The impact of climate change on phytoplankton – bacterioplankton interactions

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Doctoral Thesis

by

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SUMMARY

Global warming has already and is continuing to impact the global oceans and its inhabitants in various ways. Half of the global primary production is performed by phytoplankton in the oceans and about half of this marine primary production is utilised by heterotrophic bacteria. This way the heterotrophic marine bacteria channel a substantial amount of primary organic carbon through the microbial loop and hence represent an important part of the marine carbon and nutrient cycles. Understanding the influence of climate change on these important processes is therefore essential for an assessment of the vulnerability of the carbon cycle and possible feedbacks.

The presented work was conducted as part of the Kiel AQUASHIFT mesocosm cluster, which set out to investigate the impacts of climate change on the spring succession of plankton communities in moderately deep, well mixed water bodies such as the Kiel Bight. This thesis reports results from investigations on the temperature dependent coupling between phytoplankton and bacterioplankton, with respect to additional effects of light intensity and inorganic nutrient concentrations. During four consecutive years, mesocosm experiments with natural Kiel Fjord winter plankton communities investigated the influences of increasing water temperatures of up to ΔT +6°C and different light intensities between 16 and 100% of natural incident light. In an additional microcosm experiment with a single algal species and the natural bacterial community, a full factorial combination of three different temperatures and two inorganic nutrient concentrations was used, in order to evaluate the combined effects of both parameters on the algal-bacterial coupling. In all experiments the process of autotrophic carbon dioxide assimilation was assessed by primary production measurements. Heterotrophic bacterial organic carbon utilisation was measured by different parameters such as cell abundance, biomass production and respiration. The coupling of both processes was evaluated on the basis of timely overlap of the occurring peak development during the spring bloom succession, and by the ratios of heterotrophic to autotrophic quantities.

Summarising the results from all experiments it can be concluded, that increasing temperatures generally lead to an increased heterotrophic bacterial organic substrate utilisation relative to primary production through a combination of decreased time-lag between the two peaks and a stronger increase in the bacterial activity parameters. If a future warming trend would be accompanied by a further brightening, the supplemental promotion of primary production would increase the absolute amounts of cycled organic matter. Future increasing precipitation, leading to increased P-limitation in coastal waters would lead not only to an increased absolute amount of cycled carbon through increased primary production, but additionally to an increased relative amount of remineralised organic carbon through the microbial loop.

The results described in this work on changes in the relationship between autotrophic carbon fixation and its utilisation by heterotrophic bacteria under warmer, brighter and more P-limited marine environments demonstrate how the marine organic matter cycling could be substantially altered in a future climate. An increased organic matter transfer through the microbial loop has the potential to alter the whole structure and functioning of the marine food web and the biological sequestration of carbon to depth. In essence, an increase in the trophic levels facilitates a reduced transfer of energy and matter to higher trophic levels and, together with a generally increased respiration, leads to a substantial enhancement of CO_2 emissions and hence represents a positive feedback loop to the global climate change problem.

ZUSAMMENFASSUNG

Die globale Klimaerwärmung hat die Ozeane der Welt bereits in vielfältiger Weise beeinflusst und dieser Einfluss dauert an. Die Hälfte der globalen Primärproduktion findet in den Ozeanen statt und wiederum circa die Hälfte dieser Primärproduktion wird von heterotrophen Bakterien genutzt. Auf diese Weise schleusen die heterotrophen marinen Bakterien eine erhebliche Menge an primärproduziertem organischen Kohlenstoff durch die mikrobielle Schleife und repräsentieren daher einen wichtigen Teil der marinen Kohlenstoff- und Nährstoffkreisläufe. Es ist daher von enormer Wichtigkeit, den Einfluss des Klimawandels auf diese wichtigen Prozesse zu verstehen, um eine Einschätzung der Anfälligkeit des Kohlenstoffkreislaufes und mögliche Rückkopplungsmechanismen geben zu können.

Die hier vorgelegte Arbeit wurde als Teil des Kieler AQUASHIFT Mesokosmen Clusters durchgeführt, welches die Einflüsse des Klimawandels auf die Frühjahrssukzession des Planktons in mäßig tiefen, durchmischten Wasserkörpern, wie der Kieler Bucht, untersucht. Diese Dissertation legt die Ergebnisse von Untersuchungen über die temperaturabhängige Kopplung zwischen Phytoplankton und Bakterienplankton dar, unter zusätzlicher Berücksichtigung der Einflüsse unterschiedlicher Lichtintensitäten sowie verschiedener anorganischer Nährstoffkonzentrationen. In vier aufeinander folgenden Jahren wurden Mesokosmosexperimente mit natürlichen überwinterten Planktongemeinschaften aus der Kieler Förde durchgeführt und die Einflüsse einer Erwärmung um bis zu +6℃ und von Lichtverhältnissen zwischen 16 und 100% der natürlichen Lichteinstrahlung untersucht. In einem zusätzlichen Mikrokosmosexperiment, mit einer einzelnen Algenart und einer natürlichen Bakteriengemeinschaft, wurde in einer faktoriellen Kombination der kombinierte Einfluss von drei Temperaturen und zwei anorganischen Nährstoffkonzentrationen auf die Algen-Bakterien Kopplung beurteilt. In allen Experimenten wurde der Prozess der autotrophen Kohlenstoffassimilation als Primärproduktion gemessen. Die heterotrophe Kohlenstoffverwertung durch Bakterien wurde anhand verschiedener Parameter, wie der Bakterienabundanz,

Biomasseproduktion und Respiration bestimmt. Die Kopplung beider Prozesse wurde auf Basis der zeitlichen Überlappung der Peaks während der Entwicklung der Frühjahrsblüte beurteilt, sowie über die Verhältnisse von heterotrophen zu autotrophen Quantitäten.

Zusammenfassend für alle Experimente kann abgeleitet werden, dass steigende Temperaturen im Allgemeinen zu einer erhöhten heterotrophen bakteriellen Verwertung organischen Materials führte, relativ zur Primärproduktion, folgernd aus einer Kombination aus verringerter Zeitversetzung der beiden Peaks und einer relativ stärkeren Förderung bakterieller Aktivitätsparameter. Würde ein zukünftiger Trend zur Erwärmung von einem weiteren Anstieg der Lichtintensität begleitet, würde eine zusätzliche Förderung der Primärproduktion die absolute Menge an organischem Kohlenstoff im Kreislauf erhöhen. Eine erhöhte Niederschlagsmenge, wie vorhergesagt, könnte zu steigender P-limitation in Küstengewässern führen. Dadurch würde nicht nur, über erhöhte Primärproduktion, die beschriebene absolute Menge an Kohlenstoff im Kreislauf steigern, aber zusätzlich auch die relative Menge dieses organischen Materials erhöhen, die in der mikrobiellen Schleife remineralisiert würde.

Die in dieser Arbeit beschriebenen Ergebnisse zu den Veränderungen im Verhältnis zwischen autotropher Kohlenstofffixierung und heterotropher Verwertung unter wärmeren, helleren und stärker P-limitierten Verhältnissen in marinen Lebensräumen demonstriert, wie der marine organische Kohlenstoffkreislauf in einem zukünftigen Klima substantiell beeinflusst sein könnte. Ein verstärkter Fluss organischen Materials durch die mikrobielle Schleife hat das Potential die gesamte Struktur und Funktion des marinen Nahrungsnetzes zu verändern, ebenso wie die biologische Sequestration von Kohlenstoff in die Tiefen der Ozeane. Im Wesentlichen bewirkt eine Erhöhung der trophischen Ebenen einen reduzierten Transfer von Energie und Material zu höheren trophischen Ebenen und führt, zusammen mit einer generell verstärkten Respiration, zu einer erheblichen Ausweitung der CO_2 Emissionen und damit zu einer positiven Rückkopplung der globalen Klimawandelproblematik.

INTRODUCTION

The World Ocean comprises the bulk of the global hydrosphere and covers about 71% of the earth's surface. The oceans govern global water balance and climate, control the global nutrient matter cycles and contribute essentially to mankind's livelihood through their abiotic and biotic resources. Global warming has already impacted the global oceans and its inhabitants in various ways and is continuing to do so. The direct and indirect influences of warming on processes like the ocean's currents, stratification and nutrient supply are widely unknown and feedback loops complicate the situation. Concerning the biotic resources, about 50 % of the total global annual primary production is performed by phytoplankton in the oceans. The phytoplankton spring bloom is the major and most important biological event in the temperate climate zones, giving the essential seasonal pulse of primary production at the base of the whole marine food web. However, phytoplankton C-fixation is counteracted by degradation processes, as marine heterotrophic bacteria utilise up to 50% of this marine primary production, thereby channelling a substantial amount of organic carbon through the microbial loop (Azam et al. 1983, Hagström et al. 1988). This demonstrates impressively the relevance of marine heterotrophic bacteria for the marine carbon and nutrient cycles. Understanding the influence of climate change on these fundamental processes is therefore essential for the evaluation of the vulnerability of the carbon cycle and possible feedback reactions. The presented work is part of a large study on the impact of increasing temperatures and light variability on the Baltic Sea spring bloom succession. In this introduction I will therefore describe the framework for the presented studies, concerning global warming, the marine microbial world and interacting processes between carbon fixation and CO_2 recycling processes.

Global warming – background of the study

It is now widely accepted that human greenhouse gas emissions (mainly carbon dioxide, but also methane, nitrous oxide and halocarbons) due to fossil fuel burning, changes in land use and deforestation are responsible for the global

temperature increase the Earth is currently experiencing (IPCC 2007). The present CO_2 concentration of 380 ppm is the highest compared to the last 420,000 years as inferred from ice-cores (Petit et al. 1999). Also the speed of increase in greenhouse gases caused by human activities is faster than any time before, with a rise of 70% between 1970 and 2004 (IPCC 2007). Due to the response time of the Earth's climate, the observed warming is predicted to continue for centuries to come, even if emissions would be stabilised at the current levels (IPCC 2007). Hansen et al. (2006) report a rise in global mean temperature of approximately 0.8℃ during the last century, with a recent increase in the speed of warming to 0.2℃ per decade for the last 30 years. Furthermore, the global average temperature increase in the upper 3000 m of the oceans is estimated to be 0.037℃ for the years 1955 - 1998 (Levitus et al. 2005). The changes in the abiotic and biotic world as a consequence of global warming can already be observed manifold and there is worldwide effort in trying to predict future consequences to the natural world and ultimately human livelihood. In this sense, the presented work is part of this effort, assessing possible consequences for a future oceanic carbon cycle.

Abiotic changes

In any case, abiotic and biotic changes in the oceans will directly and indirectly influence the marine microbes and their interaction. For example, abiotic changes include thermal expansion of the oceans and progressive sea-ice melt, which lead to rising sea levels. Changes in atmospheric circulation (due to differing temperature changes over land and oceans) can have consequences for storm frequencies, precipitation patterns and increased upwelling events. High-latitude oceans usually exhibit a deep winter mixing, which represents a light limiting situation for phytoplankton that is only released through the thermal stratification occurring in spring. Global warming is expected to lead to increased stratification, which is likely to enhance primary production by increased light availability and by prolonging the growing season (Behrenfeld et al 2006). The expansive stratified low-latitude oceans on the other hand, are expected to show the opposite reaction, because increased thermal stratification and a deepening of the

thermocline prevent necessary nutrient input into the light-saturated euphotic layer, hence reducing primary production (Behrenfeld et al 2006, Harley et al. 2006). In this context, the results from the presented study on the different effects of light intensities and inorganic nutrient concentrations might reveal general response patterns of the planktonic microbial community, which are important and applicable to different oceanic regions and the oceans in general.

Increased CO_2 does not only act as greenhouse gas, but also leads to ocean acidification. About 50% of the increased CO_2 between 1800 and 1994 has already been taken up by the oceans (Sabine et al. 2004), leading to a decrease in ocean pH by 0.1 units (IPCC 2007). Estimates of future development suggest pH to drop for a further 0.3 to 0.5 units (0.14 – 0.35, IPCC 2007) until the end of the century, changing the saturation horizons of aragonite, calcite and other minerals essential to calcifying organisms (Feely et al. 2004).

As light is the primary energy source for primary production, possible future changes of solar radiation on Earth have to be considered in the context of global warming and its consequences. Changes in global solar radiation can have profound effects on surface temperature, the hydrological cycle and ecosystems via primary production. In the literature, two different periods of solar radiation variability are described, namely the “dimming” period before 1990 and the “brightening” period since 1985 (Wild et al. 2005, Norris & Wild 2007, Wild 2009). Global solar radiation decreases during the dimming period are estimated to have been 1.6 - 5.1 W m^{-2} per decade between 1960’s and 1990’s, values for Europe between 2.0 and 10.0 W m^{-2} per decade are reported (Wild et al. 2005, Wild 2009 and references therein). Global increases on the other hand, between 1980’s and 2000’s range between 2.2 and 6.6 W m^{-2} per decade, for Europe between 1.4 and 4.9 W m^{-2} per decade (Wild 2009 and references therein). Absolute solar radiation at Stockholm station, for example, was 112 – 119 W m^{-2} between 1980 and 2000. The recent increase is attributed to a decrease in aerosol burden due to more effective clean-air regulations and, for Europe, in the decline in economy connected to the political changes in the late 1980’s. The described trends in brightening are valid for all-sky as well as for clear-sky conditions, supporting the notion that cloud cover did not substantially contribute to the observed changes in

solar radiation (Wild et al. 2005, Wild 2009). It is suggested, that the dimming period might have balanced some of the global warming trends, while the brightening might actually contribute to increasing temperatures (Wild et al. 2005). Wild et al (2009) also showed how increased surface net radiation is quantitatively consistent with the observed substantial increase in land precipitation (3.5 mm y^{-1} between 1986 – 2000) and the associated intensification of the land-based hydrological cycle. As described, solar radiation variability is mainly dependent on anthropogenic air pollution and hence will future changes depend on future anthropogenic emissions. These in turn are mainly dependent on global socio-economic development and predictions are afflicted with great uncertainties. Possibilities include a future global dimming through increased emissions in Southeast Asia (Stier et al. 2006), no change over Europe due to stabilised aerosol levels since about 2000, or an increased global brightening due to globally effective air pollution regulations (Wild 2009 and references therein). Very recent measurements still show a brightening trend at the moment (A. Macke, personal communication). On the background of recent brightening events, light variability was considered as a variable in our experiments.

Biotic changes

Biotic changes in consequence of the described changes in the physical and chemical environment can be assessed on different levels and the IPCC (2007) states that there is *high confidence* that observed changes in marine and freshwater systems are associated with rising water temperatures and related physico-chemical parameters. Direct effects of changes in temperature are influencing individual's performance at various stages in their life cycle (Harley et al. 2006). On the population level climate change affects recruitment and dispersal, while on the community level abundances and species interactions are affected. In effect, climate change alters species distributions, biodiversity, productivity and microevolutionary processes (Harley et al. 2006). The IPCC report (2007) attributes shifts in ranges and changes in algal, plankton and fish abundance in high-latitude oceans, increases in algal and zooplankton abundance in high-latitude and high-altitude lakes and range changes and earlier fish

migrations in rivers with *high confidence* to rising water temperatures and related physico-chemical parameters.

Responses to temperature are different between and within species. Generally, the closer a species or an ontogenetic stage already lives to their physiological temperature limit, the more susceptible it is. Reef-building corals for example react very sensitively to increasing temperatures, which leads to coral bleaching and mortality (McWilliams et al. 2005). Temperature induced shifts in the timing of life-cycle events can lead to temporal mismatches with predators or prey. Temperature induced earlier spawning of *Macoma balthica* for example has led to a mismatch of larvae with their food, as phytoplankton did not show earlier blooms (Philippart et al. 2003). On the community level, the sea star *Pisaster ochraceus* could eliminate large sections of mussel beds through temperature induced increased abundance and increased consumption rate (Sanford 1999). The possible changes in the timing of events of the spring plankton succession, together with temperature induced shifts in species ranges and consequences for activity patterns are described in this study.

Predictions

The latest IPCC report (Intergovernmental Panel on climate change, 4th assessment report, 2007) makes predictions on future global warming based on different CO_2 emission scenarios. All of the different scenarios have in common, that CO_2 levels will either stabilise at current levels or continue to rise and consequently global warming will progress, reaching increases of between +1.1℃ (B1 scenario) and +6.4℃ (A1Fl scenario) until the end of this century (Figure 1). The applied temperature scenarios described in this study are based on these predictions and therefore include experimental warming of between ΔT +2℃ and ΔT +6℃ on top of the *in situ* baseline.

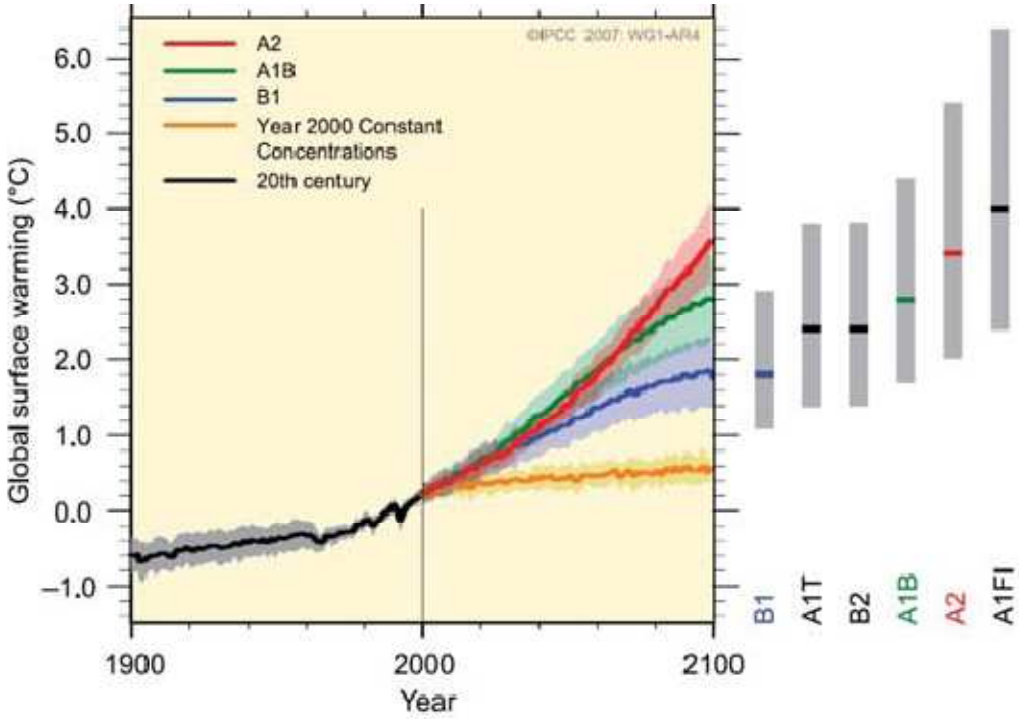


Figure 1. Atmosphere-Ocean General Circulation Model projections of surface warming. Solid lines are multi-model global averages of surface warming (relative to 1980-1999) for the SRES (Special Report on Emission Scenarios, 2000) scenarios A2, A1B and B1, shown as continuations of the 20th century simulations. The orange line is for the experiment where concentrations were held constant at year 2000 values. The bars to the right of the figure indicate the best estimate (solid line within each bar) and the likely range assessed for the six SRES marker scenarios at 2090-2099 relative to 1980-1999.

…in the Baltic Sea

Biotic and abiotic changes are also expected in the boreal seas like the Baltic, which is where this study is based. The Baltic Sea formed as a consequence of the retreating border of the ice-shield after the last glacial time, creating small Fjords like the Kiel Fjord, with relatively shallow water depths (Kiel Bight average depth of 17 m). The Kiel Bight is characterised by low salinity surface water influxes from Fehmarn Belt and high salinity bottom water, average salinity ranges between 14 and 24 psu.

While the global temperature increase during the last century was 0.4 – 0.8℃ (IPCC 2007), the rise was higher for the Baltic Sea Region with 0.85℃ (BALTEX 2007). The additional increase for this region can possibly be attributed to an enhanced solar radiation due to the decreasing air pollution over Europe. Also,

the Baltic Sea is a relatively small boarder-sea, which is surrounded by land masses which themselves are prone to increased warming compared to water masses. Also, as predicted in the IPCC report (2007), winter and spring temperatures have been shown to increase stronger, compared to the other seasons. The predictions for further increases until the end of the century range between 3 and 6℃ (BALTEX 2007) and 4 – 10℃ (IPCC 2007), again prognosticating increases to be relatively stronger during winter/spring compared to summer. Hence we can expect influences of increasing temperatures to be especially pronounced on the sensitive event of the spring bloom succession, which is assessed in the work at hand.

Variability in precipitation can influence inorganic nutrient availability for organisms in border-seas like the Baltic. Between 1900 and 2005 precipitation over Northern Europe has increased significantly (IPCC 2007). Regional projections foresee increased amounts of precipitation *very likely* in high-latitudes (~ 10-20% increase in 2090-2099 relative to 1980-99 period), continuing observed patterns in recent trends (IPCC 2007). Runoff is projected with *high confidence* to increase by 10 to 40% by mid-century at higher latitudes. This will have consequences for the nutrient input also for the Baltic Sea. Generally N (nitrogen in form of nitrate, nitrite and ammonium) is the limiting nutrient for phytoplankton primary production in most oceanic areas, as found in the Baltic Proper (Andersson et al. 1996). However, some coastal regions can also be P-limited (phosphorus in form of phosphate) as documented for example for the Finnish and Botnian Bay (Andersson et al 1996, Rivkin & Anderson 1997, Zweifel et al. 1993). Coastal regions are strongly influenced by riverine inflow and land-runoff. Rivers carry a high load of N through the extensive use of N-fertilisers plus atmospheric input (Jickells 1998). The P-load is mainly based on chemical weathering of rocks and aeolian dust deposition as well as detergents. In an effort to reduce nutrient loads of rivers it was possible to reduce P-fluxes significantly, but not so much for N, resulting in increased N:P ratios of river runoff reaching coastal areas (Jickells 1998). Hence in the light of future increasing precipitation and consequently increased high-N freshwater inflow, it can be expected that the

spread of P-limited border-sea regions will increase in the future. Therefore the possible consequences of changing inorganic nutrient availability are also considered as part of this study.

The microbial loop

The above described processes and developments impact substantially on the organisms at the base of the food web, which are the focus of this work. Primary production by phytoplankton is the process of assimilation of dissolved inorganic carbon (DIC) in form of CO_2 and build-up of particulate organic carbon (POC). This autotrophic process forms the basis for all life in the oceans and provides the organic matter for all further trophic levels. DIC represents the largest of the carbon pools in the oceans and the equilibrium between the water surface and the air is sustained by CO_2 diffusion. The model of the classical food chain describes the grazing of POC by zooplankton and further by higher trophic levels. The biological pump concept describes the transformation of POC aggregates into sinking particles, which are exported into depth, together with faecal material from zooplankton grazing (Longhurst 1991). This export process supplies all organisms in the aphotic zone with substrate. However, this classical food chain and export process is complemented by the so called “microbial loop” (Azam et al. 1983, Figure 2). This concept describes the direct utilisation of a fraction of the dissolved organic matter from primary production by heterotrophic bacteria (Sherr & Sherr 1988). The so formed particulate organic matter is subsequently re-entered into the classical food chain via grazing by heterotrophic nanoflagellates and/ or ciliates. Viruses influence the viability of all trophic levels. Considering that about 95% of total organic carbon is of the dissolved fraction (Wetzel 1984), the importance of this loop for marine organic matter cycling becomes evident. Actually up to 50% of primary production is channelled through the microbial loop (Longhurst 1991, Williams 2000). Dissolved organic matter becomes available to the heterotrophic bacteria via direct exudation by healthy growing cells (Björnsen 1988) and indirect processes like sloppy feeding by zooplankton, lysis by viruses and disintegration of dying cells in the late phase of a phytoplankton bloom (Nagata 2000, Chrost et al 1989, Middelboe et al 1995).

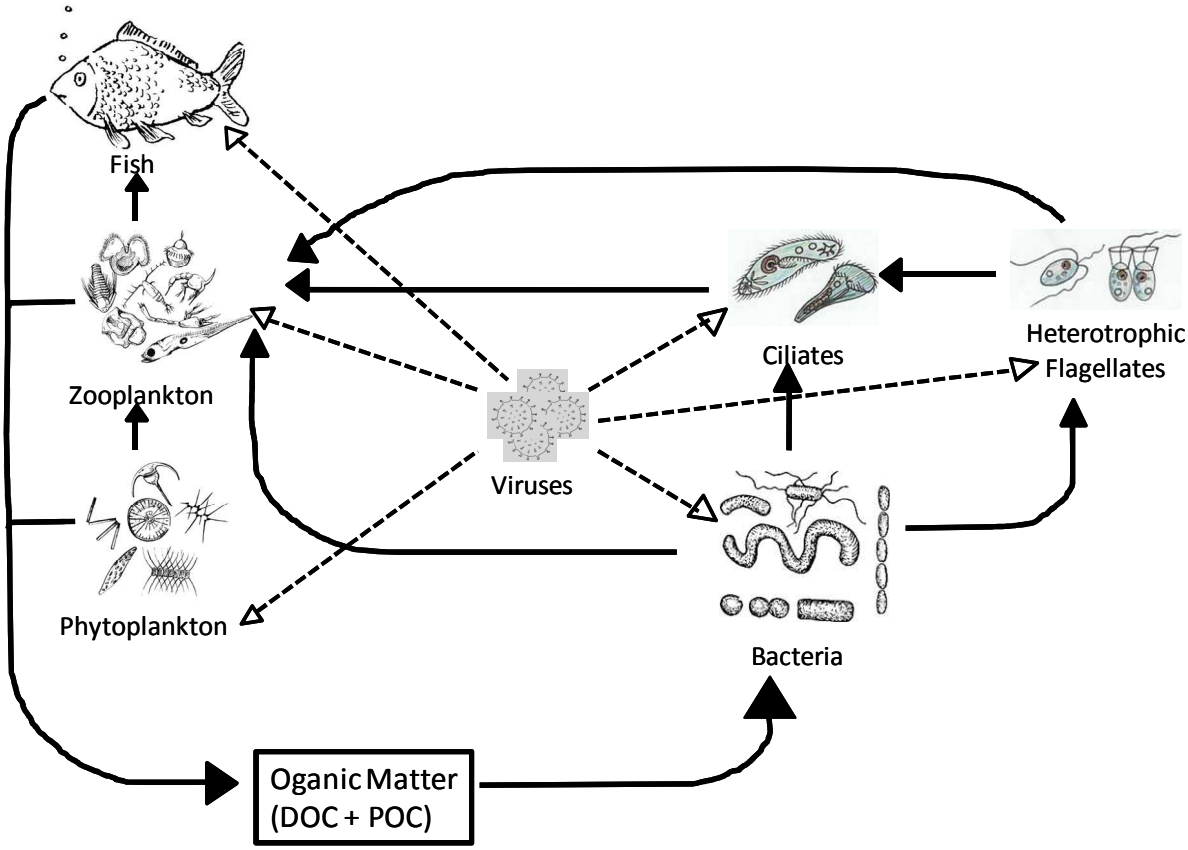


Figure 2. The marine microbial loop. Simplified illustration of the marine microbial loop. On the left hand side the classical food chain of phytoplankton, zooplankton and fish, which all contribute to the formation of the organic matter pool. This pool is utilised by bacteria, forming the basis of the microbial food web. Bacteria are grazed upon by ciliates and heterotrophic flagellates. Ciliates graze upon heterotrophic flagellates, while all groups are ingested by various members of the zooplankton, forming the link back to the classical food chain. Viruses operate on all members of the food chain. The simplified illustration does not account for further complexities within the food web, like for example auto- and mixotrophy of heterotrophic flagellates.

The exudation as percent extracellular release (PER) of dissolved components by living phytoplankton cells can have large ranges and depend on, for example, species specific differences, nutritional status of the cells and external influences like for example light intensity and temperature (Baines & Pace 1991). The authors summarised available data into an average PER of 13%. The importance of the microbial loop for the utilisation of organic matter is thought to be lower in highly productive areas with large spring blooms, where the classical food chain dominates and export is large due to a temporal decoupling of phytoplankton and

their grazers. The microbial loop is more important in low-latitude oligotrophic oceans where heterotrophic bacteria constitute the main consumers of DOM and hence dominate organic matter fluxes (Gasol et al. 1997). Additionally a second pathway via heterotrophic bacteria becomes obvious here, namely the utilisation of the particulate organic carbon pool. Through extracellular enzyme activity, particle-attached bacteria (and free-living bacteria) are able to utilise large organic molecules (Hoppe et al. 1988) and hence actually become the key mediators of particle solubilisation and decomposition and hence directly control the efficiency of the biological carbon pump (Cho & Azam 1988, Smith et al. 1992). Considering, that only 10-20% of the dissolved organic matter pool is directly utilisable monomers, the importance of extracellular enzyme activity also for the remaining 80-90% of DOC is highlighted. With all the organic material going into the microbial loop it has to be kept in mind however, that the efficiency of the organic matter transfer to higher trophic levels is also directly dependent on the bacterial growth efficiency. This parameter describes the amount of carbon that is assigned to bacterial secondary production relative to the total amount that is assimilated. The remains are simply respired and hence leave the system as inorganic CO_2, which contributes to the CO_2 pool in the water and consequently influences air-sea exchange of this important greenhouse gas. The BGE is typically < 30%, showing that most of the primary production that is channelled through the microbial loop is actually respired (Del Giorgio & Cole 1998, Bjornsen 1986, Reinthaler & Herndl 2005).

The overall efficiency of the microbial loop is dependent on a variety of abiotic and biotic factors, which directly or indirectly impact bacterial survival and performance. In coastal regions with high productivity top-down factors are more important, while in low productivity regions the influence of bottom-up factors is larger. Top-down factors include the grazing pressure by zooplankton (Wright & Coffin 1984) and the infection by viruses (Weinbauer & Höfle 1998). Bottom-up factors are for example the quantity and quality of the organic matter available from phytoplankton and the availability of additional inorganic nutrients (Kuparinen & Heinänen 1993). Several authors have demonstrated, that organic substrates like organic carbon and organic nitrate are limiting factors for bacteria (Sala et al

2002, Thingstad et al 2005, Kirchman 1990). Inorganic phosphorus and nitrogen have also been shown to stimulate bacterial growth (Zweifel et al. 1993) and heterotrophic bacteria can even outcompete phytoplankton for inorganic nutrients (Rhee 1972, Currie & Kalff 1984, Suttle et al 1990). In the Mediterranean Sea there is evidence that P limitation affects both primary production and bacterial uptake of dissolved organic carbon (Thingstad & Rassoulzadegan 1995) and Obernosterer & Herndl (1995) demonstrated that exudates released from P-limited algae could not be utilised by bacteria due to their own P-limitation for growth. On theoretical grounds it has been suggested that substrate concentration should not be limiting to heterotrophic bacteria in the upper mixed layer but Nedwell (1999) argued that heterotrophic bacteria in natural waters are often presented with sub-optimal concentrations of substrates (and limiting temperature extremes).

Based on the above described relationships within the marine microbial food web, we tried to assess the direct and indirect influences of abiotic factors like temperature, light intensity and inorganic nutrient concentrations on the phytoplankton and heterotrophic bacteria compartments. We did this by assessing activity and quantification parameters like PER and BGE as is described in detail in the respective Chapters of this work.

Bacteria and temperature

One of the most important abiotic factors influencing bacterial performance is temperature (Wiebe et al. 1993, Pomeroy & Deibel 1986), and in the light of current and future global warming an important factor to investigate. The positive correlation of bacterial metabolic processes like bacterial secondary production and respiration with temperature, in temperate waters, has been described manifold (e.g. Pomeroy & Wiebe 2001, Kirchman et al. 2005, Felip et al. 1996, Lopez-Urrutia et al. 2006). As a measure for the temperature dependence of different processes the Q_{10} value is generally used, which represents the increase in a rate for an increase in temperature by 10°C. Published Q_{10} values for heterotrophic bacterial processes are between 2 and 3 (Pomeroy & Wiebe 2001). However, the influence of increasing temperatures is not the same for bacterial

production compared to bacterial respiration. As Rivkin and Legendre (2001) reviewed, there is an inverse relationship between temperature and growth efficiency (BGE), demonstrating that the temperature effect is stronger on bacterial respiration, which increases more than bacterial production with increasing temperatures. Investigations from Chesapeake Bay (USA) support these results, showing a strong negative temperature response of bacterial growth efficiency due to significantly different temperature dependences of bacterial production and respiration (Apple et al 2006).

Vazquez-Dominguez et al. (2007) on the other hand, showed in the Mediterranean, that an increase in temperature by 2.5℃ did increase the bacterial carbon demand (bacterial production + respiration), but left the bacterial growth efficiency unchanged. The influence of temperature increase was hence the same for both parameters, but due to the generally low BGE (< 30 %, see above), according to the authors this would mean increased CO_2 emissions under future warming conditions. Reinthaler & Herndl (2005) report from the North Sea, that while bacterial production varied over 1 order of magnitude over the seasonal cycle, bacterial respiration varied only 2-fold, resulting in a higher mean BGE at increased temperatures in spring and summer. Jiménez-Mercado et al (2007) demonstrated in continuous cultures of marine bacterioplankton maximum BGE values at higher temperatures. Del Giorgio and Cole (1998) show in their review contrasting results of increased, decreased or unchanged BGE at increasing temperatures and argue that environmental factors such as substrate quality and quantity are more important in determining growth efficiencies.

Notwithstanding the described temperature-activity relationships with a focus on temperate areas, values of >10 are reported from arctic bacterial strains (Pomeroy & Deibel 1986), demonstrating that substantial bacterial activity is possible even at very low temperature. Rapid bacterial growth was found at temperatures below 2℃ in Antarctic waters (Fuhrman & Azam 1980) and several other studies in polar seas and sea ice communities revealed high bacterial activities, with normal Q_{10} factors, even at subzero temperatures (Li & Dickie 1987, Robinson & Williams 1993, Rivkin et al. 1996). Several authors described, how substrate supply could partly compensate temperature limitation at low water

temperatures in cold water bacterial strains and how algal production during arctic phytoplankton blooms met enhanced substrate requirements and hence overcame temperature limitation (Nedwell & Ruttner 1994, Pomeroy et al. 1991, Pomeroy & Wiebe 2001, Wiebe et al. 1992). Nedwell (1999) proposed that decreased membrane fluidity and efficiency of membrane transport proteins decreases the affinity of bacteria for substrates below their optimum growth temperature. These studies suggest, that temperature seems to be one important factor in the regulation of the structure of the bacterial assemblage, with bacteria with lower temperature optima forming communities at the respective temperatures. Global warming can therefore be expected to promote a shift from more cold-adapted species to a community of warmer-adapted species. Different species will display different enzymatic features and hence different organic matter decomposition properties can be expected (Martinez et al 1996). These prospects highlight that the temperature response of a bacterial assemblage cannot be fully assessed without knowledge on the community composition. Altogether it can be stated that the effect of temperature on bacteria is complex and cannot be generalised, which is why it is a special focus in the work at hand.

Coupling of phytoplankton and bacteria in the Baltic Sea spring bloom – now and in the future

As described above, the marine microbial loop is an important part of the marine carbon cycle and its relative importance is dependent on abiotic factors like temperature, light and nutrients. The so-called coupling between the heterotrophic bacteria and the substrate-delivering phytoplankton is interpreted in terms of the relative carbon flow between the two compartments in this study. Timing dependent overlap of peaks of phytoplankton and heterotrophic bacteria determine this coupling, as well as the direct influences of the investigated factors on the relative quantities of the peaks. As described above, heterotrophic bacteria show a strong response to temperature. Phytoplankton on the other hand is mainly controlled by light (and nutrient availability) and light limited photosynthesis is even temperature independent (Tilzer et al. 1986). While the Q_{10} values of heterotrophic processes are typically between 2 and 3 (see above), autotrophic

primary production Q_{10} displays values between 1 and 2 (Tilzer et al. 1986). Generally in aquatic systems the phytoplankton bloom can start as soon as light conditions are favourable (Sommer & Lengfellner 2008, Sommer 1996, Sommer et al. 1986). According to Sverdrup's critical mixing depth concept (Sverdrup 1953), the temperature stratification in deep waters in spring restricts phytoplankton to an upper water layer, where they receive on average enough light to trigger the phytoplankton bloom. This way the influences of temperature and light for the start of the phytoplankton bloom are coupled. In moderately deep water bodies like the Kiel Bight, phytoplankton is restricted to a shallow water depth anyway, so that increasing light levels in spring alone are responsible for the start of the phytoplankton bloom, independently of the temperature conditions. Consequently, the spring bloom in Kiel Fjord occurs at usually very low water temperatures (10 year average for early February is 2.4℃). At these low temperatures heterotrophic bacterial activity is still very restricted, representing a mismatch situation and leading to a decoupling of autotrophic carbon assimilation and heterotrophic organic carbon utilisation, leaving a large portion of the algal derived organic carbon unused (Pomeroy & Wiebe 2001 and references therein).
Future increasing winter/ spring temperatures (see above) can have profound effects on this decoupled situation. Bacterial growth is expected to increase earlier at higher water temperatures, decreasing the contemporary time lag to the peak of primary production. Also an increase in bacterial growth and respiration can be predicted. Together this will likely lead to an increasing amount of organic carbon being channelled through the microbial loop, consequently reducing sedimentation and export to depth (Hoppe et al. 2002, Kirchman et al. 1995, Legendre & Lefevre 1995).

The AQUASHIFT experimental model system

The above descriptions show the complexity of ecosystems, of the interactions on different levels of organisation and with the abiotic environment. We can deduce from this knowledge that it is of major importance to assess possible effects of future climate change on as many levels as possible. The ideal way of doing so would be the ability to experimentally manipulate only one factor, as for example

temperature, and keep all other factors as naturally as possible. Because the experimental temperature manipulation within oceanic areas is not feasible, we have switched to a more manageable system. Large (1500 L) mesocosms give us the advantage of maintaining the natural overwintering plankton population of the Kiel Fjord and at the same time conducting experiments under clearly defined conditions of interest, like temperature or light intensity. The disadvantage however, is the restricted transferability of results to the field, because of the artificial components in the experimental system and natural factors that just can't be mimicked in an experimental setup. Nevertheless, experiments are a vital tool in order to study and explain basic processes and connections.

As part of the DFG priority program AQUASHIFT, the Kiel cluster conducted mesocosm experiments in annual intervals since 2005. The Kiel AQUASHIFT mesocosm facility (IFM-GEOMAR, Kiel) consists of four climate chambers, which were each stocked with two to three 1500 L mesocosms. The mesocosms were 1 m in height and consisted of food safe polyethylene. A sophisticated lighting system provided natural day light considering quality and quantity, with day lengths and sunrise / sunset regulated according to outside real conditions. Temperature in the four different chambers could be regulated to within ± 0.5°C, but it has to be mentioned however, that due to an inherent variance in the cooling system the mesocosms in one chamber differed slightly in temperature from each other. One problem we encountered in some of the experiments was the substantial development of wall-growth after a certain experimental period, usually after the bloom development. A biofilm of benthic algae and its influence on nutrient cycling and bacterial utilisation was not quantifiable satisfactorily and hence experiments were either stopped at that time point or data was excluded from this time on for the presented work. On the other hand, due to the relatively low height of the mesocosms, sinking aggregated material from the collapse of the phytoplankton bloom sank to the bottom and was not out of the water column as it would be in nature. Hence it has to be kept in mind, that for example heterotrophic recycling processes by bacteria might have occurred and in turn have influenced processes in the water column, but again only at a very late time

point in the experiment. This work mainly focuses on the peak bloom period and hence this problem can be neglected for our analyses.

As described in detail in Sommer et al (2007), we can confidently say that the mesocosm experiments are a suitable tool for the assessment of climate change impacts on the spring succession in the Kiel Fjord. This way Kiel Fjord can serve as a model system for moderately deep water bodies as in the Baltic Sea, the North Sea and shallow lakes. The basic phytoplankton – bacterioplankton interactions can however also be transferred to situations of the restricted upper water layer in the open ocean.

Thesis outline

The presented work was conducted as part of the Kiel AQUASHIFT mesocosm cluster, which set out to investigate the impacts of climate change on the spring succession of plankton communities in moderately deep, well mixed water bodies such as the Kiel Bight. A series of five mesocosm experiments, and one additional bottle experiment, were conducted between 2005 and 2008 at the mesocosm facility and a collaboration of several working groups engaged on answering the question on different levels. The investigation presented in this thesis focussed on the phytoplankton – bacterioplankton coupling and how this is affected by increasing water temperatures. Additional factors investigated included the influence of different light intensities as well as inorganic nutrient levels. We assessed the quantity of autotrophic primary production and bacterial secondary production, bacterial abundance, as well as respiration. In order to assess the influence of the described factors on the relative importance of the microbial loop for the total carbon flow and hence CO_2 emissions and particle segmentation, a special focus was put on the relations of bacterial to autotrophic activities.

The **first chapter** (“Temperature dependence of the coupling between phyto- and bacterioplankton during early spring bloom conditions in the western Baltic Sea – a mesocosm study”) focuses on the results from one mesocosm experiment, which was conducted under relatively high light conditions. Four different temperature settings were applied and the development of a phytoplankton bloom succession was followed for 30 days. The light and temperature settings were

kept constant throughout the course of the experiment, which is different from the experiments described in chapter 3. Primary production as well as bacterial abundance, production and respiration were measured. The coupling between the autotrophic and heterotrophic compartments was assessed on a timing and on a quantitative level.

The **second chapter** ("The influence of temperature and light on phytoplankton-bacterioplankton interactions during the spring bloom – recurring patterns from four years of mesocosm experiments") summarises the recurring patterns of phytoplankton- heterotrophic bacteria interactions that were found in different mesocosm experiments. Four different experiments in four years were conducted, all encompassing the same temperature conditions, but differing in the light intensities that were applied. In all cases the light and temperature settings followed the natural development over the experimental period. A special focus is on the 2008 experiment, which represented a full factorial combination of two temperature and three light treatments.

Chapter three ("The combined effects of temperature and nutrients on the phytoplankton-bacterioplankton coupling") describes an additional bottle experiment, that was conducted in the summer of 2007. A full factorial setting of three temperature and two inorganic nutrient levels (constant settings) was set up in 25 L carboys, in order to assess the influence of different nutrient levels on the coupling between a typical spring bloom phytoplankton species and a natural bacterial community.

Finally the results of the thesis will be summarised, conclusions drawn and future implications of this work will be outlined.

CHAPTER 1

Temperature dependence of the coupling between phyto- and bacterioplankton during early spring bloom conditions in the western Baltic Sea - a mesocosm study -

Introduction

The relationship between autotrophic production and heterotrophic microbial degradation of organic matter is an important regulating factor of the marine carbon cycle. Heterotrophic microorganisms recycle up to 50% of organic matter (dissolved, DOM and particulate, POM) produced by phytoplankton via the microbial loop (Azam et al. 1983) through a combination of biomass production and respiration (CO_2 recycling) and form the basis of the heterotrophic food chain in the oceans (Azam 1998, Ducklow 1999).

The seasonal timing of events is a species specific response to environmental conditions like for example temperature or photoperiodicity. The right timing is vital to maximise synchronisation of predator and prey and species-specific shifts in phenology can result in so called mismatch situations with temporal asynchrony (Cushing 1972). These mismatch situations can consequently lead to a reduction in energy flow through the food web.

The spring bloom of phytoplankton in aquatic systems is initiated by favourable light conditions (Sommer & Lengfellner 2008, Sommer 1996, Sommer et al. 1986). In deeper water bodies these conditions are dependent on the thermal stratification (e.g. Lake Constance; Scheffer et al. 2001), which limits the algae to an upper water layer with an overall sufficient light dose ("critical mixing depth concept" sensu Sverdrup 1953) hence coupling the influence of light and temperature. In moderately deep water bodies like the Kiel Bight, the onset of the spring bloom is independent of thermal stratification and can hence occur at very low water temperatures as soon as the light conditions are favourable in late winter/early spring. And while the light limited photosynthesis is basically independent of temperature (Tilzer et al. 1986), the general temperature dependence of planktonic bacterial growth and activities is well documented (White et al. 1991, Hoch & Kirchman 1993, Shia & Ducklow 1994). Several authors have been able to show that bacterial production and respiration are highly temperature dependent (Felip et al. 1996, Pomeroy & Wiebe 2001, Kirchman et al. 2005). As it is known from published Q_{10} values, autotrophic processes such as primary production are less affected by temperature increases

(Q_{10} 1-2) than are heterotrophic processes (Q_{10} 2-3, Pomeroy & Wiebe 2001, Tilzer et al. 1986).

So due to the low temperatures at the spring bloom, as usually found in Kiel Bight, heterotrophic processes are thought to be initially low, leaving a large portion of the algal derived carbon unused (Pomeroy & Wiebe 2001 and references therein). The two processes of autotrophic carbon fixation and heterotrophic bacterial utilisation are mostly decoupled, representing a mismatch situation. Increasing winter water temperatures, as predicted by the IPCC (IPCC 2007), can be expected to increase heterotrophic processes relative to autotrophic processes, changing this mismatch situation. Hence, while the onset of the spring bloom is dependent on light, bacterial growth will increase earlier, when temperatures are favourable. An earlier bacterial production peak decreases the lag time with the peak of primary production, resulting in more organic matter being available for remineralisation before sinking out of the photic zone, reducing sedimentation (Hoppe et al. 2002, Kirchman et al. 1995, Legendre & Lefevre 1995). This effect is supposedly combined with a quantitative increase in bacterial production and respiration rates, again resulting in more utilisation of the available organic matter pool.

Additionally we know from previous studies, that the influence of increasing temperatures is not the same on bacterial respiration compared to bacterial production. Rivkin and Legendre (2001) reviewed the available literature and found a significant inverse relation between temperature and growth efficiency. Also, Apple et al. (2006) revealed significantly different temperature dependences of bacterial production and respiration in Chesapeake Bay (USA), which lead to a strong negative temperature response of bacterial growth efficiency. Hence an increased coupling of phyto- and bacterioplankton and the relatively higher respiration rates would represent a positive feedback loop to the greenhouse gas problem.

Combined with this is the effect of increasing temperatures on the rest of the food web. Micro-zooplankton directly profits from the warmer temperatures and consequently increases the grazing pressure on phytoplankton (Sommer &

Lengfellner 2008, Lengfellner 2008), contributing to a reduction in sedimenting organic matter. Keller et al. (1999), in a similar mesocosm experiment in Narraganset Bay, found a low standing stock of phytoplankton associated with high zooplankton abundance and low sedimentation, at warmer temperatures. Indirectly, zooplankton can profit from increased availability of bacteria through an increased carbon transfer through the microbial loop, via heterotrophic nanoflagellates and ciliates and back into the classical food chain.

In view of the predicted temperature increase we hypothesise that increasing temperatures will lead to an increased organic matter transfer via the microbial food web through a combination of two factors: (1) a shift in the timing of events, leading to a reduction in the time lag between autotrophic production and heterotrophic microbial degradation of organic matter, (2) an increase in the quantity of bacterial organic matter utilisation relative to its production. In order to test our hypotheses, temperature dependent changes in plankton communities were investigated by indoor mesocosms at between 2.5℃ (in situ) and 8.5 ℃. Water from Kiel Fjord was incubated under artificial light conditions to induce phytoplankton bloom development. The coupling of phytoplankton and heterotrophic bacteria was assessed under the aspects of timing of events (1) and quantity of carbon flow through the different compartments (2). This setup has been shown to be feasible for reproducing the typical pattern of in situ spring bloom succession and evaluating the influence of future water temperature changes (Sommer et al. 2007, Hoppe et al. 2008).

Material and Methods

Experimental setup

The experiment was performed between 6th January and 5th February 2006. Eight mesocosms were setup pair wise in four climate chambers. The *in situ* treatment was run at 2.5℃ which corresponds to the ten year mean (1993 – 2002) for the Kiel Fjord for this time of year. The other three climate chambers were adjusted to 4.5, 6.5 and 8.5℃ (for the realised temperatures s ee Table 1 and Fig. 1). The

mesocosms were allowed to adapt to the chosen temperatures for four days before the first sampling. Temperatures were kept constant throughout the experiment.

The mesocosms were synchronously filled with 1400 L of unfiltered Kiel Fjord water from 6 m depth outside the IFM-GEOMAR (salinity 18 psu), containing the over-wintering populations of phytoplankton, bacteria and protozoa. Mesozooplankton from net catches was added in natural over-wintering densities (~ 10 ind. L^{-1}). The water was gently stirred at all times, preventing light particles to sink down to the bottom, while at the same time allowing heavier particles to drop out of the water column. Due to the unusually low nitrate concentration of 8 µM, a further 13 µM of nitrate was added at the beginning of the experiment in order to achieve similar nutrient conditions of 21 µM compared to a previous experiment in 2005 (Sommer et al. 2007, Hoppe et al. 2008) and in order to ensure bloom development.

Water samples were taken daily or every other day by siphoning approximately ten litres of water through a silicone tube from the middle of each mesocosm directly into 20 L pre-washed carboys. Withdrawn water was not replaced in order to prevent nutrient pulses and the addition of organisms. Subsamples for the determination of the different parameters were taken from the carboys after gentle mixing. Only for the determination of respiration rates, water was taken directly from the mesocosms in order to prevent mixing and stirring influence on oxygen content of the samples.

Light was provided by fluorescent tubes (a mixture of JBL Solar Tropic and JBL Solar Natur) from the top of the mesocosms. The daily light cycle followed a triangular curve between 6 am and 6 pm, with the maximum light intensity at 12 noon, hence providing a constant 12:12 hour light:dark cycle throughout the experiment. The integrated daily light intensity was calculated to be 29 kWh m^{-2}, which is comparable to a cloudless day at the beginning of April (according to the model of Brock 1981 for Kiel).

Particulate and dissolved primary production

Particulate primary production measurements were performed using ^{14}C bicarbonate incubations following the methods of Gargas (1975) and Steeman Nielsen (1952). For each mesocosm three aliquots of 30 ml each were incubated with 100 µl of a 4 µCi / 100 µl ^{14}C-bicarbonate solution. The blank treatment was kept dark during incubation. Incubation took place at approximately half depth inside the respective mesocosm, ensuring a mean light exposure and in situ temperature conditions. After 4-5 hours of incubation, aliquots of 10 ml were filtered onto 0.2 µm cellulose nitrate filters. The filtrate was collected for measurement of dissolved primary production. The filters were subsequently fumed with 37 % HCl fumes in a closed box for 5-10 min and then measured in 4 ml of Scintillation cocktail (Lumagel Plus) using a Packard Tricarb counter.

The filtrate received 100 µl of 1 N HCl and was stored in an exsiccator under vacuum for 8 days. For collecting the expelled CO_2 the exsiccator contained 1 N NaOH. Preliminary experiments had shown that this treatment guaranteed maximum outgassing of remaining inorganic ^{14}C from the samples. After this storage time 10 ml of Scintillation cocktail (Aquasol) was added and the radioactivity of the samples counted.

Particulate and dissolved primary production were calculated for the 12 hour light day by considering the amount of light received during the incubation period relative to the total daily light quantity. The two variables are presented as µg C L^{-1} d^{-1}.

The original CO_2 concentration of the water sample was determined according to the method and dissociation constants described in Stumm & Morgan (1981).

Bacterial production

Bacterial protein production

Bacterial protein production measurements were conducted following the protocol of Simon & Azam (1989). Four aliquots (3 replicates and one blank) of 10 ml of water were each incubated with 50 µl of a 1 µCi / 10 µl ^{3}H-leucine solution (specific activity: 160 µCi $nmol^{-1}$) plus 50 µl of a 2 nmol / 100 µl unlabeled leucine solution. This resulted in a total concentration of 103 nmol L^{-1} of leucine in the

sample, which is known to be saturating under the conditions found in the Kiel Fjord (Giesenhagen, unpublished data).

All samples were incubated in the respective climate chambers at in situ temperature in the dark for 1.5 - 3 hours. Incubation was terminated by the addition of formaldehyde (1 % v / v) and 5 ml aliquots were separately filtered onto 3.0 µm (particle-attached bacteria) and 0.2 µm (total bacteria) polycarbonate filters. The filters were subsequently rinsed with ice cold 5 % TCA (trichloro acetic acid) solution, before being radio-assayed in 4 ml of scintillation cocktail (Lumagel Plus). Results in terms of pM h^{-1} bacterial protein production were transferred into µg C L^{-1} d^{-1} biomass production using a theoretical conversion factor of 3.091 x 10^{-3} kg C mol^{-1} leucine (Simon & Azam 1989).

Bacterial Cell Production

Incorporation of ^{3}H-methyl-thymidine for the determination of bacterial cell production was done slightly modified after Fuhrman & Azam (1982). For each sample, three replicates and one blank (treated with 1 % v / v formaldehyde) of 10 mL of water were each incubated with 50 µL of a 1 µCi / 10 µL ^{3}H-methyl-thymidine solution (specific activity: 63 µCi $nmol^{-1}$), resulting in a final and saturating concentration of 8 nmol L^{-1}.

Samples were treated as described for ^{3}H-leucine above (including fractionated filtration onto 3 µm and 0.2 µm filters). Results in terms of pM h^{-1} bacterial production were transferred into µg C L^{-1} h^{-1} biomass production using a self-determined empirical conversion factor of 30.87 kg C mol^{-1} thymidine.

The conversion factor for ^{3}H-methyl-thymidine incorporation was determined by adding 400 ml of unfiltered water to 1600 ml of 0.2 µm filtered water. The determination was performed between day 9 and 15 of the experiment, for one of the coldest (2.5 ℃) and one of the warmest (6.5 ℃) mesocosms. Samples for bacterial abundance and bacterial production measurements were taken at 8 – 24 h intervals (depending on the development of the bacterial abundance) and treated as described above for the respective parameters. The calculations are based on assuming a mean cell volume of 0.045 $µm^3$ (average cell volume in the actual experiment was 0.032 $µm^3$, but because it was not determined separately

for the conditions during the conversion factor experiments we decided to stick to the literature values, also for comparability) and cell carbon calculation of cell carbon (fg C $cell^{-1}$) = 218 x $V^{0.86}$ (Loferer-Krößbacher et al. 1998), resulting in 15.14 fg C $cell^{-1}$.

Respiration

Respiration was determined using Winkler Titration (Winkler 1888) with automated photometrical endpoint detection. For each mesocosm six 100 ml glass bottles were filled with unfiltered water for determination of total community respiration, another six bottles were filled with 3 µm pre-filtered water (always < 200 mbar) for determination of respiration assigned mainly to bacteria. Total community respiration (unfiltered water) incorporates dark phytoplankton respiration, respiration by zooplankton and total bacteria. Bacterial respiration represents free-living bacteria and bacteria attached to particles <3µm but does exclude bacteria attached to particles >3µm. Three flasks of each set were immediately fixed and the other three replicates were incubated for 48 h at in situ temperature in the climate chambers, in the dark and submersed in water. Respiration in terms of O_2 uptake (mg L^{-1} h^{-1}) was multiplied by a recommended factor of 0.32 (based on RQ of 0.85, Ogura 1972) to calculate C-utilisation for respiration in terms of mg C L^{-1} d^{-1}.

Total bacterial number

For determination of bacterial abundance (cells ml^{-1}) aliquots of 100 ml of water were fixed with formaldehyde to a final concentration of 2 % (vol / vol) and stored at 4°C until filtration. Filtration of 6 ml aliquots onto black 0.2 µm polycarbonate filters was performed within 7 days of fixation. Cells were stained using DAPI (4´-6-diamino-2-phenylindole) to a final concentration of 100 µg ml^{-1} and frozen at -20°C until being counted under an epifluorescence microscope (Axioskop2mote plus, Zeiss, Germany). At 1000x magnification, using a NewPorton G12 Grid, 20 grids or at least 400 cells were counted.

Data analysis and statistics

The timing of the peaks in relation to temperature was computed from the regression between the days when these peaks occurred and the temperatures of the respective mesocosms. The slopes of the linear regressions between the day of peak and the temperature correspond to the acceleration that the respective parameter experiences in days per each 1℃ warming. The slopes were compared using ANCOVA.

In order to examine the relationship between temperature and the quantities of the measured parameters during the algal bloom, we quantified each individual peak (for bacterial abundance and bacterial production this meant focussing on the first peak). Quantification was achieved by calculating the area formed by three measuring points: one before the peak, one after and the peak itself – covering a time period of seven days around the peak. For dissolved primary production the measuring point of the peak and one after were chosen, to ensure continuity for all mesocosms. Each calculated area value was plotted against its respective temperature and linear regression lines fitted through the data using SigmaPlot. Increases in percent for a temperature increase of 6 ℃ was calculated by using first 2.5 ℃ (in situ = 100%) and then 8.5 ℃ in th e linear equations.

The total amount of carbon required by bacteria for growth and respiration (bacterial carbon demand, BCD) was calculated by adding bacterial production and bacterial respiration (BCD = BP + BR). Because BP incorporates all bacteria while BR does not take into account the respiration of bacteria attached to particles >3 µm, the relative amount of particle-attached bacteria was calculated from the BP >3µm measurement and added to the BR measurements accordingly (“corrected BR”, only for BCD and BGE). Also, in cases where the peaks of BP and BR were not at the same time, the BP peak was chosen as the shared time period. Due to the two described calculation methods for BCD, the integrated BCD peak value can be different to the sum of the individual BR and BP values. In order to assess the relative amount (percentage) of carbon being used by bacteria for growth in relation to the total carbon demand, the bacterial growth efficiency (BGE) was calculated by dividing bacterial production by BCD and multiplying by 100 (BGE = BP/ BCD).

The ratios were calculated from the areas described above, thus comparing each individual peak, excluding the effect of different peak timings. For ratios including dissolved primary production, the area was adjusted to the shorter peak length of this parameter.

Linear regressions were performed using SigmaPlot software (Systat Software Inc., USA), statistical analyses was performed using Statistica data analysis software (StatSoft Inc., USA).

Results

Physico-chemical parameters

Temperatures were fairly constant during the course of the experiment, except for some slightly stronger fluctuations in the coldest treatment (Figure 1).

Table 1. Temperature treatments and realised temperatures during the experiment in the eight mesocosms.

Mesocosm No.	Treatment (℃)	Realised Temperature (℃, mean ± sd)
1	2.5	2.1 ± 0.2
2		2.4 ± 0.3
3	4.5	4.1 ± 0.2
4		4.8 ± 0.2
5	6.5	5.9 ± 0.1
6		6.5 ± 0.2
7	8.5	7.0 ± 0.2
8		8.0 ± 0.2

Deviations between the temperatures of the two replicate mesocosms were due to the temperature regulating system emitting cold air in the front of the large room and resulting in slightly warmer conditions for the mesocosm, which was situated

in the back of the room. Therefore, for all statistical analyses the realised temperatures for each mesocosm were taken (Table 1).

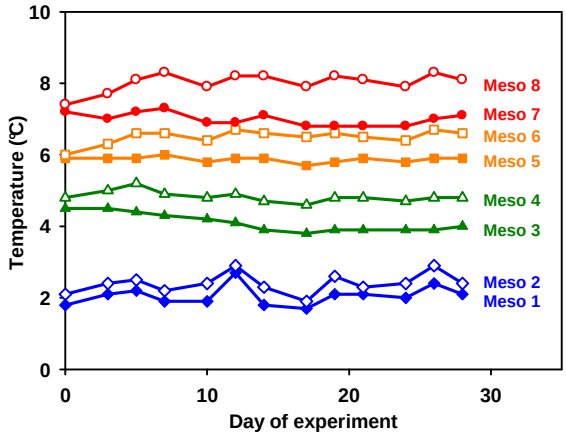


Figure 1. Time series of temperatures in the eight mesocosms during the course of the experiment.

Nutrient concentrations at the start of the experiment were as follows: phosphate 0.9 µM, nitrate 21 µM, ammonium 5.6 µM, silicate 20 µM. None of the nutrients was the single limiting factor at one point, as N, P and Si always fell below detection limit on the same days for each mesocosm. These were day 17-18 for mesocosm 1, day 13-17 for mesocosm 2 & 3, day 11-12 for mesocosm 4, day 10 for mesocosm 5 & 6, day 11-12 for mesocosm 7 and day 9-10 for mesocosm 8 (Wohlers et al. 2009).

Time courses and quantities

Autotrophic and heterotrophic parameters

The time courses of the particulate primary production (PPP) data show the development of a phytoplankton bloom in all 8 mesocosms (Fig. 2, A). The bloom was mainly composed of the diatom *Skeletonema costatum* in all mesocosms (U. Sommer, personal communication). Peak values ranged from 475 (mesocosm 7, 6.5 °C treatment) to 776 µg C L^{-1} d^{-1} (mesocosm 1, 2.5 °C treatment), with no apparent temperature effect. Peaks were reached earlier in warmer treatments compared to the colder ones: mesocosms 7 and 8 (8.5 °C) reached their peak value on day 17, mesocosms 5 and 6 (6.5 °C) on days 14 and 11 respectively, while mesocosms 3 and 4 (4.5°C) peaked on day 11 and the two warmest mesocosms (1 and 2, 2.5 °C) on day 13 and 10.

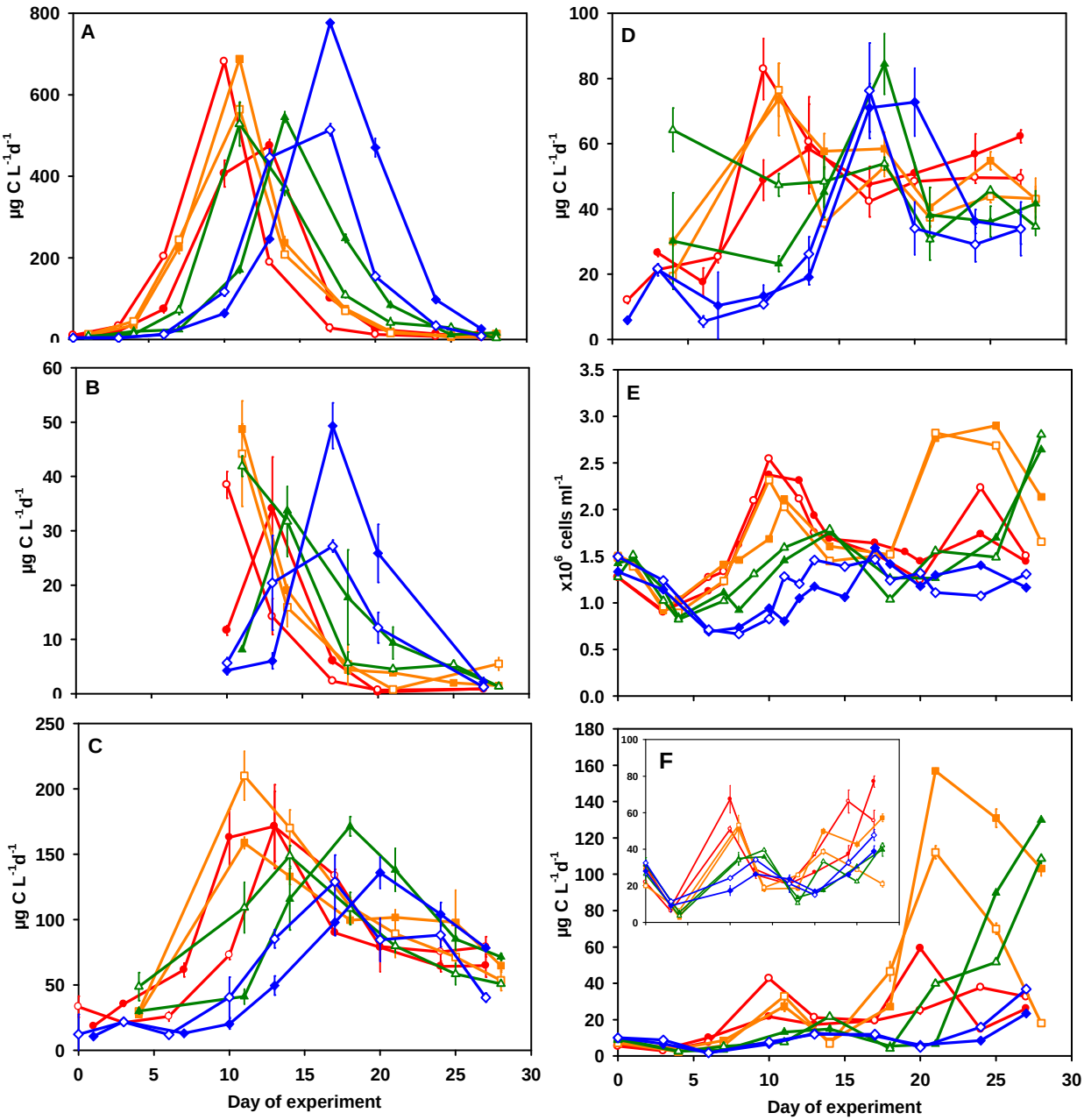


Figure 2. Development of particulate primary production (A) dissolved primary production (B), community respiration (C), bacterial respiration (D), total bacterial numbers (E) and bacterial production from ^{3}H-thymidine (F) and ^{3}H-leucine (insert, F) over the course of the experiment. Same colours represent the two replicate mesocosms run at the same temperature: blue: in situ temperature (2.5℃), gre en: 4.5℃, orange: 6.5℃, red: 8.5℃. Bars represent ±1 SD.

Measurement of dissolved primary production (DPP) started on day 10, and from the development we can see, that it followed the dynamics of PPP closely (Fig. 2, B). Because the peaks for mesocosms 1, 2, 3 and 7 show the same timing as

PPP, we can confidently assume that the remaining mesocosms also peaked at the same respective days. DPP maxima ranged from 27.2 (mesocosm 2) to 49.3 µg C L^{-1} d^{-1} (mesocosm 1) and the peaks showed a highly significant positive relationship with PPP (linear regression of peak period, R^2=0.9, p<0.0001, data not shown). The percent extracellular release (PER = DPP / DPP+PPP) ranged between 2.4 and 7.9 % during the bloom (days 10-18) and increased up to 28.4% (mesocosm 6) during the degradation phase of the bloom (overall average 8.7%, data not shown). There was no effect of temperature apparent on PER (repeated measures ANOVA: F=3.5, p=0.13, data was marginally not normally distributed).

The total community respiration (CR) development was closely associated with the primary production peaks (Fig. 2, C), concerning the timing. Here, higher maximal values were observed in the warmer treatments compared to the colder ones, ranging between 129 µg C L^{-1} d^{-1} (mesocosm 2) and 210 µg C L^{-1} d^{-1} (mesocosm 6). Community respiration levels were still elevated at between 40.4 and 79.3 µg C L^{-1} d^{-1} towards the end of the experiment. The decrease was higher in the community respiration compared to bacterial respiration, where in some cases the values were actually rising again when the experiment was terminated.

Bacterial respiration (BR) increased in the two warmer treatments, forming a peak on day 11 (mesocosms 5 and 6) and on days 13 and 10 (mesocosms 7 and 8, respectively, Fig. 2, D). The development was slower in the two colder treatments (mesocosms 1 - 4), showing peaks on days 20, 17, 18 and 18 for the respective mesocosms. The height of the peaks did not seem to be influenced by the temperature treatments. Peak values ranged from 53.9 (mesocosm 4) to 84.4 µg C L^{-1} d^{-1} (mesocosm 3) and remained on a relatively high level during the degradation phase of the bloom at 33.9 – 62.3 µg C L^{-1} d^{-1}, with higher values in the two warmer treatments compared to the two colder ones. The average contribution of bacterial to community respiration was 58%. This contribution was higher before and after the peak of community respiration (41 - 100%), while being lower during the maximum of community respiration (21 - 72%). At the respiration peak the contribution of bacterial to community respiration was highest in the coldest treatments (53 and 59% at 2.5°C, compared to 35% at 8.5°C).

Total bacterial numbers (TBN) showed an initial decline, before increasing towards a first peak, which coincided with the phytoplankton bloom (PPP). Peak values were reached on day 17 for the coldest treatments, on day 14 for mesocosms 3 & 4, day 11 and 10 for mesocosms 5 & 6 and on day 10 for the warmest treatments (Fig.2, E). Higher peak numbers of bacteria were counted in the warmer treatments compared to the colder treatments (maximum 2.54 x 10^6 cells ml^{-1} at 8.5 ℃, 1.46 x 10^6 cells ml^{-1} at 2.5℃ for the first peak). During the degradation phase of the phytoplankton bloom bacterial numbers increased again, forming a second peak in the two warmer treatments, the same development was indicated but not completed for the two colder treatments within the time frame of the experiment. Peak values of the second peak were higher compared to the first peak for mesocosms 3 – 6.

The development of bacterial secondary production (BP) was very synchronous for both methods and showed a first peak on day 13 and 14 for the two colder treatments and on day 11 and 10 for the two warmer treatments for both ^{3}H-thymidine (Fig.2, F) and ^{3}H-leucine (Fig.2, F insert) incorporation methods. Maximum values of 12.0 – 42.6 µg C L^{-1} d^{-1} (mesocosms 2 and 8) for ^{3}H-thymidine incorporation and between 26.3 and 67.3 µg C $L^{-1}d^{-1}$ (mesocosms 1 and 8) for ^{3}H-leucine incorporation were calculated, and the higher values were always reached in the warmer treatments. In both cases the development of a distinct second peak was detected in the two warmer treatments, while the development was not completed in the two colder treatments by the end of the experiment. For ^{3}H-leucine incorporation similar quantities were reached by the end of the experiment, compared to the first peak. Results from the ^{3}H-thymidine incorporation method showed much higher values for the second peak for mesocosms 5 and 6 (157 and 112 µg C $L^{-1}d^{-1}$) as well as mesocosms 3 and 4. The contribution of particle-attached bacteria to total bacterial production (% 3.0 µm in 0.2 µm, data not shown) increased from values between 1.4 and 11.1 % during the peak to values between 15 and 39 % during the degradation phase (^{3}H-thymidine).

There was a highly significant linear relationship between the results of both methods (linear regression, R^2=0.21, p=0.0001) and repeated measures ANOVA

confirmed that there was no significant difference between the two methods (F=2.43, p=0.14). The ratio of ^{3}H-leucine to ^{3}H-thymidine (based on pM raw data) was on average 19 and showed a general tendency to decrease during the course of the experiment (data not shown). Starting values ranged between 28-46 (mean: 34) and end values ranged between 3 and 29 (mean: 12), with no peak developments. Ratios in the colder treatments were significantly larger than ratios in the warmer treatments for most of the course of the experiment (repeated measures ANOVA, F=7.04, p=0.045).

As we determined a conversion factor for the ^{3}H-thymidine incorporation method for the actual experiment, but have to rely on literature values for the ^{3}H-leucine incorporation method, all further calculations related to the carbon flow in the experiment are based on the results of the ^{3}H-thymidine incorporation method only (if not stated otherwise).

Bacterial carbon demand and growth efficiency

Concerning the performance of the microbial community under increased temperature conditions, two derived values are of special interest, the bacterial carbon demand (BCD) and the bacterial growth efficiency (BGE). BCD is a combination of bacterial respiration and production measurements and hence reflects a combination of both parameters (Fig. 3, A). A first peak was observed with maxima on day 10 and 13 for the warmest mesocosms, day 11 for the 4.5℃ treatment, day 18 for mesocosms 6 and 7 respectively and on days 17 and 20 for the coldest treatments (significant peak acceleration with temperature, R^2=0.76, p=0.004). Peak values ranged between 78 and 127 µg C $L^{-1}d^{-1}$ and showed no response to temperature (linear regression, R^2=0.01 , p=0.84). As BR shows about double the quantities during the first peak compared to BP, the BCD time course reflects mostly the development of BR. A second peak was observed in the two warmer treatments, the same development was indicated but not completed in the two colder treatments. The peak values of the second peak were higher for the 4.5 and 6.5℃ treatments. The time course of the second peak was mainly determined by the quantity of BP because BR was on relatively low levels during the degradation phase of the bloom (see Fig. 2).

First peaks of BGE occurred between days 6 and 14, with no temperature influence (linear regression, R^2=0.21, p=0.25) (Fig. 3, B). Maximal values between 27 and 41% were not significantly related to temperature (linear regression, R^2=0.32, p=0.14). Values increased towards a second peak, which displayed large values compared to the first peak in all treatments, especially in the 4.5 and 6.5℃ treatments. As described for BCD above, BP was much higher than BR during the degradation phase of the bloom, leading to the very high BGE values in this phase of the experiment.

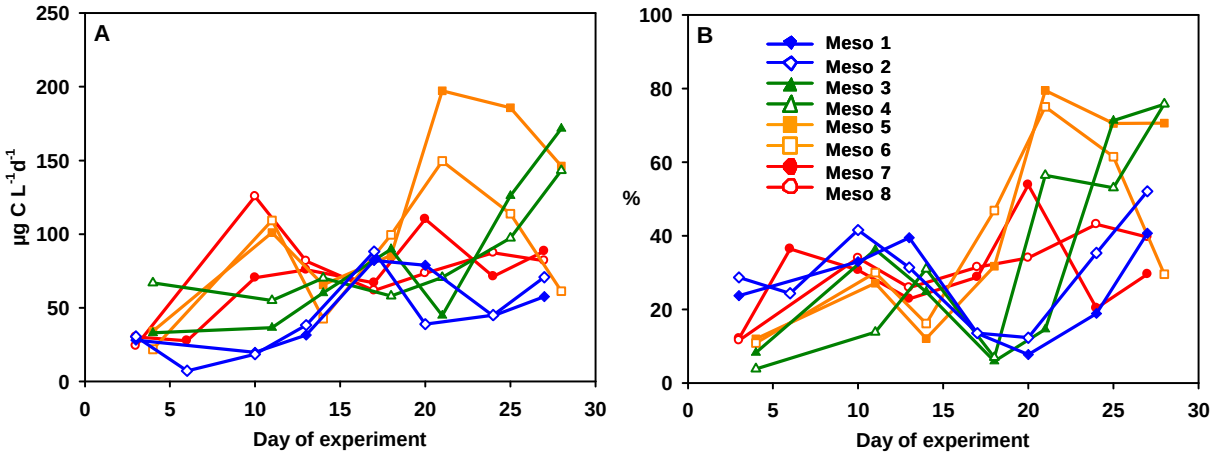


Figure 3. Dynamics of bacterial carbon demand (A) and bacterial growth efficiency (B) during the course of the experiment. Same colours represent the two replicate mesocosms run at the same temperature: blue: in situ temperature (2.5℃), green: 4.5℃, orange: 6.5℃, red: 8.5℃.

Peak timings

In order to assess the influence of increased temperatures on the timing of events we focused on the first peak of each parameter. The development of a second peak in bacterial abundance and bacterial secondary production could only give an indication of the influence on the further development but was not sufficient for a quantitative assessment because the experiment was terminated before the peak was reached in all temperature treatments. When plotting the day of each first peak against its respective temperature, the slope of the regression line indicates the acceleration of the development of each parameter. The negative

slope value equals the number of days the peak moves forward in time for one degree Celsius of warming.

All of the parameters showed a significant acceleration of development at elevated temperatures (Figure 4). The influence of increasing temperatures was similar for all the parameters (ANCOVA: comparison of slopes, F=1.9, p=0.14), accelerating the peak between – 1.13 and –1.60 d per ℃ (Table 2), with the acceleration for bacterial production being somewhat smaller, only reaching –0.62 d per ℃.

Table 2. Linear regression between each parameter's peak and its respective temperature, for particulate primary production, total bacterial number, community and bacterial respiration, and bacterial production (for ^{3}H-thymidine and ^{3}H-leucine alike, see text). The negative slope of the linear regression line (see Figure 3) corresponds to the acceleration of the parameter's peak (day of peak, DOP) in days for each 1℃ increase in temperature (DOP_{+1}), n=8.

Parameter	Equation	R^2	p
Particulate primary production	DOP_{+1} = -**1.13** DOP + 18.77	0.76	0.004*
Total bacterial number	DOP_{+1} = -**1.38** DOP + 19.89	0.94	<0.0001*
Community respiration	DOP_{+1} = -**1.29** DOP + 21.21	0.69	0.01*
Bacterial respiration	DOP_{+1} = -**1.60** DOP + 22.89	0.76	0.004*
Bacterial production	DOP_{+1} = -**0.62** DOP + 15.17	0.62	0.02*

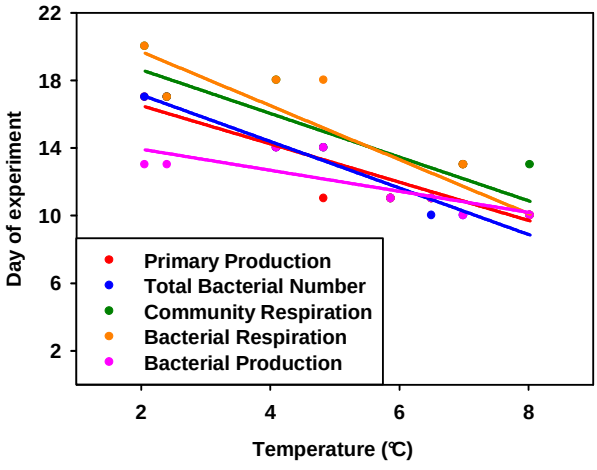


Figure 4. Linear regression between each parameter's peak and its respective temperature, for particulate primary production, total bacterial number, community and bacterial respiration, and bacterial production (for ^{3}H-thymidine and ^{3}H-leucine alike, see text). For equations see Table 2, all relationships were significant at the p<0.05 level

Temperature effects on integrated quantity of autotrophic and heterotrophic parameters

The areas defined by each parameter's peak show the quantitative responses of the determined parameters to increasing temperatures. Particulate primary production showed a trend towards decreasing values with increasing temperatures, while dissolved primary production quantity was only weakly affected by temperature (+19% and +10% decrease respectively from 2.5℃ to 8.5℃, with the value at 2.5℃ taken as 100%, Fig. 5, A). All other parameters (community and bacterial respiration, bacterial abundance, bacterial production) increased with increasing temperatures (Fig. 5, B, C). This increase was statistically significant for bacterial abundance (+46 %) and bacterial production (+148 % for ^{3}H-thymidine and +73 % for ^{3}H-leucine incorporation, Table 3), while it was only trend for community respiration (+32 %) and very weak for bacterial respiration (+3 %).

Specific bacterial respiration (bacterial respiration divided by bacterial numbers) was little affected by temperature change (-8 %, Table 3). Specific bacterial production (bulk production divided by total bacterial numbers) showed a significant increase with increasing temperatures (+53 %) for ^{3}H-thymidine incorporation and an insignificant decrease by –15 % for ^{3}H-leucine incorporation (Table 3).

The BCD increased significantly by 68 % (^{3}H-thymidine) and 75 % (^{3}H-leucine), the BGE increased by 43 % for ^{3}H-thymidine incorporation and was unaffected (-2 %) for ^{3}H-leucine incorporation (Table 3). The bacterial growth efficiency displayed values between 19 and 27 % and between 34.4 and 33.7 %, respectively.

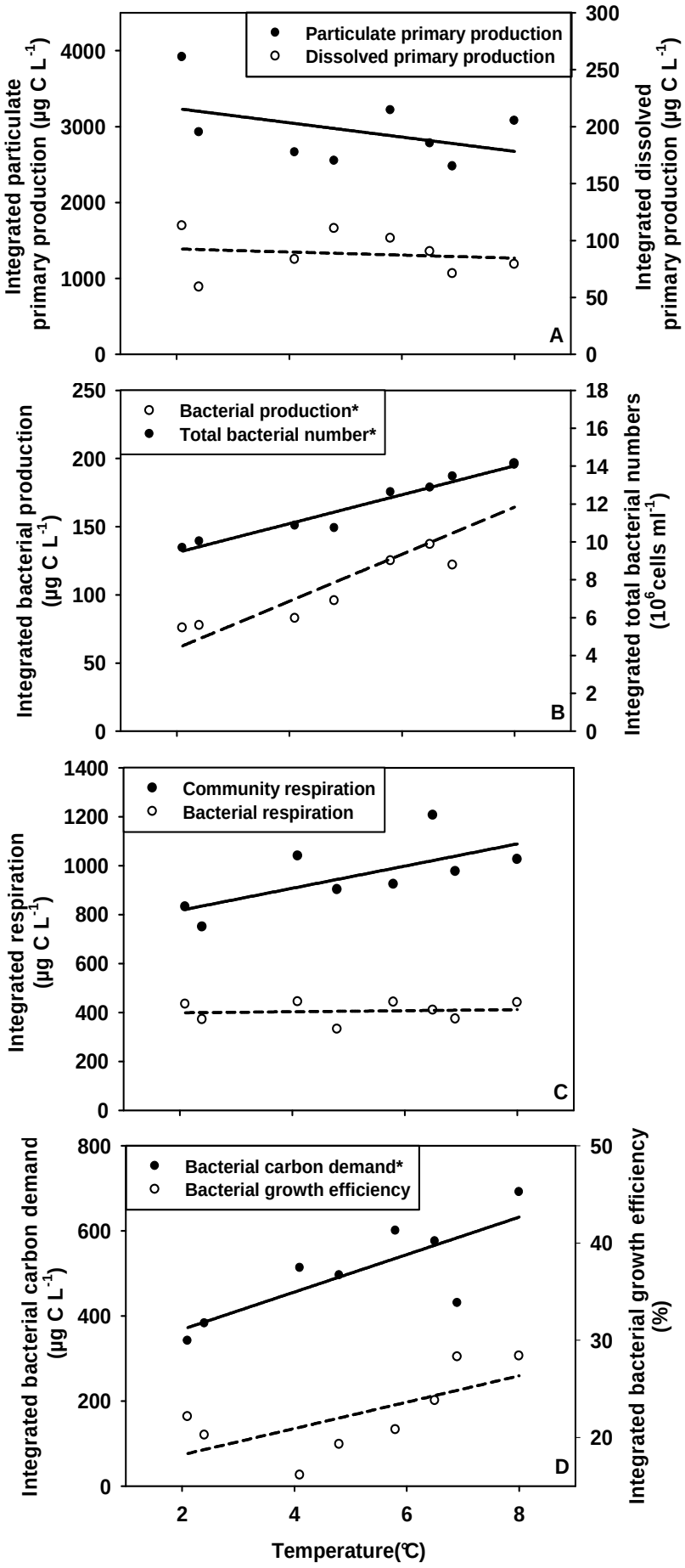

Particulate primary production
Dissolved primary production
Integrated particulate primary production (µg C L-1)
Integrated dissolved primary production (µg C L-1)
A
Bacterial production*
Total bacterial number*
Integrated bacterial production (µg C L-1)
Integrated total bacterial numbers (10^6cells ml-1)
B
Community respiration
Bacterial respiration
Integrated respiration (µg C L-1)
C
Bacterial carbon demand*
Bacterial growth efficiency
Integrated bacterial carbon demand (µg C L-1)
Integrated bacterial growth efficiency (%)
D
Temperature(℃)

Figure 5. Relationships between temperature and particulate – and dissolved primary production (PPP & DPP) (A), bacterial production and total bacterial number (BP & TBN) (B), community – and bacterial respiration (CR & BR) (C) and bacterial carbon demand (BCD) and bacterial growth efficiency (BGE) (D) integrated over the first peak period (for description of calculations refer to material and methods section). Significant relationships ($p < 0.05$) are indicated by asterisks, fitted lines represent the linear regression, for equations see Table 3.

Table 3. Relationships between temperature and particulate – and dissolved primary production, bacterial production and specific bacterial production, total bacterial number, community – and bacterial respiration and specific bacterial respiration, bacterial carbon demand and bacterial growth efficiency integrated over the first peak period (for description of calculations refer to material and methods section), n=8.

Parameter	Equation	R^2	p
Particulate primary production	PPP = -94.17 T + 3227.12	0.18	0.29
Dissolved primary production	DPP = -1.35 T + 95.22	0.02	0.72
Bacterial production (^{3}H-thymidine)	BP = 17.24 T + 26.39	0.81	0.002*
Bacterial production (^{3}H-leucine)	BP = 22.22 T + 126.73	0.69	0.01*
Specific bacterial production (^{3}H-thymidine)	BP_s = 4.61 T + 40.26	0.59	0.02*
Specific bacterial production (^{3}H-leucine)	BP_s = -3.85 T + 160.05	0.20	0.26
Total bacterial numbers	TBN = 0.76 T + 7.91	0.95	<0.0001*
Community respiration	CR = 45.37 T + 725.86	0.47	0.06
Bacterial respiration	BR = 2.10 T + 394.52	0.01	0.8
Specific bacterial respiration	BR_s = -8.09 T + 649.39	0.03	0.66
Bacterial carbon demand (^{3}H-thymidine)	BCD = 44.03 T + 280.2	0.64	0.02*
Bacterial carbon demand (^{3}H-leucine)	BCD = 66.70 T + 369.94	0.87	0.0008*
Bacterial growth efficiency (^{3}H-thymidine)	BGE = 1.35 T + 15.51	0.45	0.07
Bacterial growth efficiency (^{3}H-leucine)	BGE = -0.12 T + 34.70	0.002	0.91

Ratios

For a further assessment of the influence of temperature on the relative carbon flow between phytoplankton and bacteria during the bloom period, ratios of

bacterial production (BP) and bacterial carbon demand (BCD) to primary production (particulate and dissolved, PPP and DPP) were calculated from the individual area values used in Fig. 5. The ratios were then plotted against the respective temperatures (Fig. 6). A significant increase of the BP to PPP and BCD to PPP ratios with temperature was found (Table 4). The BP : PPP ratio for ^{3}H-thymidine incorporation increased from 2.2 to 6.1% (from 2.5 to 8.5℃, +177% with the value at 2.5℃ taken as 100%) (Fig. 6) and from 6.0 to 11.7 % for ^{3}H-leucine incorporation (+95%, Table 4), while the BCD : PPP ratio increased from 12.9 to 23.5 % (+82%) and from 17.7 to 34.0 % (+92%), respectively, albeit these ratios are rather low. Compared to that, the ratios resulting from dividing BP and BCD by DPP are much higher. Here we see an increase from 48.3 to 93.2 % for ^{3}H-thymidine incorporation (+93%, Fig. 6) and from 121.1 to 147.4 % for ^{3}H-leucine incorporation in the BP : DPP ratio (+22%, Table 4). The BCD : DPP ratio decreased from 348.7 to 324.6 % (-7%) and from 445.4 to 439.4 % (-1.3%), respectively. All ratios with DPP only show a trend, as they are not statistically significant (Table 4).

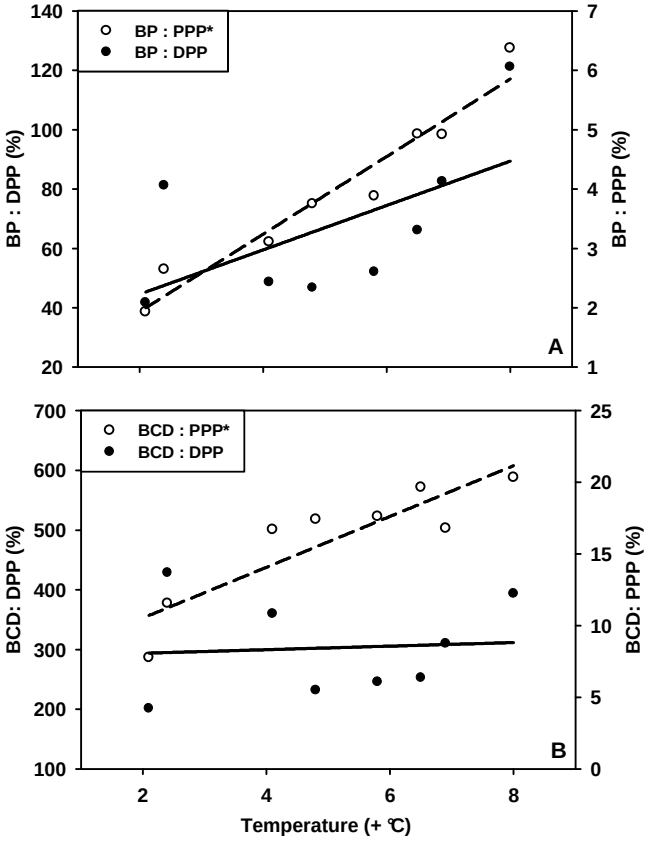


Figure 6. Relationships between temperature and the ratios (in %) of bacterial production (^{3}H-thymidine) to particulate and dissolved primary production (BP : PPP, BP : DPP) (A) and of bacterial carbon demand (^{3}H-thymidine) to particulate and dissolved primary production (BCD : PPP, BCD : DPP) (B) during the bloom period. Significant relationships ($p < 0.05$) are indicated by asterisks, fitted lines represent the linear regression, for equations see Table 4.

Table 4. Relationships between temperature and the ratios (in %) of bacterial production to particulate and dissolved primary production and of bacterial carbon demand to particulate and dissolved primary production during the bloom period, n=8.

Parameter	Equation	R^2	p
Community respiration : particulate primary production	CR:PPP = 2.31 T + 21.56	0.41	0.08
Bacterial production (^{3}H-thymidine): particulate primary production	BP:PPP = 0.65 T + 0.62	0.94	p<0.0001*
Bacterial production (^{3}H-leucine): particulate primary production	BP:PPP = 0.95 T + 3.58	0.53	0.04*
Bacterial production (^{3}H-thymidine): dissolved primary production	BP:DPP = 7.48 T + 29.59	0.35	0.12
Bacterial production (^{3}H-leucine): dissolved primary production	BP:DPP = 4.38 T + 110.20	0.04	0.61
Bacterial carbon demand (^{3}H-thymidine): particulate primary production	BCD:PPP = 1.76 T + 8.53	0.70	0.01*
Bacterial carbon demand (^{3}H-leucine): particulate primary production	BCD:PPP = 2.71 T + 10.94	0.79	0.003*
Bacterial carbon demand (^{3}H-thymidine): dissolved primary production	BCD:DPP = -4.01 T + 358.7	0.01	0.83
Bacterial carbon demand (^{3}H-leucine): dissolved primary production	BCD:DPP = -1.0 T + 447.91	0.0004	0.96

Discussion

The presented study has shown that the advanced mesocosm setup is able to reproduce a typical spring succession pattern with a phytoplankton bloom accompanied by bacterial degradation of organic matter, demonstrating that

mesocosm experiments of this type can be used to assess biological phenomena associated with future climate change (Sommer et al. 2007, Hoppe et al. 2008). Our mesocosm approach with water from the Kiel Bight served as a model system for moderately deep water bodies, where the spring bloom can start before the onset of thermal stratification and hence the influence of temperature and light are decoupled. In this respect our results are directly transferable to moderately deep water bodies in the temperate and boreal climate zone. Nevertheless, even in the open ocean of the temperate and high latitude regions, where high nutrient levels after winter sustain the typical spring blooms, autotrophic and heterotrophic processes are to some extent decoupled due to the different temperature responses of the two compartments. The interplay of the direct temperature effects, as demonstrated here, with indirect effects via increased surface layer stratification is expected to have a strong impact especially in these regions (Wohlers et al. 2009). Still, as described by Sommer & Lengfellner (2008), our experiment can only mimic the typical spring bloom of temperate and boreal waters, where the primary trigger for the phytoplankton spring bloom is the release from physical controls (light, temperature, stratification).

Time courses and quantities

There was no significant influence of temperature on the composition and the quantity of the phytoplankton bloom development, which was similarly dominated by the diatom *Skeletonema costatum* in all mesocosms. The exudation of dissolved organic carbon by the growing phytoplankton followed the bloom development closely. Baines and Pace (1991) estimated PER to be on average 13% of total fixation, Maranon et al. (2004) measured an average PER of 19 % in a coastal system off Spain and values between 7 and 20 %, in a comparison of different oceanic regions, were reported by Moran et al. (2002). Our results for the bloom period are at the lower end of these values (2.4 – 7.9 %), but increase to higher values during the degradation phase of the bloom, when PPP is low. Due to methodological reasons, DOC production might have been underestimated. Concerning the determination of dissolved primary production it has to be kept in mind that the measurement cannot take into account the amount of labelled

photosynthate that is taken up by bacteria during the incubation period. Banes and Pace (1991) reviewed the available literature and calculated that on average about half of the radioactivity released during ^{14}C incubations is found in the bacteria. On the other hand one has to consider that probably not the entire DOC released is readily available for bacteria to utilise and that a fraction will remain unused due to its refractory nature.

The timing of the first peaks of all bacterial parameters (TBN, BP, BR) coincided with that of primary production (PPP and DPP) which indicates the direct utilisation of dissolved organic matter (DOM) from phytoplankton. Authors like Cole et al. (1988), White et al. (1991) and Gasol & Duarte (2000) have proposed that the covariation between biomass and activity of phytoplankton and bacterioplankton is based on the direct bacterial use of algae-produced labile dissolved organic carbon (DOC). The second peak in bacterial numbers and production, which developed during the degradation phase of the bloom, indicates increased utilisation of particulate organic material (POM) of dying phytoplankton cells, together with DOM, which is released through sloppy feeding by zooplankton and disintegration of dying cells. Pomeroy & Wiebe (2001) proposed viral lysis, nutrient deficiency lysis and the excretion, defecation and sloppy feeding by micro-zooplankton and protists as additional connections for organic matter transfer from auto- to heterotrophs. In 1988, Cho and Azam demonstrated that bacteria, rather than the particle-feeding zooplankton are the principal mediators of organic particle decomposition in the mesopelagial. The switch from utilisation of mainly dissolved to more particulate organic carbon is supported by the results of bacterial production in the >3 µm fraction (particle-attached bacteria), which increased during this degradation phase of the bloom. Becquevort et al (1998) and Middelboe et al (1995) demonstrated the relevance of particle-attached bacteria in the collapse of phytoplankton blooms through the degradation of particulate organic material via the use of extracellular enzymes (Hoppe et al. 1993). During this phase the bacterial respiration was still at elevated levels, but the dramatic increase in bacterial production lead to rather high values of bacterial carbon demand and consequently also of bacterial growth efficiency.

Although both incorporation methods for the determination of bacterial production can be expressed in carbon equivalents, they differ in the growth processes they assess. Because ^{3}H-thymidine is incorporated into DNA, it is used as a proxy for DNA replication and consequently cell division (Fuhrman & Azam 1982). The incorporation of ^{3}H-leucine into proteins, on the other hand, can be used as an indication for cell growth through the build-up of protein and hence cell biomass (Simon & Azam 1989). The second peak of bacterial production from ^{3}H-thymidine incorporation shows much larger values than the first peak for most of the mesocosms, indicating the increasing importance of cell division during the degradation phase of the bloom. The increase in ^{3}H-thymidine bacterial production for the second peak is directly reflected in the bacterial numbers (see especially the two medium temperature treatments), which supports the proxy value in terms of cell division. The rate of ^{3}H-leucine bacterial production was similar for the first and the second peak, although it has to be noted, that for most of the mesocosms values were still increasing by the end of the experiment. This could be an indication that bacterial protein production was less affected by the different conditions (i.e. switch from more DOC to more POC utilisation) than was cell division, or, was reacting slower.

When directly comparing the results of both methods in terms of carbon-turnover, one has to keep in mind that the choice of the conversion factor influences the absolute results and hence hampers comparability. Our empirical conversion factor for ^{3}H-thymidine is higher compared to the frequently used factor of 17.86 kg C mol^{-1} thymidine by Riemann et al. (1987) but is in the range of the factors for nearshore and offshore waters of 25.74 and 36.34 kg C mol^{-1} thymidine respectively, proposed by Fuhrman & Azam (1982) and Ducklow & Carlson (1992) who proposed a factor of 30.28 kg C mol^{-1}. Wikner & Hagström (1999) found a conversion factor of 22.71 kg C mol^{-1} thymidine for an estuary in the Northern Baltic Sea, Carlson et al. (1996) found a value of 25.35 kg C mol^{-1} thymidine in the Sargasso Sea, Li et al. (1992) determined 24.98 kg C mol^{-1} thymidine in the North West Atlantic, and Ducklow et al. (1992) an average CV of 19.20 kg C mol^{-1} thymidine in the North East Atlantic. Nevertheless, as the factors do not change, comparison is feasible and showed that the results of both

methods were significantly positively correlated and showed no significant differences over the course of the experiment.

Direct comparisons, as in the leucine: thymidine ratio, are based on the pM raw data. When rates of protein- and DNA-synthesis are uncoupled (i.e. a change in the ratio over a given time) growth is unbalanced (Chin-Leo & Kirchman 1990) and variability in the ratio has been interpreted as a change in the growth state of bacteria by several authors (Chin-Leo & Kirchman 1990, Shia & Ducklow 1997, Pomroy & Joint 1999). These changes can occur over temporal and spatial scales and are influenced by environmental factors such as substrate supply and temperature (Chin-Leo & Kirchman 1990, Shia & Ducklow 1997, Pomroy & Joint 1999, Tibbles 1996).

In our experiment, the rates of thymidine and leucine incorporation were significantly correlated, although the correlation was not very high (R^2=0.21, p=0.0001). Previous studies have shown that there is usually a high correlation between the two incorporation rates (Chin-Leo & Kirchman 1988, Kirchman & Hoch 1988), which suggests balanced growth of bacterial assemblages. Other authors (e.g. McDonough et al. 1986) have found a lack of covariance due to unbalanced growth, but also methodological problems like non-specific incorporation of thymidine into protein have to be taken into account. Although not statistically significant, we did detect a change in the ratio over the course of the experiment suggesting a tendency towards unbalanced growth towards the end of the experiment, where DNA incorporation increased faster than protein incorporation, suggesting a response of the bacterial assemblage to changing environmental conditions (like substrate availability) (Chin-Leo & Kirchman 1990). Shiah & Ducklow (1997) suggest a lower ratio to occur under favourable environmental conditions, when bacteria optimise DNA production to maximise reproduction. This same process could explain the differential response to temperature which was also detected in our experiment, with significantly higher leucine: thymidine incorporation rates in the cold compared to the warm treatments. The same pattern was found by Shiah & Ducklow (1997) in both temperature manipulation experiments and *in situ*. On the other hand, Tibbles (1996) observed positive correlations between temperature and leu: thy ratios.

Potential top-down effects

The decreases in bacterial numbers (TBN) and bacterial production (BP) in between the two peaks might be explained by grazing due to heterotrophic nanoflagellates (HNF). Indeed, low bacterial abundance always coincided with peak values in HNFs and vice versa, so that a significant negative linear relationship could be established between TBN and HNF (data in Walther 2009). These results have to be taken into account when interpreting the development of the bacterial parameters. It emphasises, that temperature and nutrients (DOC + POC from phytoplankton) might have significant bottom-up effects on the bacterial community, but that grazers might also influence the development in a top-down manner. Due to the nature and complexity of the experiment, we cannot conclude on the relative importance of both effects.

When considering top-down effects, one has to take into account also the influence of viruses. Walther (2009) reported higher numbers of virus-like particles (VLP) at colder temperatures, which might eventually have contributed to lower TBN in these conditions.

In contrast to bacterial respiration the total community respiration showed a response to increased temperatures. This measurement incorporates the respiration by zooplankton, which might have been responsible for the temperature response. Walther (2009) does indeed report significantly higher meta-zooplankton numbers in the warmer treatments. At the peak of respiration the contribution of bacteria <3µm to community respiration was highest in the coldest treatments because of decreased community respiration in the cold. Respiration as a loss process for primary produced organic carbon has been shown to be dominated by heterotrophic bacterioplankton. Blight et al (1995) report a contribution of up to 70% by heterotrophic bacteria to total respiration measurements, Williams (1981) attributes a substantial contribution (> 50 %) of plankton respiration to organisms < 5 µm and Harrison (1986) also found over 50% of respiration associated with organisms < 1 µm. These values correspond well with the 53 – 59 % of bacterial contribution to total community respiration in our cold treatments (2.5℃). The smaller proportion of only 35 % in the warm

treatments (8.5℃) can be attributed to generally higher community respiration due to increased zooplankton activity as described above.

Peak timings

A similar acceleration of around 1 day per 1 ℃ temperature increase indicates a close association of autotrophic and heterotrophic development. This is contradictory to our hypothesis, that primary production would be less affected than bacteria. Both processes are affected in a similar way by increasing temperature, concerning the timing of events. This could be due to the high light levels in this experiment, which were saturating for photosynthesis, hence making primary production temperature dependent. Tilzer et al. (1986) found that light saturated photosynthesis exhibited a Q_{10} value of 4.2, while showing a Q_{10} of 2.6 under light-limited conditions, at temperatures between –1.5 and +2℃. These differences in Q_{10} might explain the different results, concerning the effect on the timing of events, between the two experiments. On the other hand this would not explain the different responses to temperature concerning the quantity of the described parameters' rates. But still, temperature-dependent primary production does not necessarily mean we would see higher production at warmer temperatures, if we take increased grazing and increased phytoplankton respiration (i.e. reduced growth efficiency) into account. Lengfellner (2008) could show in similar experiments, that reduced phytoplankton biomass at warmer temperatures coincided with increased copepod abundance and Aberle et al (2007), in a similar study from 2005, demonstrated increased grazing rates on phytoplankton by ciliates and copepods at warmer temperatures.

There are only few reports concerning the temperature-dependent changes in the temporal coupling between phytoplankton and bacteria (Hoppe et al. 2008 and references therein). In a previous experiment, which was performed at low light levels, Hoppe et al. (2008), found no influence of increasing temperatures on the peak timing of primary production and an acceleration of approximately 2 days per 1℃ warming for bacterial secondary production. The increased temporal coupling in this case can be assumed to contribute to increased carbon cycling through bacteria. The difference to our experiment can partially be explained by the

different light conditions for the algae, as well as different starting conditions concerning the quality and quantity of the overwintering phytoplankton population (Sommer & Lengfellner 2008, Gaedke et al. 2009). Wohlers et al. (2008), for the same experiment, confirmed our results for the phytoplankton timing from Chl a measurements. Sommer & Lengfellner (2008) also found a peak acceleration of around one day per 1°C temperature increase for phytoplankton biomass in similar experiments.

Temperature effects on integrated quantity of autotrophic and heterotrophic parameters during the bloom

There occurred no significant change in integrated primary production for the bloom period at the different temperature regimes. The trend towards higher particulate primary production in colder treatments may be due to increased grazing in warmer treatments as was already observed in a similar experiment (Lengfellner 2008, Sommer et al. 2007) as well as by other authors (Wiltshire et al. 2008, Keller 1999). Lengfellner (2008) showed that phytoplankton production based on biomass measurements was indeed significantly diminished at warmer temperatures, likely due to enhanced grazing by ciliates and copepods.

Dissolved primary production showed no response to temperature, providing the same amount of exudates for bacterial utilisation in all treatments. Hence increased bacterial parameters at warmer temperatures were probably not linked to increased or decreased DOC availability, but rather a temperature response.

The significant increases in bacterial parameters like BP (148 % and 73 %, for ^{3}H-thymidine and ^{3}H-leucine respectively), TBN (46 %) and BCD (68 % and 75 %) for a temperature increase of +6 °C indicate an increasingly heterotrophic system, compared to the autotrophic compartment, which was not influenced by temperature. This may have an important impact on the total amount of carbon being cycled through the microbial loop. Also the increasing community respiration (33 %) indicates an increased heterotrophy in the system, which means that more organic carbon is being respired, leading to increased CO_2 emissions, possibly creating a positive feedback effect concerning the effect of CO_2 on global temperature. Berglund et al (2007), in mesocosm experiments with

northern Baltic Sea water, demonstrated that a bacteria-based foodweb displays a significantly reduced food-web efficiency due to the extra trophic levels in the microbial loop. They conclude that such a foodweb, which will be favoured by increased organic nutrient supply in a future climate, through increased precipitation and river runoff, will reduce pelagic productivity at higher trophic levels. Considering the results from our study for increased temperatures, this effect would be enhanced even further.

The results confirm the expectations, concerning the different effect of temperature on autotrophic and heterotrophic processes. According to Pomeroy and Wiebe (2001), at the lower limits of growth, which was given here at the low temperature treatments, Q_{10} values for heterotrophs can even reach double digits. This would even enhance the possible differences in responses and increase possible temperature effects.

Temperature effects on bacterial growth efficiency (BGE)

The integrated calculation of BGE for the bloom period showed the percentage to vary between 19 and 27 % (34.4 – 33.7 % for ^{3}H-leucine). This is in accordance with values of 10 – 30 % as reported by Bjornsen (1986) from continuous plankton cultures and values of 20 – 27 % as reported by Bell & Kuparinen (1984) from freshwater systems, or the median ocean value of 22 % given by del Giorgio and Cole (1998). Reinthaler & Herndl (2005) report a mean annual BGE of 20% for the North Sea.

The maximal values of the first peak of BGE, as shown in the development over the course of the experiment, ranged between 27 and 40% and are in accordance with the integrated bloom period values. The higher values, which were observed for the second peak of BGE, reflect the development of bacterial production, which was increasing towards the end of the experiment, while bacterial respiration (BR as well as “corrected BR”) showed elevated but lower than peak values.

In contrast to our expectations the BGE did not show any significant response to increases in temperature, neither for the peak maxima, nor for the integrated bloom period values. If any, there was a trend towards increasing integrated

bloom period values with increasing temperature for the results from the thymidine incorporation, which is in contrast to published results by authors such as Rivkin & Legendre (2001) or Apple et al (2006). On the other hand Reinthaler & Herndl (2005) report from the North Sea, that while BP varied over 1 order of magnitude over the seasonal cycle, BR varied only 2-fold, resulting in a higher mean BGE at increased temperatures in spring and summer. Jiménez-Mercado et al (2007) demonstrated in continuous cultures of marine bacterioplankton maximum BGE values at higher temperatures. Del Giorgio and Cole (1998) show in their review contrasting results of increased, decreased or unchanged BGE at increasing temperatures and argue that environmental factors such as substrate quality and quantity are more important in determining growth efficiencies. Our results can be attributed to the missing effect of temperature increase on bacterial respiration (the original BR as well as the "corrected" BR, which incorporated bacteria attached to particles >3µm). This shows that increasing temperatures lead to more carbon being transferred into bacterial biomass, rather than being respired. This could be an indication, that bacteria were in a favourable condition at higher temperatures, rather than being stressed. Organic carbon utilisation is more efficient and less respiration is necessary for the same amount of growth at higher temperatures, respectively. This discussion highlights that parameters like BGE can not solely be assessed by looking at temperature effects only and ideally other environmental factors have to be taken into account (del Giorgio & Cole 1998).

We can confidently assume that the determination of BGE was based on good quality parameter measurements. In general, as can be seen from the raw data in Fig. 2, standard deviations were relatively low and replicate mesocosms showed similar patterns. Nevertheless, BCD and BGE were finally based on calculated BR, to account for particle-attached bacteria. The calculations were based on BP measurements and hence presume a constant relationship between bacterial production and respiration, which is clearly not the case. Still, we assume a more realistic and correct illustration of BGE development, correcting for this bacterial fraction. Still, during the relevant bloom phase, particle-attached bacteria only

accounted for a few percent to total BR, and increased only during the degradation phase of the bloom.

One has to also take into account that our measurements were obtained within a very narrow temperature frame, in contrast to authors such as Rivkin & Legendre (2001), who assessed BGE for temperatures between 1.7 and 29℃. Probably the temperature differences in our experiment were not large enough to detect possible effects on bacterial respiration and hence BGE.

Ratios

The ratios of the heterotrophic rates (CR, BR, BP, and BCD) in relation to primary production were calculated to assess the degree of coupling between autotrophic and heterotrophic processes. The strong increase of the community respiration to particulate primary production ratio indicates a strong transition towards a more heterotrophic system, with 51% more carbon being respired (relative to production) and released as CO_2 for an increase of 6℃ in temperature (see Table 4).

Also, bacterial production and bacterial carbon demand increase relative to primary production (PPP and DPP), supporting the observation of a higher relative amount of organic carbon being utilised at higher temperatures, compared to the amount produced. Nevertheless the ratios of BP: PPP between 2 and 6% (and 6 – 12 %) are very low and do not allow the conclusion of a serious impact on organic carbon cycling. However, other authors have found similarly low values. Hoppe et al. (2002) reported a ratio of 2 – 10 % for cold and temperate regions and Moran et al. (2002) measured consistently low BP: PP (total primary production) ratios of between 0.3 and 4.1 % in different oceanic regions. It also has to be taken into account that our numbers arise from calculations of a very narrow time frame during the maximum of particulate primary production and not over a longer time period (in our case, the average ratio over the whole time of the experiment is 100%!). Under the controlled experimental conditions phytoplankton can be expected to be in healthy and active condition, releasing relatively little DOC, while under field conditions a mixture of algae in different metabolic conditions with increased DOC release are more realistic. Also, obviously the ratio changed

dramatically during the degradation phase of the bloom, when bacterial production was high and primary production decreased.

For further analysis we chose an additional way of looking at the topic by comparing the bacterial production and bacterial carbon demand with dissolved primary production, because bacterial production is mainly dependent on dissolved organic matter from phytoplankton especially during the bloom phase (Norrman et al. 1995). For the BP: DPP ratio from ^{3}H-thymidine incorporation an increase up to a ratio of 93 % does indeed show the relevant impact of higher temperatures. For ^{3}H-leucine incorporation the ratio was also always above 100 %, showing that in this case the demand for protein production was not sufficiently supplied by DOC (see also below). The BCD: DPP ratio seemed unaffected by temperature, but the ratio always lay above 100%, revealing that exudation was at no point able to satisfy the carbon demand by bacteria. The DPP: BCD ratio (^{3}H-thymidine incorporation) showed, that on average, the fraction of bacterial carbon requirements, which extracellular release can meet, was only 32%. Cole et al. (1982) reported the fraction of bacterial carbon requirements, which extracellular release (i.e. dissolved primary production) can meet, to be around 40 %. Banes and Pace (1991) calculated the fraction to be 32% on average, assuming a BGE of 50% (and only 13% for a BGE of 20%). If BCD is much higher than DPP, then bacteria must have other sources of carbon for maintenance and growth (Moran et al. 2002, Banes and Pace 1991). Pomeroy & Wiebe (2001) have described that the potentially rapid transfer of dissolved organic carbon from auto- to heterotrophs usually falls short of the demand of bacteria for growth. Hence additional connections have been proposed, such as viral lysis, nutrient deficiency lysis and the excretion, defecation and sloppy feeding by micro-zooplankton and protists. Although measurements of dissolved primary production cannot distinguish between exudation of labelled DOC by healthy cells and via cell lysis by viruses or due to cells dying, it can be assumed that the effect of zooplankton, via sloppy feeding and excretion is underestimated due to the small sample sizes (it would only be by chance to have included copepods in the samples). Additionally the dissolved organic matter is not the only source for bacteria, which

also use extracellular enzyme activity in order to degrade and utilise particulate organic matter, such as dead phytoplankton cells.
In this context, one has to be aware, that the influences of substrate quantity and quality and temperature on the planktonic food web cannot be assessed sufficiently if considered separately. Further studies, which disentangle the combined effects of nutrient supply and temperature, are needed and one example will be presented as part of this thesis. Additionally the influences of shifts in the bacterial community composition have to be taken into account. Hall et al (2008) showed how the often unclear relationships between temperature and bacterial metabolism can be understood by allowing for changes in the relative contributions of thermally differently adapted species to the total community reaction. The influence of temperature on the bacterial community composition was assessed as part of this experiment and is described in the thesis by Walther (2009).

Summary and Conclusions

We hypothesised that increasing temperatures would lead to an increased transfer of organic matter via the microbial food web due to a decreased lag time between the autotrophic production and heterotrophic microbial degradation in combination with an increased heterotrophic microbial activity.

Our results show that the lag time between carbon fixation by phytoplankton and its utilisation by bacteria was not influenced by the temperature increase. Both processes were closely coupled and bacteria utilised dissolved and particulate organic carbon from phytoplankton during the bloom and then again increasingly during the degradation phase of the bloom. Additionally all bacterial parameters were significantly quantitatively increased at elevated temperatures, while primary production was unaffected by the temperature increase. BGE showed a trend towards increased values with increasing temperatures, revealing that bacterial production increased stronger than bacterial respiration under warmer conditions, indicating improved growth conditions rather than a stressful environment for

bacteria. Ratios of bacterial parameters to primary production revealed the increasing organic carbon transfer via bacteria with increasing temperatures. It also demonstrated that dissolved primary production did not suffice to supply enough readily available organic carbon for bacterial carbon demand, with an increasing deficit under warmer conditions.

In summary, relative to the autotrophic production, more organic matter was transferred through the microbial loop and respired to CO_2. In a future scenario of winter warming conditions, these results predict an increasing importance of the microbial loop in organic carbon cycling, leading to an overall more heterotrophic planktonic system. More CO_2 will be released directly by bacteria and indirectly by the members of the complex food web, leaving less organic carbon for aggregation and sinking and representing a positive feedback loop for the CO_2 climate problem.

CHAPTER 2

The influence of temperature and light on phytoplankton – bacterioplankton interactions during the spring bloom – recurring patterns from four years of mesocosm experiments

Introduction

Oceanic phytoplankton primary production contributes to about 50% of global CO_2 fixation from the atmosphere. This autotrophic process of organic matter build-up is directly dependent on the availability of light and inorganic nutrients. Indirectly it depends on temperature via stratification, which regulates mean light and nutrient availability in the photic zone (Behrenfeld et al. 2006). The resulting particulate organic matter is ingested by zooplankton and subsequently higher trophic levels, leaving the remains for aggregation and sinking (classical food chain). Up to 50% of primary production, however, is cycled through the microbial loop in temperate waters (Azam et al. 1983), up to 102 – 188% for example in the equatorial Indian Ocean and this process is mainly dependent on temperature (Pomeroy & Wiebe 2001). This important link between the physical environment and biological functions in the ocean highlights the urgency of studying the effects of predicted climate change in terms of solar irradiance and temperature on the coupling between phytoplankton carbon fixation and heterotrophic carbon remineralisation and hence the marine carbon cycle. Also, the combined effects of temperature and light on the marine carbon cycle have rarely been considered together (Rochelle-Newall et al. 2008).

Current situation

In moderately deep water bodies like the Kiel Bight, the influence of temperature and light on the onset of the spring bloom are decoupled. All plankton is physically restricted to a shallow water depth and as soon as light conditions are favourable, this triggers the phytoplankton spring bloom (Sverdrup 1953, Sommer et al 1986, Sommer & Lengfellner 2008). Because of this light-dependence of phytoplankton, the spring bloom is usually associated with cold water temperatures (2.4℃ as the 10 year mean in early February). At these temperatures, heterotrophic activity is still very low, so that the autotrophic carbon fixation by phytoplankton and the heterotrophic bacterial utilisation are mainly decoupled during this time. Consequently the remineralisation of organic matter is low and a large portion is lost to sedimentation (Pomeroy & Wiebe 2001).

Climate predictions

The IPCC report (IPCC 2007) predicts an increase in winter temperatures for north-central Europe of up to 8.2 ℃ until the end of the century (annual mean: 2.3 – 5.3℃). At the same time, confirmed prognosis on the development of light conditions is not available (Wild 2009). However, a tendency towards increasing irradiation between the 1980s and 2000s by 1.4 and 4.9 W m^{-2} per decade was observed for Europe, which was mainly attributed to changes in anthropogenic aerosol emission leading to less scattering and adsorption of radiation (Wild 2009). Predictions on future irradiation changes are associated with great uncertainties because they have to account for future development of anthropogenic aerosol emissions, which is coupled to economic advancement as well as the effectiveness of air pollution regulations. Recent measurements however, still confirm the continuing brightening trend at the moment (A. Macke, personal communication). Apart from the global irradiance, an indirect effect of water column warming is predicted to be an increased light availability in the temperate climate zone (Behrenfeld et al 2006). In deep water columns, increasing winter water temperatures are expected to increase thermal stratification and hence increase the light availability for phytoplankton as well as prolonging the growing season (Behrenfeld et al 2006). As described above, our mesocosm system represents a model for moderately deep water bodies. Nevertheless, the basic mechanisms, as found for example for future increasing light availabilities, might well be transferable to deep water bodies.

Possible future changes

While light-limited phytoplankton is mainly independent of temperature (Tilzer et al 1986), the temperature dependence of bacterial processes like bacterial secondary production and bacterial respiration has been described manifold (e.g. Shia & Ducklow 1994, Pomeroy & Wiebe 2001, Kirchman et al. 2005). Published Q_{10} values of 1 - 2 for auto- and 2 - 3 for heterotrophic processes (Pomeroy & Wiebe 2001, Tilzer et al. 1986) suggest that a temperature increase would mainly favour bacteria over phytoplankton. For phytoplankton on the other hand, increasing light availability would result in earlier phytoplankton blooms, as the

critical light quantum for the onset of the bloom is reached earlier in the year. Also the process of carbon fixation is expected to be enhanced at higher light availabilities.

For predictions on a future phytoplankton-bacterioplankton coupling and the associated carbon cycling through the microbial loop, several different possibilities can be devised. These possibilities can be considered on two different levels: **a.** the absolute amount of organic carbon that is fixed and consequently cycled through the microbial loop and **b**. the relative amount of primary produced organic matter that is utilised by heterotrophic bacteria. In the context of a predicted future warming and possible continuing brightening, the current situation is termed as a "cold" and "dim" spring situation.

Considering the above-described current time-lag between autotrophic carbon-fixation and heterotrophic carbon remineralisation, future warming can be expected to shift bacterial activity forwards in time, diminishing the time-lag und creating a (increasing) timely overlap with the phytoplankton bloom, thereby increasing the substrate availability for bacteria. Because phytoplankton would not be affected in their timing by the increased temperature, the relative amount of organic carbon going through the microbial loop would increase. A closer timely coupling of bacteria to phytoplankton will hence increase bacterial production and consequently favour the degradation of organic matter in the euphotic zone and the recycling of CO_2 to the atmosphere and leave less matter for sedimentation processes. In the case of future brightening, however, it can be expected, that the phytoplankton spring bloom will start even earlier in the year, this way increasing the time-gap to bacteria, hence decreasing the relative amount of recycled carbon. The combined effects of "warming" and "brightening" cannot be deduced from this scenario as the result depends on the relative effects of light on phytoplankton and temperature on bacteria. If both effects are the same for the timing of the bloom, then no change compared to the current situation will be expected. Also, the consequences of a timely overlap are obviously directly dependent on the effects of temperature and / or light on the primary and secondary production quantities.

For the quantity of primary and secondary production a similar basic scenario can be devised. Increasing temperatures are expected to increase the quantity of bacterial activity, while it will affect phytoplankton to a lesser extent. This will lead to an increased relative amount of primary produced organic matter (i.e. increased BP:PP). Increasing solar irradiance on the other hand will enhance primary production while it will not affect bacterial activity and hence the relative amount of organic carbon going through the microbial loop will diminish (i.e. decreased BP:PP ratio). In a future scenario of both "warming" and "brightening", where both processes are positively affected, we can expect the absolute amount of primary produced organic matter that is recycled by heterotrophic bacteria to increase. However, we cannot predict how the relative amount will change, because the effect of light on phytoplankton and temperature on bacteria might not be the same.

Obviously these considerations are a simplification of the much more complex food-web interactions. Temperature can have small effects on primary production (Q_{10}=1 - 2) while there is no direct effect of light on heterotrophic bacteria. Other trophic levels have to be taken into account, like for example increased zooplankton grazing on phytoplankton can reduce phytoplankton primary production in warmer conditions (Lengfellner 2008).

The research questions which follow up from these considerations are the following:

1. Will there be an increased coupling of phyto- and bacterioplankton at higher temperatures?
2. What will be the influence of light on this coupling?

We will draw conclusions from indoor mesocosm experiments, which were conducted under different experimental settings over the course of four years. In each year, winter water from Kiel Fjord, containing the overwintering plankton communities, was exposed to different temperature settings, including the current in situ "cold" situation and warming scenarios of up to ΔT +6℃ (future "warming" scenario). In the subsequent experiments different natural light scenarios were

chosen, representing current “dim” and future “brightening” of light conditions. Autotrophic and heterotrophic parameters were determined during the development of the spring phytoplankton bloom in order to assess the coupling between phytoplankton and bacteria and to interpret the results in terms of a possible future “warming” and “brightening” scenario.

Materials and Methods

Experimental setup

The experiments were performed in early spring in the years 2005, 2006, 2007 and 2008. For the experiments in 2005 – 2007, eight mesocosms were set up pairwise in four climate chambers, thus creating two replicates per temperature treatment. The *in situ* treatment was run at 2.4℃ (ΔT+0℃). This corresponds to the ten years mean (1993 – 2002) for the Kiel Fjord for the 4th of February (Julian day 35), which was chosen as the virtual starting point. The other three climate chambers were adjusted to 4.4, 6.4 and 8.4℃ (ΔT+2℃, +4℃ and +6℃, respectively). In 2008 two climate chambers were run at ΔT+0℃ and the other two at ΔT+6℃ (see Table 1 for an overview over the different experimental settings).

The mesocosms were allowed to adapt to the chosen temperatures before the first sampling. Temperatures were adjusted according to the decadal mean temperature model (Fig. 1).

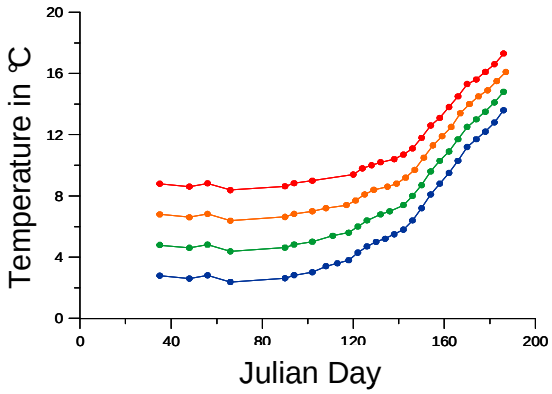


Figure 1. Spring temperature model. The blue line represents the baseline treatment (ΔT+0℃) and corresponds to the decadal mean of the Kiel Bight water temperatures between 1993 – 2002. Climate warming regimes were elevated by ΔT+2℃ (green), ΔT+4℃ (orange) and ΔT+6℃ (red).

The mesocosms were synchronously filled with 1400 L of unfiltered Kiel Fjord water from 6 m depth outside the IFM-GEOMAR, containing the overwintering populations of phytoplankton, bacteria and protozoa. Mesozooplankton from net catches was added in natural overwintering densities. The water was gently stirred at all times, preventing light particles to sink down to the bottom, while at the same time allowing heavier particles to drop out of the water column. The starting mesozooplankton concentrations and nutrient conditions for all years can be found in Table 1.

Water samples were taken in regular intervals into 20 L pre-washed carboys. Subsamples for the determination of the different parameters were taken from the carboys after gentle mixing. Only for the determination of respiration rates, water was taken directly from the mesocosms in order to prevent mixing and stirring influence on oxygen content of the samples.

Light was provided by fluorescent tubes (a mixture of JBL Solar Tropic and JBL Solar Natur) from the top of the mesocosms. The light units were computer controlled (GHL Groß Hard- und Softwarelösungen, Lamp unit HL3700 and ProfiluxII). Daily light cycles (i.e. sunrise and sunset) were adjusted according to the natural light conditions in the Kiel Bight and were transformed to triangular light curves with integrated daily intensities. These daily intensities were calculated according to the geographical position of Kiel after the model described in Brock (1981). Therein I_0 represents the natural daily integrated solar irradiation reaching the water surface on a cloudless day. This theoretical 100% I_0 level was subsequently reduced to levels of between 16 and 64% in the different experiments, in order to simulate current natural dim spring situations as well as possible future brightening scenarios. In the three experiments in 2005, 2006 and 2007, one light level each was applied (with four temperature levels), while in 2008 a combination of two temperatures with three different light levels was applied (repeating light levels were termed “b” ,see Table 1). The theoretical 100 % I_0 light level ranges between 1.40 kWh m^{-2} on the 4th February and 9.08 kWh m^{-2} for example on the 28th February (average: 4.16 kWh m^{-2}). Measurements of solar irradiation actually reaching the surface, performed at IFM-GEOMAR for

February 2008, showed values of between 0.30 and 2.25 kWh m^{-2} (average: 1.05 kWh m^{-2}). Calculated for the entire year, on average only about 20 % of the theoretical irradiance actually reaches the surface. Hence the performed experiments with 16 and 32% I_0 can be conceived as representing current dim spring situations, while the other treatments (48% and 64% I_0) represent possible future brightening scenarios.

Table 1: Overview over the different experiments and their respective experimental settings. I represents the % of natural light intensity without cloud cover (I_0). ΔT is the initial temperature elevation relative to the long-term mean (1993-2000). Mesozooplankton was added from net catches in actual over wintering densities. Natural starting nutrient conditions were different between the different years.

	2005	**2006**	**2007**	**2008**
Light (I in % I_0)	16	64	32	32 b, 48, 64 b
Temperature (ΔT +°C)	0, 2, 4, 6	0, 2, 4, 6	0, 2, 4, 6	0, 6
Mesozooplankton (individuals L^{-1})	12 - 20	7 - 10	3 - 6	10
Nutrients (µM)				
Phosphate	0.8	0.7	1.1	0.9
Nitrate	21.5	8.7	31.9	10.6
Ammonium	2.2	1.7	4.4	1.3
Silicate	24.7	18.9	32.5	30

Particulate primary production

Particulate primary production (PPP) measurements were performed using ^{14}C bicarbonate incubations following the methods of Gargas (1975) and Steeman Nielsen (1952) (in 2008 primary production was conducted by Aleksandra Lewandowska). For each mesocosm three aliquots of 30 ml each were incubated with 100 µl of a 4 µCi / 100 µl ^{14}C-bicarbonate solution. The blank treatment was kept dark during incubation. Incubation took place at approximately half depth inside the respective mesocosm, ensuring a mean light exposure and in situ temperature conditions. After 4-5 hours of incubation, aliquots of 10 ml were

filtered onto 0.2 µm cellulose nitrate filters. The filters were subsequently fumed with 37% HCl fumes in a closed box for 5-10 min and then measured in 4 ml of Scintillation cocktail (Lumagel Plus) using a Packard Tricarb counter.

Particulate primary production were calculated for the light day by considering the amount of light received during the incubation period relative to the total daily light quantity. The variable is presented as µg C L^{-1} d^{-1}. In the text the term “primary production” will be used with reference to particulate primary production.

The original CO_2 concentration of the water sample was determined according to the method and dissociation constants described in Stumm & Morgan (1981).

Bacterial production

Bacterial protein production

Bacterial protein production (BP) measurements were conducted following the protocol of Simon & Azam (1989). Four aliquots (3 replicates and one blank) of 10 ml of water were each incubated with 50 µl of a 1 µCi / 10 µl ^{3}H-leucine solution plus 50 µl of a 2 nmol / 100 µl unlabeled leucine solution. This resulted in a total concentration of >100 nmol L^{-1} of leucine in the sample in all years, which is known to be saturating under the conditions found in the Kiel Fjord (Giesenhagen, unpublished data).

All samples were incubated in the respective climate chambers at in situ temperature in the dark for 1.5 - 3 hours. Incubation was terminated by the addition of formaldehyde (1 % v / v) and 5 ml aliquots were separately filtered onto 3.0 µm (particle-attached bacteria) and 0.2 µm (total bacteria) polycarbonate filters. The filters were subsequently rinsed with ice cold 5 % TCA (trichloro acetic acid) solution, before being radio-assayed in 4 ml of scintillation cocktail (Lumagel Plus). Results in terms of pM h^{-1} bacterial protein production were transferred into µg C L^{-1} d^{-1} biomass production using a theoretical conversion factor of 3.091 x 10^{-3} kg C mol^{-1} leucine (Simon & Azam 1989).

Bacterial Cell Production

Incorporation of ^{3}H-methyl-thymidine for the determination of bacterial cell production (BP) was done slightly modified after Fuhrman & Azam (1982). For each sample, three replicates and one blank (treated with 1 % v / v formaldehyde) of 10 mL of water were each incubated with 50 µL of a 1 µCi / 10 µL ^{3}H-methyl-thymidine solution resulting in a final and saturating concentration of 8.2 nmol L^{-1} (2005) and 7.9 nmol L^{-1} (all other years).

Samples were treated as described for ^{3}H-leucine above (including fractionated filtration onto 3 µm and 0.2 µm filters). Results in terms of pM h^{-1} bacterial production were transferred into µg C L^{-1} h^{-1} biomass production using empirical conversion factors of 30.87 kg C mol^{-1} thymidine (2006) and 12.12 kg C mol^{-1} (2007) – for a description of the determination of conversion factor see Chapter 1. In 2005, no conversion factor was established, so a literature value of 17.32 kg C mol^{-1} (Riemann et al. 1987) was used.

Respiration

Respiration was determined using Winkler Titration (Winkler 1888) with automated photometrical endpoint detection. For each mesocosm six 100 ml glass bottles were filled with unfiltered water for determination of total community respiration, another six bottles were filled with 3 µm pre-filtered water (always < 200 mbar) for determination of respiration assigned mainly to bacteria. Total community respiration (CR, unfiltered water) incorporates dark phytoplankton respiration, respiration by zooplankton and total bacteria. Bacterial respiration (BR) represents free-living bacteria and bacteria attached to particles <3µm but does exclude bacteria attached to particles >3µm. Three flasks of each set were immediately fixed and the other three replicates were incubated for 48 h at in situ temperature in the climate chambers in the dark, and submersed in water. Respiration in terms of O_2 uptake (mg L^{-1} h^{-1}) was multiplied by a recommended factor of 0.32 (based on RQ of 0.85, Ogura 1972) to calculate C-utilisation for respiration in terms of mg C L^{-1} d^{-1}.

Total bacterial number

For determination of bacterial abundance (TBN) (cells ml^{-1}) aliquots of 100 ml of water were fixed with formaldehyde to a final concentration of 2 % (vol / vol) and stored at 4℃ until filtration. Filtration of 6 ml aliquots onto black 0.2 µm polycarbonate filters was performed within 7 days of fixation. Cells were stained using DAPI (4´-6-diamino-2-phenylindole) to a final concentration of 100 µg ml^{-1} and frozen at -20℃ until being counted under an epifluorescence microscope (Axioskop2mote plus, Zeiss, Germany). At 1000x magnification, using a NewPorton G12 Grid, 20 grids or at least 400 cells were counted.

Data analysis and statistics

The timing of the peaks in relation to temperature (and for 2008 also light intensity) was computed from the regression between the days when these peaks occurred and the temperatures (ΔT) or light intensities (% I_0) of the respective mesocosms. The slopes of the linear regressions between the day of peak and the temperature or light correspond to the acceleration that the respective parameter experienced in days per each 1℃ warming or 1% increase in light intensity. The slopes were compared using ANCOVA.

In order to establish the relationships between temperature and the quantities of the measured parameters I quantified each individual peak by calculating its mean. The individual peaks were determined from the start until the end of exponential increase. Where no exponential increase occurred, the first and last days of a substantial increase in the respective parameter was determined by eye. The mean value allows for the direct comparison of the different parameters from different experiments, irrespective of different length and height of the individual peaks. Each calculated mean value was plotted against its respective temperature and linear regression lines fitted through the data using SigmaPlot.

The total amount of carbon required by bacteria for growth and respiration (bacterial carbon demand, BCD) was calculated by adding bacterial production and bacterial respiration (BCD = BP + BR). Because BP incorporates all bacteria while BR does not take into account the respiration of bacteria attached to particles >3 µm, the relative amount of particle-attached bacteria was calculated

from the BP >3µm measurement and added to the BR measurements accordingly ("corrected BR", only for BCD and BGE). The relative amount (percentage) of carbon being used by bacteria for growth in relation to the total carbon demand, the bacterial growth efficiency (BGE) was calculated by dividing bacterial production by BCD and multiplying by 100 (BGE = BP/ BCD).

Percent increases in any of the parameters with temperature were assessed from the equations of the linear regressions by using first 2.4℃ (in situ = 100%) and then 8.4℃ in the equations. For light intensity, I used first 32 % or 48 % (where appropriate) and then 64% in the linear equations.

Because the experiments with different light treatments were performed in different years, a joint statistical analysis is not possible. When comparing the results it has to be kept in mind that different starting conditions, concerning the relative quantities and compositions of the planktonic community, were present in the different years. Due to the full factorial combination of temperature and light treatments in the 2008 experiment, an analysis of the above described parameters (timing, mean quantity, BCD, BGE and ratios BP: PPP and BCD: PPP) in relation to light additionally to temperature was feasible. Multiple linear regression was performed in order to assess the relative influences of light and temperature on the parameters. In the results section the experiments from 2005 - 2007 are therefore always described separately from the 2008 experiment.

Linear regressions were performed using SigmaPlot software (Systat Software Inc., USA), statistical analyses (ANCOVA comparison of slopes, multiple linear regression) was performed using Statistica data analysis software (StatSoft Inc., USA).

Results

Time courses

In order to give an overview over the development of autotrophic and heterotrophic parameters, the time courses of primary production (PPP) and bacterial production (BP) at the two extreme temperature regimes (ΔT +0℃ and +6℃) are displayed in Figures 2 and 3. The results for PP show, that in each single experiment, i.e. treatment, a phytoplankton bloom developed.

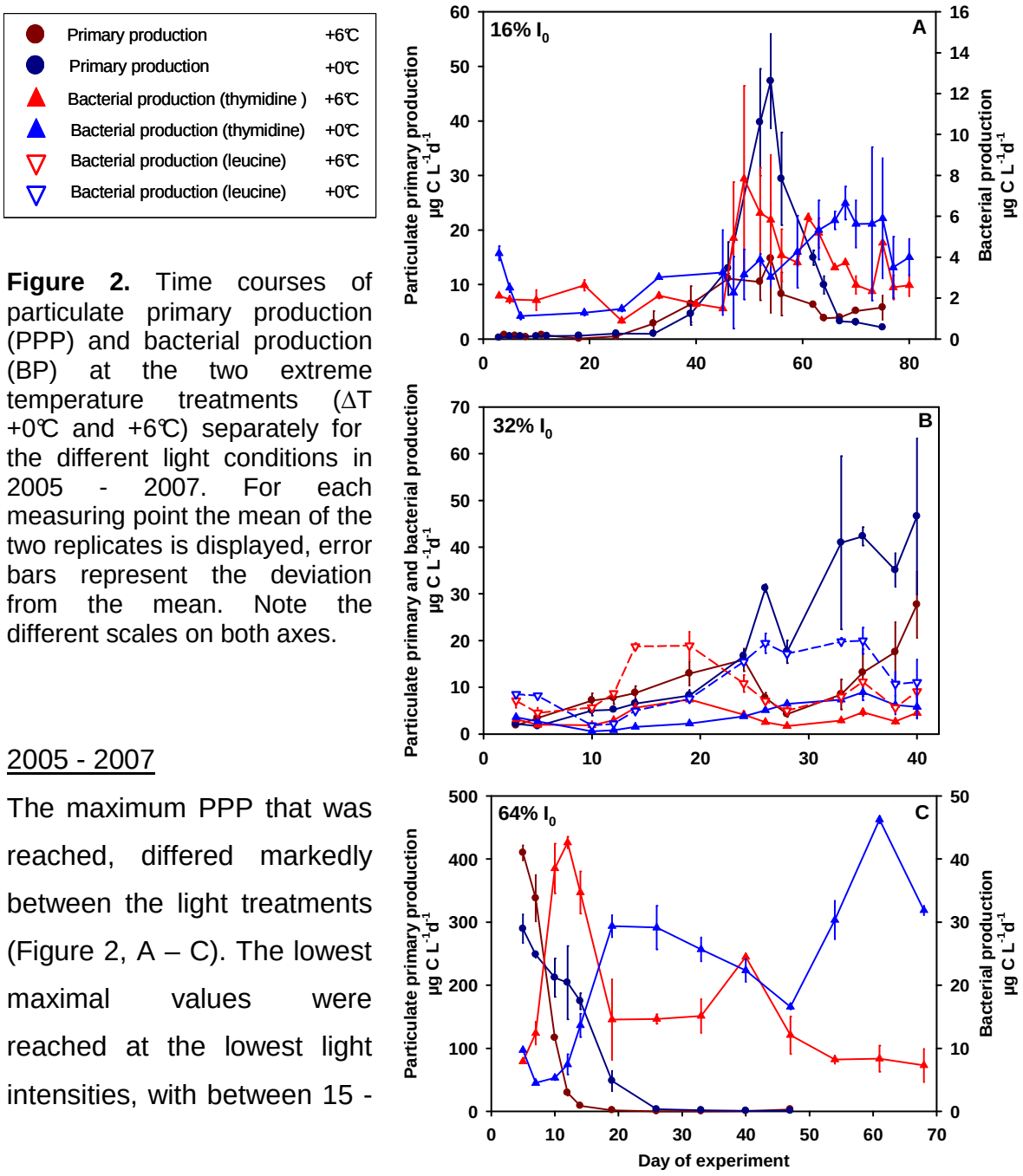
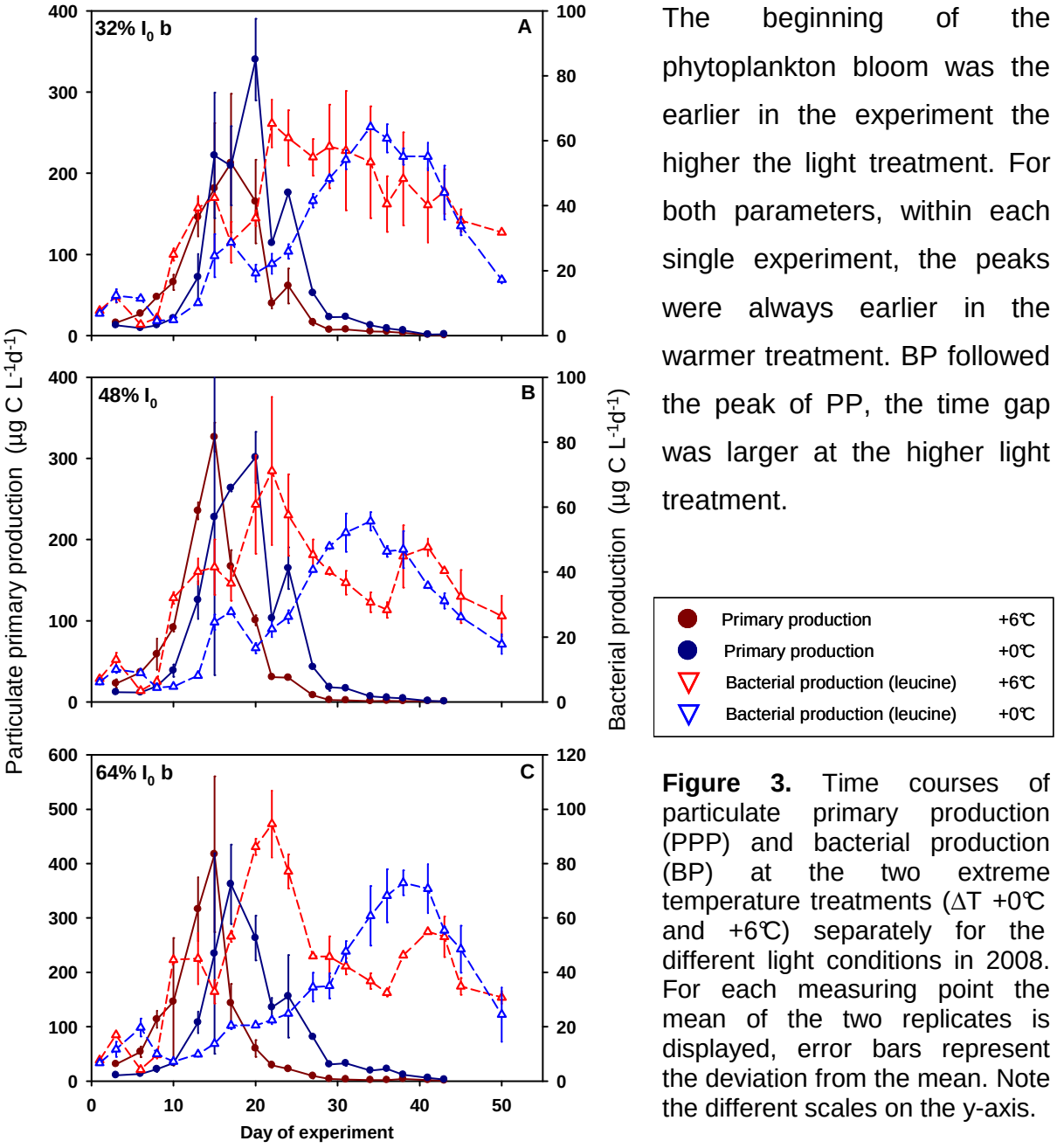


Figure 2. Time courses of particulate primary production (PPP) and bacterial production (BP) at the two extreme temperature treatments (ΔT +0℃ and +6℃) separately for the different light conditions in 2005 - 2007. For each measuring point the mean of the two replicates is displayed, error bars represent the deviation from the mean. Note the different scales on both axes.

2005 - 2007

The maximum PPP that was reached, differed markedly between the light treatments (Figure 2, A – C). The lowest maximal values were reached at the lowest light intensities, with between 15 -

47 µg C $L^{-1}d^{-1}$ at 16% I_0 and 20 – 50 µg C $L^{-1}d^{-1}$ at 32 % I_0. At 64% I_0, PPP reached between 289 and 410 µg C $L^{-1}d^{-1}$. As seen for PPP, the lowest maximal values of BP were found at 16% and 32% I_0, with between 6.6 - 7.8 and 7.4 – 8.9 µg C $L^{-1}d^{-1}$ (^{3}H-leucine incorporation) respectively. For ^{3}H-thymidine incorporation, at 32% I_0, between 18.8 and 19.8 µg C $L^{-1}d^{-1}$ were measured. At 64%, measurements with ^{3}H-leucine incorporation showed a production of 29.4 – 42.6 µg C $L^{-1}d^{-1}$.



The beginning of the phytoplankton bloom was the earlier in the experiment the higher the light treatment. For both parameters, within each single experiment, the peaks were always earlier in the warmer treatment. BP followed the peak of PP, the time gap was larger at the higher light treatment.

Figure 3. Time courses of particulate primary production (PPP) and bacterial production (BP) at the two extreme temperature treatments (ΔT +0℃ and +6℃) separately for the different light conditions in 2008. For each measuring point the mean of the two replicates is displayed, error bars represent the deviation from the mean. Note the different scales on the y-axis.

2008

PPP in 2008 (32 % b, 48% and 64% b I_0) was higher than in 2005 and 2007 (lower light treatments), but at a comparable level as in 2006 (64% I_0). However, here also the maximal values increased with increasing light intensities, with between 213 and 340 µg C $L^{-1}d^{-1}$ at 32% b I_0, between 301 and 326 µg C $L^{-1}d^{-1}$ at 48 % I_0 and between 362 and 417 µg C $L^{-1}d^{-1}$ at 64% b I_0.

In all light treatments of the 2008 experiment (32% b, 48% and 64% b I_0) only BP measurements using ^{3}H-leucine incorporation were performed, yielding increasing maximal values with increasing light intensity at the warmest temperature (64.3 - 65.3 µg C $L^{-1}d^{-1}$ at 32% b I_0, 55.6 - 71.1 µg C $L^{-1}d^{-1}$ at 48% I_0 and 72.9 - 94.6 µg C $L^{-1}d^{-1}$ at 64% b I_0).

In all light treatments the peaks of the two parameters were always earlier in the warmer treatments. BP peaks were always after the peaks of PPP.

Summary

- Absolute maximal values of both parameters, PPP and BP, differed between the different years, values were generally higher in the 2008 experiment
- PPP and BP maximal values increased with increasing light intensity in the 2005-2007 and within the 2008 experiment
- Peaks in the warmer treatments were always earlier than in the colder treatments (both parameters)
- The peaks of BP occurred after the PPP in most cases
- Peaks of BP were always higher in the warmer treatments, there was no such pattern for PPP

A detailed assessment of the influence of light and temperature on the timing and quantity of the measured parameters can be found in the following sections.

Timing

In order to assess the influence of temperature and light on the timing of the bloom event dynamics, I plotted the day of each peak against its respective

temperature. As above there is a plot for each light treatment and the 2008 experiment separately from the other years. The equations for the linear regressions can be found in Annex Table 1, where the slope of the equation represents the number of days acceleration (or retardation) of the peak for a temperature increase of 1℃, and the difference of each parameters' peak relative to the peak of autotrophic carbon fixation (PPP) is displayed.

2005 - 2007

A comparison between the different light levels revealed a large difference in the timing of the bloom (PPP). At the highest light treatment (64% I_0) the peak started almost immediately at the beginning of the experiment, while it was around day 22 for the 32% I_0 treatment and only around day 51 at the lowest light level (16% I_0).
Within the 16% I_0 light experiment (Figure 4, A) PPP was slightly accelerated by 0.8 days by increasing temperature, while BP was accelerated by 2.2 days, which would lead to a decrease of the gap between autotrophic and heterotrophic production by over 8 days for a total temperature increase of ΔT +6℃. Bacterial abundance (TBN) showed basically no correlation with temperature (R^2 = 0.02). Statistically the accelerations were not significantly different from each other (ANCOVA comparison of slopes, F=0.92, p=0.42).
PPP was significantly but only very weakly accelerated by the temperature increase at 32% I_0 (0.35 days) (Figure 4, B). Bacterial and community respiration (BR and CR) were basically unaffected. BP (^{3}H-leucine: 1.6, ^{3}H-thymidine: 2.42 days) and TBN (2.35 days) were (significantly) stronger accelerated, so that the gap between the bacterial parameters and PPP was reduced by 7 and 12 days, respectively. All bacterial parameters even "overtook" the PPP peak. Statistically, CR and BR accelerations were similar (ANCOVA, F=0.39, p=0.54), TBN and BP (both methods) were similar (F=0.91, p=0.42) and PPP was different from all the others (for CR+BR: F=3.55, p=0.05; for all other parameters: F=5.34, p=0.006).
There was no influence of temperature on the timing of the PPP peak at 64% I_0 (Figure 4, C). All measured bacterial parameters were significantly accelerated at warmer temperatures, similarly around 1.8 days for TBN and BP and stronger for BR (3.8 days). These accelerations would lead to a reduction of the gap between

auto- and heterotrophs of 11 and 22 days for a temperature increase of ΔT +6℃. ANCOVA comparison of slopes revealed that BP (^{3}H-thymidine), BR and TBN accelerations were statistically not significantly different from each other (F=2.59, p=0.10), while PPP was different from all of these (F=5.96, p=0.002).

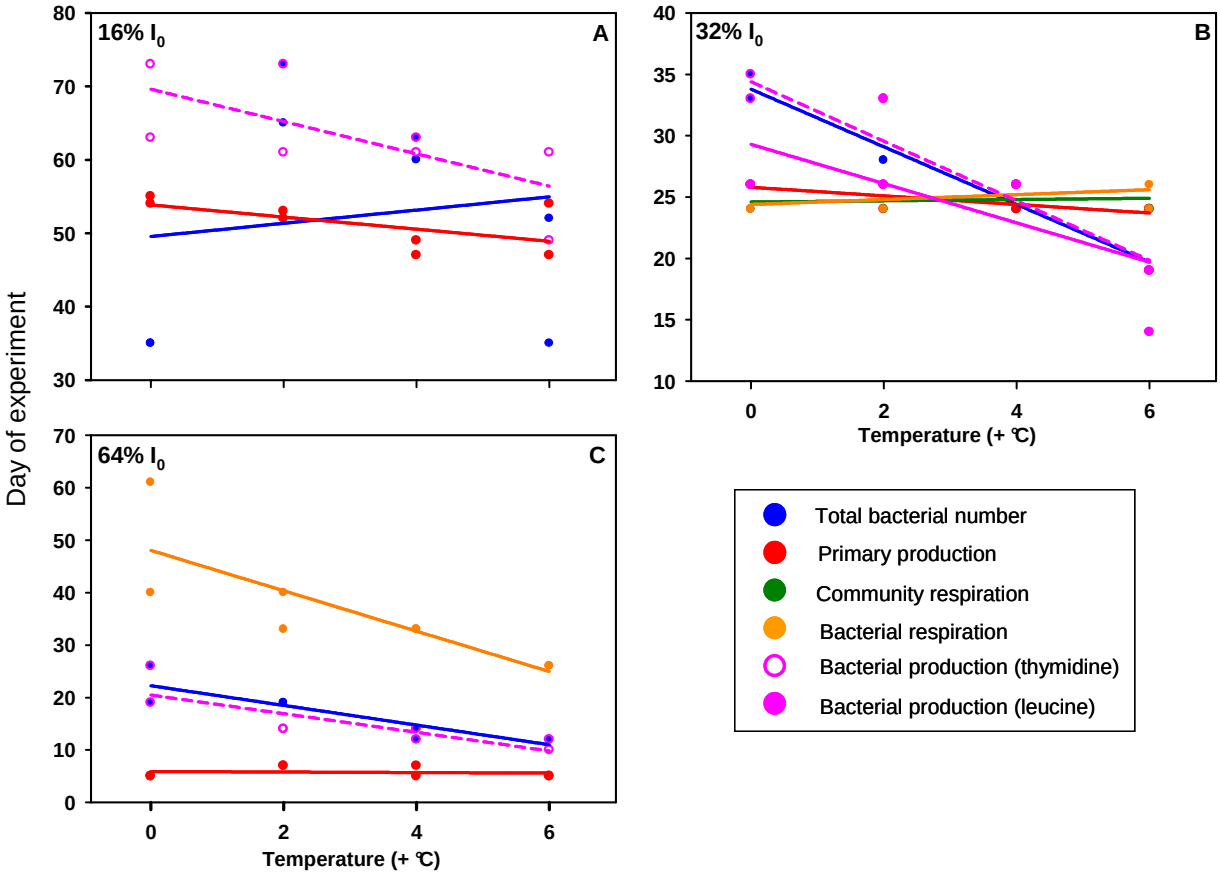


Figure 4: Acceleration of peaks of the different parameters for the temperature increase of ΔT +0℃ to +6℃ at the respective light treatments f rom the 2005 – 2007 experiments. The day of each peak is plotted against its respective temperature and the relationship is assessed by linear regression. For equations see Annex Table 1. Note the different scales on the y-axis.

2008

For the 2008 treatments (32% b, 48% and 64% b I_0) there were only data available for two temperature treatments (ΔT +0 and +6℃). The patterns of peak accelerations for the light treatments in this experiment were very similar (Figure 5). PPP and both respiration parameters (BR, CR) were always only little affected by the temperature increase (all ≤ 1 day). BP showed a strong acceleration (1.4,

1.6 and 3.1 days respectively), leading to a reduction of the gap to PPP of 4.5, 7 and 15 days at the different light settings. TBN showed the strongest peak acceleration with increasing temperatures, which was also very similar between the different light treatments (4.5 - 4.7 days). The strong acceleration led to a decrease in the difference of the peak timing to PPP of 23-25 days, so that the peak of TBN would even be earlier than that of the algae. ANCOVA comparison of slopes shows for the two lower light levels, that TBN acceleration was statistically different from all other parameters (F=19.17, p=0.002 and F=24.52, p=0.00). At 64% b I_0 the accelerations were similar for BR, CR and PPP (F=0.95, p=0.44) and TBN and BP (^{3}H-leucine) were additionally also different from each other (F=17, p=0.001).

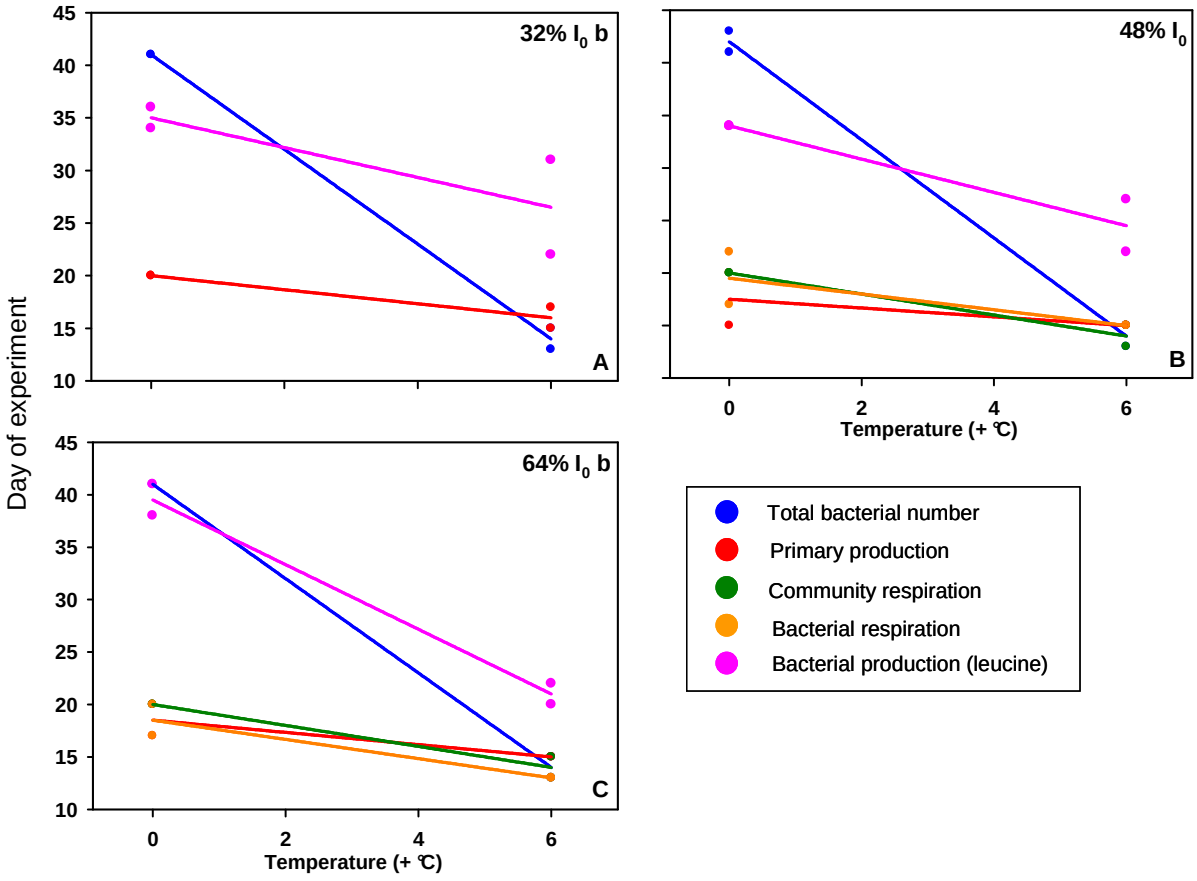


Figure 5: Acceleration of peaks of the different parameters for the temperature increase of ΔT +0℃ to +6℃ at the respective light treatments f rom the 2008 experiment. The day of each peak is plotted against its respective temperature and the relationship is assessed by linear regression. For equations see Annex Table 1.

Due to the full factorial experimental design in 2008, a direct and statistical comparison of the different influences of temperature (ΔT +0℃ and +6℃) and light (32% b, 48% and 64% b I_0) was feasible. Hence I plotted all data points of each parameter at the same temperature together in Figure 6. This graph shows that the overall acceleration of peak timing of PPP, irrespective of the light intensity, only measured a total of 0.56 days per 1℃ temperature increase (Annex Table 2). CR and BR peaks coincided with the peak of PPP and were only slightly stronger accelerated by 0.8 and 1 day. The difference in acceleration to BP was stronger (2 days), leading to an overall reduction in the gap to PPP by almost 9 days (original difference 14 days at ΔT +0℃). The strongest acceleration was seen for TBN with 4.6 days and a difference to the acceleration of the algae of 24 days for a temperature increase of ΔT +6℃, leading to an overall earlier peak of TBN compared to the algae (PPP). All accelerations were statistically significant and ANCOVA comparison of slopes showed that PPP, CR and BR showed statistically the same acceleration (F=1.89, p=0.18), while TBN and BP were different from the others as well as different from each other (F=50.7, p=0.00).

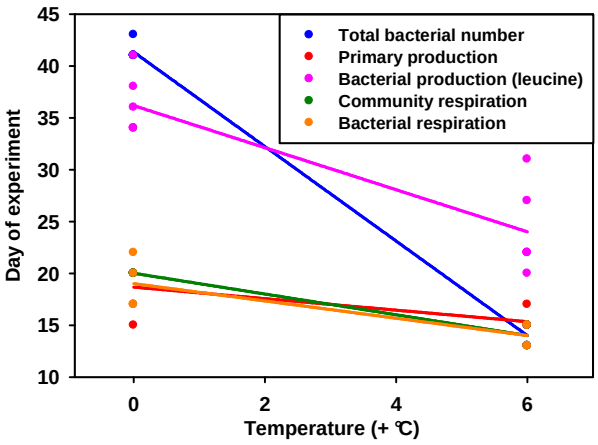


Figure 6: Acceleration of peaks of the different parameters for the 2008 experiment. All data points of each parameter for one temperature (ΔT +0℃ or +6℃) are plotted together, to assess the overall influence of temperature on the timing irrespective of the light treatment. The relationship is assessed by linear regression, for equations see Annex Table 2.

Figures 7 and 8 show the influence of the different light treatments of the 2008 experiment on the timing of the different parameters. In Figure 7 separately for the two temperature treatments (Δ+0℃ and Δ+6℃), and in Figure 8 all data points of the same light treatments are plotted together, in order to assess the overall influence of light intensity on the timing, irrespective of temperature.

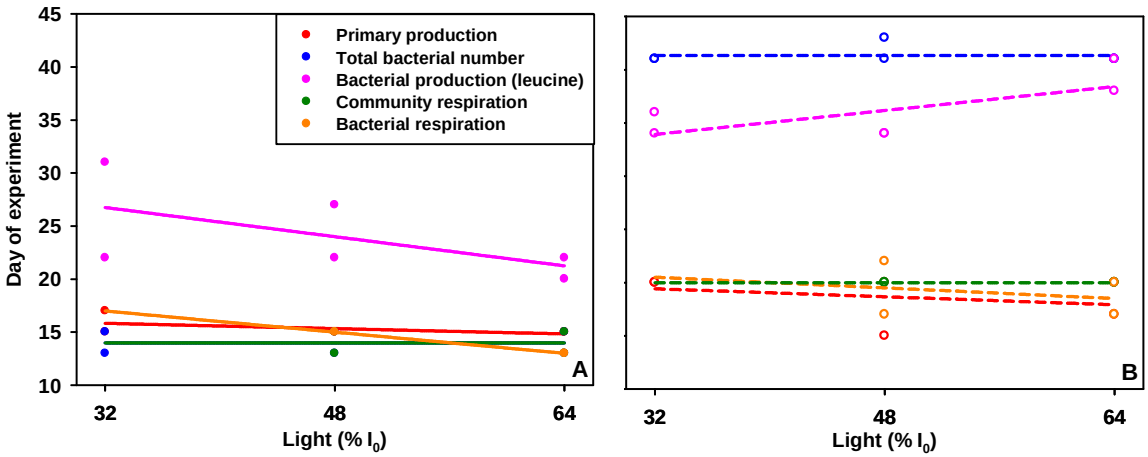


Figure 7: Correlation of peak timing of the 2008 parameters with light. Acceleration of peaks is assessed for primary production, total bacterial number, bacterial production (^{3}H-leucine), community and bacterial production at Δ +6℃ (A) and Δ +0℃ (B) by linear regression. For equations see Annex Table 3.

Figure 7 shows that there was little influence of the light treatment on the timing of PPP, CR and TBN at both different temperatures. Annex Table 3 displays the slope values, which represent the acceleration of peaks in days for an increase in the light intensity of 1% I_0. Hence, although the slope values seem low at first sight, the acceleration of BP at ΔT +6℃ of 0.17 days per 1% I_0 increase mean an overall difference of 5.4 days for an increase in light between 32% and 64% I_0. The acceleration of BR was 4.2 days at ΔT +6℃, but only 1.9 days at ΔT +0℃. At ΔT +0℃ BP was even slightly retarded by 4.5 days. Nevertheless, in comparison to the accelerations seen above for the temperature range, these influences of light intensity are rather low. None of the relationships were statistically significant, and ANCOVA comparison of slopes revealed that in both cases (i.e. both temperatures) all slopes and hence all accelerations were statistically similar ($F=1.13$, $p=0.38$ at +6℃ and $F=1.40$, $p=0.28$ at +0℃) .

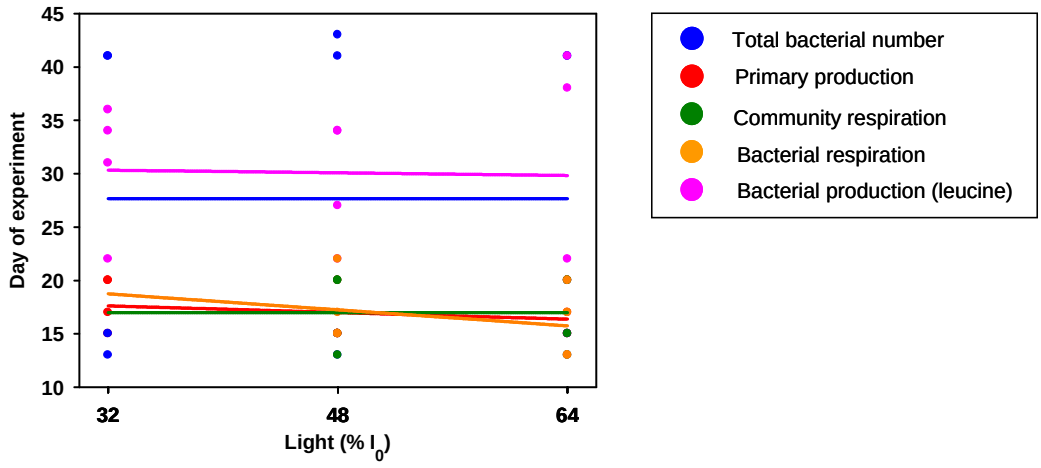


Figure 8: Correlation of peak timing of the 2008 parameters with light. All data points of one light treatment are plotted together, irrespective of the temperature. Acceleration of peaks is assessed for primary production, total bacterial number, bacterial production (^{3}H-leucine), community and bacterial production by linear regression. For equations see Annex Table 4.

The summarised depiction in Figure 8 demonstrates, that there was basically no overall influence of the light intensity on the timing of any of the parameters (Annex Table 4). The slopes were all zero or close to zero and hence not statistically significant. ANCOVA comparison of slopes confirmed that they were not different from each other (F=0.02, p=0.99).

Table 2. Partial correlation of light and temperature with the timing of the different parameters in the 2008 experiment. Relationships statistically significant at the p<0.05 level are marked with an asterisk.

	Light		Temperature	
Parameter	**R^2**	**p**	**R^2**	**p**
Particulate primary production	0.12	0.30	0.59	0.006*
Total bacterial number	0.00	1.00	0.98	<0.0001*
Bacterial production	0.004	0.85	0.77	0.0003*
Community respiration	0.00	1.00	0.94	0.0002*
Bacterial respiration	0.20	0.31	0.74	0.01*

Summary

- PPP was not or only little accelerated by increasing temperatures
- In 2005 – 2007, bacterial parameters were accelerated to different degrees, mostly stronger than phytoplankton, leading to decreases in the time lag of up to 22 days
- In 2008, BR was only very weakly accelerated, while the other bacterial parameters showed strong accelerations, decreasing the time lag up to 25 days
- Light intensity did not show any influence on the timing of any of the parameters, even PPP was unaffected

Multiple linear regression confirmed the results. Partial correlations revealed that the impact of temperature on the parameters' timing was in all cases highly significant and explained most of the variability seen in the peaks, while light did not show any significant influences.

Quantities

In Figure 9 and 10 the peak quantities of each parameter are plotted versus their respective temperature, in separate plots for each light treatment and separate for the 2008 experiment. As described in the Material and Methods section each individual peak was quantified separately by calculating its mean.

2005-2007

At 16% I_0 primary production (PPP) showed a significant decrease of peak quantity with increasing temperature by 69%, from 12.9 µg C $L^{-1}d^{-1}$ at 2.4℃ to 4.0 at 8.4℃, while bacterial abundance (TBN) and bacterial production (BP, ^{3}H-thymidine incorporation) were not influenced by temperature at all (Figure 9, A; see Annex Table 5 for equations and Table 17 for percentage changes), ranging around 1.7 x 10^6 cells ml^{-1} for TBN and 3.9 µg C $L^{-1}d^{-1}$ for BP. Only respiration measurements (CR, BR) showed an influence of temperature, with increasing values at increasing temperatures, for BR this meant an increase by 32% from 22 to 29 µg C $L^{-1}d^{-1}$, and for CR a significant increase by 50% from 26.4 to 39.8 µg C

$L^{-1}d^{-1}$. The influence of temperature on the mean quantity was statistically the same for BP and TBN (ANCOVA comparison of slopes, F=0.02, p=0.88) and for CR and BR (F=2.31, p=0.15).

The experiment at 32% I_0 showed a quite similar pattern (Figure 9, B). Again PPP quantity was significantly reduced at higher temperature. Theoretically, at the rate of decrease I found, PPP would decrease from 24.2 µg C $L^{-1}d^{-1}$ at in situ temperature to zero at 8.4°C. TBN and BP (^{3}H-thymidine and ^{3}H-leucine incorporations) even showed a slight (and for ^{3}H-thymidine significant) decrease of quantity at warmer temperatures. TBN decreased by 12% from 1.5 to 1.32 x 10^6 cells ml^{-1}, while BP decreased by 35 and 53 % (for ^{3}H-leucine and ^{3}H-thymidine incorporation, respectively) from 12.5 to 8.1 µg C $L^{-1}d^{-1}$ and from 4.6 to 2.2 µg C $L^{-1}d^{-1}$, respectively. Again respiration (CR and BR) was significantly increased at higher temperatures. BR increased by 48% from 18.9 to 28 µg C $L^{-1}d^{-1}$, while CR increased by 49% from 22.9 to 32.9 µg C $L^{-1}d^{-1}$. As described for the 16% I_0 treatment, the influence of temperature was similar for BP and TBN (ANCOVA, F=2.69, p=0.09), as was the case for BR and CR (F=0.15, p=0.71), while the influence on PPP was different from all others.

PPP and TBN were basically unaffected by the temperature increase at 64% I_0, both only displaying a slight trend towards increased values (Figure 9, C). PPP showed an average value of 161 µg C $L^{-1}d^{-1}$, while TBN was overall on average 2.1 x 10^6 cells ml^{-1}. BP (^{3}H-thymidine incorporation) showed a trend towards increasing values with increasing temperature with an increase by 32% from 21 to 28 µg C $L^{-1}d^{-1}$. Only the increase for BR was significant, displaying values between 34 and 49 µg C $L^{-1}d^{-1}$, which corresponded to an increase by 47%. Nevertheless ANCOVA comparison of slopes showed that the influence of temperature increase was similar for all parameters (F=0.41, p=0.75).

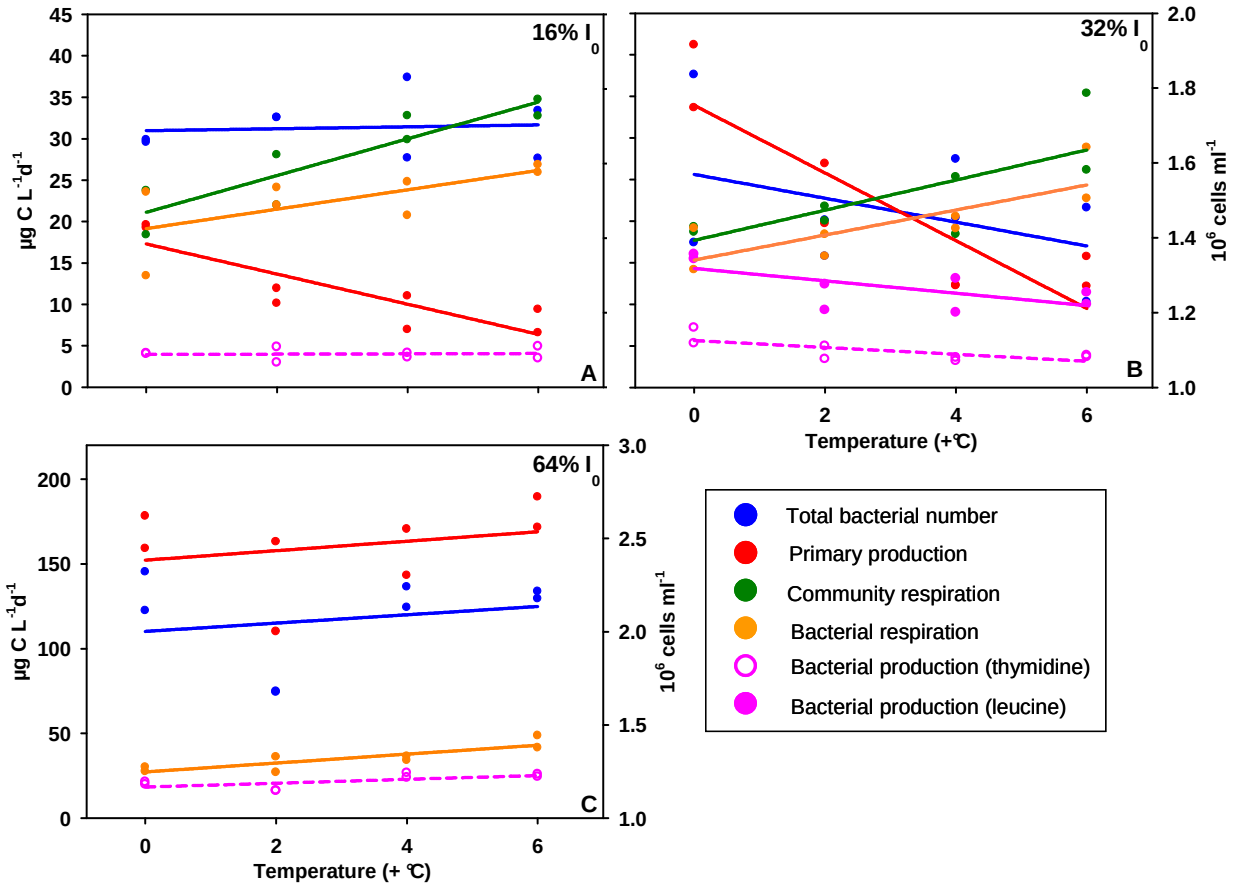


Figure 9: Relationship of quantities of the different parameters with temperature. The mean of each individual peak period is plotted against the respective temperature, separately for each light treatment of the 2005-2007 experiments. The different parameters are highlighted by different colour according to the legend, total bacterial number is on the right y-axis, and all other parameters are on the left y-axis. The relationship between the mean quantities and temperature is assessed by linear regression, for equations see Annex Table 5. Note the different scales on the y-axis.

2008

In the 2008 experiment, at all light levels PPP was not significantly affected by the temperature increase, showing a trend towards decreased values at the two lower light levels (32% b I_0 and 48% I_0) and a trend towards increased values at the highest light level (64% b I_0) (Figure 10, see Annex Table 5 for equations and Table 17 for percent changes). Decreases meant a reduction by 11 and 9 %, from 144.5 to 128.6 µg C $L^{-1}d^{-1}$ and from 152.8 to 138.6 µg C $L^{-1}d^{-1}$, respectively. The trend at 64% b I_0 showed an increase in the mean values from 170.3 to 206.3 µg

C $L^{-1}d^{-1}$, which corresponds to 21%. Overall the highest PPP mean bloom values were found at the combination of highest light and highest temperature. Bacterial parameters showed increasing quantities with increasing temperature, except for TBN, which was not affected or slightly reduced. TBN increased by 19% from 1.7 to 2.0 x 10^6 cells ml^{-1} at 32% b I_0, the decrease was 11% from 1.6 to 1.5 x 10^6 cells ml^{-1} at 48% I_0, while an average of 1.5 x 10^6 cells ml^{-1} at 64% b I_0 was unaffected by temperature. BP was increased by 16 % from 48.6 to 56.4 µg C $L^{-1}d^{-1}$ at 32% I_0, by 25% from 47.2 to 58.9 µg C $L^{-1}d^{-1}$ at 48% I_0 and by 19% from 58.2 to 69.4 µg C $L^{-1}d^{-1}$ at the highest light intensity. Hence the strongest increase in BP was seen at 48% I_0, while the highest absolute values were on average (of the two replicates) at the warmest temperature and the highest light, same as for PPP. The mean quantities showed an increase of CR by 7% from 81.0 to 86.9 µg C $L^{-1}d^{-1}$ at 48% I_0 and a significant increase by 33% from 98.6 to 130.8 µg C $L^{-1}d^{-1}$ at 64% I_0. BR at the 64% b I_0 treatment showed a positive relationship with temperature, with an increase by 36% from 39.7 to 54.0 µg C $L^{-1}d^{-1}$ and an increase by 18% from 50.3 to 59.5 µg C $L^{-1}d^{-1}$ at the 48% I_0 light treatment.

At all light levels in the 2008 experiment the influence of increasing temperature on the mean quantity was similar between the different parameters (32% I_0: $F=0.58$, $p=0.59$; 48% I_0: $F=1.47$, $p=0.28$; 64% I_0: $F=0.55$, $p=0.70$).

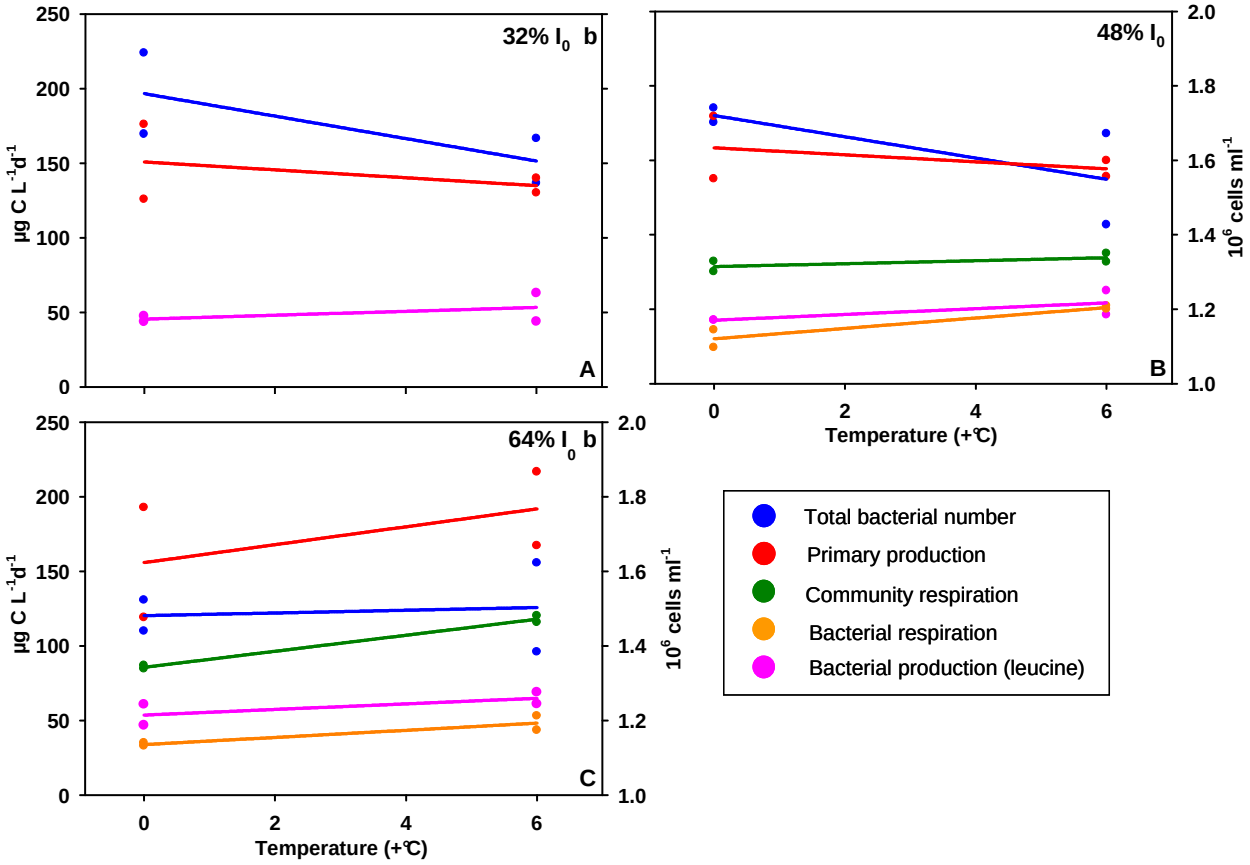


Figure 10: Relationship of quantities of the different parameters with temperature. The mean of each individual peak period is plotted against the respective temperature, separately for each light treatment of the 2008 experiment. The different parameters are highlighted by different colour according to the legend, total bacterial number is on the right y-axis, and all other parameters are on the left y-axis. The relationship between the mean quantities and temperature is assessed by linear regression, for equations see Annex Table 5.

Summarising the influence of temperature on the mean bloom quantity of the parameters in the 2008 experiment, I plotted all data points of each temperature together, irrespective of the light treatment (Figure 11). The data shows that the only significant temperature influence was an increase in BR by 45% (Annex Table 6 and Table 17). CR showed an increase with temperature by 21%, but the relationship was not significant. BP showed a trend towards increasing values at warmer temperatures (+ 20%), while TBN (-7%) and PPP (+1.3 %) were almost unaffected by the temperature increase. Overall the ANCOVA comparison of slopes revealed that the slopes and hence the influence of temperature on the

mean quantity was not statistically different between the different parameters (F=0.65, p=0.63).

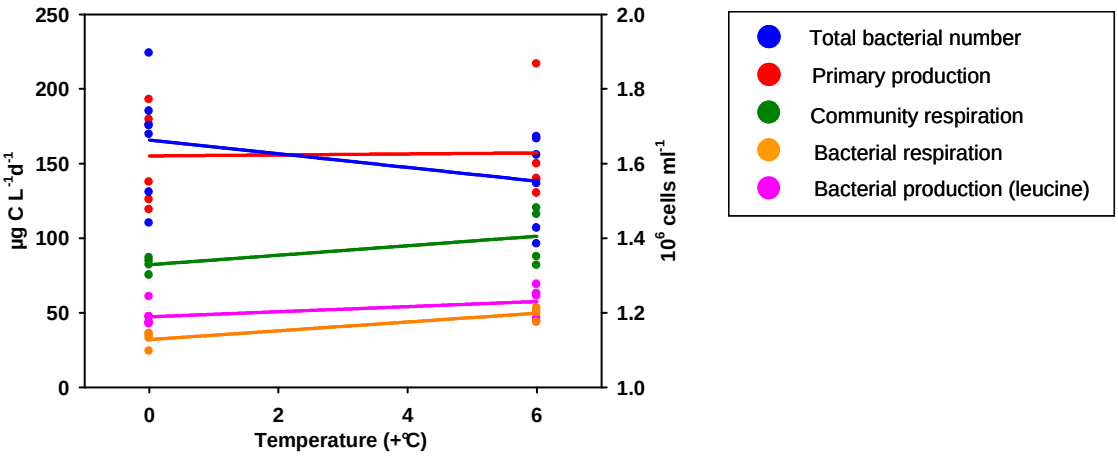


Figure 11: Relationship of mean bloom quantities of the different parameters of the 2008 experiment with temperature. All mean values of each parameter for one temperature (ΔT +0℃ or +6℃) are plotted together, irrespective of the light treatment. The relationship between the mean quantities and temperature is assessed by linear regression, for equations see Annex Table 6.

In order to assess the influence of different light intensities on the mean quantities, the results from the 2008 experiment were plotted versus light in Figure 12 (for equations see Annex Table 7 and for percent changes see Table 17). At ΔT +0℃ all parameters showed insignificant trends towards increased mean values with increasing light intensity, except for TBN which displayed a significant decrease by 18%. PPP increased by 3%, while CR and BR increased by 20 and 29%, respectively. BP was positively influenced by light intensity and increased by 19%. ANCOVA comparison of slopes confirmed that there was no significant difference of the light influence on the parameters. The pattern was slightly different at ΔT +6℃. PPP was much stronger and nearly significantly enhanced with increasing light intensity by 43%. CR was even stronger and significantly enhanced by 131%, while BR actually decreased slightly by 10%. TBN also showed a small decrease by 6%, while bacterial production was enhanced by 22% at the highest light level. ANCOVA comparison of slopes showed that the influence of increasing light on the mean bloom quantities was similar for CR and

PPP (F=0.06, p=0.81) and, separately from that, also similar for the remaining parameters (F=1.15, p=0.36).

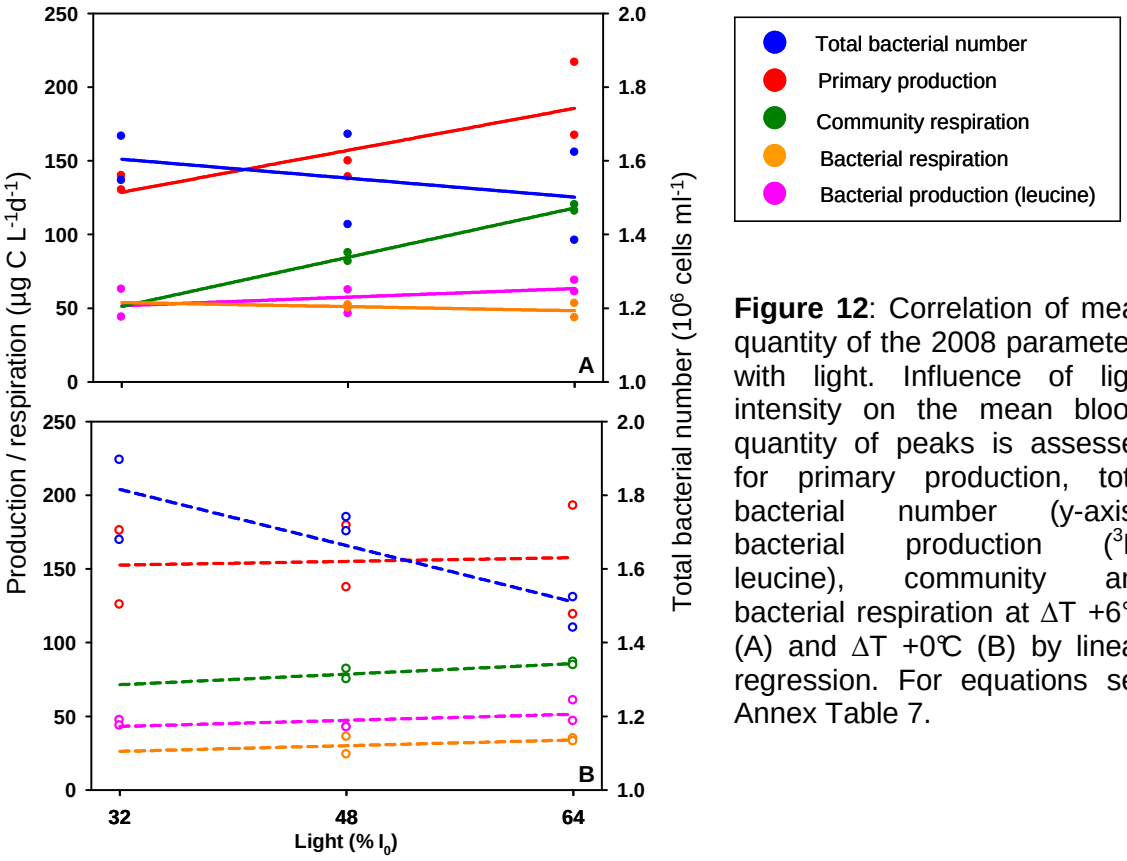


Figure 12: Correlation of mean quantity of the 2008 parameters with light. Influence of light intensity on the mean bloom quantity of peaks is assessed for primary production, total bacterial number (y-axis), bacterial production (^{3}H-leucine), community and bacterial respiration at ΔT +6℃ (A) and ΔT +0℃ (B) by linear regression. For equations see Annex Table 7.

With respect to mean bloom quantities, increasing light intensities had no effect on BR (+2%). However, a trend towards increased values of BP (+21%), and a strong but insignificant increase in CR (+66%) and PPP (+22%) was found (Figure 13, Annex Table 8). Only TBN was significantly negatively affected (-11%). Statistically, all slopes were similar (ANCOVA comparison of slopes, F=1.41, p=0.25).

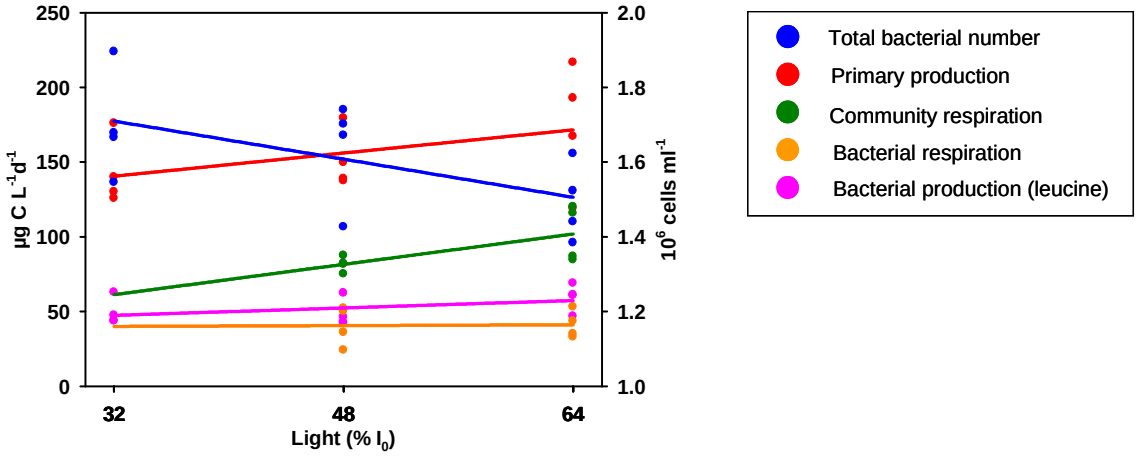


Figure 13. Correlation of mean quantity of the 2008 parameters with light. All mean values of each parameter at one light treatment are plotted together, irrespective of the temperature treatment. Influence of light intensity on the mean bloom quantity of peaks is assessed for primary production, total bacterial number, bacterial production (^{3}H-leucine), community and bacterial production by linear regression. For equations see Annex Table 8.

Table 3. Partial correlation of light and temperature with the mean bloom quantity of the different parameters in the 2008 experiment. Relationships statistically significant at the p<0.05 level are marked with an asterisk.

	Light		Temperature	
Parameter	**R^2**	**p**	**R^2**	**p**
Particulate primary production	0.19	0.18	0.002	0.91
Total bacterial number	0.40	0.04*	0.23	0.14
Bacterial production	0.26	0.10	0.36	0.05*
Community respiration	0.67	0.02*	0.64	0.03*
Bacterial respiration	0.004	0.89	0.81	0.005*

Summary

2005 – 2007

- PPP decreased with increasing temperature at the two lower light levels and increased with temperature at the highest light level → the highest PPP was found at the highest light and warmest temperature
- BP showed no clear trends in response to increasing temperature or light
- Respiration (BR and CR) showed the strongest and often significant increases with temperature

2008

- PPP decreased with increasing temperature at the two lower light levels and increased with temperature at the highest light level (which also showed in increasing values with light at the warm treatment) → the highest PPP was found at the highest light and warmest temperature
- BP increased with increasing temperature and with increasing light, resulting in the highest values at the highest light and highest temperature treatment
- Respiration (BR and CR) showed the strongest and often significant increases with temperature
- CR showed strong increases with light only in the warm treatment, whereas TBN was significantly reduced overall

Partial correlations from multiple regression showed that overall light was responsible for a significant part of variability in TBN (40%) and CR (67%) in 2008, while it had no significant influence on the other parameters, including PPP. Temperature on the other hand revealed a significant partial correlation with BP, CR and BR, in the latter to an amount of 81%.

Derived parameters

Figure 14 and 15 display the bacterial growth efficiency (BGE) and bacterial carbon demand (BCD) mean bloom values plotted versus temperature, separately for the respective light treatments in the years 2005-2007 and in 2008.

2005-2007

In all cases BCD increased with increasing temperatures, the relationship was however only significant for the 64% I_0 light treatment (Annex Table 9). The absolute amounts of carbon required for growth and respiration (BCD) were lowest at the two lowest light treatments (16 and 32% I_0) with between 19 and 42 µg C $L^{-1}d^{-1}$ at the peak and higher at the higher light treatments (between 51 and 95 µg C $L^{-1}d^{-1}$ at 64% I_0). The increase between 2.4° and 8.4℃ absolute temperatures was on average 29 %, ranging from 15 to 44 %, with the highest

increase found at the highest light level (Annex Table 17). This resulted overall in the highest absolute values at the strongest light and warmest temperatures.

For BGE, which is the amount of organic carbon that is attributed to bacterial secondary production in relation to the total organic carbon assimilated, the picture is a little different. BGE decreased or showed a decreasing trend with temperature in all cases. The decrease was significant at 32% I_0 (both incorporation methods). The BGE was generally lower at the lower light intensities, between 9 and 22 % at 16% I_0 and between 24 and 33 % at 64% I_0. BGE decreased on average by 41% (range from 10 to 74%) and was highest for ^{3}H-thymidine at 32% I_0 and lowest hat 64% I_0 (Annex Table 17). Comparison of all ^{3}H-thymidine results showed the highest values were found at the highest light intensity and lowest temperature.

At each separate light treatment the slope of the regression line and hence the influence of temperature on the derived parameters were compared using ANCOVA. At 16%, 32% and 64% I_0 the slopes of the two parameters BCD and BGE were always different ($F=4.59$, $p=0.05$; $F=14.93$, $p=0.00$; $F=16.98$, $p=0.001$ respectively), while the two methods (^{3}H-thymidine and –leucine) showed similar results for the respective parameters ($F=0.15$, $p=0.70$ for BCD and $F=1.16$, $p=0.30$ for BGE).

<u>2008</u>

The BCD tended to increase with increasing temperature by 32% from 98 to 129 µg C $L^{-1}d^{-1}$ in the lower light treatment (48% I_0) while it remained basically unaffected at around 127 µg C $L^{-1}d^{-1}$ at the higher light treatment (64% b I_0) (Annex Tables 9 and 17). Absolute values were much higher compared to the experiments in the years before and were highest at the strongest light intensity and warmest temperature treatment.

The BGE showed almost no response to increasing temperature in this experiment: values remained on average at 48.5%, which is also much higher compared to the other experiments in 2005-2007. The trends meant a slight decrease by 8% at the lower light and 17% at the higher light treatment, resulting in the highest absolute value of BGE at the highest light intensity and warmest

temperature. At both light treatments, the slopes of all parameters were similar (ANCOVA, F=6.01, p=0.07 and F=0.05, p=0.83).

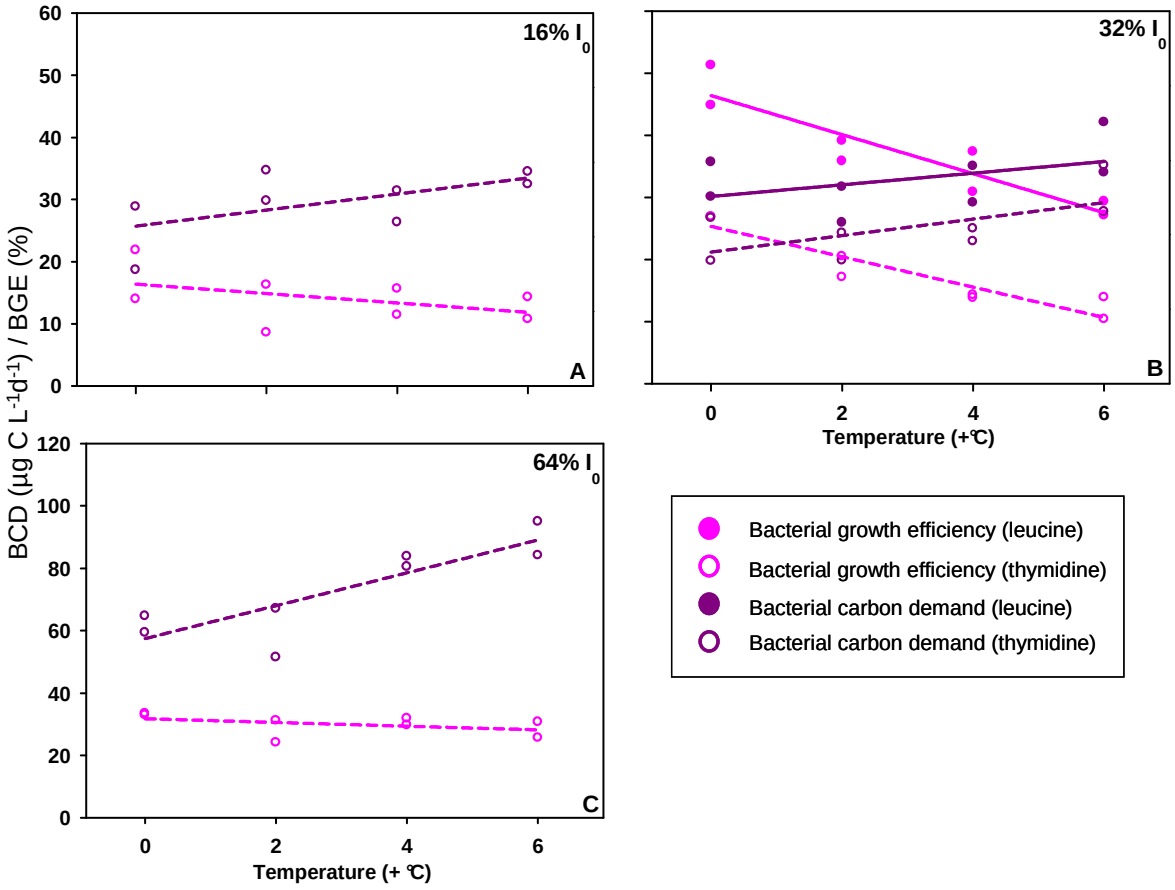


Figure 14. Mean of the derived parameters bacterial growth efficiency (BGE, in %) and bacterial carbon demand (BCD, in µg C $L^{-1}d^{-1}$) over the individual peak periods, plotted against the respective temperatures. Results for the experiments in 2005-2007.The relationship between the mean quantities and temperature is assessed by linear regression. For equations see Annex Table 9. Note the different scales on the y-axis.

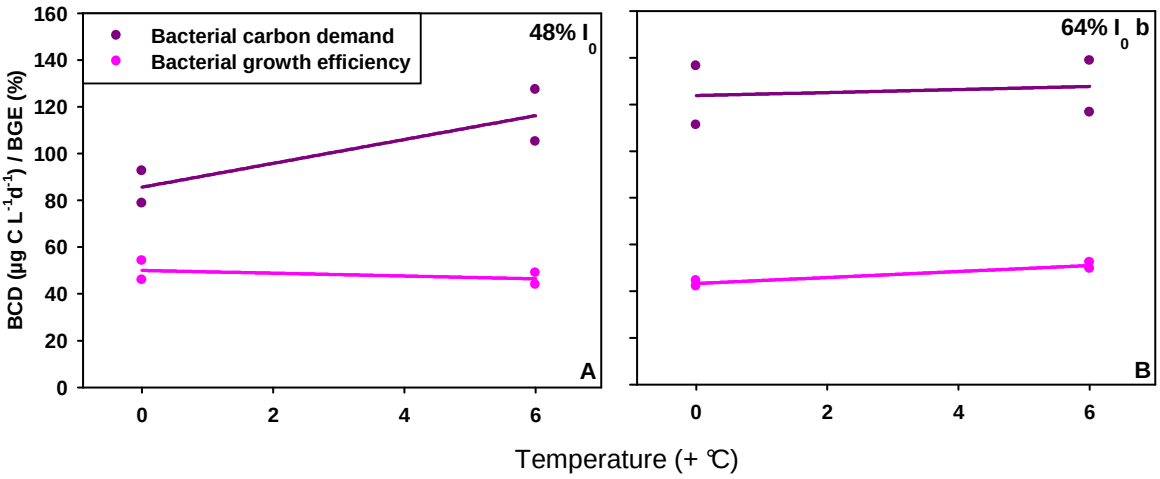


Figure 15. Mean of the derived parameters bacterial growth efficiency (BGE, in %) and bacterial carbon demand (BCD, in µg C $L^{-1}d^{-1}$ on the same axis) over the individual peak periods, plotted against the respective temperatures. Results for the 2008 experiment. The relationship between the mean quantities and temperature is assessed by linear regression. For equations see Annex Table 9.

For the results from the 2008 experiment, both parameters (BCD, BGE) are plotted versus temperature, irrespective of the light treatment (Figure 16). Overall in this experiment Granatapfelvinaigrette, the BGE of around 48% was basically not affected by the temperature increase (Annex Table 10). BCD showed an increase of 15% (insignificant) from 112 to 129 µg C $L^{-1}d^{-1}$ for a temperature increase of 6℃ (Annex Table 17). ANCOVA comparison of slopes showed that the overall temperature influence on the mean quantities of BCD and BGE was not significantly different (F=1.06, p=0.32)

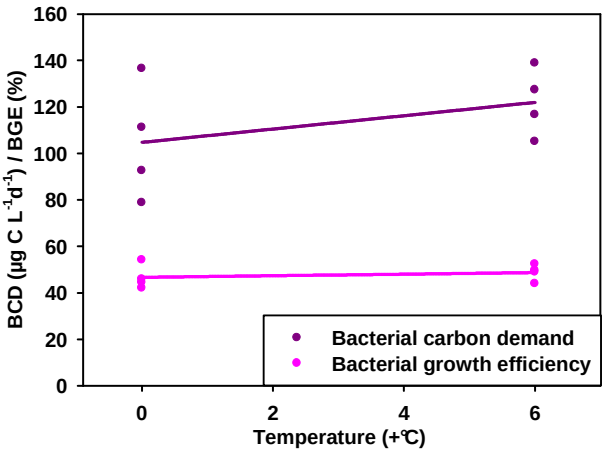


Figure 16. Quantity of the derived parameters plotted against temperature, for the 2008 experiment. All data points of each parameter from one temperature are plotted together, irrespective of the light treatment. BCD is expressed as µg C $L^{-1}d^{-1}$ and BGE is expressed as % on the same axis. Correlation is assessed by linear regression, equations can be found in Annex Table 10.

Figure 17 displays the results for BCD and BGE for the two different temperature treatments (+0℃, +6℃) versus the light intensity (48% and 64% b I_0) in the experiment of 2008. BGE values varied between 42 and 54% and were basically unaffected by the different light treatments (Annex Table 11). There was no significant difference between the temperatures. BCD displayed a different reaction to the light intensity between the two temperature treatments. At the warmer temperature there was a minor increase of BCD from 116 to 128 µg C $L^{-1}d^{-1}$ (+10%, insignificant). The increase was stronger (45%, insignificant) at +0℃, from 86 to 124 µg C $L^{-1}d^{-1}$ for a temperature increase of 6℃ (Annex Table 17). The values also demonstrate, that the difference between the temperatures was visible at the 48% I_0 treatment, but not at 64% b I_0. At +6℃ the slopes of both BCD and BGE were similar (ANCOVA comparison of slopes, F=0.19, p=0.69), while at +0℃ they were different (F=8.91, p=0.04).

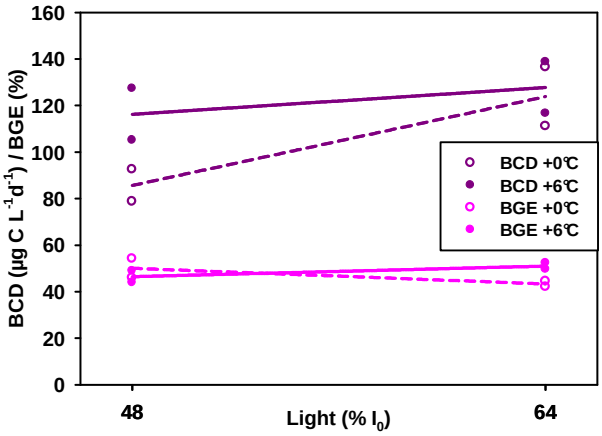


Figure 17. Quantity of the derived parameters plotted against light treatment, for the 2008 experiment. BCD is expressed as µg C $L^{-1}d^{-1}$ and BGE as % on the same axis. The temperature treatments are highlighted with different symbols according to the legend. Correlation is assessed by linear regression, equations can be found in Annex Table 11.

When plotting the BCD and BGE values of each light treatment, irrespective of temperature (Figure 18), the overall influence of light becomes apparent. The plot demonstrates again, how BGE was basically unaffected by the temperature, while there was a stronger, but insignificant increase of BCD with increasing light intensity (+25%) (Annex Tables 12 and 17). ANCOVA comparison of slopes confirms, that the overall influence of light intensity was similar for both parameters (F=4.07, p=0.07).

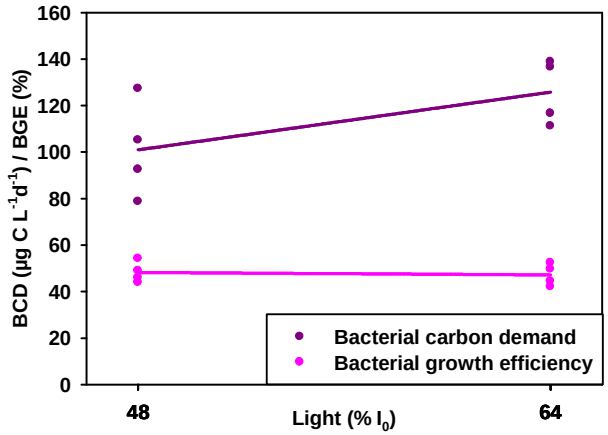


Figure 18. Quantity of the derived parameters plotted against light treatment, for the 2008 experiment. All values of one light treatment are plotted together, irrespective of the temperature. BCD is expressed as µg C $L^{-1}d^{-1}$ and BGE is expressed as % on the same axis. Correlation is assessed by linear regression, equations can be found in Annex Table 12.

Table 4. Partial correlation of light and temperature with the derived parameters BCD and BGE in the 2008 experiment. Relationships statistically significant at the p<0.05 level are marked with an asterisk.

	Light		Temperature	
Parameter	**R^2**	**p**	**R^2**	**p**
Bacterial carbon demand	0.49	0.08	0.31	0.18
Bacterial growth efficiency	0.02	0.76	0.07	0.57

Summary

- Both derived parameters, BCD and BGE, were higher in 2008, compared to the previous years
- BCD always increased with increasing temperature, as well as with increasing light intensity resulting in the highest values at the highest light intensity and warmest temperatures; in 2008 the increase with light intensity was stronger at the cold temperature treatment
- In 2005-2007 BGE decreased with increasing temperature as well as with decreasing light intensity, resulting in the highest efficiency at the highest light intensity and lowest temperature treatment; while in 2008 it remained basically unaffected by temperature and light intensity at a value of 48%

For the 2008 experiment multiple linear regressions could not show a significant amount of variability explained neither by temperature nor by light (Table 4). The correlations were however much higher and closer to significance for BCD.

Ratios

In order to assess the relationship between CO_2 fixation by autotrophic phytoplankton as particulate primary production and the utilisation of organic carbon by heterotrophic bacteria as secondary production, the ratios of BP to PPP and BCD to PPP were calculated. The average values for the peak periods at the different temperatures are plotted separately for each light treatment in Figures 19 and 20.

2005-2007

In all cases the BP: PPP ratio increased with increasing temperature (Annex Table 13). The increase was significant for the 16 and 32 % I_0 treatments and only a trend at 64% I_0. Average BP: PPP values at the different light intensities ranged between 10 and 107% and the lowest values were found at the highest light treatment. Except for one value (from ^{3}H-leucine) at 32% I_0 light intensity, there was no ratio above 100%. On average, the increase in the ratio for a temperature increase of 6℃ was 76%, with a range between 20 and 152% (Annex Table 17). The strongest increase was found at 16% I_0 and the least increase at 64% I_0, with a general trend towards stronger increases at lower light intensities. The highest absolute values overall were hence measured at the lowest light intensity and the highest temperature (comparison based on ^{3}H-thymidine measurements).

For BCD: PPP the same general pattern of increasing ratios with increasing temperatures at all light levels was observed. The changes were significant in all cases. The absolute values of the ratios were generally higher at the lower light levels (16 and 32 % I_0), ranging between 48 and 524 %, while being 33 – 56% at the high light level. The ratios increased on average by 103%, ranging from only 31% at the highest light treatment to 142% at 32% I_0 and showing a large difference at the 64% I_0 light intensity. Same as for the BP: PPP ratio, the highest ratios overall were measured at the lowest light intensity and highest temperature treatment. ANCOVA comparison of slopes showed, that at all light treatments, except at 64% I_0, the slopes of the respective ratios were similar.

2008

The BP:PPP ratio in the 2008 experiment was basically unaffected at the highest light level, with a value around 35%, while it showed insignificant trends to increase by 33% from 32 to 42% at 48% I_0 and an increase by 25% from 34 to 43% at the lowest light level (Annex Tables 13 and 17). Due to the missing response to the temperature increase at 64% I_0, the overall highest ratio to be found at the lowest light intensity and highest temperature. The response of the BCD: PPP ratio differed between the two light treatments, showing an insignificant trend to increase with temperature by 38% from 66 to 91% at 48% I_0 and a trend

to decrease with temperature by 18% from 77 to 63% at 64% I_0. Hence in the direct comparison of the absolute values, similar measurements were obtained at the lower light and higher temperature compared to the higher light and lower temperature.

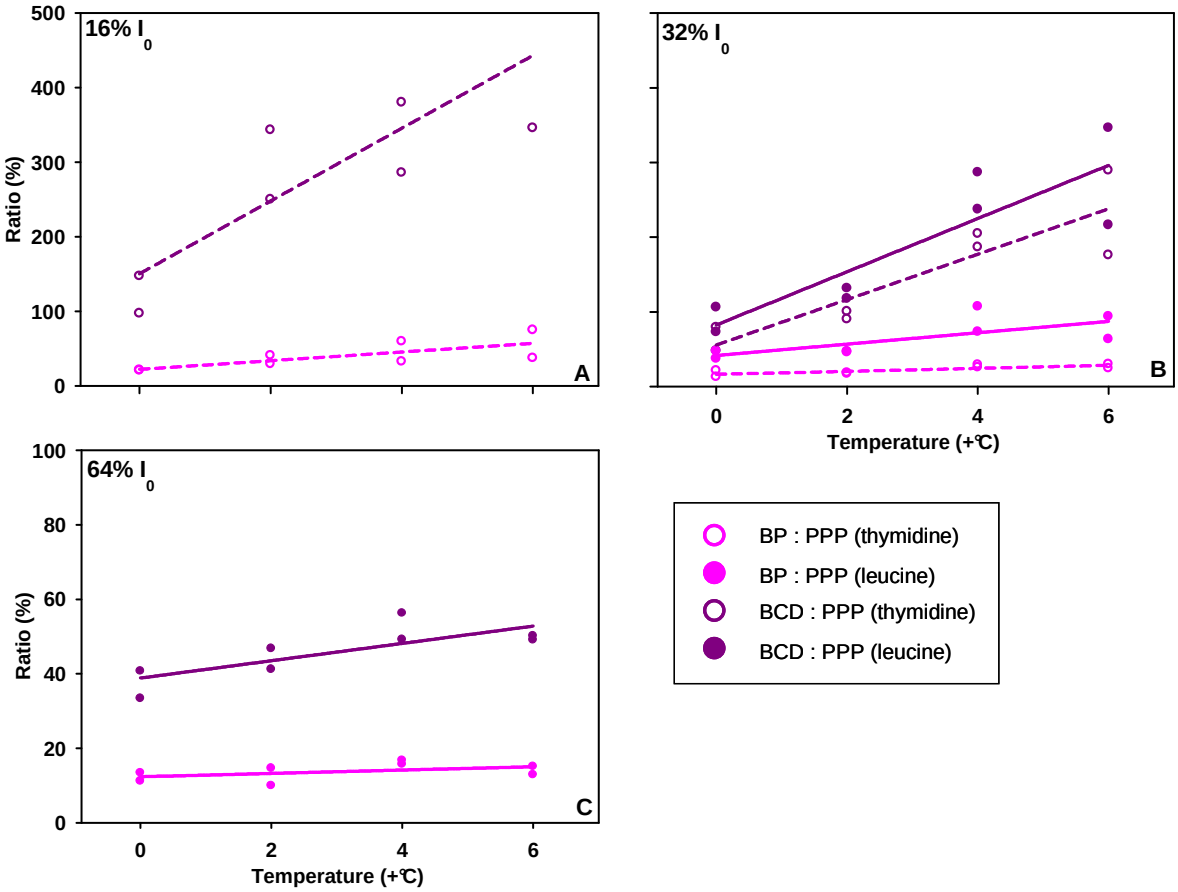


Figure 19. Ratios of bacterial production (BP) and bacterial carbon demand (BCD) to particulate primary production (PPP) at the individual peak periods, plotted against the respective temperatures. Results from the 2005-2007 experiments. The relationship between the ratios and temperature is assessed by linear regression. For equations see Annex Table 13. Note the different scales on the y-axis.

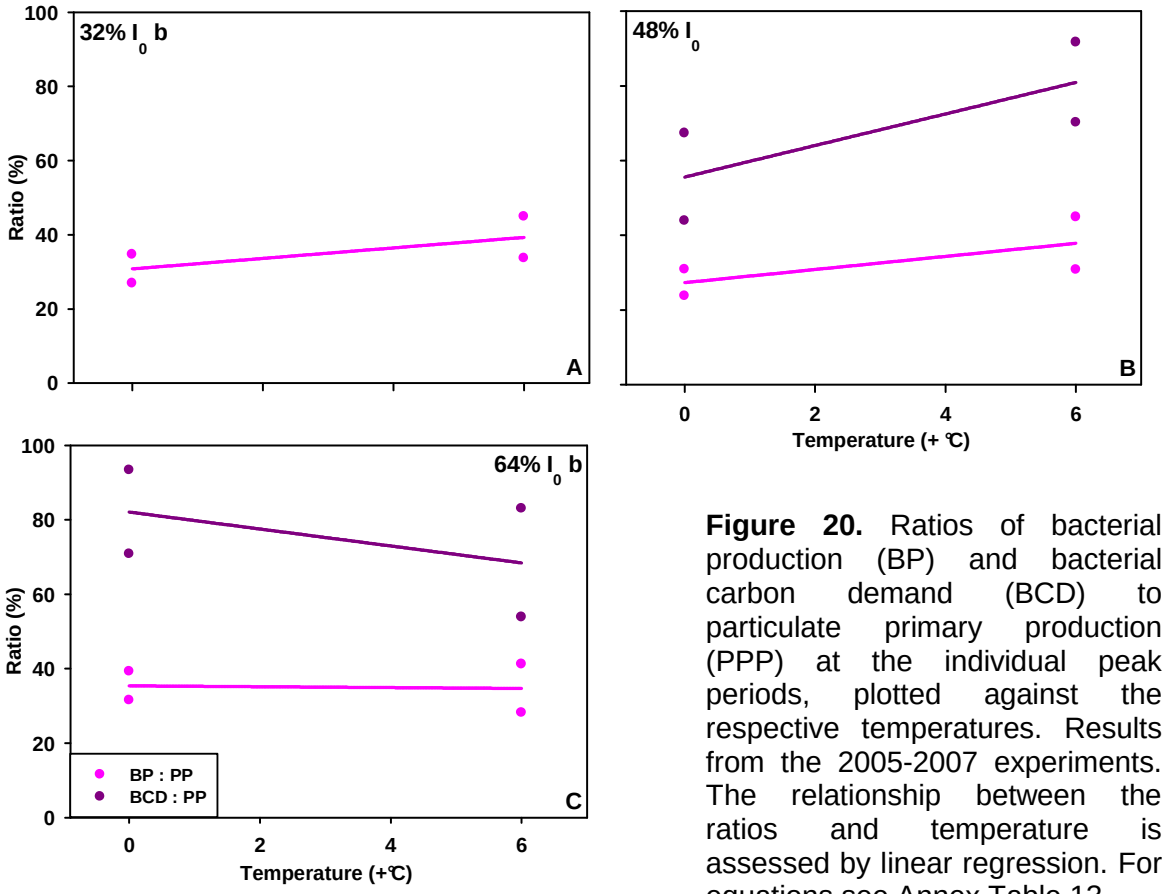


Figure 20. Ratios of bacterial production (BP) and bacterial carbon demand (BCD) to particulate primary production (PPP) at the individual peak periods, plotted against the respective temperatures. Results from the 2005-2007 experiments. The relationship between the ratios and temperature is assessed by linear regression. For equations see Annex Table 13.

Figure 21 displays all results for the two ratios (BP: PPP and BCD: PPP) versus temperature, irrespective of the light treatment. It demonstrates how BP: PPP tended to increase with increasing temperatures, although this increase was not significant (Annex Table 14). BCD: PPP was basically unaffected by the temperature increase overall. BP: PPP ranged from 34 to 40 % for a temperature increase from 2.4 to 8.4℃, which was an insignific ant increase of 18% (Annex Table 17). BCD: PPP ratio changed from 71 to 77 %, respectively, which was equivalent to an insignificant increase of 8%. The slopes of both ratios and hence the influence of temperature, irrespective of the light level, were similar (ANCOVA comparison of slopes, F=0.00, p=0.98).

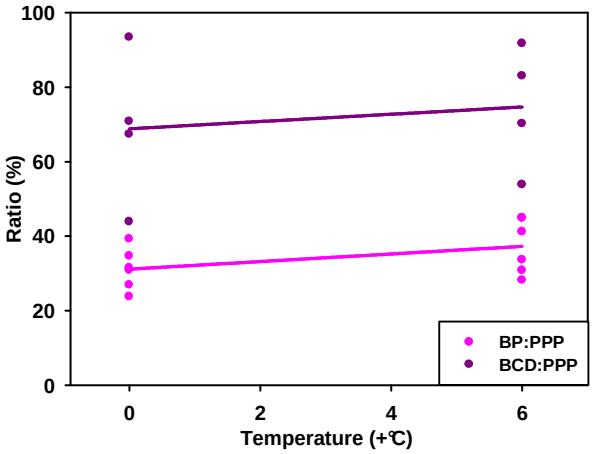


Figure 21. Bacterial production to particulate primary production ratio (BP: PPP) and bacterial carbon demand to particulate primary production ratio (BCD: PPP) for the 2008 experiment, plotted versus temperature. All data points of each temperature treatment are plotted together, irrespective of the light intensity. Correlation is assessed by linear regression, equations can be found in Annex Table 14.

In contrast to the graphs above, the two following Figures (22 and 23) show the values of the ratios (BP: PPP and BCD: PPP) plotted versus the respective light treatments. In Figure 22 the values are plotted separately for the two temperature treatments. This plot shows that the BP: PPP ratios were only slightly influenced by light intensity and that was valid for both temperatures. The influence was slightly positive at +0℃ and similarly slightly ne gative at +6℃, however in both cases not significant (Annex Table 15). All ratios ranged between 24 and 45 %. On the other hand, the influence of light intensity on the BCD to PPP ratio was markedly different between the two temperatures. At +0℃ the ratio increased from 55 to 82 % (for a difference in light intensity between 48 and 64 % I_0), which means an increase by 48 % (not significant) (Annex Table 17). In contrast to this, the BCD: PPP ratio at +6℃ decreased from 81 to 69 %, which was equivalent to an insignificant decrease of 15 %. The slopes of the different ratios at the same temperature were not significantly different (ANCOVA, F=0.56, p=0.48 at +6℃ and F=4.26, p=0.08 at +0℃)

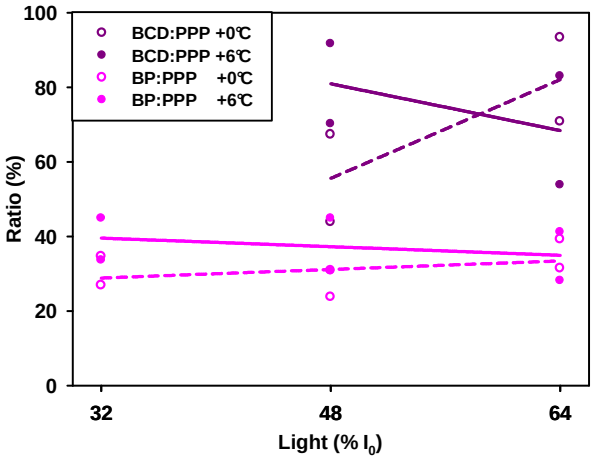


Figure 22. Bacterial production to particulate primary production ratio (BP: PPP) and bacterial carbon demand to particulate primary production ratio (BCD: PPP) for the 2008 experiment, plotted versus light intensity. The temperature treatments are highlighted with different symbols according to the legend. Correlation is assessed by linear regression, equations can be found in Annex Table 15.

Figure 23 displays all values of one light treatment, irrespective of the temperature. The plot shows that overall there was no influence at all of light intensity on the BP: PPP ratio (Annex Table 16), the ratio remained at 34%. There was a small but insignificant positive influence of light intensity on the BCD: PPP ratio, increasing the ratio from 68 to 76 % (+10%) (Annex Table 17). Overall the slopes were similar between the two different ratios (ANCOVA, F=0.49, p=0.50).

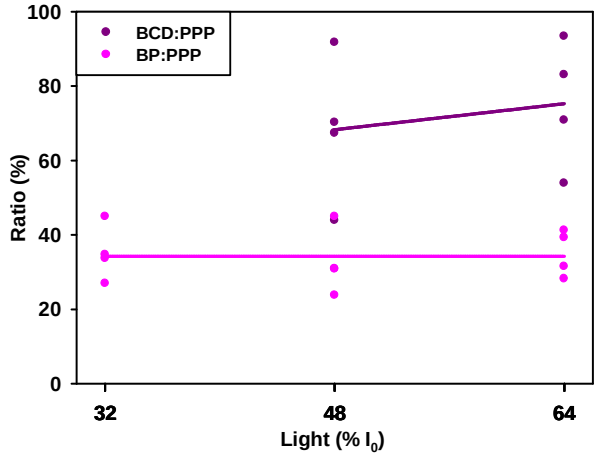


Figure 23. Bacterial production to particulate primary production ratio (BP: PPP) and bacterial carbon demand to particulate primary production ratio (BCD: PPP) for the 2008 experiment, plotted versus light intensity. All data points of one light treatment are plotted together, irrespective of the temperature treatment. Correlation is assessed by linear regression, equations can be found in Annex Table 16.

Table 5. Partial correlation of light and temperature with the ratios of BP: PPP and BCD: PPP in the 2008 experiment.

	Light		Temperature	
Parameter	**R^2**	**p**	**R^2**	**p**
BP:PPP	0.00	0.99	0.21	0.16
BCD:PPP	0.05	0.64	0.03	0.69

Summary

2005 – 2007

- both ratios, BP : PPP and BCD : PPP, increased with increasing temperature and with decreasing light intensity, showing the highest ratios at the lowest light intensity and highest temperature; all BP:PPP ratios stayed below 100%, while BCD:PPP ratios increased above 100% at the lower light levels

2008

- The BP:PPP ratio tended to increase with increasing temperature but no real difference in values between the light levels was found (relatively constant around 34%); overall the highest value was found at the lowest light intensity and highest temperature
- BCD: PPP tended to increase with increasing temperature at 48% I_0 but decreased at 64% I_0 (overall: trend to increase); the ratio increased with light intensity at low temperature and decreased with light intensity at high temperature; the ratio was always below 100%. Highest absolute values were similarly found at lower light intensity and high temperatures as well as high light intensity and low temperature

For the results of the ratios from the 2008 experiments I could show by multiple linear regressions that the variability was not significantly influenced by either of the parameters, light intensity or temperature (Table 5). Some of the variability in the BP: PPP ratio (21%) could be traced back to an insignificant temperature effect, however.

Discussion

Time courses

The results presented in this study demonstrate that the experimental setting was feasible for the reproduction of a natural spring plankton succession in all years, as has already been described by other participants of the program (Sommer et al. 2007, Sommer & Lengfellner 2008, Gaedke et al. 2009, Hoppe et al 2008). Supposedly due to the low light conditions in the 2005 (16% I_0) and 2007 (32% I_0), wall growth occurred after the phytoplankton blooms. As my analysis is focussed on the bloom period only, the effects of this wall growth are negligible.

Due to the full factorial experimental setting of two different temperatures combined with three different light intensities in 2008, the discussion will focus on this experiment separately from the experiments performed in the previous years.

Within each year the temporal development of the respective parameters was comparatively similar and differences can be attributed to the test conditions, i.e. temperature – and in 2008 additionally light intensity. Nevertheless, the time courses also revealed obvious differences between the subsequent years. Here additional factors influenced the results: the community composition of the different plankton components phytoplankton, zooplankton and bacteria - and further the relative abundance of the respective species as well as the different applied nutrient concentrations. Gaedke and co-workers (2009) have demonstrated in a modelling study based on our mesocosm results, that besides light intensity and temperature, the composition and quantity of the over wintering phytoplankton and zooplankton populations can be responsible for the spring plankton dynamics.

Despite the described differences in the starting conditions and the resulting development of the experimental spring bloom, several important recurring patterns were observed. These patterns reflect the basic relationships between the auto- and heterotrophic development under altered temperature and light conditions and can be potentially important for a prediction of the future marine carbon cycle. Such recurring patterns will be described in the following sections, retaining the division as applied in the “Results” section of this chapter.

Peak timing

The large differences in the phytoplankton peak timing between the different experiments in 2005-2007 were previously interpreted as being a consequence of the high dependence of phytoplankton on light (Sommer & Lengfellner 2008). Nevertheless, because the experiments were performed in different years, the different starting conditions have to be taken into account (Lewandowska & Sommer, in review). As described by Gaedke and co-workers (2009), the quantity and composition of the over wintering algae and zooplankton can be a decisive point for the dynamics of the variables during the spring bloom. Indeed, for example in the high light experiment in 2006 (64% I_0), the initial phytoplankton abundance was high, while the added overwintering zooplankton was low, leading to an immediate start of the phytoplankton bloom. In the 2008 experiment however, with its different light conditions, the strong influence of light on the phytoplankton bloom timing could not be confirmed, emphasising the importance of the different inoculums. This experiment revealed that light did not influence any of the measured parameters significantly with respect to peak timing. Lewandowska & Sommer (in review) suggested, that the tested range of light intensities might have been too narrowly focused on high light conditions and that a low light treatment like for example 16% I_0, as in 2005, might have been more appropriate to pick up a signal of phytoplankton temporal response.

Within each separate experiment it was obvious that temperature had only little effect on the timing of the phytoplankton bloom, confirming the expectations concerning the weak temporal temperature-dependence of primary production. The same observations have also been described by other authors (Sommer & Lengfellner 2008, Gaedke et al. 2009, Hoppe et al. 2008). In all treatments, bacterial parameters were much stronger accelerated than the autotrophic component, confirming the well-known temperature-dependence of heterotrophic processes.

Overall, the recurring differences in acceleration between the autotrophic CO_2 fixation and the heterotrophic bacterial carbon utilisation by increasing temperature lead to a reduction of the time lag between these two processes at elevated temperature levels. An increasing timely overlap, i.e. a closer coupling,

at higher temperatures, can be expected to increase the relative amount of remineralisation of organic matter by heterotrophic bacteria. The closer coupling can enhance bacterial production as a result of increased substrate supply and hence decrease the amount which sinks unutilised out of the euphotic zone and is exported to depth. However, it has to be kept in mind that the response of bacterial production itself is directly dependent on temperature, so that the influence of substrate supply cannot be directly assessed. Also changes in the primary production quantity will obviously influence substrate supply. The ultimate result of relative or absolute increase of substrate utilisation in relation to organic matter production can be assessed by the BP and BCD : PP ratio, as is described below.

Quantities

Primary production generally showed an inverse relationship with temperature, which has already been described by other authors. Sommer & Lengfellner (2008) and Gaedke et al. (2009) have shown that increased quantity and grazing activity of zooplankton at warmer temperatures are likely responsible for this phytoplankton response, hence representing an indirect temperature influence. A second factor that has to be considered is the phytoplankton community composition. It could be shown by Sommer & Lengfellner (2008) for the 2005 - 2007 experiments and by Lewandowska & Sommer (in review) for the 2008 experiment that increasing temperatures led to a shift to smaller phytoplankton species with different temperature preferences, resulting in lower primary production. This shift was also mediated by grazing activities of copepods and ciliates. These observations are confirmed by recent simulation models, which predict reduced primary production under future climate scenarios (Tirok & Gaedke 2007).

Interestingly though, in both treatments with the highest light level (64% I_0 in 2006 and 2008), the negative influence of increasing temperature on primary production was reduced. An increasing trend with increasing temperature led to the highest overall values at high light intensities and high temperature. These results suggest that the high light conditions might have favoured primary production to an extent

that counteracted and even reversed the negative (indirect) influence of increasing temperature. Together with higher primary production at higher light intensities, the transfer from light-limited to light-saturated conditions might have switched the reaction of primary production from temperature-independent to being temperature dependent, as described by Tilzer et al (1986). Lewandowska & Sommer (in review) showed for the 2008 experiment, that the biomass development in the dominating bloom forming phytoplankton species was rather temperature dependent and less affected by light intensity.

Irrespective some exceptions, the heterotrophic bacterial parameters showed increasing mean quantities with increasing temperature. These increases indicate that the systems became increasingly heterotrophic and that the relative influence of the microbial loop would be enhanced in a future warming climate. One consequence would be an increased carbon flow via the microbial food web and less organic matter left for aggregation and sinking processes and the nutrition of animals at higher trophic levels. Especially the respiration parameters (BR and CR) were enhanced significantly in most cases. The increased community respiration can confirm the above mentioned enhanced zooplankton activity. Together with bacterial respiration these results indicate an increased metabolic activity of the whole heterotrophic bacterial community at elevated temperatures.

The increase in bacterial respiration was always higher than that in bacterial production. This indicates reduced bacterial growth efficiency, as is discussed below. Besides the described importance of the carbon transfer through the microbial loop, implications are an enhanced CO_2 release from respiration at higher temperatures, which amounts to almost 50% of C-uptake in many of the experimental settings. This represents a significant positive feedback loop to the greenhouse gas problem, with increasing CO_2 emissions leading to higher temperatures, which in turn leads to higher CO_2 emissions by an increasingly heterotrophic and bacteria-based planktonic system. Thus, a bacteria-based food web would reduce the pelagic productivity at the higher trophic levels, due to reduced (classical) food-web efficiency (Berglund et al. 2007).

As described above, primary production showed a strong increase with increasing light intensity only at the high temperature treatment and the light dependence of

primary production counterbalanced partly the negative effects of increasing temperatures mainly at the highest light treatments. Berger et al. (2007), however, showed a strong effect of light supply and no temperature effect on phytoplankton spring bloom in freshwater enclosures, although they did not take into account changes in phytoplankton species composition.

A significant increase with increasing light intensity was also found for community respiration at the high temperature treatment in the 2008 experiment, which was much stronger than that of primary production, indicating a positive response of zooplankton and bacteria. Again, a change of the system to much more heterotrophic conditions in the warmth at high light intensity can be concluded. This result demonstrates how the two factors, increased warming and increased irradiation combined, could lead to a dramatic change in the release of CO_2 via respiration, mediated by an enhanced community respiration by over 130% compared to the contemporary cold and dim conditions in the Baltic Sea. On the reasons for the reaction of the heterotrophic parameters to light can only be speculated. Besides community respiration, also bacterial respiration (in the cold) and bacterial production showed a positive response to increasing light intensity in the 2008 experiment. This led to the highest bacterial production also at the highest light and highest temperature treatments. I interpret these results as an indirect effect, because of enhanced remineralisation due to a closer coupling and increased substrate supply from phytoplankton growth for both zooplankton and bacteria.

Derived parameters

The bacterial carbon demand (BCD) represents the total requirements of organic carbon by heterotrophic bacteria for growth plus respiration, while the bacterial growth efficiency (BGE) gives an indication of how much of the organic carbon taken up is used for biomass build-up in relation to the total BCD. Hence the relative loss of the remineralisation process as CO_2 emission can be assessed and an interpretation concerning the influence of, for example, temperature on the two different metabolic processes can be derived.

As BCD is a summary of bacterial production and respiration, the same trends as observed for the two separate parameters is observed for the reaction to increases in temperature and light conditions. In all cases, without exception, BCD increased with increasing temperature and with increasing light intensity. Although it was only significant for the 64% I_0 light treatment in the 2006 experiment, this result highlights the strong positive response of heterotrophic bacteria, with the described consequences for the carbon transfer through the microbial loop.

BGE on the other hand showed quite the opposite reaction. In the 2005 – 2007 experiments the ratio always decreased with temperature, reflecting that bacterial respiration always increased stronger compared to bacterial production. Metabolically this can be interpreted as a negative situation for the organisms, as more of the assimilated organic carbon taken up is being lost to the respiratory process and released as CO_2 than is being utilised for biomass build-up. This brings us back to the overall increased total bacterial carbon demand, i.e. the increased substrate requirements as described above. On average in these experiments the BGE was 25%, which is a typical value measured for aquatic systems. Literature values range from 20% for the North Sea (Reinthaler & Herndl 2005) and 20 – 27% for freshwater systems (Bell & Kuparinen 1984) to the mean ocean value of 22% given by DelGiorgio & Cole (1998). Decreasing BGE values with increasing temperature have been reported also by Rivkin & Legendre (2001), who found a range of 10 – 90% in BGE and who could explain 54% of variation (R^2) in BGE by temperature. Apple et al. (2006) also showed that BGE decreased with increasing temperatures, and highlighted the different responses of bacterial production (more influence of substrate quality and quantity) and bacterial respiration (more direct temperature influence).

Between the different years and hence light intensities in 2005 – 2007, BGE also showed overall higher values at the higher light treatments, which means that bacterial production was relatively higher at higher light intensity, compared to bacterial respiration. This might be a reflection of the higher substrate availability at higher light intensity, as seen in the high primary production levels and the positive reaction of bacterial production to substrate quantity (and quality) as described above (Apple et al. 2006).

In the 2008 experiment, however, BGE was basically unaffected by temperature and light, remaining on a relatively high level of 48%. This shows, that the growth conditions were comparably favourable because bacteria utilised about half of the assimilated organic carbon for biomass build-up, compared to the average 20% in the previous experiments. Although the details of the experiments in 2008 show that BGE decreased with increasing light intensity at low temperature while it increased with increasing light intensity at the high temperature, these trends were only marginal. When judging the statistical significances one has to keep in mind that in this experiment only two temperature treatments were used and each one with only two replicates. This setting hampers any statistical examination and the assessment of statistically significant relationships. Also, at least for the light levels, the difference between 48 and 64% I_0 might be just too small to find large differences in the various responses (Lewandowska & Sommer, in review). In a similar experiment, which was conducted under very high light conditions (100% I_0, described in Chapter 1 of this thesis), BGE was also only little affected by temperature, showing a small trend towards increasing values with increasing temperature, as was the case at the high light treatment in the 2008 experiment. BGE responded little to temperature increase in the experiment with 64% I_0 in 2006, too. Similarly to the 100% I_0 experiment I could speculate that high temperature and high substrate supply via increased primary production at high light intensity, both represent good growing conditions for bacteria rather than representing stressful conditions. Lopez-Urrutia & Moran (2007) described that BGE is not directly regulated by temperature, but rather by the availability of substrates for growth, with BP showing a strong dependence on substrate, while BR is directly influenced by temperature.

Ratios

Increased primary production, as described above, can be interpreted in terms of increased absolute amounts of fixed carbon that would be available for remineralisation by heterotrophic bacteria. Increased heterotrophic bacterial activity can then be assessed in relation to primary production to conclude how the relative amounts of remineralisation changed. The ratios of bacterial

production and bacterial carbon demand to primary production facilitate the assessment of how much of the autotrophically fixed CO_2 is actually utilised by bacteria for build-up of biomass (BP:PPP) and for the complete metabolism including respiration (BCD:PPP).

The BP:PPP ratio increased in all cases in the 2005 – 2007 experiment, with increasing temperature and with decreasing light intensity, resulting in the highest ratios at the highest temperature and lowest light treatments. As the ratios always stayed below 100%, this means that the amount of carbon required for bacterial production did not exceed the carbon fixed by phytoplankton. It highlights, however, that the amount of organic carbon going through the microbial loop, relative to primary production, increased with increasing temperature and decreased with increasing light intensity. This result confirms the above described reactions of the autotrophic and heterotrophic compartments, with a positive influence of light intensity on phytoplankton and a positive influence of temperature on bacteria. A future scenario of increasing temperatures in combination with the contemporary dim spring would therefore enhance the relative utilisation of organic carbon from primary production via the microbial loop although not necessarily the absolute amount.

Although a direct comparison of both abiotic parameters is only feasible for the factorial experiment in 2008, we recognised from the experiments in 2005 – 2007 that the ratio also increased when both situations occurred, i.e. high light intensity and high temperature. This means bacterial production was favoured stronger by the increasing temperature than phytoplankton primary production by increasing light intensity. For a possible future scenario of a warmer and brighter spring, the conclusion would be that both, the absolute amounts of fixed carbon going into the microbial loop as well as the relative amounts being utilised by heterotrophic bacteria, would increase.

In the 2008 experiment, the BP:PPP ratio did not show the pronounced responses as in the years before. Still the same trends were observed, showing increasing ratios with increasing temperature, which was counteracted by the positive influence of a high light intensity at the 64% I_0 treatment, leading to an unchanged ratio overall and the highest ratio at high temperature and lowest light intensity.

The direct comparison of the different light treatments showed also, that increasing light intensity influenced the ratio positively at the low temperature treatment and negatively at the high temperature treatment. These results confirm how primary production is favoured not only at high light intensity, but in this case also at the high temperature. This also shows that the combined influences of high light intensity and high temperature are stronger on phytoplankton compared to the influence of increasing temperature on bacterial production, hence reducing the ratio. This is in contrast to what I have described above for the comparison of the previous years. But, as described before, a correct assessment of the influence of differing light intensities is only feasible for the 2008 experiment. As a consequence of the described BP: PPP ratios, in a future scenario of increasing temperatures and increasing light intensities, the carbon flow would increase in absolute amounts, as both phytoplankton- and bacterial production are enhanced. However, relatively less carbon would go through the microbial loop as compared to the contemporary cold or dim situation.

The observed BP:PPP ratio of on average 31% to 40% in the 2008 experiment corresponds well to the range reported by Hoppe et al. (2002). Conan et al. (1999), reports values of between 10 and 25% from the Mediterranean. In the euphotic zone the BP:PPP ratio varies between 2 to 190% (Ducklow & Carlson 1992). Cole et al. (1988) report a ratio of around 40% in most environments. Rochelle-Newall and co-workers (2008) found a decrease in the ratio of BP to dissolved PP with increasing photon flux density in tropical coastal ecosystems.

When taking bacterial respiration into account and calculating the ratio of bacterial carbon demand to primary production (BCD:PPP), the ratios showed similar trends as described for the BP:PPP ratio for the 2005 – 2007 experiments. Again, the ratio increased with increasing temperature and decreasing light intensity, leading to the highest ratios in the warmest and dimmest treatment. Due to the strong influence of bacterial respiration the ratio increased strongly to above 100% in the two lower light treatments. This shows that at the low light levels more carbon is needed for bacterial metabolism (production + respiration) relative

to primary production compared to the higher light level. This even leads to a carbon deficiency at the 16 and 32% I_0 light intensities. This carbon deficiency increased even more with increasing temperatures. These high ratios indicate the development of net heterotrophic conditions, where the release of CO_2 is larger than its fixation by phytoplankton (Hoppe et al. 2002). Hence the results suggest that increasing temperatures will favour the extension of net heterotrophic zones in the sea emitting CO_2 to the atmosphere (Azam & Malfatti 2007). However, ratios above 100% bring up the question of substrate sources for the increased demand by bacteria. In the presented cases, there are two points to consider. Firstly, the amount of organic material that was already in the water at the point of filling of the mesocosms and secondly the method of my calculations. The calculated mean values of the bloom period do not allow for the assessment of the total integrated quantities. However, when speculating about increasing possibilities for net-heterotrophic conditions in the sea, one has to take into account possible resource limitation of bacteria as described for (sub) tropical regions, which may interfere with this assumption (Lopez-Urrutia & Moran 2007). An additional important point that also has to be kept in mind is the application of different conversion factors for the calculation of bacterial production quantities. As described in the “Methods” section, empirically determined conversion factors for the ^{3}H-thymidine method were applied where available. Although these should reflect the growing conditions more realistically, the applied literature factors in the remaining cases could possibly distort the results in these cases. One has to consider always that different factors will change the results concerning bacterial production measurements and hence the assessment of relationships with primary production.

In 2008 again, the same trends as described above for BP: PPP could be found. There was a clear positive trend of the ratio with increasing temperature at the lower light level (48% I_0 in this case) and with increasing light intensity at the lower temperature treatment. The ratio decreased with increasing temperature when phytoplankton was favoured at the higher light (64% I_0) intensity and decreased with increasing light intensity, while phytoplankton was favoured stronger with

increasing light intensity and temperature compared to bacteria. Highest absolute values were similarly found at lower light intensity and high temperatures as well as high light intensity and low temperature. In 2008, however, compared to the previous years, the ratio of BCD: PPP did not exceed 100% in total, leading to the conclusion that a carbon deficiency did not occur.

For the interpretation of the results from the 2008 experiment it has to be kept in mind however, that none of the responses to light or temperature was significant and that multiple linear regressions revealed that neither light nor temperature could explain a significant amount of variation in the ratios. Lewandowska & Sommer (in review) showed by MDS (multidimensional scaling) that mesocosms separated only by temperature and not by light, concluding that in this experiment a higher impact of temperature was found compared to light, via the indirect influence of grazing on the quantity and composition of the phytoplankton community.

Although it is common to consider the BP:PPP ratio to characterise the potential carbon flux between the phytoplankton and bacterial compartments (Ducklow & Carlson 1992), when interpreting the results of BP:PPP or BCD:PPP ratios it has to be considered that we are comparing bacterial parameters to particulate primary production. Heterotrophic bacteria are able to utilise particulate organic material e.g. during the degradation phase of phytoplankton blooms. Most importantly though they utilise dissolved organic matter which is directly (via exudation) or indirectly available (via autolysis and disintegration of phytoplankton cells through for example sloppy feeding by zooplankton) (Azam et al. 1998). Considering that exudation is smaller than the particulate primary production (0-80% Conan et al. 1999 and references therein), this highlights even more the importance of possible carbon deficiencies as measured at increasing temperatures at the lower light levels.

Summary and Conclusions

The most challenging aspect of understanding variability in biological processes is associating detected changes with the responsible environmental forcings

(Behrenfeld et al. 2006). Recent global warming is caused by anthropogenic green house gas emissions. One of the predicted consequences of this warming is in turn a reduction of carbon sinks on land and in the oceans and hence a further increase in CO_2 concentration in the atmosphere (Sarmiento 2000). Also, in the expansive stratified low latitude oceans, warming is expected to increase thermal stratification and hence reduce the nutrient supply for phytoplankton. This would result in decreased ocean primary production, while the opposite effect is expected for high-latitude oceans where increased stratification would release phytoplankton from light-limitation and would extend the growing season (Behrenfeld 2006). The continuation of the currently observed trend in "brightening", i.e. an increase in light intensity, can be expected to further enhance primary production, especially in the temperate climate zone (Wild 2009, Pinker et al. 2005). The combination of increasing CO_2 concentrations, warming and brightening trends might have fundamental influences on the relationship between carbon fixation and utilisation.

In our experiments, where we investigated the consequences of future warming and brightening scenarios on the coupling of phytoplankton carbon fixation and the utilisation of organic carbon by heterotrophic bacteria, a few striking and recurring patterns emerged. Primary production was always enhanced directly at higher light intensities and indirectly at higher temperatures (only under high light conditions), resulting in the highest values at warm and bright conditions. The same result could be seen for bacterial production and most of the respiration parameters. This consequently resulted in the highest bacterial carbon demand under these conditions. Bacterial growth efficiency revealed firstly that bacterial respiration was favoured stronger by increasing temperatures (lower BGE) than bacterial production as long as light was dim, while higher light levels favoured bacterial production more (higher BGE), presumably due to increased organic substrate supply. **Concluding from the combined results on BP: PPP and BCD: PPP ratios from our experiments, the highest relative carbon remineralisation would consequently occur in a future scenario of a dim and warm winter/spring, while the highest absolute amount would be utilised in a situation of bright and warm winter/spring.**

Consequences for a future warming ocean would in any case be a decreased sedimentation of organic matter below the photic zone as a matter of long term storage of CO_2. On the contrary, due to the enhanced importance of the microbial loop, more organic carbon will be available for higher trophic levels hence reducing the efficiency of the food web and at the same time increasing the release of CO_2 to the atmosphere and therefore representing a positive feedback loop to the greenhouse gas problem. In the case of a continued brightening trend, these relative shifts will switch to absolute amounts of increased organic carbon cycling.

CHAPTER 3

The combined effects of temperature and nutrients on the phytoplankton-bacterioplankton coupling

Introduction

The global marine carbon cycle is largely determined by the autotrophic primary production by phytoplankton and the subsequent heterotrophic utilisation of dissolved and particulate organic carbon by bacteria. Heterotrophic bacteria can channel up to 50 % of primary production through the microbial loop, via a combination of biomass build-up and respiration (Azam et al. 1983). The importance of the microbial loop determines the efficiency of the biological pump. The relative distribution among these pathways of CO_2 fixation and recycling is determined by the interactions of biological, physical and chemical forces.

Bacterial degradation of organic carbon is influenced by bottom-up factors like temperature and inorganic nutrients (Pomeroy & Wiebe 2001, Baines & Pace 1991, Chrzanowski et al. 1995) as well as the quality and quantity of organic carbon (Kirchman 1990). Top-down factors are predation by heterotrophic nanoflagellates and lysis by viruses.

One of the most important direct effects of climate change is ascribed to increases in water temperature, with most pronounced effects during winter in northern Europe (IPCC 2007). Concerning the effect of temperature, it can be assumed that heterotrophic bacteria will be affected differently by increasing temperatures compared to phytoplankton, hence influencing the coupling between auto- and heterotrophs with reference to organic matter cycling. Published Q_{10} values (i.e. factorial increase in a rate for a 10℃ increase in temperature) for bacterial heterotrophic activities lie between 2 and 3 (Pomeroy and Wiebe 2001), while phytoplankton growth and photosynthesis show only a low temperature sensitivity ($1 < Q_{10} < 2$) under light-limited conditions (Tilzer et al. 1986). Because phytoplankton blooms are primarily controlled by light intensity they can occur at the lowest water temperatures, at which bacterial activities might be reduced. In temperate latitudes bacteria may approach no-growth temperature in winter, but they may also be limited by lower rates of production of DOC and POC by phytoplankton in winter as the result of lower light intensity and deep mixing, and these limiting factors may interact synergistically at lower temperature (Pomeroy & Wiebe 2001).

The general temperature dependence of planktonic bacterial growth and activities is well documented (White et al. 1991, Hoch & Kirchman 1993, Shiah & Ducklow 1994). Results from temperate waters indicate a close positive correlation between temperature and bacterial production and respiration (Felip et al. 1996, Pomeroy & Wiebe 2001, Kirchman et al. 2005, Lopez-Urrutia et al. 2006). Pomeroy & Deibel (1986) showed that bacterial activity and respiration were more inhibited at temperatures below 4°C than phytoplankton photosynthesis and arctic bacterial strains displayed Q_{10} values of up to >10. A temporal de-coupling between the early spring phytoplankton bloom and bacterial development has been documented for different marine systems including the Baltic Sea (Blight et al. 1995, Bird & Karl 1999, Lignell et al. 1993). However, there is also contradictory evidence regarding the effect of temperature. Rapid bacterial growth was found at temperatures below 2°C in antarctic waters (Fuhrman & Azam 1980, Hanson et al. 1983) and several other studies in polar seas and sea ice communities revealed high bacterial activities, with normal Q_{10} factors even at subzero temperatures (Li & Dickie 1987, Robinson & Williams 1993, Rivkin et al. 1996). In this context, the adaptation of bacterial communities concerning the community composition with respect to psychrophilic species has to be considered.

From these results it became obvious that additional factors have to be taken into account, as temperature and substrate supply are interacting factors for bacteria (Felip et al. 1996). Nedwell (1999) argued that decreasing membrane fluidity and efficiency of membrane transport proteins reduces the affinity of bacteria for substrates below the optimum growth temperature. This could explain increased substrate requirements and why substrate supply could partly compensate temperature limitation at low temperatures in cold water bacterial strains (Nedwell & Ruttner 1994, Pomeroy et al. 1991, Pomeroy & Wiebe 2001, Wiebe et al. 1992). The bioavailability of organic carbon and inorganic phosphate has been found to be a limiting factor for bacteria (Zweifel et al. 1993, Sala et al. 2002, Thingstad et al. 2005), and Kirchman (1990) could show that organic nitrate is highly stimulating marine bacterial growth in subarctic waters. Moreover, other studies have shown that heterotrophic bacteria can outcompete phytoplankton for

inorganic nutrients (freshwater, P: Currie & Kalff 1984, marine, NH_4: Suttle et al. 1990), which would imply that bacteria are rarely limited by the supply of ammonium and phosphate. In the Mediterranean Sea, there is evidence that P limitation affects both primary production and bacterial uptake of dissolved organic carbon (Thingstad & Rassoulzadegan 1995). Additionally, Obernosterer & Herndl (1995) demonstrated that exudates released from P-limited algae could not be utilised by bacteria due to their own P-limitation for growth.

These results clearly show that temperature and nutrients have to be considered together in order to describe potentially limiting effects on marine heterotrophic bacteria (Nedwell & Ruttner 1994, Reay et al. 1999, Pomeroy & Wiebe 2001). In order to disentangle the different effects of temperature and nutrients on the phytoplankton-bacterioplankton interactions, we designed a factorial experiment with a combination of three different temperatures and two nutrient levels. One high-N nutrient level was chosen, which represents the typical winter/spring situation in the Kiel Fjord and is potentially P-limiting for growth. The second, low-N nutrient level, was adjusted to be potentially N-limiting, and represents the typical open ocean situation, which is also found for example in the Baltic proper in summer (Andersson et al 1996). Current N-limiting conditions in the oceans could potentially shift to P-limiting conditions in coastal areas through increased N-rich and P-limited runoff from land and river inflow (freshwater being usually P-limited) under future climate conditions, as has already been reported for the Finnish and Bothnian Bay (Andersson et al 1996, Rivkin & Anderson 1997). The IPCC report (2007) predicts increases in land and river runoff for northern Europe during the winter season. This could increase oceanic P-limited regions or even intensify the deficiency of already P-limited coastal areas, like the Kiel Fjord.

Temperatures were selected to consider close to winter/spring values (4°C) and predicted climate change increases (8°C) and also to facilitate comparability with previous mesocosm experiments (Hoppe et al. 2008, Sommer et al. 2007, Sommer & Lengfellner 2008). One third elevated temperature (12°C) was added for further comparability with summer values. In order to elucidate the temperature dependence of algae-bacteria interactions without the complex food web effects, we conducted the experiment using an axenic culture of a dominant

phytoplankton species (*Skeletonema costatum*) from previous mesocosm experiments and the natural bacterial assemblage from Kiel Fjord.

The different nutrient setups were chosen in order to:

a. create different quantities of phytoplankton particulate and dissolved primary production, i.e. different quantities of substrate for bacterial growth
b. create different qualities of organic substrate for bacteria (from dissolved primary production), through different exudates from phytoplankton under P- and N-limitation
c. assess the competition of phytoplankton and bacteria for inorganic primary nutrients

Using this setup, we tried to answer the question, if the effects of different nutrient levels will influence bacteria directly or indirectly via algal primary production. The interacting influences of temperature and organic substrate quantity and competition with phytoplankton for inorganic nutrients will be assessed in this chapter, while the influence of organic substrate quality is discussed by Wohlers (Wohlers 2009).

Materials and Methods

The experiment was performed between 5th July and 16th August 2007. Low nutrient Baltic Sea water was collected from Booknis Eck, Kiel Bight, on 16th May 2007 at 10 m depth. The water was allowed to age at IFM-GEOMAR in the dark at 15°C until the beginning of the experiment, in order to reduce organic carbon in the water as much as possible. The aged water was filtered directly into 25 L autoclaved carboys (polycarbonate) one day before the start of the experiment using a cascade of a combusted (5h, 450°C) GF/F prefilter followed by a 0.45 µm cellulose acetate filter. This was done in order to remove heterotrophic nanoflagellates and all larger grazers, and at the same time to keep the natural bacterial community as complete as possible. The filter set was renewed after each individual carboy, the first 1-2 L were discarded, and then exactly 24 L filtered into the carboy. Randomly taken samples during the filtration process confirmed the absence of flagellates and almost no reduction in bacterial numbers.

A total of 18 carboys were filled and 6 each were stored in one of 3 climate chambers, which were set to the temperatures: 4℃, 8℃, 12℃. Carboys received light from fluorescent bulbs, positioned directly above the horizontally aligned carboys. Light was supplied with a light: dark cycle of 12:12 hours, increasing to a maximum of ~388 µE $m^{-2}s^{-1}$ at noon. These settings represent non-limiting light conditions for algal growth. An axenic *Skeletonema costatum* (strain CCMP 1332) culture was grown in the aged seawater with nutrients added in f/2 amounts (Guillard and Ryther 1962, Guillard 1975), at 20℃ and ~250 µE $m^{-2}s^{-1}$ ahead of the experiment. On the evening before the start of the experiment 3 subcultures were transferred to each of the experiments' temperatures, to allow acclimatisation. On the day of the experiments' start, the respective culture was added to the 6 carboys of each temperature in a final concentration of ~700 cells ml^{-1}. Together with the cultures, phosphate and nitrate were added to the seawater, in order to create two different N:P ratios (see below). Silicate was added to avoid distortion of results with respect to silicate limitation at any time of the experiment. Trace metals, vitamins and selenium were added in f/10 amounts in order to avoid any limitation. Each carboy was gently stirred for 1 min. each day ahead of sampling. The sampling frequency for all other parameters was individually adjusted to the biomass development in the respective samples. Samples were drawn by casting 1.5 L of water directly into a clean measuring cylinder, from which subsamples for the respective measurements were taken.

An overview over the sample numbers, temperatures and the respective N:P ratios is given in Table 1.

Table 1: Sample numbers, temperatures and N:P ratios of the treatments used in the experiment

Sample No.	Temperature (℃)	N:P
1, 2, 3	12 ± 0.5	29
7, 8, 9	8 ± 0.5	29
13, 14, 15	4 ± 0.5	29
4, 5, 6	12 ± 0.5	8
10, 11, 12	8 ± 0.5	8
16, 17, 18	4 ± 0.5	8

Nutrients

The salinity of the water was 15.1. The nutrient levels in the original aged seawater and the two different nutrient treatments is shown in Table 2. The phosphate level was uniformly increased to 0.87 ± 0.06 µM, a realistic level to facilitate development of a phytoplankton bloom and for comparability in all treatments. This resulted in an N:P ratio of 8 (total N = 7.1, representing possible N-limiting conditions). In order to create a possible P-limiting situation nitrate was added to a final concentration of 23.8 ± 1.7 µM, resulting in an N:P of 29 (total N = 25.5). This setup represents the typical winter/spring situation in the Kiel Fjord and was observed repeatedly in previous experiments (Hoppe et al. 2008, Sommer et al. 2007). At constant P concentration, the high-N treatment would therefore theoretically provide for a 2 times higher phytoplankton biomass compared to the low-N treatment as based on the Redfield ratio (C:N:P = 106:16:1), because at low-N the total N concentration of 7.1 µM is decisive and at high-N the P concentration of 0.87 µM is decisive for the maximally expected buildup of organic C (47 and 92 µM C, respectively).

Table 2: Nutrient concentrations in the original aged water and in the respective treatments (concentrations in µM) after nutrient addition, at the start of the experiment.

	Nitrate	Ammonium	Phosphate	Silicate
original	5.8	1.9	0.3	5.9
29	23.8 ± 1.7	1.7 ± 0.1	0.87 ± 0.06	43.8 ± 0.4
8	5.4 ± 0.7	1.7 ± 0.1	0.87 ± 0.06	43.8 ± 0.4

Nitrate, phosphate and silicate were determined according to the protocol of Hansen and Koroleff (1999) after water samples had been prefiltered through 5.0 µm cellulose acetate filters. For the assessment of ammonium (Holmes et al. 1999), unfiltered water samples were used. All measurements were performed on the day of sampling.

Particulate and dissolved primary production

Particulate primary production measurements were performed using ^{14}C bicarbonate incubations following the methods of Gargas (1975) and Steeman Nielsen (1952). For each mesocosm three aliquots of 20 ml each were incubated with 70 µl of a 4 µCi / 100 µl ^{14}C-bicarbonate solution. The blank treatment was kept dark during incubation. Incubation took place next to the respective carboys, ensuring the same light exposure and in situ temperature conditions. After 4-5 hours of incubation, the samples were filtered onto 0.2 µm cellulose nitrate filters. The filtrate was collected for measurement of dissolved primary production. The filters were subsequently fumed with 37 % HCl fumes in a closed box for 5-10 min and then measured in 4 ml of Scintillation cocktail (Lumagel Plus) using a Packard Tricarb counter.

Aliquots of 10 ml filtrate received 100 µl of a 1 N HCl solution and were stored in an exsiccator under vacuum for 8 days. For collecting the expelled CO_2, the exsiccator contained a 1 N NaOH solution. Preliminary experiments had shown that this treatment guarantees maximum outgassing of remaining inorganic ^{14}C from the samples. After this storage time, 10 ml of scintillation cocktail (Aquasol) were added and the radioactivity of the samples counted.

The original CO_2 concentration of the water sample was determined using the 40 + 10 method and dissociation constants described in Stumm & Morgan (1981).
Calculated particulate and dissolved primary production was corrected for the actual light received during the incubation period, in relation to total light during the 12 h light-day. The two variables are presented as µg C L^{-1} d^{-1}.

Bacterial Abundance

Bacterial cell numbers were determined by flow cytometry. 4 ml of a sample was fixed with 400 µl of paraformaldehyde / glutaraldehyde (1 % and 0.05 % final concentration respectively) in the dark for 1 hour at 5°C. After fixation, the samples were frozen in liquid nitrogen and subsequently stored at –80°C. Heterotrophic bacteria were stained using SYBR Green (2.5 µM final concentration, Molecular Probes) for at least 30 minutes in the dark. Cells were counted using a Becton & Dickinson FACScalibur equipped with a laser emitting at 488 nm at a constant flow rate (35 µl / min). Yellow-green latex beads (0.5 µm, Polysciences) were used as an internal standard. Bacteria were detected by their signature in a plot of side scatter (SSC) versus green fluorescence (FL 1).

Bacterial Production

Bacterial secondary production measurements were conducted following the protocol of Simon & Azam (1989). Three aliquots (2 replicates and one blank) of 10 ml of water were each incubated with 50 µl of a 1 µCi / 10 µl ^{3}H-leucine solution (specific activity: 77 µCi $nmol^{-1}$) plus 50 µl of a 2 nmol / 100 µl unlabeled leucine solution. This resulted in a total concentration of 106.49 nmol L^{-1} of leucine in the sample, which is known to be saturating under the conditions found in the Kiel Fjord (Giesenhagen, unpublished data).
All samples were incubated in the respective climate chambers at in situ temperature in the dark for 1.5 - 3 hours. Incubation was terminated by the addition of formaldehyde (1 % v / v) and cells filtered onto 0.2 µm polycarbonate filters. The filters were subsequently rinsed with ice cold 5 % TCA (trichloro acetic acid) solution, before being radio-assayed in 4 ml of scintillation cocktail (Lumagel Plus). Results in units of pM h^{-1} bacterial protein production were transferred into

µg C L^{-1} d^{-1} biomass production using a theoretical conversion factor of 3.091 x 10^{-3} kg C mol^{-1} leucine (Simon & Azam 1989).

Data handling and statistics

- Mean values for parameter quantification were obtained from individually determined peak periods.
- Ratios of bacterial production or bacterial carbon demand to primary production (particulate and dissolved) were calculated from the individual mean values.
- Bacterial respiration was calculated from the equation described in DelGiorgio & Cole (1998): BR =3.42 x $BP^{0.61}$; bacterial carbon demand (BCD) was calculated as the sum of bacterial production and bacterial respiration.
- Specific bacterial production was calculated by dividing bacterial production by the total bacterial number for each measuring point.
- Influence of temperature on mean values of variables was assessed by simple (model 1) linear regression (SigmaPlot).
- Influence of nutrient availability on mean values was assessed by T-test (accounting for normal distribution and homogeneity of variances).
- Multiple linear regressions were performed in order to assess the combined effects of temperature and nutrients on the respective parameters and partial correlations were used to assess the contribution of the respective variable to total variance while controlling for the respective other variable (Statistica). Data was tested for normal distribution (Shapiro-Wilk's W test), the model was tested for outliers (standardised residuals, Cook's distance, Mahalanobis distances) and autocorrelations (Durbin-Watson).

Results

Nutrient dynamics

Nutrient dynamics of silicate (Figure 1, A) show that there was no silicate limitation in the experiment at any time. The drawdown is clearly temperature-dependent, starting earlier at warmer temperature. There was also a difference

detectable between the nutrient treatments, showing a larger drawdown at the high-N treatments. This trend was most pronounced at 12℃ and less obvious in the other temperatures. Within the nutrient treatments, drawdown also showed a temperature effect, with the largest drawdown at the coldest temperature and vice versa.

Phosphate utilisation also showed a clear temperature-dependent development during the course of the experiment (Figure 1, B). Irrespective of the nutrient regime, all samples at 12℃ decreased strongly in phosphate concentration without delay, reaching the detection limit on day 10 (sample 3 on day 12). All samples at 8℃ showed a somewhat delayed reaction, reaching the detection limit on day 18. Only at 4℃ a slight difference between the nutrient treatments was detected, with all low-N samples reaching detection limit on day 24 and two of the three high-N treatments (samples 14 and 15) lagging behind by 3 days.

Results of ammonium determination show (Figure 1, C) that ammonium was taken up preferentially, compared to nitrate (Figure 1, D), being used up very quickly at the experiments' start. Again, the dynamics were clearly temperature dependent with minima being reached on day 8 at 12°C, days 12 – 18 at 8℃ and days 15 – 18 at 4℃.

Due to the different starting conditions, nitrate dynamics showed clear differences between the high and low-N treatments (Figure 1, D). In the low-N treatments nitrate drawdown started earliest in the 12℃ treatments, reaching the detection limit on day 10. The limit was reached on day 14 at 8℃ and on day 24 at 4℃ (day 21 for sample 18). The high-N treatments also showed temperature-dependent dynamics. Drawdown started earlier at 12°C, reaching the detection limit on day 24 (day 21 for sample 1). The limit was reached for samples 8 and 9 on day 24 at 8℃, while the third replicate (sample 7) lagged behind until day 32. The samples, which were run at 4℃, showed a clearly delayed development, reaching detection limit on day 32. Between the treatments it became obvious that nitrate was depleted earlier in the low-N compared to the high-N samples.

Concerning the limiting conditions of inorganic nitrate and phosphate, differences occurred between the high- and low-N treatments. In the high-N treatments

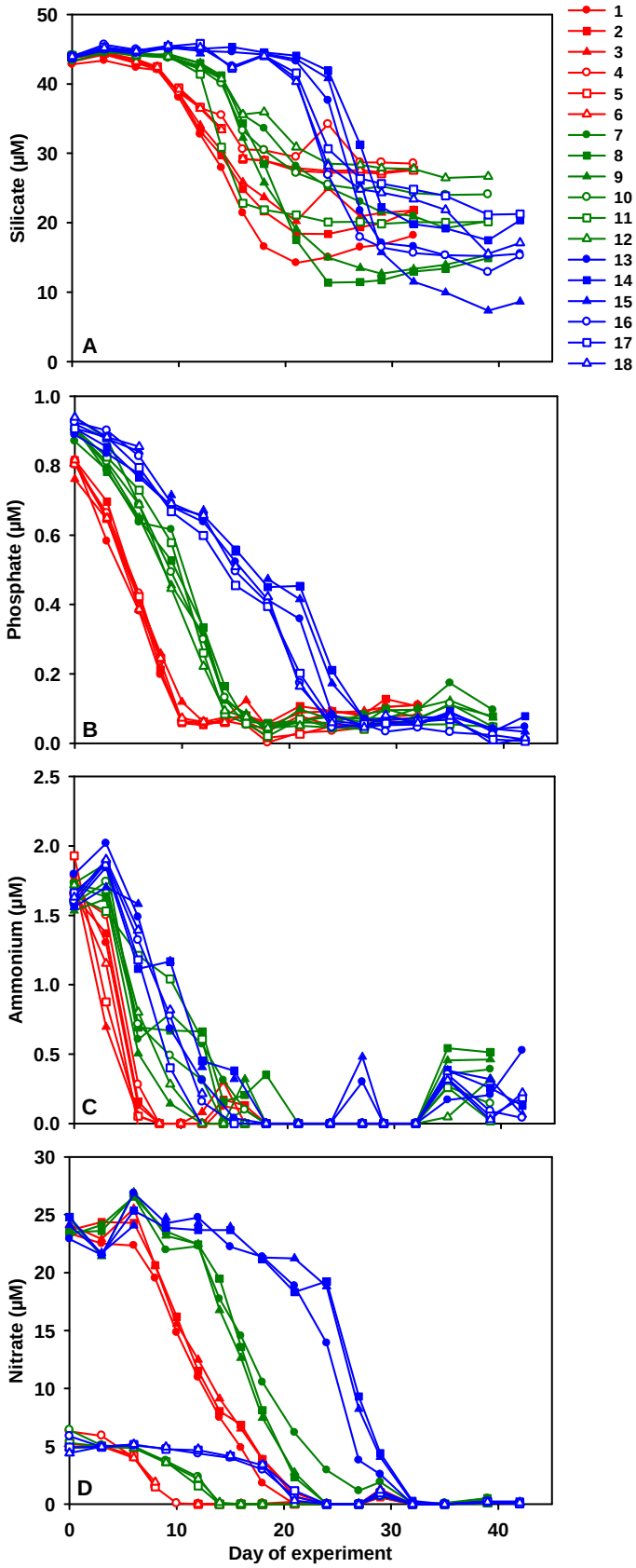


Figure 1. Nutrient dynamics during the course of the experiment. Silicate (A), phosphate (B), ammonium (C) and nitrate (D) concentrations are given in µM, red colour displays all samples at 12℃, green colour 8℃ and blue colour 4℃. Full symbols represent the high-N nutrient treatment (N: P 29) and open symbols represent the low-N nutrient treatment (N: P 8).

phosphate was exhausted before nitrate at all temperatures. This resulted in a few days of P-deficiency before nitrate reached the detection limit as well. These were days 10-21 for sample 1, days 10-24 for sample 2, days 12-24 for sample 3, days 1-32 for sample 7, days 18-24 for samples 8 and 9, days 24-32 for sample 13 and days 27-32 for samples 14 and 15. At the highest temperature in the low-N treatments, N and P were simultaneously exhausted on day 10. At 8℃, N reached the detection limit 4 days before P, resulting in N-deficient conditions between days 14 and 18. In the coldest

treatments again N and P reached the detection limit on the same day (24), except for sample 18, where N-deficiency prevailed between days 21-24.

Dynamics of phytoplankton

Particulate Primary Production (PPP)

The dynamics of particulate primary production show the development of the *Skeletonema costatum* bloom in the various treatments (Figure 2). *Skeletonema* remained the only dominating species in all microcosms (contamination with other diatoms did occur in some samples, but cell numbers stayed negligibly low). In the high-N treatments, mean particulate primary production reached between 232 and 378 µg C $L^{-1}d^{-1}$. There was a tendency towards increasing mean values with rising temperatures, but this trend was statistically not significant (Figure 3, B, Table 4). Peak timing was clearly temperature-dependent (due to the non-limiting light conditions), with a significant acceleration of 1.7 days per degree Celsius warming (Figure 3, A, Table 3). The peak was reached between days 14 and 16 at 12°C, on day 21 at 8°C and on days 27 to 29 at 4°C. P-deficiency at 4° and 8°C was reached 2-3 days before the bloom peak (see *Nutrients* above), towards the end of the exponential growth phase. At 12°C P-deficiency occurred earlier (between 2-6 days ahead of the peak), in all samples during the exponential growth phase.
The timing of the blooms in the low-N treatments showed the same acceleration of 1.7 days / 1°C warming (Figure 3, A, Table 3), but was always reached earlier than the high-N treatments. Peaks were reached on day 10 at 12°C, on days 14 to 18 at 8°C and on days 21 to 27 at 4°C. Concerning the mean quantity, there was no temperature dependence found (Figure 3, B, and Table 4). Mean values ranked between 122 and 176 µg C $L^{-1}d^{-1}$, with one unusually high value at 294 µg C $L^{-1}d^{-1}$ (sample 11). Although the results from sample 11 are not interpreted as false measurements (unusually high values on three consecutive days and confirmation from Chl a results, data see Wohlers), this sample was interpreted as a biological outlier and omitted from all further analyses (see also *Discussion*). The timing of the bloom peak in the 12°C treatments coincided with the minimum in both N and P. At 8°C the same was valid for sample 10, whereas the other two replicates (11 and 12) showed N-limitation at the peak and four days after, before

P reached the detection limit as well. At 4℃ for s ample 16 both nutrients dropped to the detection limit at the end of the exponential phase, 3 days ahead of the peak. For sample 17 this drop coincided with the peak on day 24, whereas for sample 18 N-deficiency was predominant from the peak on for 4 days, until P was exhausted as well.

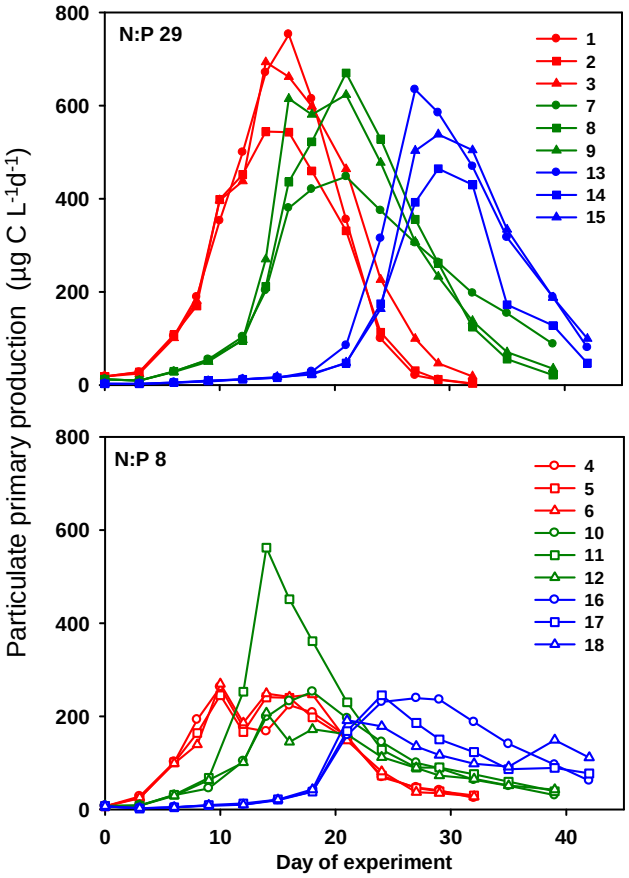


Figure 2. Dynamics of particulate primary production (µg C $L^{-1}d^{-1}$) in the high-N (N: P 29) and low-N (N: P 8) treatments during the course of the experiment.

When considering the Redfield ratio of C: N: P = 106:16:1, the theoretical ratio of biomass for the high-N to low-N phytoplankton would be 2 (see *Material and Methods)*. From the mean particulate primary production an average ratio of 2.1 could be calculated, spanning a range of 1.6 – 2.7 (data not shown). The means of the two different nutrient treatments were significantly different (p=9.5, p<0.0001, n=18). Multiple linear regression showed that 88 % of the variability in PPP could be explained by temperature and nutrient treatments (F=50.04, p<0.0001). The partial correlations revealed a 87 % contribution of the nutrient treatment (p<0.0001) and a 15 % contribution of the temperature treatment to total variance (p=0.14).

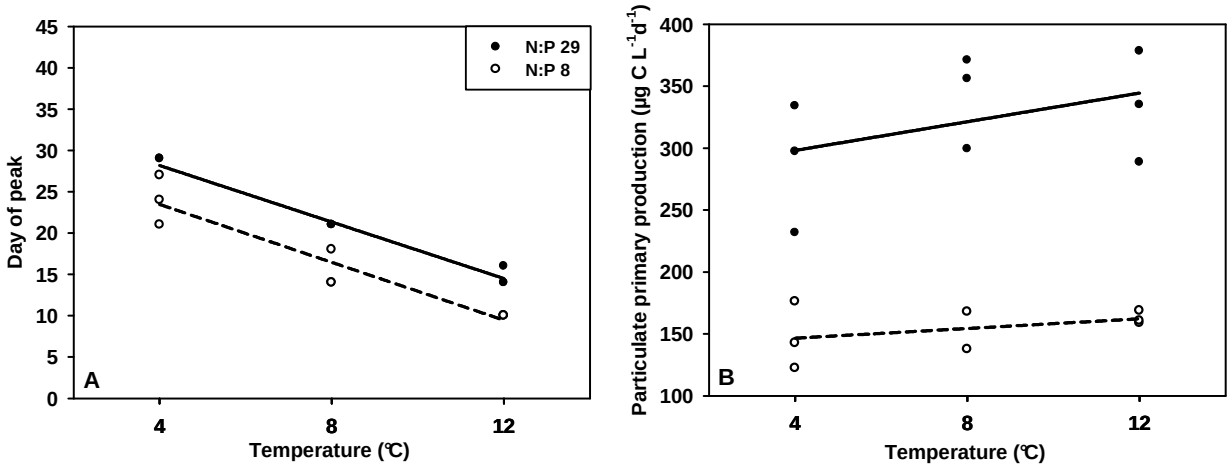


Figure 3. Peak timing (A) and mean (B) of particulate primary production peaks at the different temperatures for high-N (N: P 29) and low-N (N: P 8) treatments.
A: high-N: $Day_{(T+1)}$=-1.7$day_{(T)}$+35, R^2=0.98, p<0.0001; low-N: $Day_{(T+1)}$=-1.7$day_{(T)}$+30, R^2=0.9, p=0.0001; The slope of the linear regression line in (A) represents the days the peak is accelerated for each 1℃ warming.
B: high-N: PPP=5.8T+275, R^2=0.18, p=0.25; low-N: PPP=2.0T+139, R^2=0.16, p=0.33.

Dissolved Primary Production (DPP)

Dissolved primary production generally showed a higher variability compared to particulate primary production, both between the replicate treatments and along the course of the experiment (Figure 4).

At high-N nutrient conditions mean values reached between 2.0 and 13.9 µg C $L^{-1}d^{-1}$, and for each temperature treatment one replicate was considerably lower than the other two (Figure 4, A, Table 4). There was a trend towards higher production with decreasing temperature, which was not statistically significant (due to the one low replicate value at each temperature treatment, Figure 5, B, Table 4). The development was accelerated with increasing temperatures. Peaks at 12℃ were reached on days 14 – 21, on days 12 – 21 at 8℃ and on days 24 – 27 at 4℃. The linear model shows the significant acceleration of 1.2 days / 1℃ warming (Figure 5, A, Table 3).

Dynamics of dissolved primary production were rather variable at low-N nutrient conditions (Figure 4, B). Nevertheless some patterns emerged, for example the timing of peak development was significantly accelerated at higher temperatures by 1 day /1℃ (Figure 5, A, Table 3). Peaks were reached on days 14 – 18 at

12℃, on days 14 – 16 at 8℃ and on days 21 – 27 at 4℃. Although the linear model shows the significant acceleration, it has to be noted, however, that in both nutrient treatments, the difference in timing did only occur between 8℃ and 12℃, while no difference could be detected between 4℃ and 8℃. Also, although the acceleration at high-N was slightly higher, there was no significant difference in timing between the two nutrient treatments (ANCOVA, F=0.22, p=0.65, n=18). Mean dissolved primary production at low-N conditions ranged between 3.4 and 11.8 µg C $L^{-1}d^{-1}$ and only a small and insignificant trend towards higher values at lower temperatures could be detected (Figure 5, B, Table 4). The ratio of the mean dissolved primary production at high- to low-N treatments was on average 1.3, ranging from 0.2 to 2.4 (data not shown).

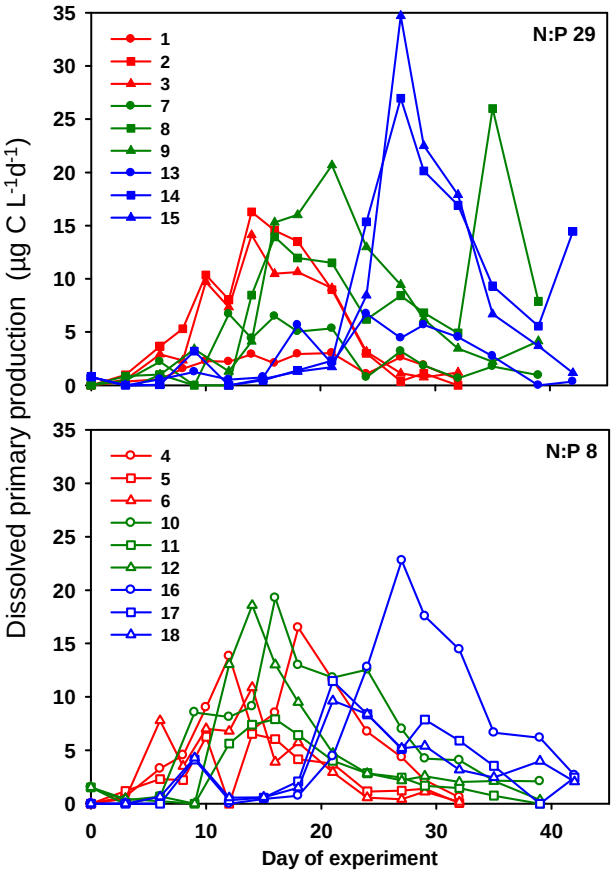


Figure 4. Dynamics of dissolved primary production (µg C $L^{-1}d^{-1}$) in the high-N (N: P 29) and low-N (N: P 8) treatments during the course of the experiment.

The mean values of dissolved primary production were not significantly different between the two different nutrient treatments (T-test, t=0.17, p=0.87, n=18). Multiple linear regression showed that only 16 % of the variability in DPP could be explained by temperature and nutrient treatment (F=1.39, p=0.001). The partial correlations revealed a 0.25 % contribution of the nutrient treatment (p=0.86) and a 15 % contribution of the temperature treatment to total variance (p=0.12).

The percent extracellular release (PER = DPP / DPP+PPP) is a measure of the amount of dissolved relative to total primary production. The average PER during

the particulate primary production bloom was 2.4 % for the high-N treatments and 4.6 % for the low-N treatments (overall average 3.5%). The difference was statistically significant (T-test, t=-2.83, p=0.01, n=18), but if determined separately for the different temperatures only the 8℃ treatment showed a significant difference. PER showed a tendency to be higher at lower temperatures, but the trend was not statistically significant (Table 4). Multiple linear regression showed that 50 % of the variability in PER could be explained by temperature and nutrient treatment (F=6.89, p=0.008). The partial correlations revealed a 41 % contribution of the nutrient treatment (p=0.008) and a 23 % contribution of the temperature treatment to total variance (p=0.06) (data not shown).

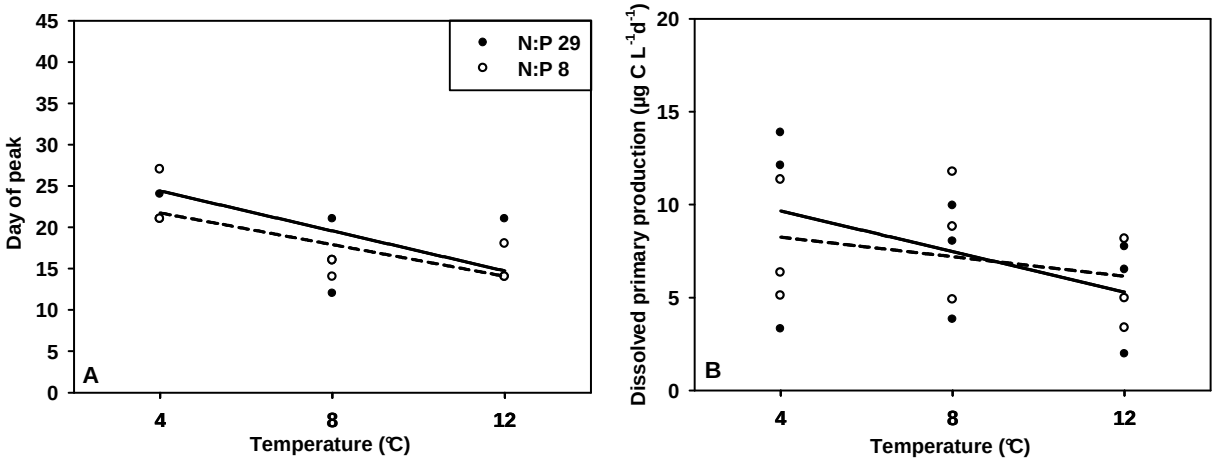


Figure 5. Peak timing (A) and maxima (B) of dissolved primary production peaks at the different temperatures for high-N (N: P 29) and low-N (N: P 8) treatments.
A: high-N: $Day_{(T+1)}$=-1.2$day_{(T)}$+29, R^2=0.53, p=0.03; low-N: $Day_{(T+1)}$=-1.0$day_{(T)}$+26, R^2=0.57, p=0.002; The slope of the linear regression line in (A) represents the number of days the peak is accelerated for each 1℃ warming.
B: high-N: DPP=-0.5T+11.8, R^2=0.22, p=0.20; low-N: DPP=-0.3T+9.3, R^2=0.09, p=0.43.

Dynamics of bacteria

Total Bacterial Number (TBN)

The dynamics of bacterial cell numbers showed distinct differences between the different temperature treatments at the high-N nutrient conditions (Figure 6, A). The replicates at 12℃ were very close together. The dynamics showed the development of an early peak before steeply decreasing and increasing again, towards the end of the experiment. This represents the main degradation phase.

The mean numbers at 12℃ reached between 1.9 and 2.0 x 10^6 cells ml^{-1}, with peak values on day 10. The dynamics at 8℃ were somewhat delayed compared to 12℃, also one replicate showed a distinctly different development. While two replicates showed their peak on day 16, the third replicate peaked much higher on day 21. Mean cell numbers ranging from 2.2 to 3.1 x 10^6 cells ml^{-1} were measured. Again, the peaks were followed by a steep decrease, before numbers rose again towards the end of the experiment. The dynamics of bacterial numbers in the coldest treatment at 4℃ showed a very different picture. No distinct peak was formed with numbers increasing almost constantly, and uniformly, until the end of the experiment. An intermediate maximum which could be interpreted as a peak was detected on day 29. Mean values at 4℃ ranged between 3.6 and 3.8 x 10^6 cells ml^{-1}. Means and peak timing were significantly negatively correlated with temperature (Figure 7, Table 3, 4). Acceleration of the peak day was 3.9 days /1℃ (Figure 7, A, Table 3).

The basic pattern in the dynamics, as described for the high-N treatments, was also found in the low-N treatments, although with some distinct deviations (Figure 6, B). The 12℃ treatments showed an early peak, then decreasing quickly. Towards the end of the experiment there was only a weak increase. The peak at 12℃ was reached also on day 10 for all replicates, mean values ranged between 1.9 and 2.0 x 10^6 cells ml^{-1}. Treatments at 8℃ showed a similar pattern, albeit one replicate (sample 11) showed a much earlier and lower peak compared to the other two. Peak values ranged between 1.5 and 2.2 x 10^6 cells ml^{-1}. The increase in bacterial numbers after the first breakdown was much lower at the low-N compared to the high-N treatments. Also, in contrast to the results described above, bacterial numbers at 4℃ did show a peak development and subsequent breakdown after the peak. Two of the replicates displayed the distinctly delayed peak on day 29, while the third replicate (sample 16) peaked on day 21. Mean values reached 2.5 to 3.7 x 10^6 cells ml^{-1}. The breakdown was not as pronounced as for the warmer treatments, and from sample 16 it is indicated that these values, too, would be increasing again towards the end of the experiment.

Mean values of bacterial abundance in the low-N treatments were significantly lower at warmer temperatures (Figure 7, B, and Table 4). The peak timing was

significantly accelerated at higher temperatures by 2 days /1℃ warming (Figure 7, B, and Table 3). Because the acceleration was 1.9 days faster at the high-N treatment, the difference in peak timing compared to the low-N treatment was highest at the coldest temperature with 15 days, lower at 8℃ with 5 days and there was no difference in the 12℃ treatment. The difference in peak acceleration with temperature was significant between the two nutrient treatments (ANCOVA, F=7.84, p=0.01, n=18).

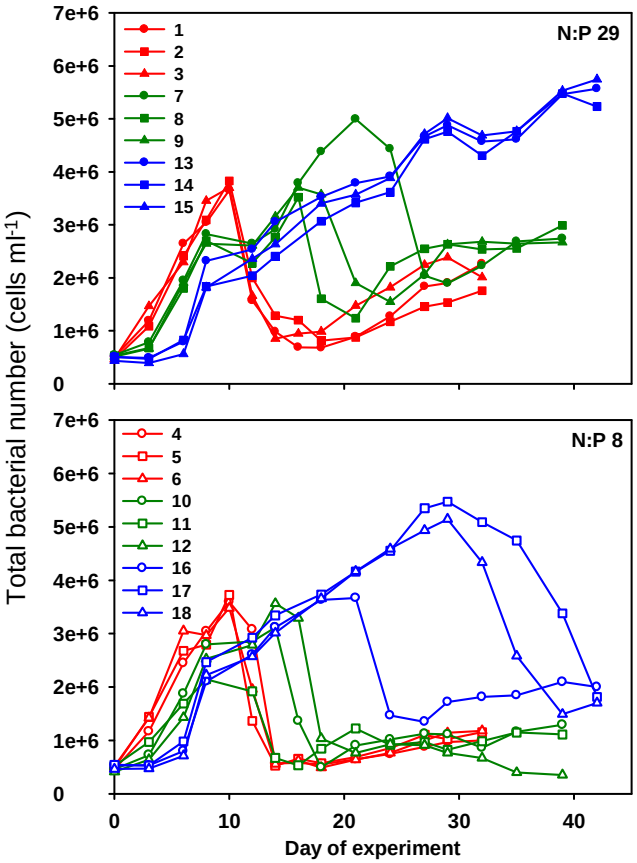


Figure 6. Dynamics of total bacterial numbers (cells ml^{-1}) during the course of the experiment in the high-N (N: P 29) and low-N (N: P 8) treatments.

The mean values were not significantly different between the two nutrient treatments over all temperatures (T-test, t=1.23, p=0.24, n=18). The ratio of high-N to low-N maximal values ranged between 0.9 and 1.6, with a mean of 1.2 (no temperature effect). Multiple linear regressions showed that 72 % of the variability in TBN could be explained by temperature and nutrient treatment (F=19.3, p<0.001). The partial correlations revealed a 23 % contribution of the nutrient treatment (p=0.05) and a 69 % contribution of the temperature treatment to total variance (p<0.0001).

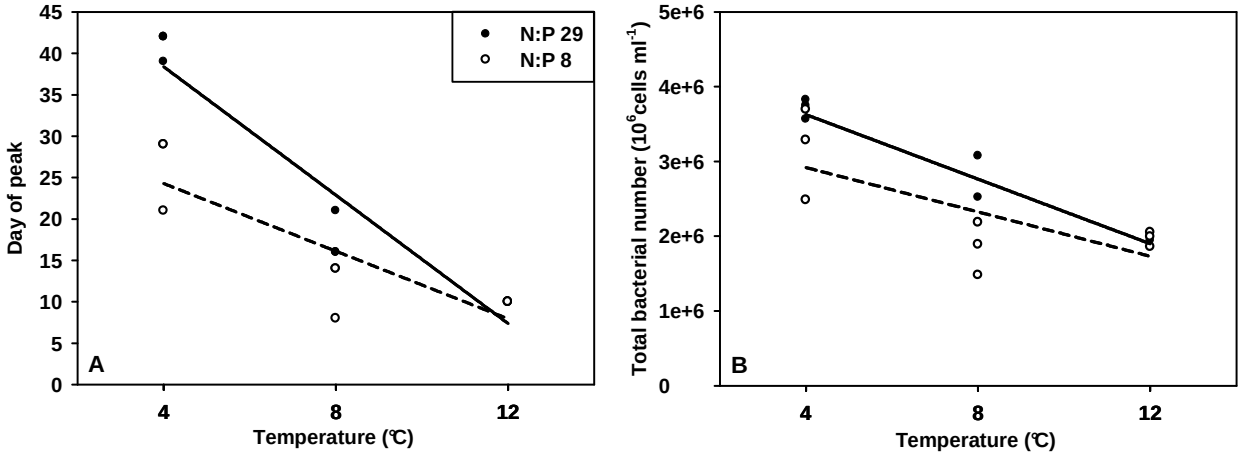


Figure 7. Peak timing (A) and maxima (B) of total bacterial number (TBN) peaks at the different temperatures for high-N (N: P 29) and low-N (N: P 8) treatments.
A: high-N: $Day_{(T+1)}$=-3.9$day_{(T)}$+54, R^2=0.91, p<0.0001; low-N: $Day_{(T+1)}$=-2.0$day_{(T)}$+32, R^2=0.74, p=0.003; The slope of the linear regression line in (A) represents the days the peak is accelerated for each 1℃ warming.
B: high-N: TBN=-2.2x10^6T+4.5x10^6, R^2=0.89, p=0.0001; low-N: TBN=-1.5x10^6T+3.5x10^6, R^2=0.51, p=0.03.

Bacterial Production (BP)

In contrast to the dynamics of bacterial abundance, the development of bacterial production did not show very distinct peaks and was more variable along the course of the experiment (Figure 8).

In the high-N treatment at 12℃, the replicates showed an initial peak on day 3, followed by a short decrease, before increasing again towards an individual peak (Figure 8, A). This peak was reached between days 12 and 27, due to the high variability, and reached mean values between 220 and 286 µg C $L^{-1}d^{-1}$. A minimum after the peak was observed in all replicates on day 29, after which all values increased again. Development at 8℃ showed delayed, but somewhat similar dynamics. Here, values also increased towards a peak, which was reached on day 35 for two of the replicates, whereas the third replicate peaked already on day 21. Mean values reached 224 and 367 µg C $L^{-1}d^{-1}$. In contrast to the other temperatures, bacterial production at 4℃ stayed on relatively low levels (mean 106 - 120 µg C $L^{-1}d^{-1}$) for most of the time and only increased towards a

low peak on day 35. A minimum directly after the peak was followed by increasing values at the end of the experiment.

The peak timing was significantly correlated with temperature, with an acceleration of 1.9 days /1℃ warming (Figure 9, A, Table 3). When plotting the mean peak values of bacterial production against the respective temperature, the linear regression model shows a trend towards increasing values with rising temperatures, which is almost significant (Figure 9, B, Table 4). It has to be noted, however, that there was no difference detected between 8 and 12℃ and only at 4℃ the mean values were considerably lower.

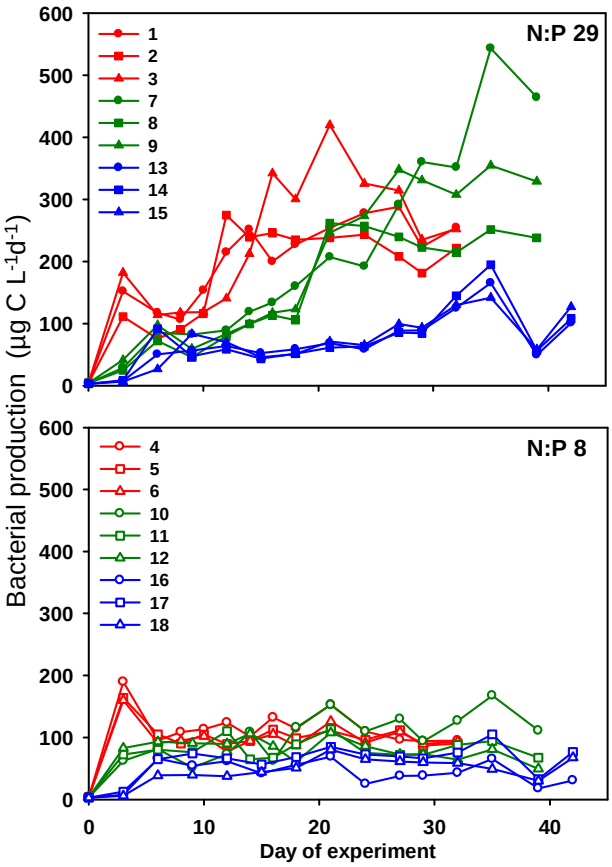


Figure 8. Dynamics of bacterial production during the course of the experiment in the high-N (A) and low-N (B) treatments.

The dynamics of bacterial production were distinctly different at low-N nutrient conditions (Figure 8, B). On day 3, a clear temperature effect emerged in the initial development, which was very similar to the high-N treatment. While at 12℃ a peak was reached, with 171 µg C $L^{-1}h^{-1}$ on average, values at 8℃ reached on average 72.4 µg C $L^{-1}h^{-1}$, and production at 4℃ was as low as 8.67 µg C $L^{-1}h^{-1}$. This first peak at 12℃ was followed by a steep decline, and mean values ranged between 97 and 111 µg C $L^{-1}h^{-1}$ with no peak development. At 8℃ the values reached on day 3 were more or less constant for the rest of the experiment. Only one replicate reached somewhat higher values, but without any peak (mean 82 – 103 µg C $L^{-1}h^{-1}$). The delayed dynamics of bacterial production at 4℃ showed a further increase until

day 6. Mean values ranged between 47 and 70 µg C $L^{-1}h^{-1}$, but again without any peak. Because there was no peak development, no correlation of peak timing with temperature could be performed. Linear regression revealed a statistically significant increase of mean values with increasing temperature (Figure 9, B, and Table 4).

The ratio of high-N to low-N values was between 1.7 and 3.6 (mean: 2.6), without any temperature effect (data not shown). Multiple linear regression showed that 83 % of the variability in BP could be explained by temperature and nutrient treatment together (F=37.6, p<0.0001). The partial correlations revealed a 77 % contribution of the nutrient treatment (p<0.0001) and a 58 % contribution of the temperature treatment to total variance (p=0.0004).

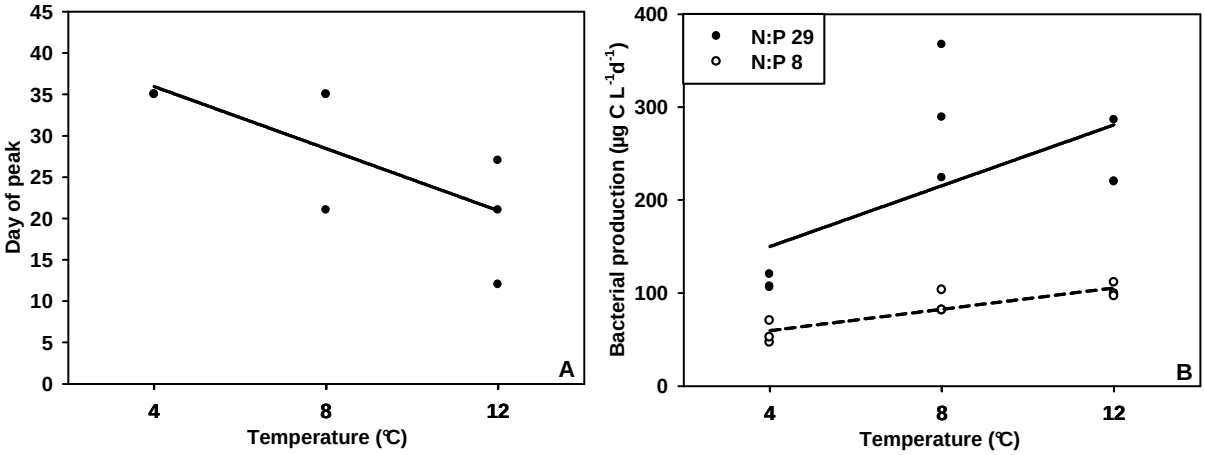


Figure 9. Peak timing (A) and maxima (B) of bacterial production peaks at the different temperatures for the high-N (N: P 29) treatments.
A: $Day_{(T+1)} = -1.88\ day_{(T)} + 43$, R^2=0.56, p=0.02; the slope of the linear regression line in A represents the days the peak is accelerated for each 1℃ warming.
B: high-N: BP = 16.4T + 84.5, R^2=0.39, p=0.07; low-N: BP = 5.7T + 36.6, R^2=0.78, p=0.002

Phytoplankton – bacteria relationships

Peak timing

Table 3 summarises the peak accelerations of all parameters at the different temperatures. The acceleration was statistically significant in all cases. Bacterial

parameters showed higher peak accelerations than primary production, but the differences were not statistically significant (comparison of slopes using ANCOVA, F=0.26, p=0.77).

Table 3: Acceleration of peaks of the different parameters in days per 1℃ temperature increase. The values were inferred from the slopes of the linear regression of peak days at the respective temperatures (original data can be found in the respective results sections above). For TBN the intermediate peak on day 29 was assessed additionally (see Results section for description). All accelerations were statistically significant on the p<0.05 level, there were no statistically significant differences between the different accelerations at the respective nutrient treatments, except for TBN 3.9.

	Slope	R^2	p
	PPP		
N:P 29	1.7	0.98	<0.0001*
N:P 8	1.7	0.90	0.0001*
	DPP		
N:P 29	1.2	0.53	0.03*
N:P 8	1.0	0.57	0.002*
	TBN		
N:P 29	3.9 (2.4)	0.91	<0.0001*
N:P 8	2.0	0.74	0.003*
	BP		
N:P 29	1.9	0.56	0.02*
N:P 8	no peaks	n.a.	n.a.

Bacterial Production to Primary Production ratio (BP : PP)

In order to assess the influence of temperature and nutrient treatments on the coupling between bacteria and phytoplankton, the ratio of bacterial production to primary production was calculated for the mean values at the respective peaks (Figure 10). For the bacterial production to particulate primary production ratio (BP:PPP), a trend towards increasing ratios with rising temperatures was detected, which was significant for the low-N treatment (Figure 10, A, Table 4). There was no difference between the nutrient treatments (T-test, t=1.24, p=0.23, n=18). The ratios show, that particulate primary production was at all times able to

satisfy the carbon demand for bacterial production, with on average 40% (N:P=29) / 39% (N:P=8) of particulate primary production being turned into bacterial biomass at 4℃ and 73% and 63% at 12℃ (Figure 11).

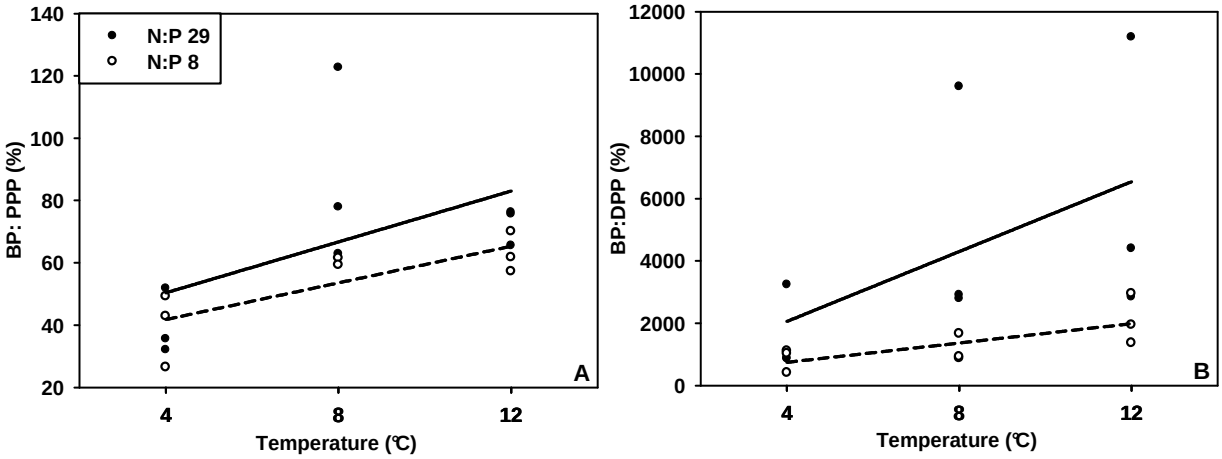


Figure 10. Correlations of the bacterial production to primary production ratio (%) with temperature for the primary production peak. Ratio of BP:PPP at high-N and low-N (A) treatments, and for BP:DPP at high-N and low-N (B) treatments.
A: high-N: BP:PPP=4.1T+34, R^2=0.27, p=0.15; low-N: BP:PPP=2.9T+30, R^2=0.63, p=0.02;
B: high-N: BP:DPP=561T-185, R^2=0.28, p=0.14; low-N: BP:DPP=155T+125, R^2=0.52, p=0.03

The bacterial production to dissolved primary production ratio (BP:DPP) also showed a trend towards increasing values with increasing temperature, which again was significant for the low-N treatment (Figure 10, B, Table 4). Values at 4℃ reached on average 1660 % at high-N conditions and 849 % at low-N conditions. For the low-N conditions it approximately doubled with an increase to 12℃ and reached 2090%, while the increase was markedly higher at high-N conditions, reaching 6146 % at 12℃. The ratios were significantly different between the nutrient treatments (T-test, t=2.73, p=0.01, n=18), which was however based only on the difference in the 8℃ treatment, if considered separately for the temperature treatments. In summary, it can be noted that dissolved primary production was always much smaller than would be needed to

supply for bacterial production and that the deficiency increased with rising temperatures, especially at high-N conditions.

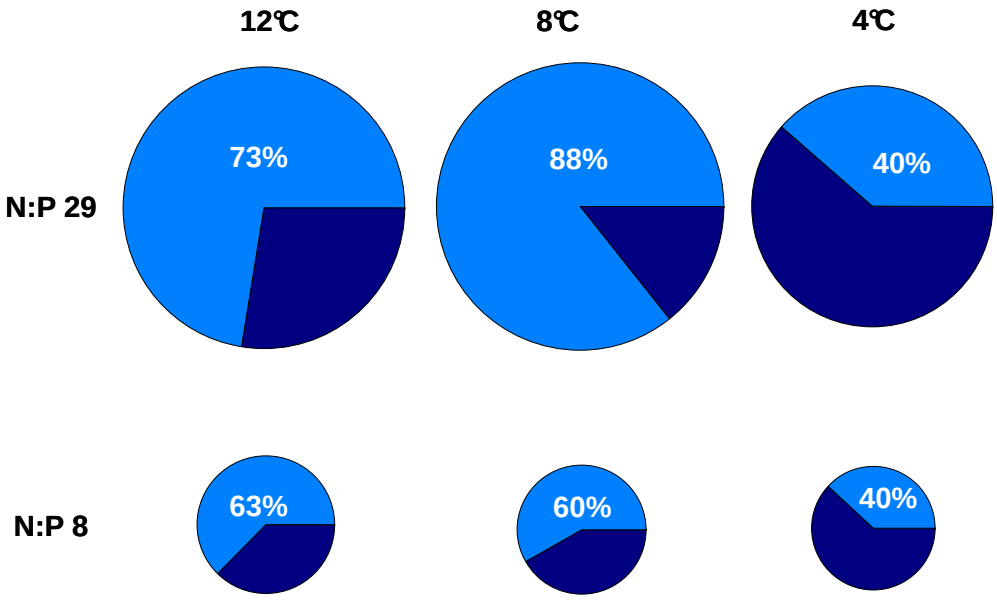


Figure 11: Pie chart of the BP:PPP ratio. The size of the pie represents the amount of PPP and the light blue slices correspond to the BP:PPP ratio (numbers in %).

Table 4: Mean values of the different parameters for their individual peak periods. PPP, DPP and BP in µg C $L^{-1}d^{-1}$; PER, BP:PPP, BP:DPP in %; TBN in 10^6 cells ml^{-1}. R^2 and p for the linear regressions of the means against temperature. The correlations were performed with the original data for each of the three replicates. Equations can be found in the respective "Results" section above.

	12℃	8℃	4℃	R^2	p
			PPP		
N:P 29	334	342	288	0.18	0.25
N:P 8	163	153	147	0.16	0.33
			DPP		
N:P 29	5.40	7.26	9.76	0.22	0.20
N:P 8	5.50	8.49	7.59	0.09	0.43
			PER		
N:P 29	1.63	2.02	3.51	0.27	0.15
N:P 8	3.26	6.28	4.77	0.19	0.28

			TBN		
N:P 29	1.98	2.60	3.71	0.89	**0.0001***
N:P 8	1.97	1.85	3.15	0.51	**0.03***
			BP		
N:P 29	242	293	111	0.39	0.07
N:P 8	102	88.8	56.4	0.78	**0.02***
			BP : PPP		
N:P 29	72.5	87.8	39.8	0.27	0.15
N:P 8	63.0	60.4	39.5	0.63	**0.02***
			BP : DPP		
N:P 29	6146	5098	1660	0.28	0.14
N:P 8	2090	1157	849	0.52	**0.03***

Bacterial Carbon Demand to Primary Production ratio (BCD : PP)

Bacterial respiration was calculated (see *Material & Methods*) in order to assess total bacterial carbon demand in relation to primary production. The values of BCD: PPP increased from an average 132 % to 191 % for the high-N nutrient treatment and from 138 % to 206 % for the low-N treatment between the temperature ranges of 4 to 12℃. In contrast to the BP: PPP ratio, here the particulate primary production could not provide for the bacterial carbon demand. There was no statistically significant difference between the ratios of the two nutrient treatments (T-test, t=-0.48, p=0.64, n=18).

Discussion

We created an experimental setup with a factorial combination of two different nutrient levels and three different temperatures in order to assess the combined effects of both factors on phytoplankton-bacterioplankton interactions during an algal bloom. Using this setup we were able to create monoalgal blooms with different quantities and temporal dynamics depending on the nutrient and temperature conditions and corresponding bacterial responses to altered

substrate supply. The details of our results will be discussed below and conclusions for possible future climate scenarios will be drawn from our results.

Dynamics of phytoplankton

As expected, particulate primary production (PPP) showed a very different quantitative development in the two nutrient treatments due to the available primary inorganic nutrients. Although algae in the high-N nutrient treatment (N: P 29) were phosphate limited during the exponential growth phase and at the peak of the bloom, PPP still continued to rise, indicating that growth was still possible due to the available nitrate. In this phase the algae could possibly utilise phosphate from cell internal reserves. Rhee (1972) showed that phytoplankton is able to build up phosphate reserves which can be used during limiting growth conditions. This is also a possible advantage in the competition with bacteria for primary inorganic nutrients, which do not have the possibility for nutrient storage under balanced growth conditions. Bacteria however, in competition, have the advantage of faster growth, during non-limiting nutrient conditions. Other possibilities include a changed element ratio in the formation of cell components or phosphatase-activity of the algae. Moreover, it is possible that remineralisation of organic compounds through heterotrophic bacteria made inorganic phosphate available, which was then used up by the algae immediately, without being detectable.

In the low-N nutrient treatment (N: P 8) nutrient limitation resulted in earlier and much lower peaks compared to the high-N treatment. The differences in mean PPP at the different nutrient treatments display a ratio of on average 2.1, which is what would be expected from the Redfield ratio (i.e. 2.0). From the daily ratio of PPP a theoretical standing stock according to Redfield cannot be inferred, but particulate organic carbon (POC) data available from Wohlers (see Wohlers 2009) shows that the observed to calculated ratios reached values, which were much higher than expected from Redfield (4.7 times higher at high-N and low-N treatments). The ratio of high-N to low-N was 1.6, which is lower than the expected ratio of 2, showing that there was also a higher POC production in the

low-N treatments relative to the high-N treatments. This result is in contrast to the ratio of 2.1, observed for the PPP ratio. It has to be noted, however, that primary production is a daily rate and does not reflect standing stocks, while the standing stocks on the other hand cannot take into account any production or loss rates. Hence these results are not directly comparable. The observed "carbon overconsumption" has been described in previous experiments (Wohlers, personal communication) and by other authors (Toggweiler 1993, Kähler & Koeve 2000, Schartau et al. 2007), possibly leading to the formation of organic material with "abnormal" C: N: P ratios. As described above, cell internal phosphate and nitrate reserves (Dortch 1982) or bacterial remineralisation could also possibly explain the observed "overconsumption" in POC in our experiment.

In the low-N nutrient treatment sample 11 showed a distinctly different development compared to the other replicates and all other treatments, and was therefore interpreted as a biological outlier and omitted from further analysis. Measurements were considered as correct, because the values were not only unusually high on one single day but on three consecutive days (and confirmed by a high chl a level on day 14, Wohlers 2009). We have no explanation however, for this distinctly different development, because the unusually high PPP was not reflected in high dissolved primary production (DPP) values, or any of the other parameters.

Irrespective of the different nutrient treatments, temperature had an influence on the temporal dynamics of primary production, accelerating peak development significantly (for comparison with temporal dynamics of bacteria see below). This could be explained by possibly saturating light levels, making photosynthesis temperature dependent. There was no significant influence of temperature on the quantity of production, but a trend towards higher particulate production at elevated temperature was observed, which could be explained by the species' temperature preference. *Skeletonema costatum* commonly dominates the spring bloom of phytoplankton in the Kiel Fjord, when temperatures are usually below 4℃ (U.Sommer, personal communication). However, multiple linear regressions confirmed that the nutrient treatment was the main factor explaining quantitative particulate primary production variation.

In both nutrient treatments, the dynamics of DPP were closely coupled with PPP concerning timing but not concerning the quantities. In contrast to the different PPP levels, DPP showed fairly similar levels in both nutrient treatments, being reflected in higher PER levels (percent extracellular release) in the low-N nutrient treatment, which could be interpreted as a result of nutrient stress. Pomeroy & Wiebe (2001) have described that nutrient-limited or -stressed phytoplankton release less and <15% of fixed carbon as DOC. With *Skeletonema* in this experiment, PER was on average only 3.5 %, with higher values observed in the low-N treatment, which is overall much lower as the described 15 %. Wolter (1982) reported that *Skeletonema costatum* released between 5.1 and 12.5% of the primary products as exudates and Myklestad (2000) states that between 2 and 10% of primary production are released as exudates during rapid growth. Nagata (2000) reports a wide range of values between 10 and 80% in various marine environments. In contrast to the statement of Pomeroy & Wiebe (2001) several authors describe that the imbalance between growth and photosynthesis, driven by nutrient deficiency, can induce/accelerate the exudation of assimilated carbon from several algal species (e.g. Puddu et al. 2003). Although mainly described for phosphate limitation, in our case the treatment with N-deficiency, or rather conjoint deficiency of N and P, was displaying the relatively higher exudation. So obviously, in our experimental setup, the algal cells at the high-N treatment were not as stressed by the apparent P-deficiency in our experimental setup, either compensating via internal P-reserves or by switching to a different organic matter composition. In the low-N treatment cells might have been more stressed by the very early combined exhaustion of both nutrients.

Although there was no significant influence of temperature on the quantity of DPP, there was a clear tendency towards higher exudation at lower temperatures within the high-N nutrient treatment (although there was always one very low replicate in each temperature treatment). One could expect this increased substrate supply to be reflected in the bacterial dynamics. However, as described below, bacterial production was very low at 4℃, so in turn the miss ing utilisation by bacteria might have been responsible for unused DPP. Probably, the quality (i.e. refractory

nature) of dissolved organic material could have played a role in this observed pattern at high-N and low temperature.

When interpreting dissolved primary production measurements, it also has to be kept in mind that, due to the nature of the method, bacteria utilise the produced dissolved organic material already during the incubation time, leading to a possible underestimation of the actual production quantity.

Dynamics of bacteria

The peak timing of total bacterial numbers (TBN) was significantly accelerated by temperature in both nutrient treatments. The peak at 12℃ in the high-N treatment was much earlier (5 days) than the peak in particulate primary production so that this peak cannot be explained by substrate availability from phytoplankton (either particulate or dissolved). Fast utilisation of available dissolved organic carbon (DOC decreases slightly in all treatments until day 15, Wohlers personal communication) from the water in these warm conditions together with successful competition for primary inorganic nutrients could possibly have fuelled this rapid growth. Possibly, the fast drawdown of ammonium in the beginning of the experiment could be partly explained by this dynamic. Nevertheless particulate primary production did not seem to have been adversely affected by this competition (possibly utilising nitrate instead of ammonium), and there was no difference compared to the other treatments. An other explanation could be fast cell division (without protein production) of the existing cells as a response to the changed ambient conditions at the start of the experiment (“bottle effect”).

Overall, the abundances were very similar in the different nutrient treatments and multiple linear regression revealed a significant influence of 69% on mean values for temperature. Increasing numbers at colder temperature could have been fuelled by the observed increasing dissolved primary production at least in the high-N treatment. It has to be noted, however, that there was always one very low replicate in all temperature treatments at high-N DPP, and this pattern was not reflected in bacterial abundance. In contrast to this, only two of the three low-N replicates at 4℃ showed higher values than all of the treatments at the different temperatures.

The constantly increasing bacterial numbers in the cold high-N treatment could have been fuelled by the high PPP, a longer availability of inorganic nutrients, and generally low, but steady generation times. Only the latter of which could also possibly be found at the low-N treatment (due to low temperature), while the first two conditions were not present.

Comparably high DPP in the low-N treatments could be responsible for similarly high bacterial abundance in these treatments, although particulate primary production was much lower. However, as shown below, DPP was much lower than the bacterial demand for production, so a significant influence of DPP on bacterial dynamics cannot really be expected. Another possible explanation could be that bacteria were able to utilise the inorganic nutrients more efficiently than phytoplankton, thus outcompeting the algae, but there is no indication of this in the algal dynamics. This is often found, as the advantage of bacteria only lies in very low concentrations of inorganic nutrients. Additionally, it would leave the source of carbon for bacteria unexplained, as well as the discrepancy with the very low bacterial production.

A possible explanation for the similarly high bacterial numbers, in view of the much lower bacterial production values in the low-N treatments, could lie in the method of acquisition. In the flow cytometer, all bacteria were grouped together and no differentiation concerning size was taken into account. So, possibly, the actual difference was not in the amount of cells, but in the biomass. The constantly increasing bacterial numbers in the high-N 4℃ treatment could thus be explained by the existence of a high number of large non-dividing cells, which were not detected by the protein production measurement of ^{3}H-leucine incorporation, while the low-N treatment was dominated by small, actively dividing cells (see also below). We checked the flow cytometry data for autofluorescence signals and could exclude a significant contribution of autotrophic cyanobacteria to the cell counts.

The sharp decreases in bacterial numbers after the respective peaks are unexpected, considering the absence of grazers. We have confirmed, however, by microscopic analysis of selected time points during the course of the experiment that possible grazers (i.e. nanoflagellates) were present, but not in

relevant numbers and not at relevant time points. Another explanation could be the lysis by viruses, which was not investigated in our experiment. Also the attachment of bacteria to particles during the bloom and post-bloom phase might have inhibited their detection in the flow cytometer.

The increase in bacterial numbers after the first breakdown in the high-N treatments at 8° and 12°C was not seen in the low-N treatments. A possible explanation could be a reaction to the degradation of the phytoplankton bloom, but there was no increase in DPP measured at that time. It could be interpreted as a result of the high bacterial production which, at both temperatures, was still high or increasing during that time.

Bacterial production (BP) in the high-N treatments displayed a significantly accelerated peak development, and the means showed a trend towards increasing values with rising temperature. The dynamics at 8° and 12°C could be interpreted as reflecting the development in primary production, but this was not valid for the 4°C treatment. Apparently, although there was enough substrate on the organic carbon level, the low temperature prevented the bacteria from displaying an increased production, i.e. increased substrate supply of DOM did not compensate for temperature suppression. In return, the low bacterial production could have been responsible for unused “leftover” dissolved organic matter. The low bacterial production at low temperature in general can be explained by the functional relationship between temperature and growth (i.e. the Q_{10} value), which becomes non-linear at temperatures near the growth limit, and in combination with low substrate even double digits are possible (Pomeroy & Wiebe 2001). This is in contrast to results from Nedwell & Ruttner (1994), Pomeroy et al. (1991), Pomeroy & Wiebe (2001) and Wiebe et al. (1992), who showed that substrate supply could partly compensate temperature limitation at low water temperatures in cold water bacterial strains. In the present case, we cannot assume a dominance of arctic or other cold water strains (see Walther 2009 for an analysis of bacterial community composition). The significant temperature-substrate interactions found by Pomeroy et al. (1991) also revealed that changes in substrate concentration of several orders of magnitude are often

necessary to maintain microbial activity at temperatures near the lower temperature limit for isolates or natural communities. Hence, at the present low temperature the available substrate pool was inaccessible for bacteria due to the temperature-related substrate affinity (Nedwell 1999). At the current high-N spring situation in the Kiel Fjord, a future increasing temperature scenario would release bacteria from the temperature suppression and enable an increased substrate utilisation.

Bacterial production at low-N stayed on constantly low levels throughout the experiment, apart from a very early small peak at 12°C, which was also seen at the high-N treatment. There was, however, a significant difference in production values between the different temperatures. There was no reflection of primary production (neither particulate nor dissolved) observed in the dynamics of bacterial production, and the dynamics are not sufficient to explain the development in bacterial cell numbers. Possibly, the bacteria in the low-N treatment were generally limited by substrate, as PPP was low (and DPP was much too low anyway, see below). The ratio of BCD: PPP was above 100% in all temperature treatments (see below), indicating organic substrate limitation. On theoretical grounds, it has been suggested that substrate concentration should not be limiting to heterotrophic bacteria in the upper mixed layer (e.g. Williams 2000) but Nedwell (1999) argued that heterotrophic bacteria in natural waters are often presented with sub-optimal concentrations of substrates (and limiting temperature extremes). Apart from substrate concentration, the substrate composition in terms of nutrient status has to be taken into consideration. As described above, the different levels of “carbon-overconsumption” might possibly lead to different nutrient compositions of algal cells at the low- compared to the high-N treatments, with possible consequences concerning bioavailability and nutritional value for the bacterial assemblage (for details on the C:N:P composition of the available POM see Wohlers 2009). Another possibility could be the limitation of bacteria by the inorganic nutrients themselves. Obernosterer & Herndl (1995) showed that P-limitation refrained bacteria from utilising the increased exudations of algae, but they used much higher ratios. We have to consider however, that P-limitation in

the high-N treatment did not result in comparably low bacterial production like in the low-N treatment (at the warmer temperatures).

Temperature had a stronger effect on bacterial production at the high-N treatment, considering the slope of increase and the absolute values. The low-N treatments at any temperature behave similar to the high-N treatment at 4°C. Under a future scenario of stronger P-limitation (see *Introduction*) in combination with increasing temperatures, this would mean an increase in bacterial production, and hence an increased remineralisation of primary produced organic matter.

The peaks in bacterial abundance at high-N were much earlier than the peaks in bacterial production, which cannot be explained by the bacterial production level. Similarly, at the low-N treatment the dynamics of bacterial abundance are not at all reflected by the development of bacterial production. As indicated above, this could possibly be explained by the different methods. Also, incorporation of ^{3}H-leucine measures the production of cellular proteins, which is an indication of individual cell growth and not directly of bacterial cell division. Pomeroy & Wiebe (2001) reported that at least some heterotrophic bacteria have been shown to have the ability to adjust their growth rate and body size according to the substrate concentration present. So maybe, at the low-N nutrient treatment, bacteria were limited to a low protein production. However, they divided nonetheless, resulting in a large amount of small cells. Total bacterial numbers between the high-N and low-N treatments were hence similar, but cells were smaller at the low-N treatment. It also has to be taken into account that bacterial production measures a growth rate and that TBN is a standing stock, so that accumulations of cells might not be reflected in actual production measurements at that time.

Concerning the competition for primary inorganic nutrients, there was no indication that bacteria were able to outcompete algae, although several authors (e.g. Rhee 1972) have shown in chemostats, that the faster growing bacteria are able to outcompete algae for phosphate. It also has to be considered that bacteria can utilise very low concentrations of inorganic nutrients, which are inaccessible by algae, so that a direct competition does not occur. On the other hand, bacteria

can fuel algal growth through the remineralisation of organic matter. We cannot assess whether the algae would have reached higher or lower production levels in the absence of bacteria, but bacteria did not show any reaction to exhaustion of inorganic nutrients in their dynamics (Fig.6 & 8).

Interaction between phytoplankton and bacteria

Although the differences were not statistically significant, we could show that the acceleration of development through increased temperature was always stronger for bacterial parameters in comparison to the autotrophic fraction (Table 3). This would mean that on a temporal basis, the bacterial peak would move towards the algal peak, closing the gap between carbon fixation and utilisation. This way, the organic carbon from phytoplankton would be available for a longer time, before sinking out of the photic zone, thus increasing the amount of organic matter that could be recycled via the microbial loop. It has to be noted here, however, that this is only valid for the bacterial production peak, as the peak timing of bacterial abundance was always at the same time or even earlier (increased acceleration) than the respective peaks of primary production anyway. The usually observed lag time between bloom and bacterial production is thought to be due to the differential response of phytoplankton and bacteria to low spring temperatures (Pomeroy & Deibel 1986), and hence would be expected to decrease with increasing temperatures, as we have demonstrated.

There was a trend towards increasing BP: PPP ratios with rising temperatures at both nutrient treatments. Although there was no statistically significant difference between the nutrient treatments in the effect of temperature on the BP: PPP ratio, the absolute values are higher and the increase in the ratio is steeper for the high-N nutrient treatment, and hence the effect of increasing temperature and increasingly P-limiting conditions (see above) could possibly lead to a relatively stronger increase in bacterial utilisation of primary produced organic matter.

A much stronger trend was observed for the ratio of BP to DPP. Again, the ratios showed increasing values with increasing temperatures, and the ratios at 4℃ already show that dissolved primary production did not play a significant role as a

substrate during the bloom. The discrepancy between demand and supply of dissolved organic matter increases with increasing temperatures and the gap grows much larger for the high-N nutrient treatment (significantly different to low-N) with the same possible consequences as described above. Pomeroy & Wiebe (2001) described that the potentially rapid transfer of dissolved organic carbon from auto- to heterotrophs usually falls short of the demand of bacteria for growth. Hence, additional connections have been proposed, such as viral lysis, nutrient deficiency lysis, the excretion, defecation, and sloppy feeding by zooplankton as well as protist grazing (the latter of which is not applicable in our experiment). Nagata (2000) supports this by stating that these processes are necessary for initiating significant bacterial activity for the microbial loop. The importance of these pathways for dissolved organic matter release become clear when considering that the direct extracellular release by phytoplankton is between 2 – 10% of primary production, but that up to 50% of photosynthetically fixed carbon circulates in the dissolved compartment as accumulated inert remains (Puddu et al 2003 and references therein).

When looking at the ratio of bacterial carbon demand to particulate primary production, a similar ratio is observed for the two nutrient treatments. This shows that including respiration in the calculations can reveal a possible carbon deficiency even from particulate carbon, which increases with increasing temperatures. It has to be kept in mind, however, that the bacterial respiration was calculated from bacterial production. Hence, the results from these calculations have to be considered carefully and can only be taken as a trend.

In general, when considering the interactions of the available organic substrate and the utilisation by bacteria, the quality of the substrate has to be taken into account. A detailed analysis of organic matter quality and an assessment of bacterial extracellular enzyme activity can be found in Wohlers (2009). Secondly, the composition of the bacterial assemblage can change over a period of days of incubation. The impact of temperature and nutrient conditions on the bacterial community composition and possible consequences of altered substrate utilisation have been assessed by Walther (2009).

Summary and Conclusions

1. We can conclude that temperature had an accelerating effect on the development of both autotrophs and heterotrophs. This acceleration was generally stronger for the heterotrophic fraction, confirming expectations and results from previous experiments. Thus, gaps between carbon fixation and utilisation would be decreased, tightening the coupling between phytoplankton and bacteria and facilitating increased carbon transfer through the microbial loop.
2. The two nutrient treatments had different effects on the quantity of particulate primary production. The absolute values were very different, reflecting the nutrient levels and the expected ratio according to Redfield. However, standing stocks (POC) indicated different non-Redfield cellular compositions. Relatively more dissolved organic compounds were exuded at low-N conditions, compared to the high-N treatment. Temperature slightly increased particulate production at the high-N nutrient treatment, while a negative temperature effect on the quantity of dissolved primary production could be observed. In a future scenario of increasing P-limitation in coastal regions (Andersson et al. 1996) we would hence expect increased phytoplankton particulate production, but not necessarily increased exudation of dissolved organic matter. Temperature increases would initially facilitate higher particulate primary production, in combination with a relatively lower exudation.
3. The increased particulate primary production at high-N obviously fuelled an increased bacterial production, compared to the low-N nutrient conditions, but only at the higher temperatures. At 4℃ the low temperature inhibited bacterial production to a level almost as low as in the low-N treatment, hence even the higher nutrient availability could not compensate the temperature inhibition. We can conclude that in comparison to the current situation of high-N and low temperature, increasing temperature will release bacteria from the temperature suppression and hence lead to a higher relative amount of primary produced organic matter being utilised. This will lead to a relatively higher CO_2 release from respiration and to decreased particulates left for aggregation and sinking.
4. This could be enhanced by an increased P-limitation in coastal regions. The comparison with the low-N nutrient treatment underscores the effects with

reference to a future open ocean. Under a future scenario of stronger P-limitation (see *Introduction*) in combination with increasing temperatures, this would mean an increase in bacterial production, and hence an increased remineralisation of primary produced organic matter. The generally low exudation of dissolved organic matter from *Skeletonema* did not play a significant role in supplying heterotrophic bacteria with substrate for growth, increasing the importance of alternative sources like sloppy feeding from zooplankton and lysis by viruses. Direct competition for primary inorganic nutrients between phytoplankton and bacteria could not be observed in our experiment.

SYNTHESIS AND FUTURE OUTLOOK

Half of the global primary production is performed by phytoplankton in the oceans and about half of this marine primary production is utilised by heterotrophic bacteria. This way the heterotrophic marine bacteria channel a substantial amount of primary organic carbon through the microbial loop and hence represent an important part of the marine carbon and nutrient cycles. The overall efficiency of the microbial loop is dependent on a variety of abiotic and biotic factors, which directly or indirectly impact phytoplankton and bacterial survival and performance and consequently the strength of the coupling between the two compartments.

In the context of global change the possible consequences of changing environmental parameters on the coupling between phyto- and bacterioplankton are of major interest. While autotrophic carbon fixation is mainly dependent on light intensity, the heterotrophic processes of carbon utilisation are temperature dependent (Tilzer et al. 1986, Pomeroy & Wiebe 2001). Hence a different influence of these changing environmental conditions and consequently a change in the coupling between the two compartments can be expected, with possibly severe consequences for the global marine carbon cycle.

Climate change predictions include the increase of winter temperatures by up to +6°C in the Northern Hemisphere until the end of the century (IPCC 2007). Combined with this are a probability of a continued brightening trend, as observed today (Wild 2009) and predictions for increases in precipitation, leading to enhanced land runoff and riverine influx of phosphate-limited waters to coastal areas (IPCC 2007).

Results presented in this work

As part of the Kiel AQUASHIFT mesocosm cluster this work investigated the temperature dependent coupling between phytoplankton and bacterioplankton, with respect to additional effects of light intensity and inorganic nutrient concentrations. In consecutive years, mesocosm experiments with natural Kiel Fjord winter plankton communities investigated the influences of warming water temperatures of up to +6°C and different light intensities between 16 and 100% of

incident light. In an additional microcosm experiment with a single algal species and the natural bacterial community a full factorial combination of different temperature and inorganic nutrient concentrations was used, in order to assess the combined effects of both parameters on the algal-bacterial coupling.
In all experiments the process of autotrophic carbon dioxide assimilation was assessed by primary production measurements. Heterotrophic bacterial organic carbon utilisation was measured by different parameters such as abundance, biomass production and respiration. The coupling of both processes was assessed on the basis of timely overlap of the occurring peak development during the spring bloom succession, and further by the ratios of heterotrophic to autotrophic quantities.

The experiment described in **Chapter 1** was conducted under high light conditions and at three elevated temperatures additionally to the in situ early spring temperature (ΔT +0°C to +6°C). We hypothesised that increasing te mperatures would lead to an increased transfer of organic matter via the microbial food web due to a decreased lag time between the autotrophic production and heterotrophic microbial degradation in combination with an increased heterotrophic microbial activity. The results showed a close timely coupling between the two compartments, where bacteria quickly utilised dissolved and particulate organic carbon from phytoplankton during the peak and the degradation phase of the bloom. As hypothesised, bacterial parameters were enhanced under increasing temperatures, showing an increase of bacterial production (from ^{3}H-thymidine) by +148%, while primary production was only little affected (-19%). This lead to an increased organic carbon transfer through the microbial loop, as displayed in increases of the ratios to 6.1% and 93.2% for bacterial production to particulate and dissolved primary production respectively. Ratios of largely over 100% for the bacterial carbon demand to dissolved primary production relationship revealed a large dissolved organic carbon deficiency. Additionally, the community respiration was enhanced under warming conditions by 51% relative to the particulate primary production, resulting in a ratio of 41%, indicating a strong shift to generally more heterotrophic conditions.

In **Chapter 2**, the results from four consecutive mesocosm experiments are described. The first three experiments were conducted under similar temperature conditions, comprising the current in situ temperature and three warming scenarios, according to the IPCC (2007) predictions (as in Chapter 1). The experiments in the different years differed in the light intensities, including current dim spring situations as well as possible future brightening scenarios (16 – 64% I_0). The fourth experiment in 2008 contained the full factorial combination of the two temperature extremes (in situ and ΔT +6°C) with three light regimes (current 32% I_0 and brightening scenarios 48, 64% I_0). The aim of this synthesis chapter was to highlight recurring patterns of changing phytoplankton-bacterioplankton interactions under warming and brightening conditions. The question, on the background of the different light levels, was not only how the relative amount of organic carbon changes, that is utilised from primary production but also how much absolute amounts of utilised organic matter would change in a future warming and brightening scenario. The experiments from different years highlighted clearly that different starting conditions due to different overwintering populations of all plankton members strongly influence the development of the plankton spring succession (Gaedke et al. 2009). Nevertheless, we could demonstrate that basic reactions to temperature and light were a consistent pattern in these populations. Autotrophic as well as heterotrophic parameters were enhanced at warmer temperatures and at higher light intensities. While bacterial production showed a relatively higher increase at warm temperature and dim light (+23% increase), the latter showed a stronger response at warm temperature and bright light conditions (43% increase). Concluding from the combined results of the heterotrophy: autotrophy ratios from our experiments, the highest relative carbon remineralisation would hence occur in a future scenario of a dim and warm winter/spring (43% BP: PP ratio), while the highest absolute amount would be utilised in a situation of bright and warm winter/spring (31% more absolute organic carbon cycling through the microbial loop, compared to dim and warm conditions).

Chapter 3 deals with a different form of experiment, where microcosms of 25 L capacity were stocked with a model algal-bacterial community and exposed to different temperature and inorganic nutrient concentrations (P-limiting and N-limiting situation). The aim was to create different quantities (and qualities) of organic substrates for bacteria via phytoplankton in order to disentangle the effects of substrate supply and temperature effects. At the same time an assessment of direct competition for inorganic nutrients was possible. The results of the assessment of peak timings showed that the time-lag between autotrophic carbon fixation and heterotrophic bacterial utilisation was somewhat diminished at warmer temperatures (between 1.4 and 4 days in the investigated temperature range). Quantity measurements showed that high-N situations lead to increased phytoplankton particulate production by around 50% according to nutrient availabilities, but in combination with a relatively lower exudation. In comparison to the current low temperature situation, warming released bacteria from the temperature suppression and hence led to a higher relative amount of primary produced organic matter being utilised. Increases in the bacterial to primary production were larger at high-N conditions (+89%) compared to low-N conditions (+59%). In a future scenario of increasing P-limitation in coastal regions in combination with warming temperatures, this would mean an increase in bacterial production and hence an increased remineralisation of, an absolutely higher, primary produced organic matter, as reflected in a bacterial to primary production ratio of 73%. Direct competition for primary inorganic nutrients between phytoplankton and bacteria could not be observed in our experiment.

Summarising the results from all experiments it can be concluded, that increasing temperatures generally lead to an increased heterotrophic bacterial organic substrate utilisation relative to primary production through a combination of a decreased time-lag between the two peaks and a stronger increase in the bacterial activity parameters. If a future warming trend would be accompanied by a further brightening, the supplemental promotion of primary production would increase the absolute amounts of cycled organic matter. Future increasing precipitation, leading to increased P-limitation in coastal waters would lead not

only to an increased absolute amount of cycled carbon (like for the high light) through increased primary production, but additionally to an increased relative amount of remineralised organic carbon through the microbial loop.

Implications of the presented results

As demonstrated in Chapter 2 (Synopsis), one of the most striking results from the consecutive years of mesocosm experiments is the reoccurrence of the observed patterns in phytoplankton-bacterioplankton coupling, despite of large differences in the starting conditions. The variety of naturally occurring differences in conditions we were presented with, from overwintering populations and water conditions to initial water temperatures, turned out to be an advantage in that we can confidently assume that the recurring response patterns we detected enable a valid prognosis for the impact of climate change on the described system. Nevertheless, one important point to keep in mind when interpreting these results is the fact that the investigated parameters temperature, light and inorganic nutrients are not always directly but also often indirectly responsible for the described reactions of phytoplankton and heterotrophic bacteria. As described in the respective chapters, indirect top-down or bottom-up effects like for example grazing by phytoplankton grazers or changes in phytoplankton community composition have to be taken into account (Sommer & Lengfellner 2008, Lewandowska & Sommer in revision, Gaedke et al. 2009). Fortunately the experimental setting of the Kiel AQUASHIFT mesocosm cluster allowed the participation of several research groups, which investigated different aspects of the spring plankton succession in the experiments. This way, additional information is available that can be included in the considerations.

Walther (2009) for example investigated the influence of increasing temperature and different inorganic nutrient compositions on the composition of the bacterial community. She could show that temperature increases lead to a change in the community composition of the heterotrophic bacteria, an effect which was intensified at high-N nutrient conditions. Changes in the species composition of the remineralising heterotrophic community can alter activity patterns as well as show differential remineralising properties, when for example psychrophilic

species disappear in future. The measured changes in bulk quantities of production or respiration, as described in this work, might hence be traced back to a different set of species, rather than a change in activities of the existing community. On the other hand, changes in community composition could be responsible for a dampened response of the community in the future, when better adapted species replace the existing community and this way maintain the existing remineralisation properties, representing an adaptation process.

Wohlers (2009) demonstrated in her work on the biogeochemical cycling of the nutrient elements, that the net DIC (dissolved inorganic carbon) uptake was significantly reduced at warmer temperatures as well as under N-deficient growth conditions. This corresponds well with the results presented in this work, on generally decreased primary production at warmer temperatures in combination with a strong increase in community respiration. Combined with this, Wohlers found an increase in DOC (dissolved organic carbon) accumulation, which was ascribed to secondary release of refractory DOC by bacteria. She concluded that the biological pump, facilitating carbon export to depth, would be weakened in a future warming ocean (and at P-limited nutrient concentrations), with a potential positive feedback mechanism to climate change, again confirming the results presented in this work.

In her work on the effects of climate warming on the phytoplankton and mesozooplankton compartments, Lengfellner (2008) found an increasing mismatch situation of the peak timing of phytoplankton and copepods offspring. This effect of warming was counteracted by the influence of increasing light supply. Ciliates and Copepods were accelerated and activity enhanced at warmer temperatures, with the implication of reduced phytoplankton biomass and changes in phytoplankton bloom composition (to smaller species). Reduced primary production at warmer temperatures was found as a general pattern in this work (except for the combination of high light and warm temperature in 2008), which might well be explained by the increased grazing and change in the algal community as described by Lengfellner. She concluded that this way temperature indirectly lead to an enhanced importance of the microbial loop via ciliates and hence reducing the efficiency of energy transfer to higher trophic levels and also a

weakening of the biological pump. Again, we can confirm these findings by our results on the activities of heterotrophic bacteria, contributing significantly to the enhanced importance of the microbial loop with the described consequences.

The temperature dependent coupling between the phytoplankton and bacterioplankton compartments has been described by various authors. Often the phytoplankton-bacterioplankton coupling is assessed as the relationship of bacterial carbon demand (BCD) to dissolved primary production (DPP), representing a lose coupling if DPP cannot meet the requirements of BCD (Moran et al. 2002 a, b). Generally this trend is found in coastal or eutrophic sites, where allochthonous sources of DOM can be important, while a tight coupling is therefore found in more open ocean sites (Cole et al. 1988, Moran et al 2002a). Teira et al. (2003) however reported uncoupling also from the oligotrophic North Atlantic, suggesting the dependence of bacterial production from alternative sources. Rochelle-Newall and co-workers (2008) assessed the phytoplankton-bacterioplankton coupling in a tropical coastal ecosystem and in most oligotrophic sites BCD was higher than the supply of dissolved PP, which was reversed only at the coastal sites, with higher inorganic and organic matter concentrations.

The direct comparison of DPP with BCD reported in this work for the high light experiment described in Chapter 1 showed a strong uncoupling because the dissolved primary production could not meet the requirements for bacterial growth, the relationship did not change with temperature, though. As described, additional sources for bacterial growth must have been available, like for example sloppy feeding by zooplankton or an existing allochthonous organic carbon background in the water. When moving away from this rather narrow definition of “coupling” we are taking particulate primary production into account, as an important source for bacterial organic carbon requirements through the use of extracellular enzymes for degradation. All described results in this work showed a positive response to temperature, with increasing BCD or BP: PPP ratios with temperature, which indicate an increased coupling.

In a recent review of phytoplankton – bacterioplankton coupling in polar oceanic regions, Kirchman and co-workers (2009) showed that the ratio of BP to PP was increased substantially at temperatures up to 4℃ and to a lesser extent at

warmer temperatures. They concluded that warming of Arctic surface waters would lead to substantially more carbon, being processed by the microbial loop and potentially less going to higher trophic levels and export to the deep sea and the benthos. They suggest however, that besides temperature, the effects of other factors like light for phytoplankton and inorganic and organic nutrients for phytoplankton and heterotrophic bacteria might have substantial influences. These results confirm our findings on the BP: PP ratios at the cold temperatures investigated in this work and also highlight the importance of our investigations on the additional factors “light” and “nutrients”.

The enhanced importance of bacterial activities in relation to primary production, as demonstrated in this work for a scenario of warming, brightening and increasing P-limitation indicate a general shift to more heterotrophic conditions in a future ocean. Berglund et al (2007), in mesocosm experiments with northern Baltic Sea water, demonstrated that a bacteria-based foodweb displays a significantly reduced food-web efficiency due to the extra trophic levels in the microbial loop. They conclude that such a foodweb, which will be favoured by increased organic nutrient supply in a future climate, through increased precipitation and river runoff, will reduce pelagic productivity at higher trophic levels.

A decrease in the ratio of BP to dissolved PP with increasing photon flux density was previously reported from tropical coastal ecosystems (Rochelle-Newall et al. 2008). This result was based on the expected increase in PPP and DPP with increasing light intensity, but also on a decrease of BP with increasing light. This is in contrast to our results, as we found BP to react to the substrate supply by phytoplankton and increase with increasing light intensity, although to a smaller extent than primary production, hence reducing the ratio at higher light intensity. Kirchman and co-workers (2009) also found the BP: PP ratio to be positively correlated with euphotic zone depth (i.e. light availability), due to the light dependence of phytoplankton.

The usually accepted N-limitation of primary production in the oceans does not hold true for all regions and a (seasonal) P-limitation has been reported for several coastal areas, like for example the Finnish and Bothnian Bay (Andersson et al 1996, Rivkin & Anderson 1997, Zweifel et al. 1993). In the light of future increasing precipitation and consequently increased high-N freshwater inflow as well as increased stratification, it can be expected that the spread of P-limited oceanic regions will increase in the future.

The results described in this work on changes in the relationship between autotrophic carbon fixation and its utilisation by heterotrophic bacteria under warmer, brighter and more P-limited marine environments demonstrate how the marine organic matter cycling could be substantially altered in the a future climate. An increased organic matter transfer through the microbial loop has the potential to alter the whole structure and functioning of the marine food web and the biological sequestration of carbon to depth. In essence, an increase in the trophic levels facilitates a reduced transfer of energy and matter to higher trophic levels and, together with a generally increased respiration, leads to a substantial enhancement of CO_2 emissions and hence represents a positive feedback loop to the global climate change problem.

Future perspectives

As we have seen in the work at hand and from related investigations, not only the direct effects of environmental factors like temperature, light or nutrients, but also the complex indirect interactions are important to assess. Apart from these indirect abiotic effects, the biotic interactions in complex food-webs are vital to understand. The Kiel mesocosm cluster has tried to approach the complexity of the marine planktonic system in a very comprehensive way, and was able to gather important information and gain valuable insight into the complex interactions and possible reactions to a changing future climate. When trying to transfer the obtained results to the natural environment the limitations, as described in the general Introduction, of the mesocosm approach have to be kept in mind.

Anyway, open questions remain and my proposals for future research to better understand this complex system are described below. Firstly I highly favour the combined assessment of such complex food-web interactions on all possible levels. As I have demonstrated in Chapter 3, an experimental setup with selected species on a microcosm scale is a very valuable method in order to find basic functional relationships between species or groups of individuals. Although not directly transferable to the natural environment, these kinds of experiments enable the full factorial combination of several abiotic or even biotic factors which can be highly controlled, and with a large enough number of replicates in order to facilitate a more comprehensive statistical analysis. As described above, mesocosms represent a link between the small scale microcosm approach and the mere observation of natural systems. In that, they enable the incorporation of larger combinations of organism groups, like for example in our case, the marine planktonic food web up to zooplankton individuals. At the same time they facilitate the experimental manipulation of desired environmental conditions. A step upwards from this experimental approach is represented by outdoor mesocosms, which have the advantage of experimentally manipulating environmental conditions for an otherwise more or less complete natural system. Examples are the University of Bergen (Norway) mesocosms, where experimental elevation of CO_2 levels revealed important information on the mechanisms in the natural planktonic system and consequences for the marine carbon cycle (Riebesell et al. 2007). Another example is the experimental manipulation of thermal stratification and consequently light conditions in lake mesocosm systems as described by Berger and co-workers (2007). Although technically very challenging our mesocosm system would ideally be transferred to a similar approach, with outdoor enclosures of the natural plankton community, which could possibly even incorporate higher trophic levels like for example jellyfish and fish larvae, and which would for example experience natural light and/or temperature conditions, while the other factors could be experimentally manipulated at the same time. Results from the described microcosm and mesocosm approaches should ideally be combined with comparative observations of the natural environment, like results gained from cruises (or comparison of different years, which naturally

display different temperature conditions in spring). Like for example in the Baltic Sea, spring blooms of more northern latitude, which occur at colder spring temperatures could be compared to spring blooms in the south, which occur at warmer temperatures. Phytoplankton-bacterioplankton coupling could be observed and compared in regions of nitrate and phosphate limited conditions.

ANNEX

Table 1. Relationships for the regression of peak timing (DOP = day of peak) with temperature at the different light treatments (2008 experiment highlighted in blue). Acceleration of peaks is the slope value in days per 1℃ temperature increase (DOP_{+1}). Accelerations statistically significant on the $p<0.05$ level are marked with an asterisk. The difference in acceleration for a temperature increase of ΔT +6℃ of the respective heterotrophic parameters in comparison to primary production is shown (Diff. to PPP).

Parameter	Equation	R^2	p	Diff. to PPP
16%				
Particulate primary production	DOP_{+1} = -**0.83** DOP + 53.85	0.37	0.11	
Total bacterial number	DOP_{+1} = **0.90** DOP + 49.55	0.02	0.74	+10.38
Bacterial production	DOP_{+1} = -**2.20** DOP + 69.6	0.47	0.06	-8.4
32%				
Particulate primary production	DOP_{+1} = -**0.35** DOP + 25.80	0.65	0.01*	
Total bacterial number	DOP_{+1} = -**2.35** DOP + 33.80	0.95	<0.0001*	-12
Community respiration	DOP_{+1} = **0.05** DOP + 24.60	0.01	0.78	+2.4
Bacterial respiration	DOP_{+1} = **0.20** DOP + 24.40	0.20	0.27	+3.3
Bacterial production (leucine)	DOP_{+1} = -**1.60** DOP + 29.30	0.46	0.07	-7.5
Bacterial production (thymidine)	DOP_{+1} = -**2.42** DOP + 34.40	0.88	0.0005*	-12.4
32% b				
Particulate primary production	DOP_{+1} = -**0.67** DOP + 20.00	0.89	0.06	
Total bacterial number	DOP_{+1} = -**4.50** DOP + 41.00	0.99	0.001*	-23.0
Bacterial production (leucine)	DOP_{+1} = -**1.42** DOP + 35.00	0.63	0.21	-4.5
48%				
Particulate primary production	DOP_{+1} = -**0.42** DOP + 17.50	0.33	0.42	
Total bacterial number	DOP_{+1} = -**4.67** DOP + 42.00	0.99	0.003*	-25.5
Community respiration	DOP_{+1} = -**1.00** DOP + 20.00	0.95	0.03*	-3.5
Bacterial respiration	DOP_{+1} = -**0.75** DOP + 19.50	0.62	0.21	-2.0
Bacterial production (leucine)	DOP_{+1} = -**1.58** DOP + 34.00	0.88	0.06	-7.0

64%				
Particulate primary production	DOP_{+1} = -**0.05** DOP + 5.90	0.01	0.78	
Total bacterial number	DOP_{+1} = -**1.88** DOP + 22.25	0.80	0.003*	-10.98
Bacterial respiration	DOP_{+1} = -**3.85** DOP + 48.05	0.67	0.01*	-22.8
Bacterial production (thymidine)	DOP_{+1} = -**1.78** DOP + 20.45	0.69	0.01*	-10.38
64% b				
Particulate primary production	DOP_{+1} = -**0.58** DOP + 18.50	0.73	0.14	
Total bacterial number	DOP_{+1} = -**4.50** DOP + 41.00	0.99	0.001*	-23.5
Community respiration	DOP_{+1} = -**1.00** DOP + 20.00	0.95	0.03*	-2.5
Bacterial respiration	DOP_{+1} = -**0.92** DOP + 18.50	0.87	0.07	-2.0
Bacterial production (leucine)	DOP_{+1} = -**3.10** DOP + 39.50	0.98	0.01*	-15.1

Table 2. Relationships for the regression of peak timing (DOP = day of peak) with temperature for the 2008 experiment. Peak days of each parameter for one temperature (ΔT +0℃ or +6℃) are taken together, irrespective of the light treatment. Acceleration of peaks is the slope value in days per 1℃ temperatur e increase (DOP_{+1}). Accelerations statistically significant on the p<0.05 level are marked with an asterisk. The difference in acceleration for a temperature increase of ΔT +6℃ of the respective heterotrophic parameters in comparison to primary production is shown (Diff. to PPP).

Parameter	**Equation**	**R^2**	**p**	**Diff. to PPP**
Particulate primary production	DOP_{+1} = -**0.56** DOP + 18.67	0.56	0.005	
Total bacterial number	DOP_{+1} = -**4.56** DOP + 41.33	0.99	<0.0001*	-24
Community respiration	DOP_{+1} = -**1.00** DOP + 20.00	0.95	<0.0001*	-2.6
Bacterial respiration	DOP_{+1} = -**0.83** DOP + 19.00	0.69	0.01*	-1.6
Bacterial production (leucine)	DOP_{+1} = -**2.03** DOP + 36.17	0.78	0.0001*	-8.8

Table 3. Relationships for the regression of peak timing (DOP = day of peak) with light at the two different temperature treatments of the 2008 experiment. Acceleration of peaks is the slope value in days per 1% increase in light intensity I_0 (DOP_{+1}). Accelerations statistically significant on the $p<0.05$ level are marked with an asterisk.

Parameter	Equation	R^2	p
+6℃			
Particulate primary production	DOP_{+1} = -**0.03** DOP + 16.83	0.30	0.26
Total bacterial number	DOP_{+1} = -**0.00** DOP + 14.00	0.00	1.00
Community respiration	DOP_{+1} = -**0.00** DOP + 14.00	0.00	1.00
Bacterial respiration	DOP_{+1} = -**0.13** DOP + 21.00	1.00	n.a.
Bacterial production (leucine)	DOP_{+1} = -**0.17** DOP + 32.25	0.35	0.21
+0℃			
Particulate primary production	DOP_{+1} = -**0.05** DOP + 20.92	0.10	0.55
Total bacterial number	DOP_{+1} = **0.00** DOP + 41.33	0.00	1.00
Community respiration	DOP_{+1} = **0.00** DOP + 20.00	1.00	n.a.
Bacterial respiration	DOP_{+1} = -**0.06** DOP + 22.50	0.06	0.76
Bacterial production (leucine)	DOP_{+1} = **0.14** DOP + 29.42	0.50	0.12

Table 4. Relationships for the regression of peak timing (DOP = day of peak) with light at the two different temperature treatments of the 2008 experiment. Peak days of each parameter at the same light treatment are taken together. Acceleration of peaks is the slope value in days per 1% increase in light intensity I_0 (DOP_{+1}).

Parameter	Equation	R^2	p
Particulate primary production	DOP_{+1} = -**0.04** DOP + 18.87	0.05	0.48
Total bacterial number	DOP_{+1} = -**0.00** DOP + 27.67	0.00	1.00
Community respiration	DOP_{+1} = -**0.00** DOP + 17.00	0.00	1.00
Bacterial respiration	DOP_{+1} = -**0.09** DOP + 21.75	0.06	0.55
Bacterial production (leucine)	DOP_{+1} = -**0.02** DOP + 30.83	0.0009	0.93

Table 5. Relationships for the regression of the mean quantities with temperature at the different light treatments (2008 experiment highlighted in blue). Correlations statistically significant on the p<0.05 level are marked with an asterisk.

Parameter	Equation	R^2	p
16%			
Particulate primary production	PPP = -1.82 T + 17.30	0.75	0.005*
Total bacterial number	TBN = 0.002 T + 1.69	0.007	0.84
Community respiration	CR = 2.22 T + 21.11	0.82	0.002*
Bacterial respiration	BR = 1.17 T + 19.15	0.44	0.07
Bacterial production (thymidine)	BP = 0.02 T + 3.95	0.005	0.87
32%			
Particulate primary production	PPP = -4.07 T + 33.94	0.77	0.004*
Total bacterial number	TBN = -0.03 T + 1.57	0.17	0.30
Community respiration	CR = 1.81 T + 17.69	0.57	0.03*
Bacterial respiration	BR = 1.51 T + 15.31	0.64	0.02*
Bacterial production (leucine)	BP = -0.74 T + 14.31	0.44	0.07
Bacterial production (thymidine)	BP = -0.41 T + 5.63	0.53	0.04*
32% b			
Particulate primary production	PPP = -2.65 T + 150.87	0.16	0.60
Total bacterial number	TBN = -0.03 T + 1.79	0.51	0.28
Bacterial production (leucine)	BP = 1.30 T + 45.49	0.24	0.50
48%			
Particulate primary production	PPP = -2.36 T + 158.45	0.18	0.58
Total bacterial number	TBN = -0.03 T + 1.72	0.49	0.30
Community respiration	CR = 0.99 T + 78.60	0.47	0.32
Bacterial respiration	BR = 3.50 T + 30.09	0.86	0.07
Bacterial production (leucine)	BP = 1.95 T + 42.56	0.51	0.29

64%			
Particulate primary production	PPP = 2.79 T + 152.24	0.07	0.51
Total bacterial number	TBN = 0.02 T + 2.00	0.05	0.61
Bacterial respiration	BR = 2.63 T + 27.30	0.73	0.007*
Bacterial production (thymidine)	BP = 1.14 T + 18.39	0.43	0.08
64% b			
Particulate primary production	PPP = 6.00 T + 155.94	0.25	0.50
Total bacterial number	TBN = 0.004 T + 1.48	0.01	0.88
Community respiration	CR = 5.37 T + 85.74	0.99	0.006*
Bacterial respiration	BR = 2.39 T + 33.94	0.81	0.10
Bacterial production (leucine)	BP = 1.87 T + 53.74	0.50	0.29

Table 6. Relationships for the regression of the mean quantities with temperature for the 2008 experiment. All mean values of each parameter for one temperature (ΔT +0℃ or +6℃) are taken together, irrespective of the light treatment. Correlations statistically significant on the $p<0.05$ level are marked with an asterisk.

Parameter	**Equation**	**R^2**	**p**
Particulate primary production	PPP = 0.33 T + 155.09	0.001	0.91
Total bacterial number	TBN = -0.02 T + 1.66	0.15	0.21
Community respiration	CR = 3.18 T + 82.16	0.37	0.11
Bacterial respiration	BR = 2.95 T + 32.01	0.81	0.002*
Bacterial production (leucine)	BP = 1.71 T + 47.26	0.30	0.07

Table 7. Relationships for the regression of mean bloom quantities with light at the two different temperature treatments of the 2008 experiment. Accelerations statistically significant on the $p<0.05$ level are marked with an asterisk.

Parameter	Equation	R^2	p
+6℃			
Particulate primary production	PPP = 1.78 I_0 + 71.63	0.64	0.06
Total bacterial number	TBN = -0.003 I_0 + 1.71	0.14	0.47
Community respiration	CR = 2.09 I_0 - 15.69	0.98	0.01*
Bacterial respiration	BR = -0.17 I_0 + 59.39	0.14	0.63
Bacterial production (leucine)	BP = 0.36 I_0 + 40.02	0.27	0.30
+0℃			
Particulate primary production	PPP = 0.16 I_0 + 147.48	0.01	0.89
Total bacterial number	TBN = -0.01 I_0 + 2.12	0.71	0.04*
Community respiration	CR = 0.45 I_0 + 57.15	0.66	0.19
Bacterial respiration	BR = 0.24 I_0 + 18.54	0.17	0.59
Bacterial production (leucine)	BP = 0.26 I_0 + 34.88	0.29	0.27

Table 8. Relationships for the regression of mean bloom quantities with light for the 2008 experiment. All mean values of one light treatments were taken together, irrespective of the temperature treatment. Accelerations statistically significant on the $p<0.05$ level are marked with an asterisk.

Parameter	Equation	R^2	p
Particulate primary production	PPP = 0.97 I_0 + 109.56	0.19	0.15
Total bacterial number	TBN = -0.006 I_0 + 1.91	0.34	0.05*
Community respiration	CR = 1.27 I_0 + 20.73	0.42	0.08
Bacterial respiration	BR = 0.03 I_0 + 38.97	0.0008	0.95
Bacterial production (leucine)	BP = 0.31 I_0 + 37.45	0.19	0.16

Table 9. Relationships for the regression of the derived parameters for the bloom period (BGE, BCD) with temperature at the different light treatments (2008 experiment highlighted in blue). Correlations statistically significant on the p<0.05 level are marked with an asterisk.

Parameter	Equation	R^2	p
16%			
Bacterial growth efficiency (thymidine)	BGE = -0.75 T + 16.36	0.20	0.27
Bacterial carbon demand (thymidine)	BCD = 1.29 T + 25.72	0.35	0.12
32%			
Bacterial growth efficiency (leucine)	BGE = -3.14 T + 46.41	0.85	0.001*
Bacterial carbon demand (leucine)	BCD = 0.94 T + 30.15	0.21	0.26
Bacterial growth efficiency (thymidine)	BGE = -2.45 T + 25.32	0.89	0.0005*
Bacterial carbon demand (thymidine)	BCD = 1.33 T + 21.19	0.41	0.09
48%			
Bacterial growth efficiency (leucine)	BGE = -0.60 T + 50.03	0.22	0.54
Bacterial carbon demand (leucine)	BCD = 5.10 T + 85.63	0.73	0.14
64%			
Bacterial growth efficiency (thymidine)	BGE = -0.59 T + 31.77	0.18	0.30
Bacterial carbon demand (thymidine)	BCD = 5.27 T + 57.45	0.72	0.007*
64% b			
Bacterial growth efficiency (leucine)	BGE = 1.29 T + 43.28	0.90	0.05
Bacterial carbon demand (leucine)	BCD = 0.65 T + 123.83	0.03	0.84

Table 10. Relationships for the regression of the derived parameters (BGE, BCD) during the bloom period of the 2008 experiment, with temperature.

Parameter	Equation	R^2	p
Bacterial growth efficiency (leucine)	BGE = 0.34 T + 46.66	0.07	0.54
Bacterial carbon demand (leucine)	BCD = 2.87 T + 104.73	0.19	0.28

Table 11. Relationships for the regression of the derived parameters (BGE, BCD) during the bloom period of the 2008 experiment, with light at the different temperature treatments.

Parameter	Equation	R^2	p
0℃			
Bacterial growth efficiency (leucine)	BGE = -0.42 I_0 + 70.31	0.55	0.26
Bacterial carbon demand (leucine)	BCD = 2.39 I_0 – 28.95	0.78	0.12
6℃			
Bacterial growth efficiency (leucine)	BGE = 0.28 I_0 + 32.75	0.56	0.25
Bacterial carbon demand (leucine)	BCD = 0.72 I_0 + 81.77	0.21	0.54

Table 12. Relationships for the regression of the derived parameters for the 2008 experiment, with light intensity. All values of one light treatment are taken together, irrespective of the temperature.

Parameter	Equation	R^2	p
Bacterial growth efficiency (leucine)	BGE = -0.07 I_0 + 51.53	0.02	0.75
Bacterial carbon demand (leucine)	BCD = 1.55 I_0 + 26.41	0.40	0.09

Table 13. Relationships for the regression of the ratios of bacterial production to particulate primary production (BP:PPP) and bacterial carbon demand to particulate primary production (BCD:PPP) with temperature at the different light treatments (2008 experiment highlighted in blue). Correlations statistically significant on the p<0.05 level are marked with an asterisk.

Parameter	Equation	R^2	p
16%			
BP : PPP (thymidine)	BP : PPP = 5.82 T + 22.05	0.54	0.04*
BCD : PPP (thymidine)	BCD : PPP = 48.77 T + 150.40	0.74	0.006*
32%			
BP : PPP (thymidine)	BP : PPP = 2.00 T + 16.44	0.63	0.02*
BCD : PPP (thymidine)	BCD : PPP = 30.37 T + 55.77	0.81	0.003*
BP : PPP (leucine)	BP : PPP = 7.60 T + 41.72	0.52	0.04*
BCD : PPP (leucine)	BCD : PPP = 35.62 T + 82.55	0.77	0.004*
32% b			
BP : PPP (leucine)	BP : PPP = 1.42 T + 30.80	0.44	0.34
48%			
BP : PPP (leucine)	BP : PPP = 1.75 T + 27.33	0.47	0.31
BCD : PPP (leucine)	BCD : PPP = 4.22 T + 55.60	0.56	0.25
64%			
BP : PPP (thymidine)	BP : PPP = 0.45 T + 12.33	0.22	0.24
BCD : PPP (thymidine)	BCD : PPP = 2.32 T + 38.84	0.61	0.02*
64% b			
BP : PPP (leucine)	BP : PPP = -0.11 T + 35.38	0.004	0.94
BCD : PPP (leucine)	BCD : PPP = -2.28 T + 82.07	0.21	0.54

Table 14. Relationships for the regression of the ratios of bacterial production to particulate primary production (BP:PPP) and bacterial carbon demand to particulate primary production (BCD:PPP) during the bloom period of the 2008 experiment, with temperature. All data points of one temperature treatment are taken together, irrespective of the light intensity.

Parameter	Equation	R^2	p
BP:PPP (leucine)	BP:PPP = 1.02 T + 31.17	0.21	0.13
BCD:PPP (leucine)	BCD:PPP = 0.97 T + 68.83	0.03	0.67

Table 15. Relationships for the regression of the ratios (BP:PPP, BCD:PPP) with light at the different temperature treatments.

Parameter	Equation	R^2	p
0℃			
BP : PPP (leucine)	BP : PPP = 0.14 I_0 + 24.29	0.14	0.47
BCD : PPP (leucine)	BCD : PPP = 1.65 I_0 - 23.82	0.57	0.24
6℃			
BP : PPP (leucine)	BP : PPP = -0.14 I_0 + 44.18	0.08	0.59
BCD : PPP (leucine)	BCD : PPP = -0.78 I_0 + 118.55	0.19	0.56

Table 16. Relationships for the regression of the ratios (BP:PPP, BCD:PPP) with light at the different temperature treatments. All data points of one light treatment are taken together, irrespective of the temperature. Correlations statistically significant on the $p<0.05$ level are marked with an asterisk.

Parameter	Equation	R^2	p
BP : PPP (leucine)	BP : PPP = -0.0002 I_0 + 34.24	0.00	1.00
BCD : PPP (leucine)	BCD : PPP = 0.44 I_0 + 47.36	0.05	0.61

Table 17. Summary of results for mean quantities, derived parameters and ratios from all experiments. Displayed are the percent increases or decreases in response to temperature increase of ΔT +6°C (from 2.4 to 8.4°C) or for an increase in li ght intensity (from 32 or 48 to 64% I_0, where appropriate), based on the regression equations in Annex Tables 5-16. The 2008 experiment is highlighted with blue headlines and additionally to the single responses the summarising responses are shown. Statistically significant changes are highlighted in bold, increases have a green and decreases a red background colour. Mind that BCD and BGE are calculated using the corrected bacterial respiration measures (as described in the Material & Methods section of Chapter 2).

	Reaction to increasing temperature						Reaction to increasing light	
	16 % I_0	32 % I_0	64 % I_0	32 % I_0	48 % I_0	64 % I_0	ΔT +0℃	ΔT +6℃
Primary production	**-69**	**(-100)**	+11	-11	-9 +1	+21	+3 +22	+43
Total bacterial number	0	-12	+6	+19	-11 -7	+1	**-18** **-11**	-6
Community respiration	**+50**	**+49**			+7 +21	**+33**	+20 +66	**+131**
Bacterial respiration	+32	**+48**	**+47**		+18 +45	+36	+29 +2	-10
Bacterial production (^{3}H-leucine)		-35		+16	+25 +20	+19	+19 +21	+22
Bacterial production (^{3}H-thymidine)	+3	**-53**	+32					
BCD (^{3}H-leucine)		+15			+32 +15	+3	+45 +25	+10
BCD (^{3}H-thymidine)	+28	+28	**+44**					
BGE (^{3}H-leucine)		**-49**			-8 +4	+17	-13 -2	+10
BGE (^{3}H-thymidine)	-33	**-74**	-10					
BP:PPP (^{3}H-leucine)		**+76**		+25	+33 +18	-2	+16 0	-11
BP:PPP (^{3}H-thymidine)	**+152**	**+56**	+20					
BCD:PPP (^{3}H-leucine)		**+130**			+39 +8	-18	+48 +10	-15
BCD:PPP (^{3}H-thymidine)	**+109**	**+142**	**+31**					

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Hiermit erkläre ich, dass diese Abhandlung – abgesehen von der Beratung durch meine Betreuer – nach Inhalt und Form meine eigene ist und keine weiteren als die angegebenen Hilfsmittel und Quellen verwendet wurden. Ich habe diese Arbeit weder ganz noch zum Teil schon an anderer Stelle im Rahmen eines Prüfungsverfahren vorgelegt, veröffentlicht oder zur Veröffentlichung eingereicht. Die Arbeit ist unter Einhaltung der Regeln guter wissenschaftlicher Praxis der Deutschen Forschungsgemeinschaft entstanden.

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DATA CONTRIBUTIONS AND PUBLICATIONS

Chapter 1:

- Temperature data was supplied by Kathrin Lengfellner
- Nutrient level data was supplied by Julia Wohlers

Derived results from data from this experiment contributed to:

Julia Wohlers, Anja Engel, Eckart Zöllner, **Petra Breithaupt**, Klaus Jürgens, Hans-Georg Hoppe, Ulrich Sommer, Ulf Riebesell (2009). Changes in biogenic carbon flow in response to sea surface warming. *Proc. Nat. Acad. Sci.* 106:7067-7072

Supplemental information (SI) is available online at *www.pnas.org/cgi/content/short/0812743106*.

Julia Wohlers (2009). Effects of sea surface warming on elemental cycling in a pelagic system. *PhD thesis.* University of Kiel

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Chapter 2:

2005 Experiment:

The experiment in 2005 was conducted before the start of this work in September 2005. Raw data from all measurements was kindly supplied to me by Prof. H.-G. Hoppe, I conducted the analyses from the raw data.

Derived results from data from this experiment contributed to:

Hans-Georg Hoppe, **Petra Breithaupt,** Katja Walther, Regine Koppe, Stephan Bleck, Ulrich Sommer, Klaus Jürgens (2008). Climate warming in winter affects the coupling between phytoplankton and bacteria during the spring bloom: a mesocosm study. Aquatic Microbial Ecology 51: 105-115.

2006 and 2007 Experiments:

- Temperature data was supplied by Kathrin Lengfellner
- Nutrient level data was supplied by Julia Wohlers

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