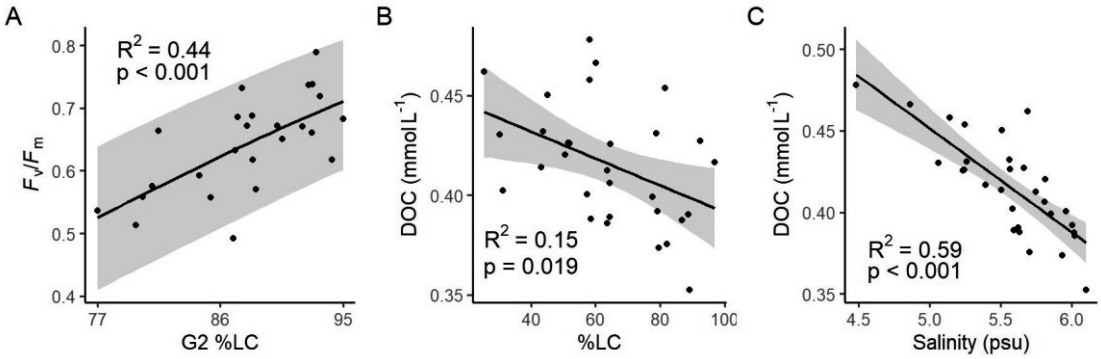

Figure 6. A: Relationship between F_v/F_m and G2 %LC in paper I. B: Relationship between %LC and DOC concentration in paper II. C: Relationship between salinity and DOC concentration in paper II. Fitted values (lines), 95% quantiles (shaded area in A) and 95% confidence intervals (shaded area in B and C) of beta regression (A) and linear regression (B, C) models.

dinoflagellate and cyanobacteria dominated. In general, all kinds of phytoplankton cells, including motile dinoflagellates, were found in the sediment traps.

Even though organic matter sedimentation was not measured during the spatial monitoring cruise, the reduced %LC at deep sampling depths suggests that the lysis of phytoplankton cells within the mixed layer may contribute to pelagic DOC release and negatively affect the sedimentation of organic matter in the form of phytoplankton biomass.

It must be noted that the Sytox Green method did not capture the relationship between initiation of cell sinking and %LC if loss of buoyancy regulation started before membrane disintegration in the cell death pathway. It is also possible that intact motile cells were brought temporarily into the sediment trap by mixing which may lead to overestimation of the %LC of sinking phytoplankton. However, %LC in surface water and sediment trap were similar even toward the end of the summer when the water column was more stratified, suggesting that mixing is not necessary for healthy cells to end up in the sediment trap.

4.4. Carbon cycling dynamics between phytoplankton and bacteria (III)

4.4.1. Phytoplankton growth and primary production

In the DOM release experiment *R. marina* grew faster to maximum density and ended the growth phase sooner than *A. malmogiense* (Figure 7A). Forward scatter of the cell populations did not change indicating that the average size of the phytoplankton cells stayed the same throughout the experiment. There was no indication of significant nutrient limitation during the experiment (see paper III for details). BA:PA ratio was most of the time between $1*10^4$ and

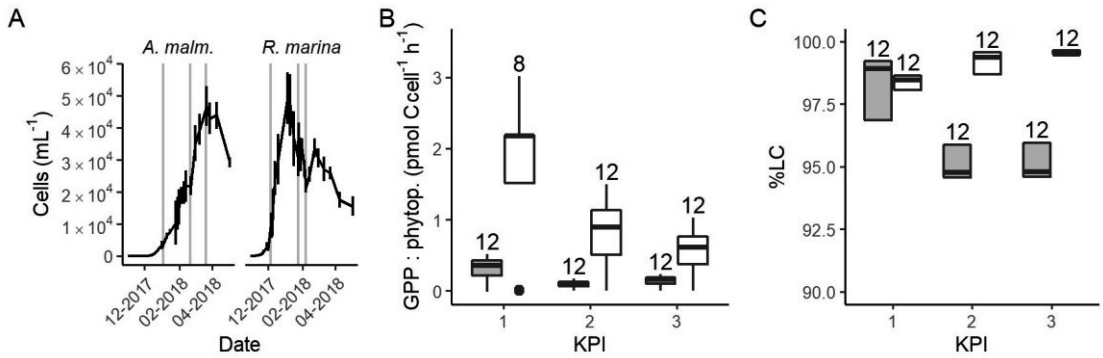

Figure 7. A: Abundance of phytoplankton (mean of three replicate units, error bars indicate one standard deviation) during the DOM release experiment. Vertical lines from left to right in each panel mark the times of 1st, 2nd and 3rd KPI. Cell specific gross primary production (B) and %LC (C) in *A. malmogiense* (white) and *R. marina* (grey) units at each KPI. Lower and upper hinges of boxes mark the first and third quartiles, respectively, while the whiskers extend to the lowest and the highest value within 1.5 times the interquartile range.

$3*10^4$ except at the 1st KPI for *R. marina*, when it was much lower. PP (gross, net and cell specific) was higher in *A. malmogiense* units (Figure 7B). *A. malmogiense* %LC was near 100% throughout the experiment, but *R. marina* %LC dropped to ~95% at the 2nd KPI (Figure 7C).

4.4.2. Bacterial production and ^{14}C transport

In the DOM release experiment BA was comparable in both phytoplankton units at the 2nd and 3rd KPI (Figure 8A). Thymidine based BP was slightly higher in *A. malmogiense* units at each KPI (Figure 8B), while leucine based BP was of equal magnitude between the species, except at the 1st KPI (Figure 8C). As a result the ratio of leucine to thymidine incorporation was higher in *R. marina* units at the 2nd and 3rd KPI (Figure 8I).

Even though PP was higher in *A. malmogiense* units, thymidine based BP was only slightly higher and leucine based BP was higher only at 1st KPI. Also, the BA:PA ratio was considerably higher in *A. malmogiense* units only at the 1st KPI even though *A. malmogiense* cells are much larger than *R. marina* cells. In the DOM consumption experiment BA increased considerably faster and BP reached higher values in *R. marina* units than in *A. malmogiense units* (Figure 8D-F). These observations suggest that *R. marina* community can support a more productive bacterial community

A. malmogiense cells were more efficient in incorporating DIC (i.e. higher PP) but they also respired more than *R. marina cells*. In the DOM release experiment community respiration was lower in *R. marina* units than in *A. malmogiense* units at 2nd and 3rd KPI and did not increase much during the experiment. In *A. malmogiense* units community respiration increased much more towards the end of the experiment.

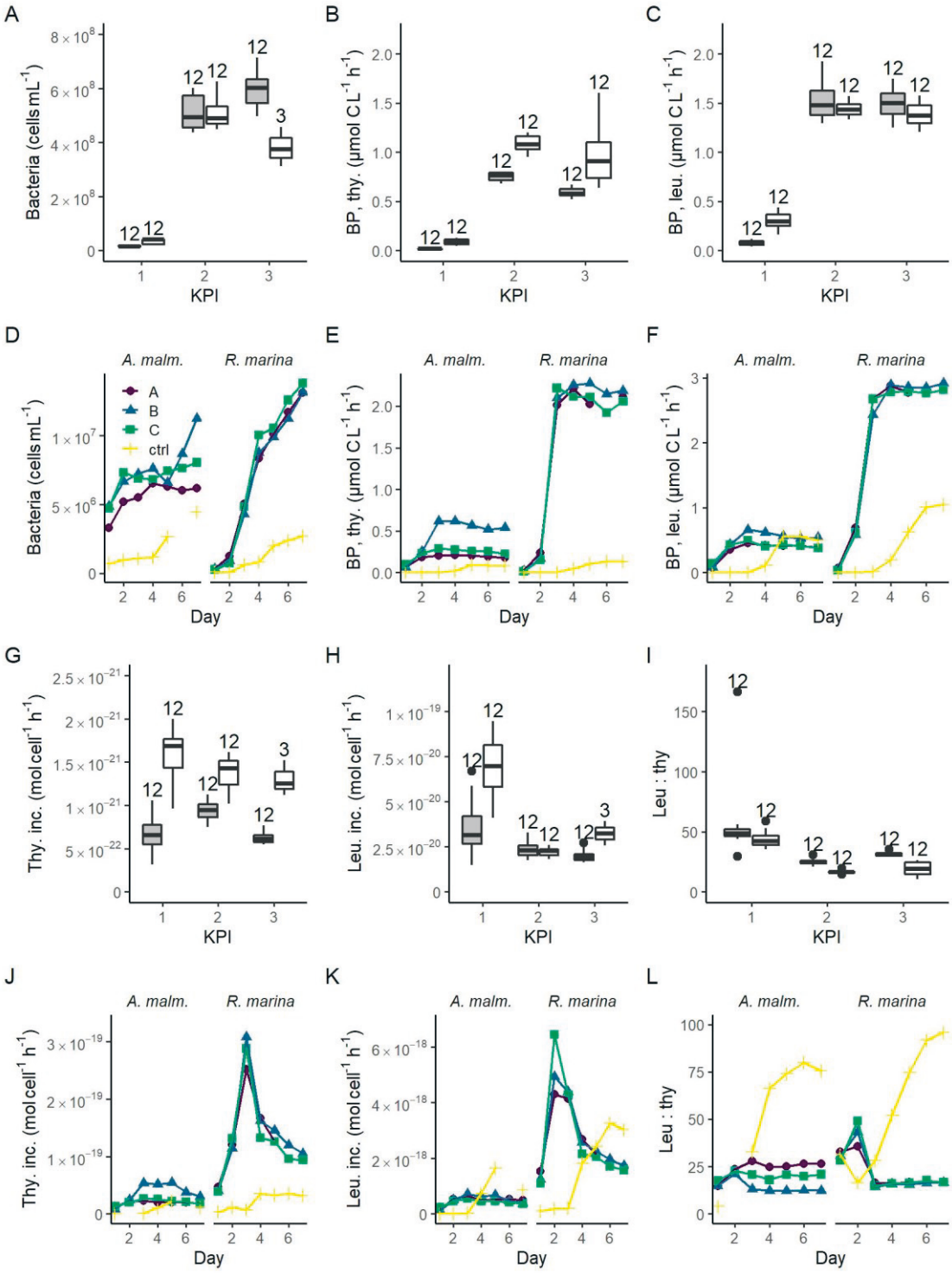
A
B
C
D
E
F
G
H
I
J
K
L
Bacteria (cells mL^{-1})
BP, thy. (µmol C L^{-1} h^{-1})
BP, leu. (µmol C L^{-1} h^{-1})
Thy. inc. (mol cell^{-1} h^{-1})
Leu. inc. (mol cell^{-1} h^{-1})
Leu : thy
KPI
Day
A. malm.
R. marina
A
B
C
ctrl

Figure 8. Bacterial abundance (A, D), thymidine (B, E) and leucine (C, F) based bacterial production, cell specific thymidine (G, J) and leucine (H, K) incorporation and leucine:thymidine incorporation ratio (I, L) at each KPI in the DOM release experiment (boxplots) and in the DOM consumption experiment (line graphs). Lines A, B and C mark different replicates (culture filtrate + sea water bacteria) and ctrl marks the control (F/2 + sea water bacteria). Lower and upper hinges of boxes mark the first and third quartiles, respectively, while the whiskers extend to the lowest and the highest value within 1.5 times the interquartile range. White boxes = *A. malmogiense*, grey boxes = *R. marina*

Community respiration divided by PA was likewise lower in *R. marina* units (Figure 9A).

In the DOM consumption experiment cell specific BR was higher in *R. marina* units (Figure 9B). While BP was higher in *R. marina* units, the lower BR in *A. malmogiense* units caused BGE to be comparable in both units (Figure 9C).

Despite the higher cell specific BR in *R. marina* units in the DOM consumption experiment the community respiration in the DOM release experiment was higher in the *A. malmogiense* units, especially during the 2nd and 3rd KPI. This was the case even though BA:PA was only slightly higher in *A. malmogiense* units at the 2nd KPI and much lower during the 3rd KPI. Therefore, the higher community respiration in *A. malmogiense* units is likely mainly caused by respiration of phytoplankton.

^{14}C-DOC, originating from ^{14}C-$NaHCO_3$, was produced by both species at each KPI. ^{14}C originating from ^{14}C-$NaHCO_3$ was incorporated in bacterial biomass at 2nd and 3rd KPI although there were considerable uncertainties with this measurement (see paper III for details). Higher PP of *A. malmogiense* led to proportionally higher incorporation of ^{14}C-$NaHCO_3$ into phytoplankton biomass (Table 4). However, a proportionally larger fraction of PP ended in filtrate in *R. marina* units. In *R. marina* units also a proportionally larger fraction of ^{14}C-organic matter was incorporated into bacterial biomass at 2nd and 3rd KPI, although the difference was very small at 3rd KPI (at the 1st KPI no activity was detected in *R. marina* units so comparisons could not be made). Of all the ^{14}C that was fixed by PP about 5 and 4 times more ended up in bacterial biomass in *R. marina* units at 2nd and 3rd KPI respectively.

Given that comparable amounts of DOC accumulated during the DOM release experiment it can be assumed that, compared to PP, relatively more carbon is directed to respiration by *A. malmogiense* and to DOC release by *R. marina*. These results also suggest that DOM released by *R. marina* is more bioavailable to bacteria than DOM released by *A. malmogiense*, and that the microbial loop is favored more strongly when DOM originates from *R. marina*.

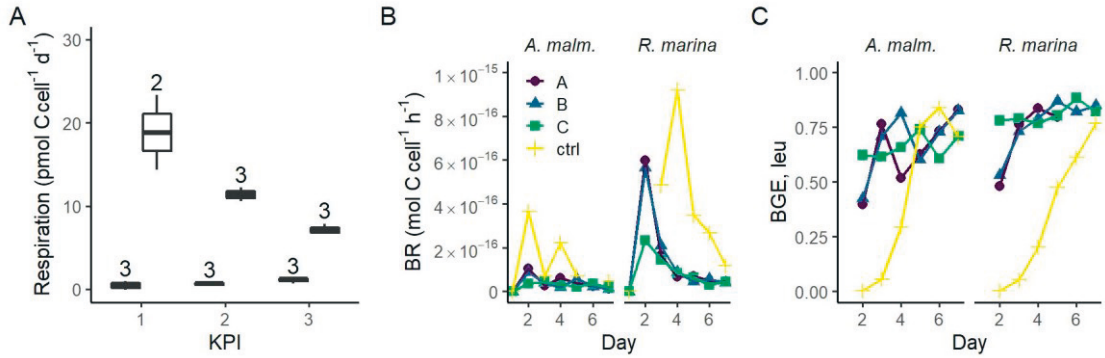

Figure 9. A: Community respiration divided by PA in *A. malmogiense* units (white) and *R. marina* units (grey) at each KPI in the DOM release experiment. Cell specific BR (B) and BGE calculated from leucine incorporation based BP (C) in the DOM consumption experiment. Lines A, B and C mark different replicates (culture filtrate + sea water bacteria) and ctrl marks the control (F/2 + sea water bacteria). Lower and upper hinges of boxes mark the first and third quartiles, respectively, while the whiskers extend to the lowest and the highest value within 1.5 times the interquartile range.

Table 4. Flow of ^{14}C between different phases of carbon cycle. Numbers are percentages of ^{14}C accumulation rates between the phases indicated in the left column (PP:TIC is an exception as PP is a rate but TIC is a concentration). Stars indicate the significance (*p*-values) at the side of the significantly higher percentage between species at the corresponding KPI, compared with Welch-ANOVA (*** < 0.001 < ** < 0.01 < * < 0.05).

species	*A. malmogiense*			*R. marina*		
KPI	1st	2nd	3rd	1st	2nd	3rd
PP:TIC	3.02*	5.24***	4.88***	0.68	0.67	0.64
DOC:PP	0.402	0.119	0.128	1.095**	0.420***	0.419***
bacteria:DOC	1.39	24.07	18.15	NA	33.87*	22.84*
bacteria:PP	0.0049	0.0283	0.0233	NA	0.1429***	0.0965**

4.4.3. Bacterial community

In the DOM release experiment classes Alphaproteobacteria, Gammaproteobacteria and Bacteroidia predominated the bacterial communities (Figure 10). Alphaproteobacteria increased and Gammaproteobacteria decreased from the 1st KPI to the 2nd KPI, whereas Bacteroidia had its peak at the 2nd KPI. The relative share of different classes differed between the units: Bacteroidia (average: 45.6%, genera *Algoriphagus* and *Polaribacter*) and Alphaproteobacteria (average: 44.7%, genera *Pseudorhodobacter* and *Sphingorhabdus*) were the most abundant classes in *A. malmogiense* units while Alpha- (average: 49.9%, genus *Pseudorhodobacter*) and Gammaproteobacteria (average: 38.8%, genera *Rheinheimera* and RS62 marine group) predominated in the *R. marina* units. Interestingly class Actinobacteria (average: 2%, genus *Candidatus Aquiluna*) appeared in *A. malmogiense*

units and slightly increased along the experiment.

In the DOM consumption experiment class Bacteroidia (average: 70%, genera Algoriphagus and Polaribacter) and Alphaproteobacteria (average: 19.9%, genera *Pseudorhodobacter*, *Sphingorhabdus* and *Seohaeicola*) predominated bacterial communities in *A. malmogiense* units (Figure 10). Congruently with DOM release experiment, class Actinobacteria (average: 5.7%, genus *Candidatus Aquiluna*) was present throughout the experiment. In the small share of class Gammaproteobacteria (average: 1.7%), the most abundant were the order Betaproteobacteriales (genera *Hydrogenophaga*, *Kerstersia*, *Limnobacter*, *Methylotenera*). In *R. marina* Alphaproteobacteria (day 1-3 average 82%, genus Pseudorhodobacter) predominated the bacterial communities until the day 3, after which they began to decrease (day 4-7 average: 36.1%) and Bacteroidia (day 4-7 average: 49.9%), genus *Flavobacterium*) increased. Also Gammaproteobacteria increased slightly towards the end of the experiment (day 4-7 average: 7.7%, genera *Shewanella*, *Marinomonas* and *Polynucleobacter*).

In both experiments, bacterial communities in seawater were distinct from those in the experimental units indicating that the bacterial communities emerged from the phytoplankton cultures (Figure 10). In the control units of the DOM consumption experiment (seawater inoculum + growth media) bacterial communities were comparable with seawater community in the beginning of the experiment in both experimental units but later developed into cultures which were different from communities in both sea water and experimental units (Figure 10).

4.4.4. DOM transformations

During the DOM release experiment DOC concentrations increased in both phytoplankton units, however, only a very small increase from 2nd to 3rd KPI was observed in *R. marina* units (Figure 11A). DOM absorbance and fluorescence generally increased during the DOM release experiment (Figure 11). The general trend in all units was the accumulation of lower molecular weight and potentially more refractory molecules, as seen by e.g. increase in $a_{CDOM}(254)$, $S_{275\text{-}295}$, humic-like DOM peak C, and humification index (HIX). While $a_{CDOM}(254)$ increased in both species during the experiment, $SUVA_{254}$ increased slightly in *R. marina* units, whereas it decreased in *A. malmogiense* units. This suggests that the increase in $a_{CDOM}(254)$ in *R. marina* units was caused mainly by the increase in DOC absorbing in UV region with relatively higher intensity than the bulk material whereas in *A. malmogiense* units the increase in $a_{CDOM}(254)$ was caused by increased absorbance due to higher bulk DOC concentration. This was also supported by the three times higher $a_{CDOM}(254)$ production by *R. marina* when the $a_{CDOM}(254)$ consumption by bacteria during the DOM consumption experiment is subtracted from the net accumulation of $a_{CDOM}(254)$ in the DOM release experiment.

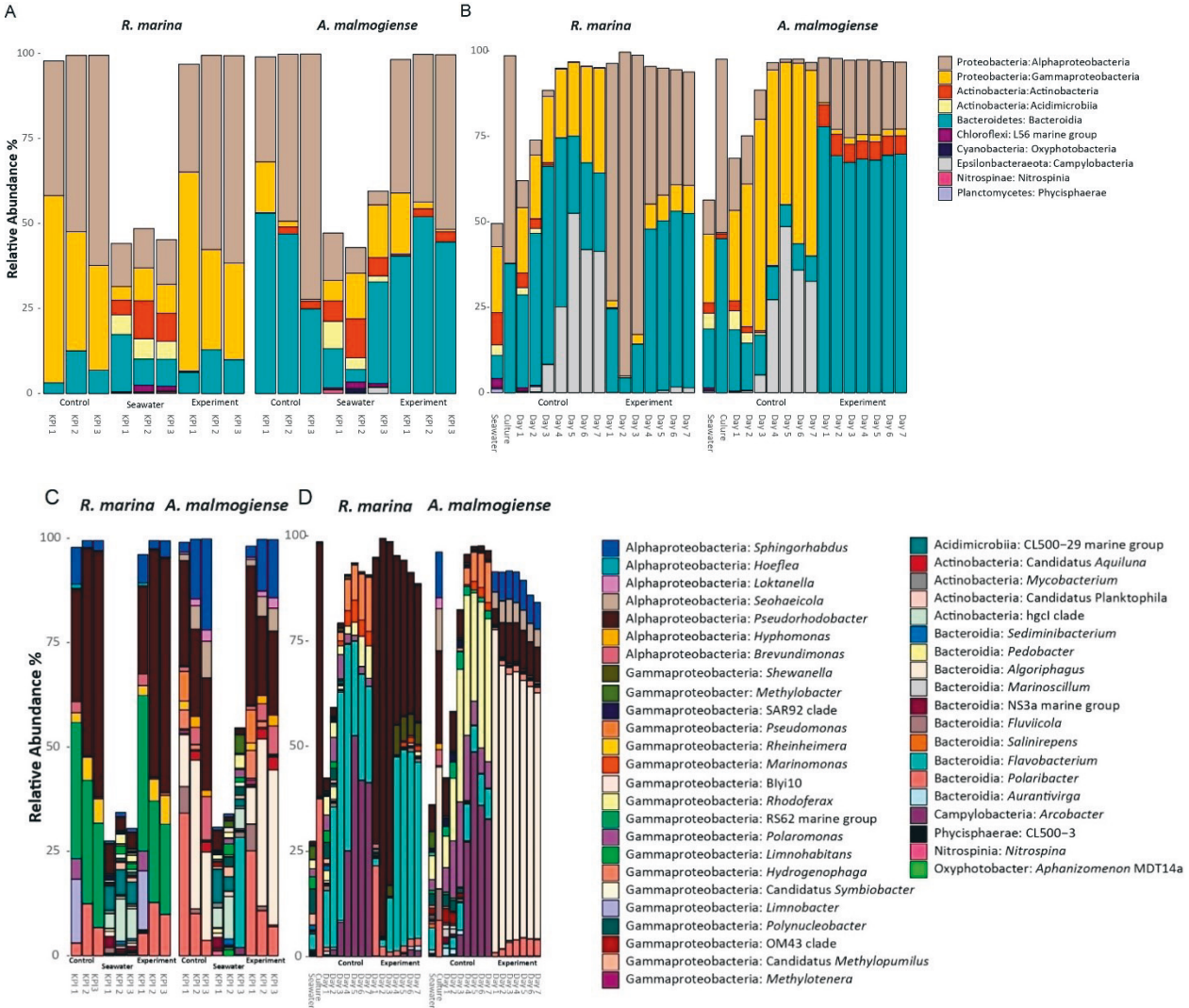

Figure 10. Class level (upper panels) and genus level (lower panels) bacterial community composition in DOM release experiment (A, C)) and in DOM consumption experiment (B, D). In plots A and C the bars labeled control refer to cultures before addition of bacterial inoculum. In plots B and D the bars labeled control refer to the control units (F/2 + sea water bacteria).

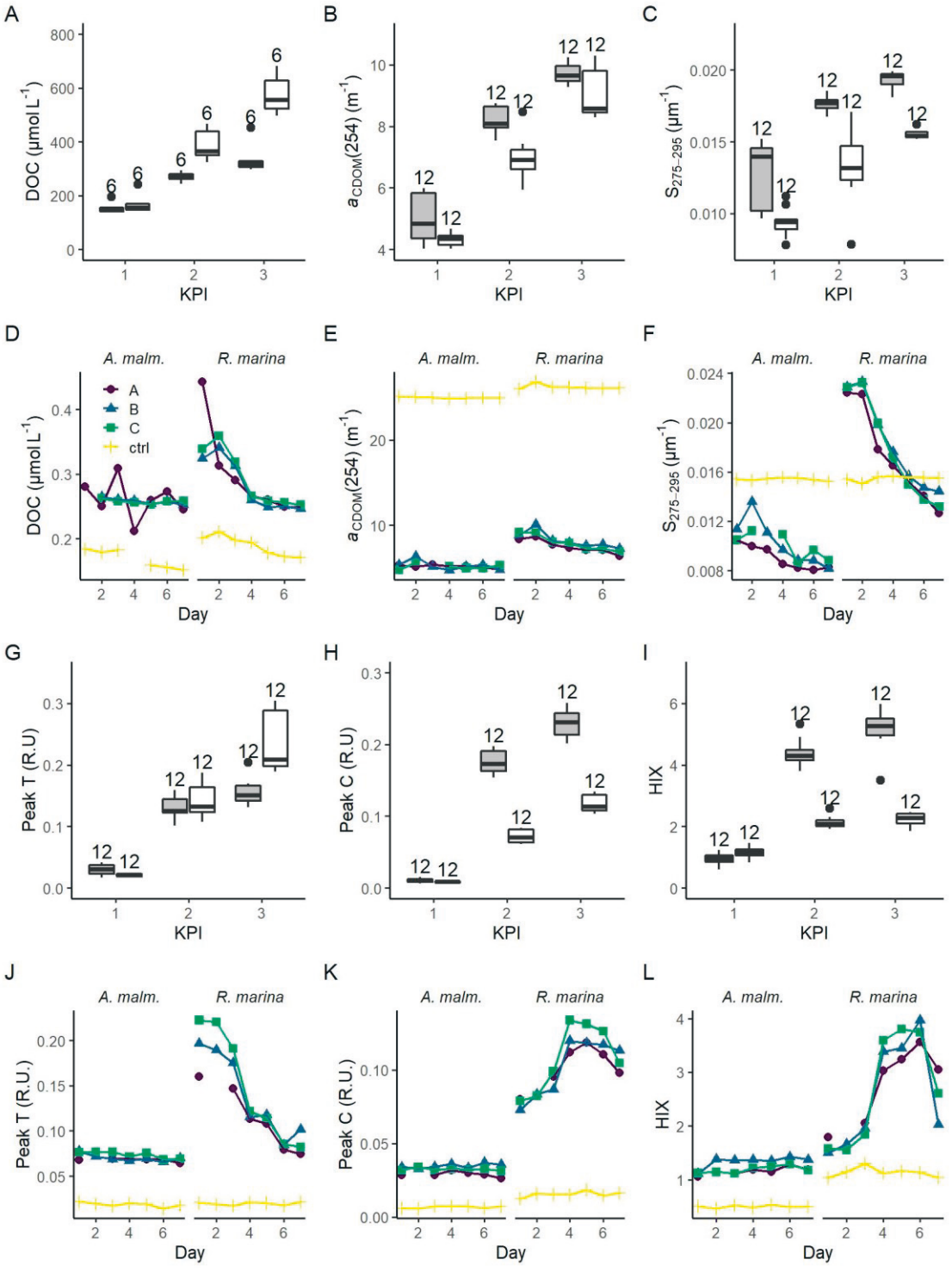
A
B
C
D
E
F
G
H
I
J
K
L
DOC (µmol L-1)
aCDOM(254) (m-1)
S275–295 (µm-1)
Peak T (R.U)
Peak C (R.U)
HIX
Peak T (R.U.)
Peak C (R.U.)
KPI
Day
A. malm.
R. marina
A
B
C
ctrl

Figure 11. DOC concentration (A, D), absorbance coefficient at 245 nm (B, E), spectral slope between 275 and 295 nm (C, F) fluorescence peaks T (G, J) and C (H, K) and humification index (I, L) at each KPI in the DOM release experiment (boxplots) and in the DOM consumption experiment (line graphs). Lines A, B and C mark different replicates (culture filtrate + sea water bacteria) and ctrl marks the control (F/2 + sea water bacteria). Lower and upper hinges of boxes mark the first and third quartiles, respectively, while the whiskers extend to the lowest and the highest value within 1.5 times the interquartile range. White boxes = *A. malmogiense*, grey boxes = *R. marina*

At the beginning of the DOM consumption experiment DOC concentrations were comparable and higher than in the control units (Figure 11D), indicating that considerable DOC production by phytoplankton had occurred in both units despite the difference in the PA before the start of the incubation (*R. marina*: ~9 x 10^4 cells mL^{-1}, *A. malmogiense*: ~1 x 10^4 cells mL^{-1}). During the incubation the DOC concentration did not change much in *A. malmogiense* units but was reduced in *R. marina* units, especially during the first four days.

Contrary to the DOM release experiment, in DOM consumption experiment DOM absorbance decreased during the incubation, although often no significant change could be detected in *A. malmogiense* units (Figure 11). Peak C and HIX increased at first, as in the DOM release experiment, but started to decline at day 4 (Figure 11K, L). Likely in the DOM release experiment the continuous production of fresh DOM by phytoplankton supplied the bacteria with bioavailable DOM which was consumed and transformed to more refractory, UV-absorbing material. In the DOM consumption experiment the phytoplankton were no longer present as a fresh DOM source so the bacteria started to use the more refractory material. This would explain why some of these variables increase in DOM release experiment but decrease in DOM consumption experiment. This would also explain the bell-shaped curves (increase until day 4 and then decrease) of peak C and HIX in the DOM consumption experiment. Until day 4 the bacteria still used more bioavailable material which was left from the phytoplankton and converted it to optically active molecules, but on day 4 this material ran out and the bacteria switched to consuming more refractory, optically active material.

5. DISCUSSION

5.1. Causes for phytoplankton cell lysis in the Baltic Sea (I, II)

There was considerable variation in PA and the %LC of phytoplankton. Although variation in PA could mostly be explained by environmental conditions, such as nutrient concentrations, the connection between %LC and environmental conditions was less straightforward. Instead there was some indication for relationship between %LC and phytoplankton community composition.

5.1.1. Comparison of trends in cell lysis in the Baltic Sea to other systems

The %LC of natural phytoplankton communities or individual phytoplankton groups in oceanic surface waters can vary from only 10% (Alonso-Laita & Agustí 2006) to almost 100% (Veldhuis et al. 2001, Agustí 2004). Almost as large variability has been observed in freshwater systems (Agustí et al. 2006, Rychtecky et al. 2014, Kozik et al. 2019). %LC in studies I and II in all sampling depths varied between 18 and 97% (I: 58-91%, II: 18-97%) and is, therefore, comparable to other studies. Therefore, it can be concluded that dying cells comprise an important proportion of the phytoplankton community in the Baltic Sea as in other systems.

Different %LC among different phytoplankton groups (I) has also been found in other studies (e.g. Veldhuis et al. 2001, Hayakawa et al. 2008, Rychtecky et al. 2014). Hayakawa et al. (2008) found that eukaryotic phytoplankton (<10 µm) had significantly lower %LC compared to *Synechococcus* sp. in the northwest Pacific Ocean. Also, Veldhuis et al. (2001) found the highest %LC in *Synechococcus* sp., with a range of 75–95% during spring. Similarly, in paper I, the %LC of picocyanobacteria (G1), mainly represented by *Synechococcus* spp. in the Baltic Sea (Kuosa 1991, Motwani & Gorokhova 2013), varied from 77 to 91%.

5.1.2. Relationship between cell lysis and abundance

The poor correlation between PA and %LC is curious but not unheard of. In cultures, phytoplankton %LC is usually high during the growth phase and starts to drop at the stationary phase (Lee & Rhee 1997, Agustí & Sánchez 2002) but %LC of a single phytoplankton group has also been shown to vary independent of the growth (Brussaard et al. 1997). This decoupling has also been observed in natural environments (Lasternas et al. 2010, Znachor et al. 2015) and not all phytoplankton taxa show similar trends between %LC and growth phase (Kozik et al. 2019). The differences in the relationship between PA and %LC among the different seasons is curious, although the small sample size limits the reliability of season specific regression analyses. What can be inferred, regardless, is that the mechanisms controlling the relationship between PA and %LC are likely not the same throughout the year. Grazing is a very likely candidate for such a mechanisms,

as high grazing may reduce PA without necessarily affecting %LC (see 5.1.4.).

Surface water %LC during the temporal monitoring campaign was the highest during the onset of 2015 spring bloom when *S. marinoi* dominated but about 15% of the cells were still membrane damaged. As conditions could be assumed optimal this fraction could be interpreted as the minimum amount of membrane damaged cells present in the *S. marinoi* community. High amount of membrane damaged cells in conditions which enable growth has also been demonstrated for another diatom *Fragilaria crotonensis* (Znachor et al. 2015). Species-specific differences in the proportion of membrane damaged cells during different growth phases might partially explain the lack of relationship between total community %LC and environmental variables in this study; a dominating species might have their %LC reduced by a specific limiting factor, e.g., nutrient concentration threshold and, therefore, affect the %LC of the whole community, whereas the %LC of another species might be unaffected by the same factor in a similar growth phase. The different control that nutrient availability exerts on PA and %LC is discussed in 5.1.5. High abundance may also lead to more harmful interactions among phytoplankton, which may affect %LC (see 5.1.6.).

During the spatial monitoring cruise there was in fact a negative correlation between G2 %LC and abundance of all nano- and pico-sized phytoplankton. No other relationships between abundance and %LC were found. The data collected during the spatial monitoring cruise do not allow for an accurate estimation of species succession, but based on observations during the temporal monitoring campaign, it could be speculated that this negative correlation may be linked to termination of a bloom. I.e. maybe the blooms of species comprising C2 were already starting to decline at the stations where high abundances were found and, therefore, also low %LC could be expected. In summary, the results, especially the conflicting trends between %LC and phytoplankton biomass between papers I and II, are in accordance with other field studies which have found it difficult to connect phytoplankton cell death to abundance or growth phases in a predictable way (Lasternas et al. 2010, Znachor et al. 2015, Kozik et al. 2019). Therefore, the health of phytoplankton communities should not be predicted from bulk measurements of PA or Chl *a* concentration.

5.1.3. Effect of removal from the photic layer on phytoplankton cell lysis

The depth-dependent variation in %LC during the spatial monitoring cruise could possibly be explained by stratification of the water column. G2 %LC at 60 m (18 stations) was slightly higher at stations where the halocline was deeper and the 60 m sample was retrieved from above the halocline. Temperature above the halocline was mostly uniform suggesting that the water column above the halocline was well mixed at most stations. This may result in uniform average %LC of the

phytoplankton when the cells are retained within the mixed layer and can be assumed to regularly reach the photic layer. However, the photic layer was always much shallower than the halocline (distance between euphotic zone and upper limit of halocline varied between 15 and 64 m). Thus, phytoplankton may be exposed to extended periods of darkness even within the mixed layer, which may explain the decreased G2 %LC in the deep end of the mixed layer (most 30 m measurements). If the cells end up below the mixed layer (as seen in 8 out of 16, 60 m depth measurements), and, therefore, permanently beyond the photic zone and the compensation depth, high decrease in %LC could be expected, as was seen for all flow cytometry-based phytoplankton groups. Any changes in %LC caused by light intensity (Segovia et al. 2003, Agustí 2004, Llabrés et al. 2010) could, therefore, be expected to be influenced by the mixed layer depth. Therefore, it has to be considered that the reduced %LC at deeper depths may be a consequence of healthy cells deteriorating after being transported below the photic zone by movement of water instead of being caused by sinking of damaged cells from shallower depths.

Because the light penetration depth in the Baltic Sea is generally low, compared to oligotrophic oceans, because of eutrophication (Fleming-lehtinen & Laamanen 2012) and high CDOM concentration (Kowalczuk et al. 2005), light limitation may be a more significant cause for phytoplankton mortality. Especially during phytoplankton blooms some phytoplankton may be expected to experience light limitation due to self-shading. Inflow of terrestrial CDOM may cause light attenuation especially in the coastal zone (Andersson et al. 2018). Any light penetration dependent niche segregation (Agustí 2004) would, therefore, have to take place within a shorter depth range. The shallower the light penetration depth, the more such niche segregation can be assumed to be disturbed by water motion, possibly making it less likely.

Non-motile species can be expected to be most vulnerable to light limitation. Some of the dominant motile spring bloom phytoplankton, dinoflagellates, are also capable of using mixotrophy to potentially compensate for light limitation (Legrand & Carlsson 1998) except maybe in total light depravation (Rintala et al. 2007). Baltic Sea spring bloom diatoms have been shown to benefit more than dinoflagellates from high light (Kremp et al. 2008). It is, therefore, tempting to postulate that increased mortality of non-motile diatoms to cell death under light limitation could be one potential mechanism behind the long term shift from diatom dominated spring blooms to dinoflagellate dominated spring blooms (Klais et al. 2011) since light attenuation can be expected to have increased due to eutrophication (Fleming-lehtinen & Laamanen 2012).

5.1.4. Effect of grazers and viruses on phytoplankton cell lysis

Grazing by zooplankton was not measured in either field studies. The effect of grazing on %LC is difficult to

predict; zooplankton might graze unselectively, prefer healthy cells or prefer damaged cells. During the spatial monitoring cruise lower abundances of all flow cytometry-based phytoplankton groups were detected in the warmer southern stations compared to the sampling sites in the north. In addition, the results suggest a negative relationship between temperature and G2 abundance which indicates that phytoplankton might have encountered higher grazing pressure in warmer waters where growth rate of zooplankton is higher (Sommer et al. 2007). Higher grazing pressure in the south could also be suggested based on the anomalous spatial distribution of G1 (including *Synechococcus* spp.) in the study area. In general, even the cold-adapted clades of *Synechococcus* spp. are more abundant in warmer waters (Paulsen et al. 2016), but during the spatial monitoring cruise, G1 (including *Synechococcus* spp.) had the highest abundance in the colder northern Baltic Sea and were either low or absent at most of the stations in the south, which could be an indication of top-down control.

Zooplankton grazing of phytoplankton at Storfjärden has previously been shown to be weak in spring (Lignell et al. 1992). In summer grazing has been shown to be high (Uitto et al. 1997) although variable depending on the composition of the planktonic community (Uitto 1996). According to these previous studies the results of paper II concerning spring likely realistically capture the relationship between %LC and environmental conditions in the absence of grazing, but caution must be taken with the interpretation of the results from summer months. The occasional co-occurrences of high BA with low %LC might not only point to increased availability of labile phytoplankton-derived DOM to bacteria (discussed in 5.2.2.) but also to possible algicidal activity by bacteria (Meyer et al. 2017) but detection of such interactions was outside the scope of paper II.

Viral infection is generally an important cause for the presence of membrane compromised cells among different phytoplankton species (Brussaard et al. 2001, Laber et al. 2018). High VLP abundance is not a direct signal of active lytic infection on the phytoplankton, especially since they also include bacteriophages not capable of infecting phytoplankton. No correlation between VLP abundance and phytoplankton %LC was found, and VLP abundance did not increase in conjunction with the decline of any of the major blooms or during events of low %LC, even though increase in VLP abundance has been linked to bloom termination in other systems (Bratbak et al. 1993, Hewson et al. 2001) and VLP abundance at Storfjärden was high compared to many aquatic systems (Sekar & Kandasamy 2013). In the light of these results, other mechanisms than viral lysis are more important drivers for variation in membrane-compromised phytoplankton cells in the study area. However, using actual viral infection indicators might lead to different conclusions as VLP abundance is a very approximate estimation of virus activity.

5.1.5. Effect of nutrients and salinity on phytoplankton cell lysis

Nutrient limitation has been shown to determine phytoplankton %LC (Agustí 2004, Agustí et al. 2006, Alonso-Laita & Agustí 2006, Rychtecky et al. 2014). Especially larger species can have low %LC in oligotrophic environments (Alonso-Laita & Agustí 2006). Direct correlations between %LC and nutrient concentrations have been observed in oligotrophic environments (Lasternas et al. 2010). Nutrient limitation can decrease the %LC cells also in phytoplankton cultures (Brussaard et al. 1997, Lee & Rhee 1997).

Contrary to these studies, the connections between nutrients and %LC in studies I and II were weak, with only very limited statistical support for the correlation between phosphate concentration and G2 %LC in paper I. This is unexpected in light of the stronger but negative correlation between phosphate concentration and G2 abundance. Nutrient affinity is tightly linked to size as the surface to volume ratio changes with a 2/3 power exponent, and the smaller sized picophytoplankton satisfy their nutritional needs at a much lower nutrient concentration (Irwin et al. 2006). In addition, with rapid nutrient turnover, the small phytoplankton cells might not experience nutrient stress even at very low inorganic nutrient concentrations. This might in part explain the high G2 abundance in low phosphate concentration, as especially the small phytoplankton gain competitive advantage against larger cells by efficiently using the recycled phosphate in nutrient depleted environment (Irwin et al. 2006). The positive relationship between phosphate and G2 %LC might then be explained by the situational advantage in otherwise poor conditions; the picoplankton are better competitors for nutrients and, therefore, more abundant in low nutrient conditions, but the low phosphate concentrations still cause them stress leading to lower %LC.

The lack of detectable correlation between %LC and nutrient concentrations on yearly scale can most likely be explained by seasonal changes in growth conditions; in winter nutrient concentrations are high but conditions are otherwise unfavorable for phytoplankton. The lack of a relationship during the productive season could perhaps be explained by effective nutrient turnover rates at the study site. High BA probably maintains high remineralization rate of riverine DOM (Asmala et al. 2013) producing enough fresh nutrients to support phytoplankton growth (Traving et al. 2017) while high phytoplankton biomass and turnover rates may nevertheless keep the nutrient concentrations (especially N) low through the productive season. High turnover rates may also mask the effect of grazer remineralization of nutrients. Zooplankton grazing and consequent nutrient remineralization may affect nutrient limitation of phytoplankton (Elser et al. 1988) and thus possibly also the effect of nutrients on %LC, but since grazing was not measured quantitative conclusions cannot be drawn. It could be speculated, however, that the results from winter and early spring bloom

samplings are unaffected by grazers since spring grazer populations start to increase later than phytoplankton (Lignell et al. 1993). In summer and fall a more tightly linked nutrient recycling between phytoplankton and their grazers may keep %LC high regardless of ambient nutrient concentrations. Regardless, during summer when the nutrient concentrations were very low, %LC was also lower than during any other season, which may indicate a very broad-scale relationship between nutrients and %LC in this system. In summer, some competition for inorganic nutrients between phytoplankton and bacteria maybe added to the nutrient limitation of phytoplankton. BA, temperature and DOC concentration were high and nitrate concentration was low suggesting that conditions might have been optimal for heterotrophic bacteria growing on DOC to outcompete phytoplankton for inorganic N (Joint et al. 2002). However, phytoplankton might have compensated the poor availability of inorganic N by uptake of DON (Bronk et al. 2007).

While the decreasing Si concentration during the spring blooms (II, data not shown) clearly indicates that diatoms quickly reduced the ambient Si concentration, it is uncertain whether Si limitation was a cause for the collapse of diatoms after the spring blooms. The Si concentration never fell below 3.5 µM and diatoms have been shown to be able to dominate in Si concentrations of 2 µM and higher (Egge & Aksnes 1992). However, *S. marinoi* may start to show Si limitation related PCD symptoms already at Si concentrations around 10 µM even though the community is still growing (Wang et al. 2017). While Wang et al. (2017) did not detect loss of membrane integrity during their 10 day experiment, since cell death eventually leads to membrane damage it could be expected that the Sytox Green analysis would have detected some Si limited *S. marinoi* cells during 2015 spring bloom when *S. marinoi* dominated and Si concentration was ~10 µM at the end of the bloom. Such cells were indeed detected but while drop in Si concentration coincided with the drop in *S. marinoi* biomass, %LC did not correlate with either even though most of the spring bloom community consisted of *S. marinoi*. In 2016 a much smaller diatom component of the mixed spring bloom caused a more drastic reduction in Si concentration, again, with no clear connection to %LC, and %LC and diatom biomass even increased again when the Si concentration was lowest. Reduction in Si concentration also coincided with the reduction in nitrate concentration in both years making it difficult to tease apart the effect of these two nutrients on the bloom collapse. The spring bloom community close to the sampling site has previously been shown to respond to Si addition but especially to the addition of both Si and nitrate (Kuuppo et al. 1998) suggesting that the diatoms at the region are principally nitrogen limited during spring blooms. In addition to the difficulties of pinpointing exact concentration where Si is limiting, linking Si concentration to diatom health and bloom termination is also complicated by complex uptake

dynamics of Si at high concentrations by diatoms (Leynaert et al. 2009).

The results demonstrate that inorganic nutrient concentrations, at least as far as they can be measured by standard methods, cannot per se be used to predict the cell lysis of phytoplankton in the studied environments. Independence between %LC and apparent growth-limiting nutrients has also been shown in other environments for total phytoplankton community (Hayakawa et al. 2008, Alou-Font et al. 2016) or for individual species (Rychtecky et al. 2014, Kozik et al. 2019).

Relating changes in %LC to changes in environmental factors such as temperature, salinity and nutrients at Storfjärden is complicated due to the estuarine nature of the site. The river does not only bring terrestrial DOM and nutrients but it also temporarily lowers surface water salinity and possibly introduces phytoplankton populations adapted or acclimated to lower salinities. Such cells may exhibit membrane integrity loss due to sudden exposure to too high or low salinity (Lancelot & Bruxelles 2011). Regardless, no significant changes in %LC during fresh water inflows were detected and the changes in salinity were modest through the sampling period (salinity range in surface: 4.3-6.2). Therefore, the conclusion is that salinity in itself did not affect %LC of phytoplankton.

5.1.6. Effect of species interactions on phytoplankton cell lysis

The question remains, what causes the presence of membrane damaged cells within the observed phytoplankton populations given that abiotic factors seem to contribute so little? During both field studies %LC was occasionally fairly low even when Chl *a* concentration was high.

One possible explanation could be the allelopathic interactions among phytoplankton themselves (Poulin et al. 2018). Among such interactions is the release of PUAs, which have been shown to induce cell death among some phytoplankton species (Casotti et al. 2005, Ribalet et al. 2007). PUA concentrations were not measured during these studies, but cautious speculation based on what is known about PUA production of different species is possible. PUAs can be produced by different phytoplankton species, but especially by diatoms (Wichard et al. 2005). For example, Taylor et al. (2009) observed increased PUA production in *Skeletonema marinoi* during increased nutrient limitation in spring in the Baltic Sea. *Skeletonema marinoi* was not present in high numbers during the spatial monitoring cruise, but at many stations diatom abundance was high (up to 5000 cells mL-1), and diatom biomass correlated negatively with G2 %LC which might indicate allelopathy, possibly mediated by PUAs. However, this interpretation is complicated by the low diatom biomass at several stations and by the positive correlation between diatom biomass and G2 abundance. A possible explanation for this inconsistency could be that the conditions were favorable for growth of

both G2 and diatoms and PUA production started only at high cell densities at the onset of diatom bloom decline, as has been demonstrated for *S. marinoi* by Vidoudez, Casotti, et al. (2011).

During the temporal monitoring *S. marinoi* dominated the 2015 spring bloom until abrupt collapse, which could be an indication of PUA mediated synchronized bloom termination, a process which has been postulated by high PUA concentrations toward the end of phytoplankton blooms (Vidoudez & Pohnert 2008, Bartual et al. 2017). *S. marinoi* were also present during the transition phase of 2016 spring bloom. At that time %LC was low which could again be speculated to have been caused by PUA release from *S. marinoi*.

Cózar et al. (2018) concluded, based on in situ measurements, that per cell release of PUAs increases with increased oligotrophy, presumably to enhance the bacterial remineralization rates of nutrients (Edwards et al. 2015). Most of the Baltic Sea is far from oligotrophic, but towards the end of the bloom the nutrient limitation might induce an increase in PUA production, which, given the high phytoplankton density, might result in a sufficiently high PUA concentration to induce a detectable reduction in %LC. Since G2 %LC also correlated negatively with the total abundance of flow cytometry based phytoplankton, the possibility of PUA mediated allelopathy among the small phytoplankton cannot be ruled out (Vidoudez, Nejstgaard, et al. 2011, Morillo-García et al. 2014). Patches of high PUA concentrations have been found at deep water layers (Bartual et al. 2018) which might provide one explanation for the low %LC at deep measurements in paper I. However, without measurements of PUA concentrations this remains speculation.

The results from the temporal monitoring campaign suggest %LC to be high in communities with low phytoplankton diversity. During early bloom high %LC might be explained by the dominating population being actively proliferating or by lack of interspecific allelopathy, as discussed earlier. Increased production of allelopathic chemicals might be induced by the presence of competing species (Ikeda et al. 2016). Given the lack of other clear causes, allelopathy presents a potential explanation for low %LC during many samplings when diversity was high. If this is the case the predicted increased competition between diatoms and dinoflagellates (Klais et al. 2011) might result in spring blooms with lower overall proportion of healthy phytoplankton cells.

It could be proposed that low %LC during low H could be because at that time the community was dominated by a species which stained poorly with Sytox Green even when dying. Such an error is unlikely, because the species which were dominant during those samplings, *S. marinoi* and *H triquetra*, stained very clearly during other samplings (Figure 2A) and because the presence-absence method of determining %LC included every Sytox Green stained cell as long as any green fluorescence was observed (see 4.1.3.).

Recent studies concerning cellular mechanisms of phytoplankton cell lysis suggest that the physiological health of phytoplankton is determined by complex interaction of biotic and abiotic mechanisms (Bidle 2015), with a significant contribution by inter- and intraspecific communication within the planktonic community. The results presented here can be interpreted according to this view; abiotic conditions alone cannot explain phytoplankton %LC even though they set strict constraints on phytoplankton productivity and bloom formation. Instead %LC seems to be high on those rare occasions when the community is strongly dominated by single phytoplankton groups, or especially, single species and, therefore, interspecific harmful interactions can be assumed to be minimal. Also Kozik et al. (2019) assumed interspecific interactions to be a potential cause for phytoplankton cell death during their field monitoring when changes in the proportions of membrane damaged cells and cells going through apoptosis could not be explained with abiotic conditions. Single cell blooms can be assumed to be common mainly during spring blooms and, therefore, it could be extrapolated that generally the highest %LC in the Baltic Sea can be found during spring blooms.

5.2. Consequences of phytoplankton cell lysis in the Baltic Sea (I, II)

The main goal of studies I and II was to investigate if natural variation in %LC of phytoplankton could be related to POC:DOC partitioning in the Baltic Sea. This was investigated by measuring ambient DOC and POC concentrations (I, II), or sedimentation of POC (II). Some connection between %LC and DOC concentrations was found in the estuarine system (II) but not in the open Baltic (I). Sedimentation rate was not affected by %LC (II) but the higher %LC at deeper open sea sites might indicate that cell lysis contributes to pelagic remineralization in the open Baltic Sea.

5.2.1. Phytoplankton cell lysis and photosynthetic efficiency

The photochemical efficiency (F_v/F_m) was found to be a good proxy for physiological state as it correlated with %LC (especially G2 %LC) and explained it better than e.g. nutrient concentrations. Photosynthesis is rapidly downregulated during stress conditions, e.g. depletion of inorganic nutrients, but there is also a taxonomic component affecting the F_v/F_m (Suggett et al. 2009). A more comprehensive %LC assessment of natural phytoplankton communities might reveal how well F_v/F_m and membrane integrity-based %LC assessments are related. This, however, can be relatively complicated because as, e.g. Franklin et al. (2009) demonstrate, a high F_v/F_m value might not be a clear sign of absence of dead cells, although cells with reduced membrane integrity likely have lower photosynthetic efficiency (Veldhuis et al. 2001). Reduced F_v/F_m might also be a transient response to stress, as phytoplankton continuously acclimate to their surroundings (Halsey & Jones 2015), and during the spring bloom in the Baltic Sea,

the PP output per Chl *a* unit (the assimilation number) is not affected by the inorganic nutrient concentration (Spilling et al. 2019). Therefore, it might be better to consider measurements of F_v/F_m and %LC as complementary assays for the physiological state of phytoplankton communities. Also Veldhuis et al. (2001) coupled membrane integrity analyses with a photosynthetic stage measurement. By using ^{14}C incorporation as a determinant of cell physiological status, they found that populations of cells containing photopigments but possessing compromised membranes were, at least partially, capable of photosynthesis, but had lower ^{14}C fixation rates. Bulk measures such as ^{14}C fixation rates, while useful in overall population assessment, inevitably integrate physiological heterogeneity within microbial populations, meaning that correlations between bulk measurements and single-cell measurements are difficult to interpret (Davey & Kell 1996). Regardless, the low F_v/F_m in connection with low %LC suggests that low %LC is also a potential indicator for lower primary productivity.

5.2.2. Effect of cell lysis on DOC release

Dead phytoplankton cells are considered an important DOC source in many, mainly oligotrophic, marine environments (e.g. Kirchman 1999, Franklin et al. 2006, Agustí & Duarte 2013). A statistically ambiguous negative correlation between %LC and DOC concentration was found during the temporal monitoring campaign, although only during spring bloom when seasons were investigated individually, (II) but not during the spatial monitoring cruise (I). The results from spatial monitoring cruise, therefore, suggest that phytoplankton cell lysis is not a significant source of DOC to the standing DOC pool during spring bloom on the open Baltic Sea. The lack of strong correlation between %LC and DOC concentration is understandable due to high ambient DOC concentration. The Baltic Sea has various DOC sources, and the influence of allochthonous DOC is strong (Sandberg et al. 2004, Alling et al. 2008, Kulinski & Pempkowiak 2008), especially in the estuaries (Hoikkala et al., 2012).

Seasonal variation in DOC and DON concentrations compared to POC and PON concentrations was minor. There was also no significant relationship between %LC and POC concentration. Together this results in surface water POC:DOC ratio being seemingly independent of %LC. Low variation in DOC and DON concentrations can maybe be explained by high background concentration of refractory DOM, with variation happening in the smaller fraction of labile or semilabile DOM (labile DOC comprises 0-9% and labile DON 0-41% of total DOC and DON, respectively, in the Gulf of Finland (Hoikkala et al. 2015)). The lysis of phytoplankton cells could be assumed to cause increase of DON concentration, as N-rich cell contents are released. However, the lack of relationship between %LC and DON concentration or %LC and DOC:DON ratio suggests that such release of N-rich molecules is either

masked by background DON concentration or consumed so quickly by heterotrophic bacteria that it cannot be detected by chemical analysis. Because the nitrate concentration was low throughout the productive season, uptake of some DON components by phytoplankton may also have occurred and complicated the interpretation of DON concentrations (Bronk et al. 2007).

At Storfjärden, detection of the release of fresh DOC from phytoplankton was complicated by the riverine DOC input. Salinity, which is a direct proxy for fresh water inflow, explained changes in DOC concentration much better than %LC. However, the most optimal model for explaining DOC concentration included both %LC and salinity. This model explained up to 64% of the changes in DOC concentration (salinity alone: 59%, %LC alone: 15%). %LC was not significantly related to salinity indicating that they produce independent impacts on DOC concentration. Given the influence of riverine inflow on DOC concentration, it is somewhat surprising that a negative relationship between DOC and %LC could be detected during spring bloom, when the inflow of smelt water can still be high. One explanation might be that the high phytoplankton biomass produced lots of DOC while BA was still so low that even labile DOC accumulated in the environment. It might also be an unrelated relationship; riverine inflow kept introducing DOC to the system but the phytoplankton bloom was already in decline due to nutrient limitation resulting in simultaneous but acausal decrease of %LC and increase of DOC concentration.

It is assumed that a large fraction of the DOM leaking from dying cells is highly bioavailable and, therefore, rapidly consumed by pelagic heterotrophic bacteria, especially when bacterial biomass is high. Therefore, during periods of high bacterial biomass the assumed increased DOC leakage from membrane damaged cells would not significantly influence the DOC pool, especially if the background DOC concentrations is high. A significant negative relationship between %LC and BA could only be found in summer but there were instances through the sampling period when BA was high and %LC was low. These could be interpreted as incorporation of fresh DOC released from phytoplankton by bacteria. However, this interpretation must be taken cautiously since during the events of low %LC also total PA was mostly low. Therefore, the phytoplankton were not necessarily an important source of DOM for bacteria during those times. Instead, high BA could be associated to earlier introduction of DOM into the system. During the spring bloom the higher contribution of HNA bacteria to the total bacterial community could indicate bacterial utilization of phytoplankton-derived DOM (Kaartokallio et al. 2016) as HNA cells have been attributed to copiotrophic bacteria capable of utilizing pulses of available organic carbon (Bouvier et al. 2007). Motile copiotrophic bacteria can be expected to especially benefit from DOM release caused by increased lysis at the

termination of the spring bloom as they can quickly move towards lysing phytoplankton cells (Smriga et al. 2016). BA was otherwise not significantly related to PA. The effect of nano- and microzooplankton grazing of bacteria, which was not quantified, could uncouple the accumulation of bacterial cells from BP and DOM consumption. The main driver for BA was likely temperature throughout the sampling period (data not shown).

As the presence of membrane damaged cells is only a rough proxy for actual DOM release from dying phytoplankton, a next step in the follow-up studies should be a closer look to what actually is released from dying phytoplankton, in what quantities and where this DOM ends up. Given the high assumed bioavailability of recently released DOM and the technical difficulties in measuring concentrations of bioavailable DOM, a more feasible approach might be to measure uptake of DOM by heterotrophic bacteria. A more thorough investigation of DOM and its processing was attempted during the latter part of the temporal monitoring campaign (2016, unpublished data) by measuring optical properties of DOM, BP, BR and extracellular activity of β-glucosidase and leucine-aminopeptidase (data not shown). None of these variables could be connected to %LC and they were excluded from paper II in order to focus on the variables which were measured throughout the monitoring period and, therefore, yielded a bigger data set. These excluded measurements nevertheless showed that the relationship between %LC and the processing of DOM by bacteria is not straightforward, especially if the focus is on the bulk processes in the water column. Instead, single cell analyses combining membrane probes or other cell death indicators to indicators of bacterial metabolic activity or perhaps single cell bacterial production (which might be possible using e.g. flow cytometric analysis of 5-bromo-2-deoxyuridine incorporation into cells (Eswaran & Khandeparker 2017)) might yield a more accurate picture of the fate of DOM released from dying phytoplankton. Also a more detailed taxonomic analysis of the bacterial community should be used to detect possible community shifts connected to changes in %LC. Taxonomic analysis could maybe be used to detect both, the presence of copiotrophic bacteria likely to benefit from fresh DOM or the presence of algicidal bacteria possibly affecting the %LC.

5.2.3. Effect of cell lysis on the sedimentation of particulate matter

%LC also was never significantly related to sedimentation rates of POC, PON or Chl *a* suggesting that cell death and lysis as a loss process is not critical for determining the fate of phytoplankton-derived carbon at Storfjärden. Instead, high %LC of phytoplankton in the sediment trap suggests that sinking is a more important loss process than lysis. Because of the estuarine nature of the sampling site the water column is dynamic and some resuspension of sedimented material into the sediment trap could be expected. The >10 m

distance from bottom to the sediment trap aimed to minimize this. The very similar %LC among surface, 20 m and the sediment trap indeed suggest that at least reintroduction of dead cells from the bottom was minimal although this does not rule out the capture of other resuspended material in the traps.

Intact diatoms accumulated in the sediment trap especially during the 2015 spring bloom and also during other seasons. Also motile phytoplankton, especially *H. triquetra* during the early fall bloom of 2015, contributed to the sinking intact cells. Generally, sinking of intact cells is associated with diatoms whereas dinoflagellates are assumed to lyse in the water column (Tamelander & Heiskanen 2004) unless there is an event of mass encystment (Heiskanen 1993) but this time at least, some healthy vegetative dinoflagellate cells was removed by sinking. These results suggest that in shallow estuaries sinking is an important loss process for most phytoplankton cells regardless of their physiological health, motility or tendency to lyse in the water column in other systems. This may highlight the importance of allochthonous riverine and terrestrial DOC for estuarine bacterial processes (Asmala et al. 2013, Kaartokallio et al. 2016).

The slightly higher %LC in sediment traps compared to surface water suggests that some of the dying cells might lyse in the water column before sinking but this fraction was assumed to be so small that it could not be related to the changes in surface water POC and DOC pools at Storfjärden. However, in the deep samples taken on the open Baltic the clearly lower %LC compared to surface waters indicated that lysis might increase as the cells sink. This may contribute to significant release of DOC from phytoplankton, and, therefore, to pelagic remineralization, especially if it is assumed that a similar trend applies also to large phytoplankton species. If the lysis of sinking cells results in cell death related PUA production, even more pronounced remineralization could be expected (Edwards et al. 2015). Lysis and remineralization in the mixed water layer may lead to increased release of CO_2 to the atmosphere compared to situation when %LC at depth is lower (Kwon et al. 2009). Conceptually this proposed idea of higher lysis of phytoplankton in the deeper water layers of the open Baltic Sea is in line with the estimated high consumption of autochthonous carbon by bacteria in the Baltic Sea (Hoikkala et al. 2015).

5.3. Carbon cycling between phytoplankton, DOC and heterotrophic bacteria (III)

Species specific differences in carbon cycling were found in both experiments of paper III; in the DOM release experiment, where simultaneous processing of DOM by phytoplankton and bacteria were investigated, and in the DOM consumption experiment, where bacterial DOM processing was investigated. Differences were clear at every level of carbon cycling: PP, flow of ^{14}C from DIC to bacterial biomass, optical properties of DOM and the response in the bacterial community.

5.3.1. DOC production, transformation and consumption

During the 2nd and 3rd KPI *R. marina* cultures were already declining which may partly explain the lower PP and some differences in DOM release. However, the trends in DOC concentration and in all of the optical variables of DOM were similar between the 1st and the 2nd and the 2nd and the 3rd KPI suggesting that there was no qualitative shift from production to consumption of any DOM fraction detected by the optical methods. Also the trends were similar between the species which further suggest that the different observed changes in the optical DOM properties were more related to total culture age than to growth phases. Of course, the optical method does not detect changes in the concentrations of optically inert molecules, such as simple carbohydrates, and there may have been growth phase dependent changes in their production (Chen & Wangersky 1996, Urbani et al. 2005).

The decline in the abundance of *R. marina* was not quick nor linear and occasionally abundance increased again, suggesting that conditions were still quite favorable for *R. marina* during all KPIs. The resumption of growth might have been due to the cells turning to heterotrophy, as some *Rhodomonas* species are known to be mixotrophs (Ballen-Segura et al. 2017). As nutrient limitation was most likely not significant, carbon limitation could be another possible cause for population decline and a switch to support growth with heterotrophy. Total dissolved carbon was high in both *R. marina* and *A. malmogiense* units even at the 3rd KPI, but since pH was not measured the relative fractions of different forms of inorganic carbon are not known. To my knowledge, the capacity of *A. malmogiense* to utilize different forms of inorganic carbon is not known, but many dinoflagellates are be able to use bicarbonate (Nimer et al. 1997) suggesting that *A. malmogiense* was likely not carbon limited. The potential for carbon limitation of *R. marina* is not clear, since the use of different forms of inorganic carbon by *R. marina* is not known. Some *Rhodomonas* species use only free CO_2 (Elzenga et al. 2000) while some also seem to use bicarbonate (Camiro-vargas et al. 2005).

During the 2nd and 3rd KPIs also %LC of *R. marina* was slightly decreased (~95%) which might have some effect on DOC release but even during these KPIs %LC was high compared to declining phytoplankton communities in cultures or natural environments (Veldhuis et al. 2001). Such a small fraction of membrane damaged cells can unlikely explain the more than twice higher ^{14}C-DOC release:PP ratio in *R. marina* units compared to *A. malmogiense* units. High %LC indicates that, when reflected to natural environment, the results of paper III related to DOC release from phytoplankton would mainly reflect peak bloom conditions when the phytoplankton communities are still healthy, and when interspecific competition is low (see 5.1.6). It may have been possible that high bacterial activity during the experiment aided in quickly disintegrating and consuming the

dying and membrane damaged cells resulting in minimal detection of membrane damaged but otherwise intact phytoplankton cells.

Usually peak T is interpreted as bioavailable material (Nieto-Cid et al. 2006), but it increased in both treatments together with the signals for less labile DOM throughout the DOM release experiment. Increase in protein-like DOM fluorescence has been connected to phytoplankton growth during simulated (Stedmon & Markager 2005) and natural (Suksomjit et al. 2009) phytoplankton blooms, but bacterial processing can decrease protein-like fluorescence while increasing humic-like fluorescence (Yamashita & Tanoue 2004b, Romera-Castillo et al. 2011). Therefore, simultaneous increase in peaks T and C likely occurred because of (1) excess production of protein-like DOM by phytoplankton, (2) production of less labile protein-like DOM by phytoplankton or (3) production of protein-like DOM by bacteria. Not all protein-like DOM fractions are equally degradable (Yamashita & Tanoue 2004a) and some protein-like FDOM can accumulate in the pelagic environment (Yamashita et al. 2017, Asmala et al. 2018). Production of peak T by bacteria might be due to bacterial reworking of initially labile (non-colored) autochthonous DOM into small, UV-absorbing molecules (Berggren et al. 2009, Asmala et al. 2018). In the case of *R. marina*, this could maybe result from the consumption of monosaccharides, which *R. marina* can produce in high amounts (Fernandes et al. 2017), as several bacterial species have been shown to produce peak T when grown on glucose (Fox et al. 2017).

Just like the simultaneous increase of most optical DOM variables in the DOM release experiment. the decrease of most of the FDOM variables towards the end of the DOM consumption experiment is somewhat surprising, given that bacterial processing of phytoplankton-derived DOM is usually connected to increase of FDOM (Romera-Castillo et al. 2011). The high abundance of *Pseudorhodobacter* might explain part of this as Rhodobacteraceae have been connected to reduced FDOM intensities when using dinoflagellate-derived DOM (Tada et al. 2017). Bacteria may also change from net source of protein-like FDOM to a net sink as bacterial activity increases (Guillemette & del Giorgio 2012). This is in line with the decreasing peak T during the DOM consumption experiment, as the higher temperature used in the DOM consumption experiment may have directly enhanced bacterial activity. Guillemette and del Giorgio (2012) also showed that production of humic-like FDOM increases with increasing BGE, which is in line with the increase of peak C and HIX concurrently with BGE until day 4, although after that the FDOM signals decreased while BGE did not. The change in DOM processing patterns on day 4, which was suspected to have been caused by the depletion of fresh labile DOM originating from phytoplankton, was interesting also because the production of humic-like DOM should increase when bacteria shift from processing labile DOM to semi-labile DOM (Jørgensen et al. 2015).

The general differences between *A. malmogiense* and *R. marina are* similar to those found by Fukuzaki et al. (2014) for dinoflagellates *Heterocapsa circularisquama* and *Alexandrium catenella* and a cryptophyte *Rhodomonas ovalis*. They observed higher biomass production for the dinoflagellates and higher apparent percentage of net photosynthetic extracellular release for *R. ovalis*. In addition to the inherent species specific physiological differences between *A. malmogiense* and *R. marina*, some fraction of the different DOM release might be caused by more general traits, such as the size difference between the species. Higher release of bioavailable DOM from *R. marina* might simply be caused by the smaller size of *R. marina* cells and, therefore, higher passive release of DOC (Bjørnsen 1988). BR was not measured in the DOM release experiment, but the higher BR, BP and DOC consumption in *R. marina* filtrate units in the DOM consumption experiment may in part explain the higher DOC accumulation in *A. malmogiense* units in the DOM release experiment, even if passive release of DOM from *R. marina* cells was suspected to be relatively higher.

Even though both of the phytoplankton species can be assumed to be mixotrophic (Rintala et al. 2007, Ballen-Segura et al. 2017) and phytoplankton can take up DOM in mixed communities (Bronk & Glibert 1993, Moneta et al. 2014), significant DOM consumption by phytoplankton during this experiment was unlikely. Uptake of organic N or P would be energetically unlikely at the presence of available inorganic N and P. Especially at the beginning of the DOM release experiment, when most of the DOM likely consisted of phytoplankton exudates and there was no sign of carbon or nutrient limitation, reuptake of their own exudates seems unlikely. Towards the end of the experiment, if the decline of *R. marina* was caused by carbon limitation, DOM consumption would have been more likely and an unknown factor of changes in the properties of DOM could maybe be attributed to reuptake and processing by phytoplankton. However, because there was a shift from increase to decrease of some optical DOM properties between the DOM release experiment and the DOM consumption experiment, the principal role of *R. marina* was likely still the production of DOM rather than its consumption throughout the DOM release experiment.

Because the observed changes in optical DOM properties seem to be independent of the BA:PA ratio, the observed changes in DOM quality have to arise primarily from the traits of individual phytoplankton species (rate and type of produced DOM (Fukuzaki et al. 2014)) or bacterial species (rate and type of consumed and produced DOM (Romera-Castillo et al. 2011, Fox et al. 2017)) instead of only from the ratio of producers to consumers. A general conclusion from DOM quality indicators is that *R. marina* produce comparatively more DOM when normalized to PP, than *A. malmogiense* and that this DOM seems to be more efficiently consumed and altered by bacteria. However, DOC release and the rate of DOC production

to PP do not necessarily reflect natural situations precisely for either phytoplankton species since the fraction of PP released as DOC from phytoplankton is generally higher in situ than in cultures (Thornton 2014).

5.3.2. Response of bacteria to DOC

The higher leu:thy incorporation ratio in *R. marina* units indicates that bacteria in these units struggled to get enough carbon and/or energy from DOM for balanced growth. A likely explanation for this is that the bacterial community in *R. marina* units efficiently depleted the medium of labile DOM and the DOM release from *R. marina* could not keep up with the demands of the bacterial community. This idea was supported by much higher BP:PP ratio in *R. marina* units during the 2nd and the 3rd KPI. In the DOM consumption experiment bacteria growing on *R. marina* filtrate invested more in thymidine incorporation (lower leu:thy ratio) than in the DOM release experiment. This was most likely caused by the dilution of BA during the filtration and further suggests that the higher leu:thy ratio in DOM release experiment was caused by intense competition for DOM among bacteria.

In the DOM consumption experiment DOC consumption was higher, bacterial growth was faster, and BP (bulk and cell specific with both methods) and BR (bulk and cell specific) were higher in communities growing in *R. marina* filtrate. This was despite the head start of BA in *A. malmogiense* units (more bacterial cells left even after filtration). All of this suggests that DOM released by *R. marina* was indeed of higher bioavailability than DOM released by *A. malmogiense*. This might simply have been caused by the smaller size of *R. marina* cells and, therefore, higher passive release of DOC (Bjørnsen 1988), or it might have been due to higher production of monosaccharides (discussed earlier) or other bioavailable molecules.

In general, Bacteroidia, Alphaproteobacteria and Gammaproteobacteria predominated bacterial communities in both DOM release and consumption experiments and the communities reflected those in the phytoplankton cultures. In the beginning of the DOM consumption experiment in *R. marina*, class Alphaproteobacteria (mostly genus *Pseudorhodobacter*) comprised 82% of the bacterial community, which was related to the high peak T and high BP. This kind of 'feast and famine' growth mode is typical for copiotrophic bacteria (Lauro et al. 2009). Alphaproteobacteria benefit from phytoplankton blooms when there is high concentration of labile DOM available (Allers et al. 2007) and they are efficient in using amino acids (Cottrell & Kirchman 2000, Gasol et al. 2008). The predicted high production of monosaccharides by *R. marina* (Fernandes et al. 2017) may explain the higher proportions of Alpha- and Gammaproteobacteria in *R. marina* units. *Pseudorhodobacter* has been detected also in a previous mesocosm study with Baltic Sea water (Camarena-Gómez et al. 2018) as well as in Baltic Sea bacterioplankton (Herlemann et al. 2011).

Generally, in *A. malmogiense* communities in both experiments the share of class Bacteroidia (genera *Algoriphagus* and *Polaribacter*) was higher than in *R. marina* communities. The more stable and less optically active DOM pool was reflected to the community in the DOM consumption experiment: class Bacteroidia comprised on average 70% of the community throughout the experiment. In the *R. marina* units class Bacteroidia (genus *Flavobacter*) became abundant only after the day 3 congruently with the drop in peak T, implicating that the ratio of labile to semi-labile DOM dropped on day 4 and caused the shift in bacterial community composition. Phylum Bacteroidetes is well known of its capability to degrade high-molecular weight DOM (Cottrell & Kirchman 2000, Romera-Castillo et al. 2011) with their polysaccharide utilizing enzymes (Grondin et al. 2017). Both Polaribacter and Flavobacterium are common moderate copiotrophs and detected from phytoplankton blooms (Teeling et al. 2012, Mühlenbruch et al. 2018). In addition, *Polaribacter* and *Algoriphagus* have been detected in previous Baltic Sea mesocosm studies (Herlemann et al. 2017, Camarena-Gómez et al. 2018). The observed pattern in the bacterial community composition support the interpretation that DOM was more labile in *R. marina* units than in *A. malmogiense*. The difference between control units and experimental replicates suggests that phytoplankton-derived DOM, not the growth medium, is the main driver for bacterial community and DOM processing dynamics.

Actinobacteria, which were present in *A. malmogiense* units, are members of autochthonous bacterioplankton in the Baltic Sea (Riemann et al. 2008) occupying several different niches and thus have likely various different functions in the Baltic Sea food web (Holmfeldt et al. 2009). They have also occurred with dinoflagellates in a previous mesocosm experiment with Baltic Sea water (Camarena-Gómez et al. 2018). In the Baltic Sea, some Actinobacteria are linked to high DOC concentrations and terrestrial DOM close to the land (Holmfeldt et al. 2009) and others are outcompeted by fast-growing copiotrophs when phytoplankton-derived DOM is available (Pérez & Sommaruga 2006). Possibly, in *A. malmogiense* units the presumably lower low to high molecular weight ratio of DOM allowed them to compete better with the copiotrophic Alpha and Gammaproteobacteria. However, it is also possible that the 0.8 µm filtration in the DOM consumption experiment caused a bias and favored them due to their small size (Hahn et al. 2003).

In the DOM consumption experiment, a shift in the preferred substrate for bacterial consumption and a concurrent shift in the bacterial community was obvious in *R. marina* units even though DOC concentration was still high after the incubation. This highlights the strong connection between phytoplankton DOM release and bacterial processes. The existing DOM pool explains only part of the mechanisms which structure the bacterial community. The fast flow of ^{14}C from DIC pool through phytoplankton to DOC pool and bacterial

biomass in DOM release experiment supports this statement. Comparable BGE in the DOM consumption experiment between the species, despite the differences in the bacterial community composition, suggests that the emerging bacterial community in both treatments consisted of bacteria well-adjusted or adapted to the DOM released from their host phytoplankton.

In both experiments the final bacterial communities were similar and seemingly unaffected by the addition of natural bacteria. This suggests that these phytoplankton-bacteria communities are somewhat deterministic and stable. This is in line with other studies which have shown stable and predictable bacterial communities associated with certain phytoplankton species (e.g. Schäfer et al. 2002, Sapp et al. 2007, Goecke et al. 2013, Buchan et al. 2014, Krohn-Molt et al. 2017, Mönnich et al. 2020). Connections between certain phytoplankton groups and a distinct bacterial community could be expected to be maintained by mechanisms such as chemotaxis or vertical transmission but the community analysis in paper III cannot be used to detect such interactions. A variety of mutualistic or algicidal interactions between bacteria and phytoplankton are known (Seymour et al. 2017) and such interactions could be assumed to have affected bacterial communities associated with phytoplankton also in this experiment. It has been proposed that diatoms promote beneficial bacteria in their close vicinity (Amin et al. 2012) and maybe such interactions shape also other phytoplankton-bacteria communities. Phytoplankton might affect bacterial community composition by producing certain amino acids (Tada et al. 2017) which may in part explain why the development of the bacterial community in the DOM consumption experiment was connected to peak T. Also possible antimicrobial activity by phytoplankton (Qin et al. 2013) or by other bacteria (Majzoub et al. 2019) may shape the bacterial community. Many of the signaling mechanisms that shape phytoplankton-bacteria communities have likely evolved to build a community with optimal nutrient use strategies for a given environment for all participants in the community and as such would select for broad selection of nutrient use strategies. Such functionally encompassing community has been found e.g. on Trichodesmium epibionts (Frischkorn et al. 2017). The minor differences in the cell specific BP between the species in the DOM release experiment despite the major differences in PP and DOC processing maybe suggests that the bacterial community is functionally optimized to grow using the DOC produced by the host phytoplankton. The minor changes in the bacterial community composition between the experiments may have been caused by the pronounced removal of particle attached bacteria (Grossart 2010) during the 0.8 μm filtration before the DOM consumption experiment. If there was a significant removal of particle attached bacteria at the start of the DOM consumption experiment the similarity of the bacterial community at the end of the DOM consumption experiment to the community in the DOM release

experiment would suggest that the majority of active bacteria in both experiments consists of free-living bacteria or bacteria with alternating free-living and particle attached lifestyles.

5.3.3. Ecological implications of species specific DOC dynamics

Baltic Sea spring blooms are shifting from traditional diatom dominance to dinoflagellate dominance (Klais et al. 2011) and the ecological consequences of this shift are currently being investigated (Spilling et al. 2018). Recent study connected a dinoflagellate community consisting of *A. malmogiense* and related species to lower BP and distinct bacterial community, compared to communities with common spring bloom diatom species (Camarena-Gómez et al. 2018). The results with *A. malmogiense* support their view that DOC released from some dinoflagellate species may lead to lower efficiency of the microbial loop.

When dinoflagellate blooms are not terminated in mass encystment they are expected to lyse in the water column and contribute to pelagic DOC pool (Spilling et al. 2018). The results of paper III indicate that blooms dominated by *A. malmogiense* indeed release high amounts of DOC but this DOC may not be readily bioavailable for bacteria coinciding with phytoplankton blooms and may, therefore, stay in the pelagic system for longer. High biomass production combined with release of less bioavailable DOC could lead to direct grazing being favored over microbial loop. In light of all this the probable long term effect of *A. malmogiense* dominance in natural communities on carbon cycling is the accumulation of less bioavailable DOC at the expense of sedimentation and microbial loop (Figure 12).

Compared to *A. malmogiense*, *R. marina* produces less phytoplankton biomass and the DOC it releases is more bioavailable. Thus, blooms dominated by R. marina may favor microbial loop and DOC processing over grazing. Since Alpha and Gammaproteobacteria, which were common in *R. marina* units, are heavily grazed by heterotrophic nanoflagellates (Alonso-Sáez et al. 2009) *R. marina* domination may increase carbon transfer through microbial loop. In addition, the higher BR in DOM release experiment may indicate that total carbon fixation is lower during such blooms. *Rhodomonas* species have not traditionally been connected to periods of high DOC release from phytoplankton (Storch & Saunders 1978) and, according to the results of paper III, this might be the result of fast bacterial consumption of DOC released by *Rhodomonas* species. The very fast consumption and transformation of DOC in *R. marina* units in the DOM consumption experiment support the assumption of fast DOC depletion in natural *R. marina* dominated blooms (Figure 12).

If the DOC release differences between *A. malmogiense* and *R. marina* are driven more by the difference in size and, therefore, passive leakage (Bjørnsen 1988), instead of only by species specific differences in physiology, similar trends could be assumed to be found in other same sized phytoplankton. While size is

not necessarily a good predictor for the proportion PP released as DOC (López-Sandoval et al. 2013), according to the results of paper III, it might be related to the quality of the released DOC. This provides a new perspective for the results of papers I and II. In paper I the focus was on small phytoplankton estimated to be of comparable size to *R. marina* or smaller. If also these small phytoplankton species release bioavailable DOC and attract copiotrophic bacterial communities which quickly deplete the available DOC, the lack of discernible contribution by these cells to DOC pool becomes easier to understand; because the bacteria respond so quickly when phytoplankton are removed (the DOM consumption experiment) it can be assumed that such a fast reaction occurs also during the mass lysis events of phytoplankton. Using the same analogy, if the larger cells investigated in the paper II produce less bioavailable DOC, it is understandable why there was a relationship (albeit weak) between %LC of these cells and DOC concentration despite the heavy bacterial processing at the estuary (Asmala et al. 2013).

However, strong extrapolations of these results to related phytoplankton species or to phytoplankton of similar size should not be made, as even much more closely related phytoplankton species may support differing bacterial communities (Grossart et al. 2005) and, consequently, different carbon cycling dynamics. Especially the direct analogy of large phytoplankton producing less bioavailable DOM cannot be taken for granted, as shown by e.g. the connection of diatoms to copiotrophic bacteria in the Baltic Sea (Camarena-Gómez et al. 2018). On the contrary, based on the results of all the studies included in this thesis, I want to highlight the importance of studying carbon cycling between individual phytoplankton species and related bacterial communities, even though mixed phytoplankton communities are the norm in natural environments. These community manipulation experiments should also include protozoan grazers as their impact on DOM composition and processing can be significant (Kujawinski et al. 2016). Grazing may, for example, alter bacterial community composition by removing groups which are less resistant to grazing (Alonso-Sáez et al. 2009), enhance DOM production (Strom et al. 1997) and affect the lability of the produced DOM (Fouilland et al. 2014), all of which can be assumed to affect cycling of DOM. Better knowledge on carbon cycling on species level will help in predicting how the large scale change in phytoplankton community composition will affect carbon cycling on ecosystem level.

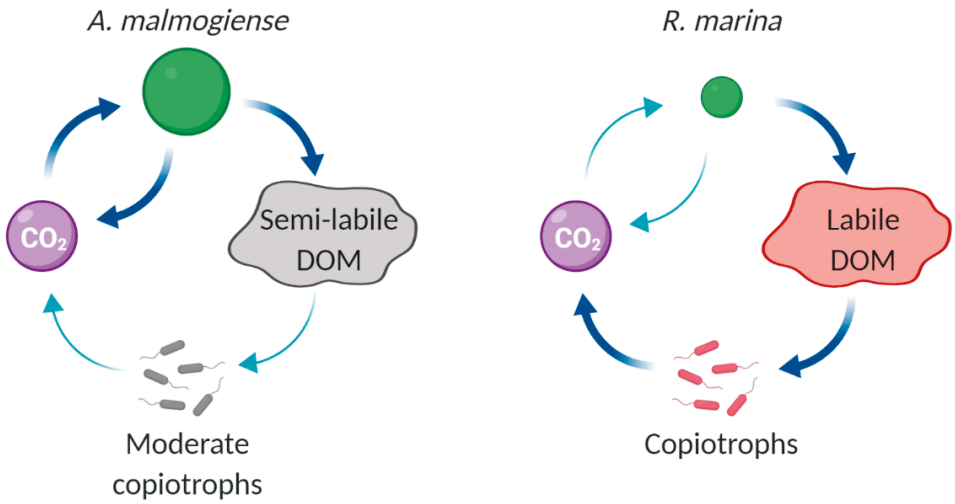

Figure 12. *A. malmogiense* and *R. marina* produce DOM of different bioavailability and attract distinctive bacterial communities specialized in utilizing that DOM. This results in different carbon cycling patterns. *A. malmogiense* cells circulate more carbon between DIC and phytoplankton biomass, while producing a pool of less labile DOC. *R. marina* cells release more labile DOC and relatively more carbon is thus directed towards the bacterial community which consume, incorporate, and respire it faster than DOC released by *A. malmogiense*. Figure created with BioRender.com.

6. CONCLUSIONS

Based on the observations of variation of %LC, the dying phytoplankton cells can be assumed to be present year around in every part of the Baltic Sea. It was demonstrated that %LC in natural phytoplankton communities in the Baltic Sea varied among different functional groups and was influenced by community composition and diversity. Dying cells were shown to contribute to spring bloom dynamics, which is the most important event of new biomass formation in the Baltic Sea, and where grazing and sinking traditionally have been regarded as the main loss factors. A higher resolution monitoring of %LC during spring bloom is called for in order to unravel the connection between %LC and the growth and demise of individual species composing the bloom community.

The main conclusions of this thesis are summarized as a conceptual model in figure 13. Abiotic factors that affect the %LC of phytoplankton communities in other more oligotrophic or oceanic environments may not be as clearly associated with phytoplankton %LC in the Baltic Sea. Also, factors affecting the growth and abundance of phytoplankton were not the same factors that affected the %LC. High %LC during periods of dominance by a single phytoplankton group and the negative correlation between diatom abundance and G2 %LC suggest interspecific competition as one potential determinant of phytoplankton %LC (Figure 13).

Based on the depth dependent variation in %LC in the open Baltic Sea, cell lysis below the photic zone but above the halocline may contribute to keeping carbon in the pelagic system, but more research is needed to confirm this. In the coastal zone %LC does not seem to influence carbon sedimentation.

Direct contribution of DOC release from dying phytoplankton to DOC concentration was found to be minor (Figure 13). This was likely influenced by high terrestrial inflow and ambient concentration of DOC. Direct incorporation of DOC into biomass of heterotrophic bacteria is assumed to be more relevant. This assumption was supported by the experimental part of this work, where rapid processing and incorporation of phytoplankton-derived DOC by associated pelagic heterotrophic bacteria were observed. The rapid changes in the DOC processing and in the bacterial community after a quick depletion of phytoplankton-derived DOC suggest that lysis-related phytoplankton loss pathways will lead to a rapid response in the bacterial community and an increased transfer of carbon to microbial loop.

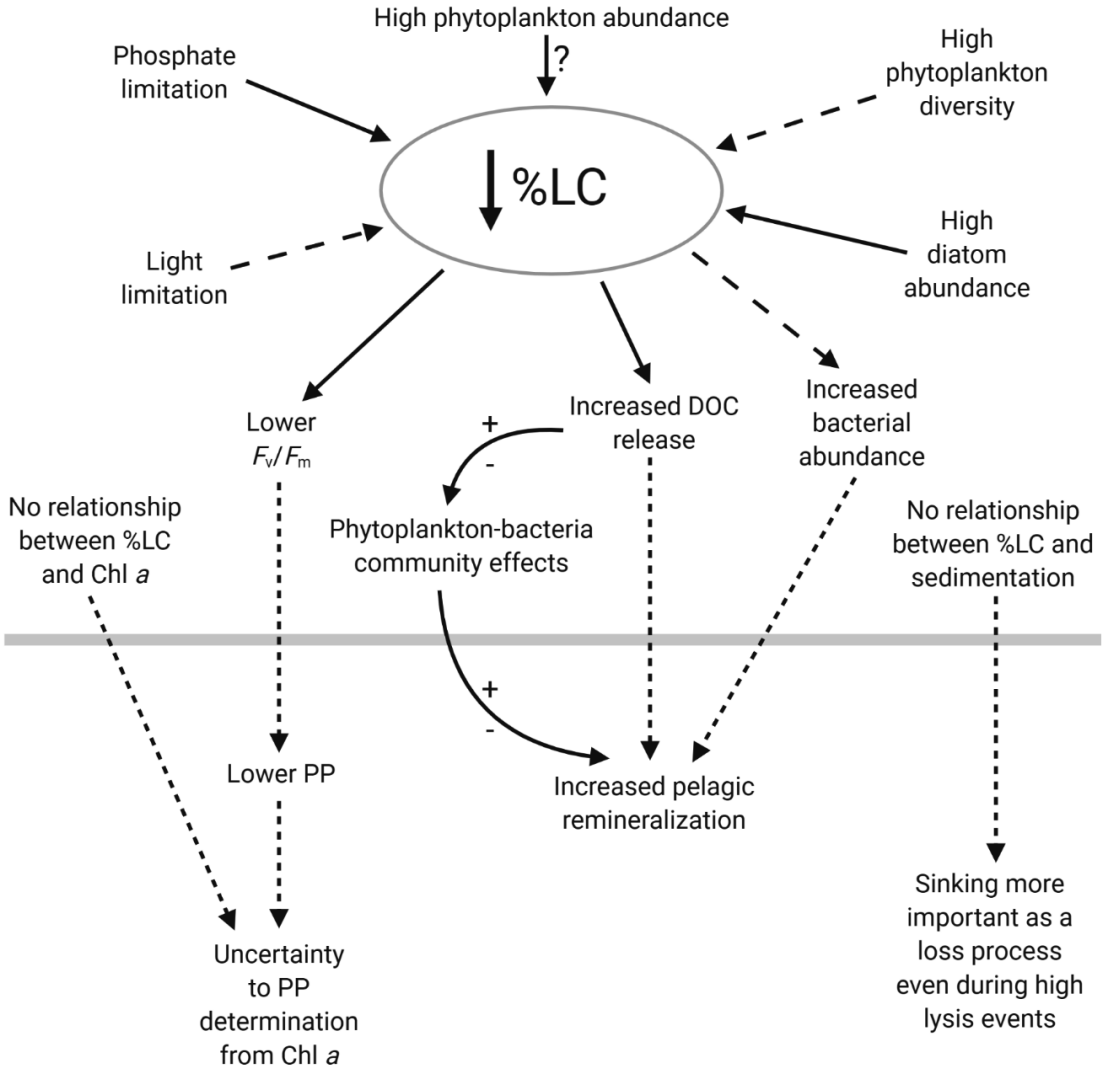

Figure 13. Simplified summary of the conclusions of this thesis; which conditions reduce %LC and what are the consequences of reduced %LC in the Baltic Sea. The mechanisms which are likely in light of the data are marked with arrows. Arrows with solid lines symbolize mechanisms for which the data provides some statistical support. Dashed line arrows with longer dashes symbolize mechanisms which the data supports only indirectly or sporadically. Dashed line arrows with shorter dashes symbolize mechanisms which are speculation based on the data. The thick grey line separates observed results (above) from speculated conclusions (below). The question mark symbolizes that the effect of phytoplankton abundance on %LC is not clear, as the relationships were contradictory between papers I and II. The arrows with the ± sign symbolize that the effect of bacteria on the remineralization of DOC depend on the properties of DOC released by the phytoplankton and may be enhanced or reduced based on the composition of the bacterial community. Figure created with BioRender.com.

The results support the other studies showing that the response of the bacteria to phytoplankton-derived DOC depends on the phytoplankton species producing that DOC in a predictable way. The phytoplankton-bacteria communities which are mediated by the DOC produced by the phytoplankton were found to be stable. From this it could be concluded that the consequences of phytoplankton lysis on carbon cycling will depend on the phytoplankton community composition and on the properties of the DOC they produce (Figure 13). In other words, it is not only necessary to know how the phytoplankton die (sensu Kirchman, (1999)), but also which phytoplankton die. This is especially relevant in the Baltic Sea because of the ongoing transition from diatom to dinoflagellate dominated spring blooms.

Because there was no relationship between Chl *a* and %LC and because there was sometimes a negative correlation with %LC and the abundance certain phytoplankton groups, caution should be exercised when phytoplankton productivity is estimated directly from Chl *a* fluorescence by, e.g., remote sensing (Figure 13). The results from this study clearly emphasize the importance of studying phytoplankton physiology at the single-cell level in addition to bulk measurements of abundance and productivity.

An important next step on the road to incorporating phytoplankton lysis in the models of carbon cycle would be to better describe how phytoplankton %LC influences PP and BP in natural systems.

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