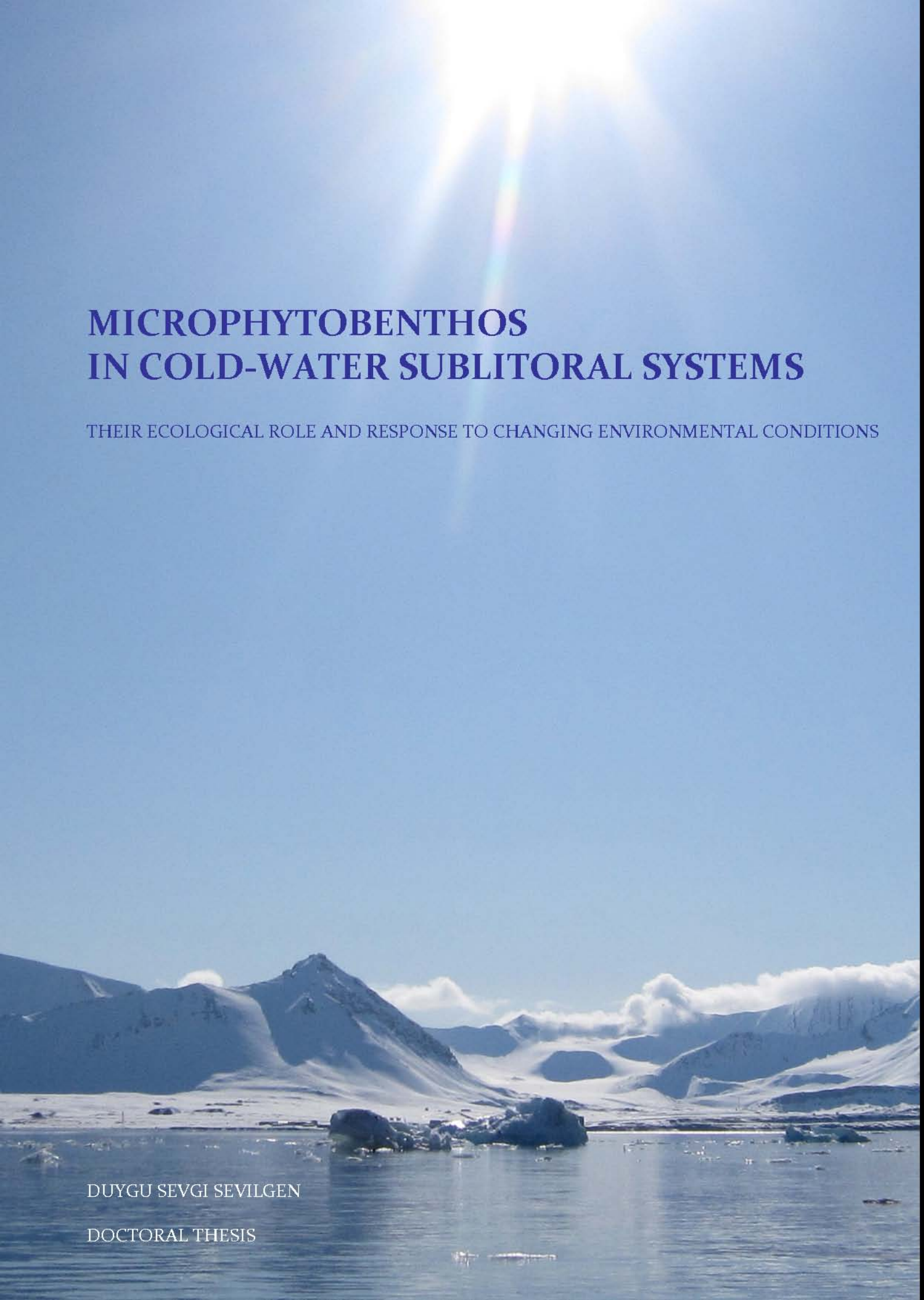
MICROPHYTOBENTHOS IN COLD-WATER SUBLITORAL SYSTEMS

THEIR ECOLOGICAL ROLE AND RESPONSE TO CHANGING ENVIRONMENTAL CONDITIONS



DUYGU SEVGI SEVILGEN

DOCTORAL THESIS

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DOCTORAL THESIS
by

DUYGU SEVGI SEVILGEN

BREMEN, FEBRUARY 2014

MIKROPHYTOBENTHOS IN SUBLITORALEN KALTWASSER REGIONEN
-
IHRE ÖKOLOGISCHE ROLLE UND REAKTION AUF SICH VERÄNDERNDE UMWELTBEDINGUNGEN

DISSERTATION
ZUR ERLANGUNG DES AKADEMISCHEN GRADES
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The present study has been realized from March 2009- March 2014 at the Alfred Wegener Institute, Helmholtz Center for Polar- and Marine Biology (AWI), Bremerhaven/Germany, the Max Planck Institute for Marine Microbiology (MPI MM), Bremen/Germany, the Biologische Anstalt Helgoland (BAH), Helgoland/Germany and the Koldewey Station of the French German Arctic Research Base (AWIPEV), Ny-Ålesund, Svalbard/Norway.
It was part of the Helmholtz Research Programme "Polar Regions and Coasts in a changing Earth System" (PACES 1.6: Ocean Warming and Acidification: Organisms and their changing Role in Marine Ecosystems) and was realized as a collaborative project between the Division of Biosciences, Section "Functional Ecology" (Prof. Dr. Thomas Brey) including the workgroup Marine Botany (Prof. Dr. Christian Wiencke) at AWI and the Microsensor Department (Dr. Dirk de Beer) within the Biogeochemistry section of MPI MM.
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"The whole is greater than the sum of its parts."

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SUMMARY

Microphytobenthos (MPB) refers to communities of phototrophic micro-organisms that live in association with substrate surfaces in aquatic habitats. They are a primary food-source for many heterotrophic levels and play a significant role in oxygen and nutrient cycling of shallow water habitats. MPB show a widespread, global distribution with habitats ranging from tropical coral reef sediments to intertidal flats, salt-marshes, rocky shores, lakes and marine subtidal sediments of Polar Regions. On soft-bottom substrates such as the illuminated sandy sediments of the continental shelves, they can add a significant share to primary production that frequently equals or exceeds phytoplankton production in the overlaying water.

The importance of MPB in ecosystem functioning has been the motivation for a multitude of studies in the last decades. However, the majority of these studies have focused on the MPB of intertidal habitats in temperate regions. MPB studies in the tropics and at high latitudes, specifically in the subtidal are much rarer. Furthermore, corresponding investigations in Polar Regions, have only recently begun. Compared to their ecological importance, this scarcity of *in situ* data and subtidal MPB community-studies represents a considerable gap in our knowledge. This is particularly highlighted when considering that in the course of global climate change, important environmental conditions such as temperature change rapidly in comparison to long-term trends. These changes will consequently influence the marine biota. Especially in high-latitudinal regions such as the Arctic, where prevailing conditions are extreme (e.g. ice/snow conditions), it is expected that these changes will have high impact and can be perceptible first. Given that the area of the Arctic continental shelf which can potentially harbor primary production represents 25% of the global shelf area, it becomes essential to develop a better understanding of MPB and its ecological function in this region.

Due to the complex nature of MPB communities, many studies addressing manipulation of environmental parameters (light, nutrient, temperature) examine responses of single species which are dominant and representative for a community. However, extrapolations from these studies are difficult as MPB is not only a complex community in itself with several interacting functional

groups and species but also part of a diverse benthic community which includes infauna organisms. Therefore, studies of single species do not suffice to predict community responses. However due to their complexity, holistic MPB community studies are rare.

In my thesis, the main aim was to study and compare subtidal MPB communities and their activity from sandy sediments of an Arctic (Spitsbergen, Svalbard) and a temperate (Helgoland, North Sea) study site. Helgoland was chosen as a comparative site because of the tight link between the North Sea and the western coast of Spitsbergen due to ocean currents. The research objective was realized in three consecutive studies.

The first study of my thesis compares the *in situ* oxygen and light dynamics, the dominant diatom species as well as the photosynthesis and respiration of MPB communities from the two study sites. I combined laboratory measurements with *in situ* data to derive daily *in situ* oxygen budgets. The two sites were similar in diatom composition and photosynthesis potential. However, sedimentary oxygen consumption rates at the Spitsbergen site were highly elevated due to increased numbers of infaunal organisms which were largely absent in Helgoland. As a result, although the MPB communities were similar overall, during our study period the *in situ* budgets showed a net heterotrophy for the Spitsbergen community and net autotrophy for the Helgoland MPB community. The study highlights the importance of observing and including the activity of the sedimentary infauna (if present) for realistic local *in situ* estimates of primary production.

In the second study I focused on the response of the MPB communities to short-term temperature changes. I studied photosynthesis and respiration under increasing temperatures and hypothesized that the Arctic community is less sensitive to short-term temperature changes as *in situ* temperature fluctuations at the Spitsbergen site are much more pronounced than in Helgoland. Overall, the communities showed no significant difference in their temperature responses, both having stronger temperature responses in respiration than net photosynthesis rates. At light-saturating conditions net autotrophy was sustained throughout all temperatures at both sites. However, I concluded that due to increased light demands *in situ*, initially both systems will develop towards net heterotrophy. This was especially valid for the

Spitsbergen site, where under consideration of the oxygen consumption of infaunal organisms, *in situ* respiration rates were highly increased. Due to large variability in the data, further studies verifying the similarity or differences between sites are suggested.

Finally, in my third study, I addressed MPB community growth at different temperatures, increased nutrient load and under mimicked *in situ* light conditions. Temperature treatments represented lowest, intermediate and highest temperatures which the communities experience naturally. The goal was to find out whether an upward shift of the temperature baseline and prolonged times of increased temperatures will change growth rates and biomass of the MPB communities. The onset of growth at the Svalbard site was more independent from additional nutrient supply than at Helgoland. At both sites, no or little growth occurred at the low temperatures. Growth-rates at intermediate and high temperatures did not differ significantly from each other or between sites. Highest final pigment biomasses however were reached at intermediate temperatures which for both sites were close to annual average temperatures. The differences in final biomasses and pigment ratios suggested that whereas a change in growth rates is unlikely to result from temperature increases, a change of the MPB community members is more likely.

Overall my thesis showed that during the study period, the MPB communities of the investigated temperate and sub-Arctic site were very similar in their structure, function and response to temperature increases. This applied despite pronounced differences in their annual average and short-term *in situ* temperature dynamics. The similarity of the MPB communities is suggested to be associated with the high number of shared and mainly cosmopolitan species. This commonality may be rooted in the tight link of similar water masses that influence the study regions. At the same time, although the MPB responses to temperature changes were similar, communities were tested within temperature ranges specifically selected for each site. Thus, the results also showed that the communities are adapted to the prevailing site conditions. However, small scale differences may be present and need study approaches of finer resolution and with multiple replicates on the community level to a) be detected, and b) enable to resolve changes within the communities over time.

ZUSAMMENFASSUNG

Der Begriff Mikrophytobenthos (MPB) beschreibt Gemeinschaften phototropher Mikroorganismen, die in Assoziation mit Substratoberflächen aquatischer Habitate leben. Sie stellen die primäre Nahrungsgrundlage für viele heterotrophe Organismen dar und haben signifikanten Einfluss auf Sauerstoff- und Nährstoffzyklen in Flachwasserhabitaten. MPB zeigt eine weit ausgedehnte, globale Verteilung mit Lebensräumen, die von den Sedimenten tropischer Korallenriffe bis hin zu Watt, Felsküsten, Seen und marinen, infralitoralen Sedimenten der Polarregionen reichen. Auf weichen Untergründen wie den sandigen Sedimenten der lichtdurchfluteten Bereiche des Kontinentalschelfs können sie einen signifikanten Anteil zur Primärproduktion beitragen, welcher häufig der Produktion des Phytoplankton im Freiwasser gleichkommt oder diese sogar überschreitet.

Die Relevanz der Funktion des MPB in Ökosystemen hat eine Vielzahl an Studien in den vergangenen Jahrzehnten motiviert. Die Mehrheit dieser Studien hat sich jedoch mit MPB in den Gezeitenzonen (dem Watt) gemäßigter Zonen befasst. Studien zu MPB aus den Tropen und höheren Breiten, besonders aus dem Infralitoral, sind wesentlich seltener. Entsprechende Untersuchungen in Polarregionen sind erst vor kurzer Zeit initiiert worden. Im Kontext ihrer ökologischen Relevanz stellt die Seltenheit von *in situ* Daten und Studien an MPB-Gemeinschaften aus dem Infralitoral eine unverhältnismäßige Wissenslücke dar. Dies wird besonders deutlich wenn man bedenkt, dass sich im Zuge des globalen Klimawandels wichtige Umweltfaktoren, wie z.B. Temperatur, im Vergleich zu Langzeittrends rapide verändern. Diese Veränderungen werden folglich die marine Biota beeinflussen. In den Regionen hoher Breitangrade wie der Arktis, mit den dort vorherrschenden extremen Umweltbedingungen (z.B. Eis/Schnee), kann man erwarten dass diese Veränderungen als erstes wahrnehmbar sind. Basierend darauf, dass die Fläche des arktischen Kontinentalschelfs welche potenziell Primärproduktion ermöglicht, 25% der globalen Schelffläche ausmacht, ist es essenziell ein besseres Verständnis des MPB und seiner ökologischen Funktion in dieser Region zu entwickeln.

Begründet in der komplexen Natur von MPB-Gemeinschaften sind viele Manipulationsstudien von Umweltparametern (Licht, Nährstoffe, Temperatur) darauf ausgerichtet einzelne Arten zu untersuchen, die dominant und repräsentativ für eine Gemeinschaft sind. Extrapolationen der Ergebnisse solcher Studien sind jedoch problematisch, da MPB selbst aus verschiedenen, interagierenden funktionellen Gruppen besteht und darüber hinaus auch Teil einer noch komplexeren, diversen benthischen Gemeinschaft ist, welche Infauna Organismen mit einschließt. Folglich reichen Studien an einzelnen Arten nicht aus um die Reaktion einer Gemeinschaft auf Veränderungen ihrer Umgebung vorauszusagen. Entsprechend der Komplexität ihrer Durchführung sind jedoch holistische MPB-Gemeinschaftsstudien selten.

Das Hauptziel in meiner Doktorarbeit war es MPB Gemeinschaften und ihre Aktivität in sandigen Sedimenten von einem arktischen (Spitzbergen, Svalbard) und einem temperaten (Helgoland, Nordsee) Standort zu untersuchen und zu vergleichen. Helgoland wurde auf Grund der engen Verbindung der Nordsee und der Westküste Spitzbergens, die mit den gleichen Meeresströmen verbunden sind als Vergleichsstandort ausgewählt. Das Forschungsziel wurde in drei aufeinander folgenden Studien umgesetzt.

Die erste Studie meiner Arbeit vergleicht die *in situ* Sauerstoff- und Lichtdynamiken, die dominanten Arten sowie die Photosynthese und Respiration der MPB-Gemeinschaften der beiden Untersuchungsstandorte. Ich habe Labormessungen mit *in situ* Daten kombiniert um *in situ* Sauerstoff-Tagesbudgets für beide Standorte zu berechnen. Die beiden Standorte waren sich in ihrer Diatomeen-Zusammensetzung und ihrem Photosynthesepotenzial ähnlich. Jedoch waren auf Grund einer hohen Anzahl an Infauna Organismen, welche in Helgoland nicht vorhanden waren, die Sauerstoff- Verbrauchsraten in den Spitzbergen-Sedimenten deutlich erhöht. Trotz der generellen Ähnlichkeit der MPB-Gemeinschaften zeigte sich folglich, dass das *in situ* Budget für die Spitzbergen Gemeinschaft netto heterotroph, hingegen für die MPB-Gemeinschaft Helgolands netto autotroph war. Diese Untersuchung unterstreicht die Wichtigkeit sedimentäre Infauna bei Anwesenheit zu erfassen und ihre Aktivität für realistische lokale Schätzungen der Primärproduktion mit einzubeziehen.

Der Fokus der zweiten Studie liegt auf der Antwort von MPB-Gemeinschaften auf Kurzzeit-Temperaturveränderungen. Die Studie basierte auf der Hypothese, dass die arktische Gemeinschaft weniger sensitiv auf Kurzzeit-Temperaturveränderungen reagiert, da sie an ihrem Standort ausgeprägteren *in situ* Temperatur Fluktuationen ausgesetzt ist als die Gemeinschaft in Helgoland. Um diese Hypothese zu testen, habe ich Photosynthese und Respiration unter zunehmenden Temperaturen untersucht. Insgesamt haben die beiden Gemeinschaften keinen signifikanten Unterschied in ihrer Reaktion auf Temperaturveränderungen gezeigt, wobei beide mit stärkerer Antwort in Respirationsraten als in den Photosyntheseraten reagierten. Unter lichtgesättigten Bedingungen waren beide Standorte bei allen Temperaturen netto autotroph. Ich habe jedoch geschlussfolgert, dass sich beide Systeme auf Grund des erhöhten Lichtbedarfs *in situ*, welcher benötigt würde um Netto-Autotrophie beizubehalten, zunächst in Richtung Netto-Heterotrophie entwickeln. Dies ist vor allem für den Standort in Spitzbergen der Fall, an welchem die Respirationsraten zusätzlich durch einen erhöhten Sauerstoffbedarf der Infauna erhöht waren. Wegen der hohen Variabilität in den Daten werden weitere Studien, die die Ähnlichkeit bzw. Unterschiede der beiden Standort verifizieren, empfohlen.

In meiner dritten Studie habe ich das Wachstum der MPB Gemeinschaften bei unterschiedlichen Temperaturen, erhöhter Nährstoffzufuhr und nachgeahmten *in situ* Lichtbedingungen untersucht. Die ausgewählten Temperaturen spiegeln die niedrigsten, mittleren und höchsten Werte wider, denen die Gemeinschaften unter natürlichen Bedingungen ausgesetzt sind. Das Ziel war es, herauszufinden, ob eine Aufwärtsverschiebung der Temperaturbasislinie und verlängerte Zeiten erhöhter Temperaturen die Wachstumsraten und die Biomasse der MPB-Gemeinschaften verändern. Der Beginn des Wachstums am Spitzbergen Standort war unabhängiger von der Nährstoffzufuhr als in Helgoland. An beiden Standorten konnte kaum oder kein Wachstum bei den niedrigsten Temperaturen festgestellt werden. Wachstumsraten bei mittleren und hohen Temperaturen waren nicht signifikant unterschiedlich, weder untereinander noch zwischen den Standorten. Höchste Endbiomasse jedoch wurde bei mittleren Temperaturen erreicht, welche für beide Standorte nah an der durchschnittlichen

Jahrestemperatur lag. Die Unterschiede in Endbiomasse und Pigmentverhältnissen legen nah, dass eine Änderung der Wachstumsraten auf Grund von Temperaturveränderungen unwahrscheinlich ist, hingehen eine Veränderung der Mitglieder der MPB-Gemeinschaft wahrscheinlicher ist.

Insgesamt hat meine Arbeit gezeigt, dass während der Zeit meiner Untersuchungen, die MPB-Gemeinschaften der untersuchten temperaten und sub-arktischen Standorte ähnlich bezüglich ihrer Struktur, Funktion und Reaktion auf Temperaturerhöhungen waren. Dies traf trotz deutlicher Unterschiede in ihren jährlichen Temperatur-Mittelwerten und kurzzeitigen Temperaturdynamiken *in situ* zu. Es ist wahrscheinlich, dass diese Ähnlichkeit der MPB Gemeinschaften mit der hohen Anzahl und den gleichen bzw. weitestgehend kosmopolitischen Arten assoziiert ist. Diese Gemeinsamkeit ist womöglich in der engen Verknüpfung der Wassermassen begründet, die die beiden Standorte beeinflussen. Obwohl jedoch die MPB Antwort auf Temperaturveränderungen ähnlich war, wurden die Gemeinschaften innerhalb der Temperaturspektren getestet, die Standort spezifisch ausgewählt wurden. Dementsprechend zeigten die Ergebnisse auch, dass die Gemeinschaften an die vorherrschenden Bedingungen angepasst sind. Es ist anzunehmen, dass kleine Unterschiede vorhanden sind, welche Untersuchungsansätze mit feinerer Auflösung und multiplen Replikate auf Gemeinschafts-Niveau verlangen um a) erkannt zu werden und b) mögliche Veränderungen über die Zeit innerhalb der Gemeinschaften aufzuschlüsseln.

i. INTRODUCTION

1. PRIMARY PRODUCTION AND THE ROLE OF MICROPHYTOBENTHOS

"Primary Production: Fuel for Life"

1.1 WHAT IS PRIMARY PRODUCTION?

Primary production generally describes the biochemical synthesis of biomass by organisms that can utilize the light energy of the sun or chemical energy of the environment to fix carbon dioxide (CO_2) or other low-energy inorganic compounds into high-energy organic matter. The pathway by which organisms sustain themselves using sunlight and CO_2 fixation, releasing oxygen (O_2) as a by-product (also called photoautotrophs, from ancient greek: *phos = light, autos = self, trophé = nutrition*), is called oxygenic photosynthesis (BOX 1) and is simply referred to as photosynthesis hereafter. This pathway stands in contrast to anoxygenic photosynthesis, which uses light but does not produce oxygen, and chemosynthesis, where other (inorganic) energy sources than sunlight are used to produce organic molecules. In the terrestrial realm of the Earth's Biosphere photoautotrophs are mainly represented as trees, grass, mosses and ferns, whereas in the oceans the photoautotrophs mainly comprise micro- and macroalgae as well as seagrasses and cyanobacteria.

Net primary production, i.e. the total (gross) production less autotrophic respiratory losses, usually defines the amount of carbon that is available at the first heterotrophic level (Field et al. 1998), which describes all organisms that consume rather than produce organic molecules, thus their own food. Net primary production therefore forms the basis of the food web, controls how much material and energy is available for the biosphere as a whole and essentially fuels all life on earth (Field et al. 1998, Buitenhuis et al. 2013). Besides this, as photosynthesis fixes CO_2, it is a major determinant of carbon sinks on land and in the oceans and profoundly affects global biogeochemical cycles and climate (Field et al. 1998, Chavez et al. 2010). Thus, as CO_2 represents a major greenhouse gas, primary production is assigned a crucial role regarding increased CO_2 contents and its removal in the context of global climate change.

BOX 1 – PHOTOSYNTHESIS

Oxygenic photosynthesis is the process during which light energy ($h \times \nu$) is used and carbon dioxide (CO_2) is fixed to drive the synthesis of carbohydrates (CH_2O). During this process, oxygen (O_2) is released as a by-product.
The simplified general equation for photosynthesis can be summarized as

$$6CO_2 + 12H_2O \xrightarrow{h \times \nu} C_6H_{12}O_6 + 6O_2 + 6H_2O$$

Photosynthesis occurs in two stages and is classically divided into the so called light-dependant reaction ("primary reaction") (Fig. 1.1) and the light-independent reaction ("secondary reaction") (Fig. 1.2).

The primary reaction takes place at the reaction centers (in the photosystems II & I) which lie within the thylakoid membrane of chloroplasts, the cell-organelles that are responsible for photosynthesis. In green plants and algae the reaction centers mostly contain the photosynthetically active pigment chlorophyll *a* and an assembly of light-absorbing and funneling antennae pigments (more chlorophyll *a* or -*b*, or other accessory pigments). The light energy is used to split water molecules into hydrogen protons and oxygen by which electrons become available. The electrons enter an electron transport chain through many enzyme-complexes which finally leads to the reduction of the coenzyme $NADP^+$ (nicotinamide-adenine-dinucleotide-phosphate) whereas the hydrogen ions are used to drive a proton pump (the enzyme ATP synthase) for the synthesis of adenosine-tri-phosphate (ATP) from adenosine-di-phosphate (ADP) and inorganic phosphate (P_i). The primary reaction summarizes as

$$H_2O + NADP^+ + 2ADP + 2P_i \rightarrow 1/2O_2 + NADPH/H^+ + 2ATP$$

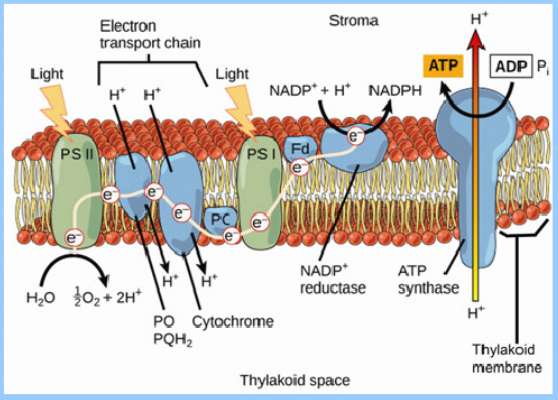


Figure 1.1 – Primary reaction of photosynthesis. Upon light-absorption, an electron travels from photosystem II to photosystem I through a series of membrane-bound proteins. The light-energy is used for the generation of the energy-carrier molecules ATP and NADPH which will be used in the secondary reaction. PS I & II: photosystem I & II, PQ (H_2): plasto (hydro) quinone, PC: plastocyanine, FD: ferrodoxin *(modified after OpenStax College. The Light-Dependent Reactions of Photosynthesis. Connexions.19.Apr. 2013, http://cnx.org/content/m45452)*

The secondary reaction (or Calvin Cycle) takes place in the stroma, outside the thylakoid membrane and is responsible for the fixation of CO_2 by the RuBisCO enzyme (Ribulose-1,5-bisphosphate-carboxylase/oxygenase) which uses ATP and $NADPH/H^+$ to reduce CO_2 and generates carbohydrates:

$$6CO_2 + 12NADPH/H^+ + 18ATP \rightarrow C_6H1_2O_6 + H_2O + 12NADP^+ + 18ADP + 18P_i$$

For the synthesis of sugars (e.g. glucose $C_6H_{12}O_6$), the energy of approximately $\Delta G = +2780$ kJ/mole is required.

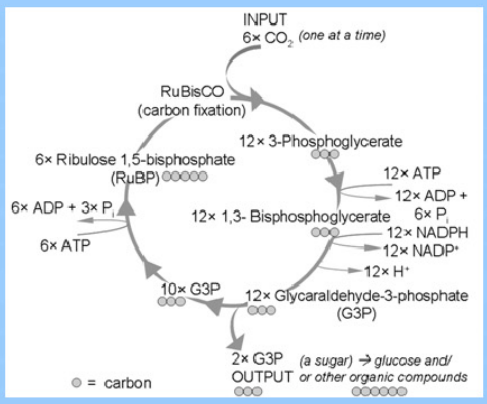


Figure 1.2 – Secondary reaction of photosynthesis. The Calvin Cycle produces carbohydrates upon CO_2 fixation and by the use of NADPH and ATP from the light reaction.

1.1.1 *Terrestrial vs. oceanic production*

Despite a high global heterogeneity and interannual changes, total global net primary production estimates have remained similar over the last 1-2 decades. Early estimates amounted to 104.9 Gt (10^9 t) C yr^{-1} of global net primary production to which the terrestrial and oceanic production had similar contributions (land: 56 Gt C yr^{-1}, oceans: 49 Gt C yr^{-1}) (Field et al. 1998; Fig. 1.3).

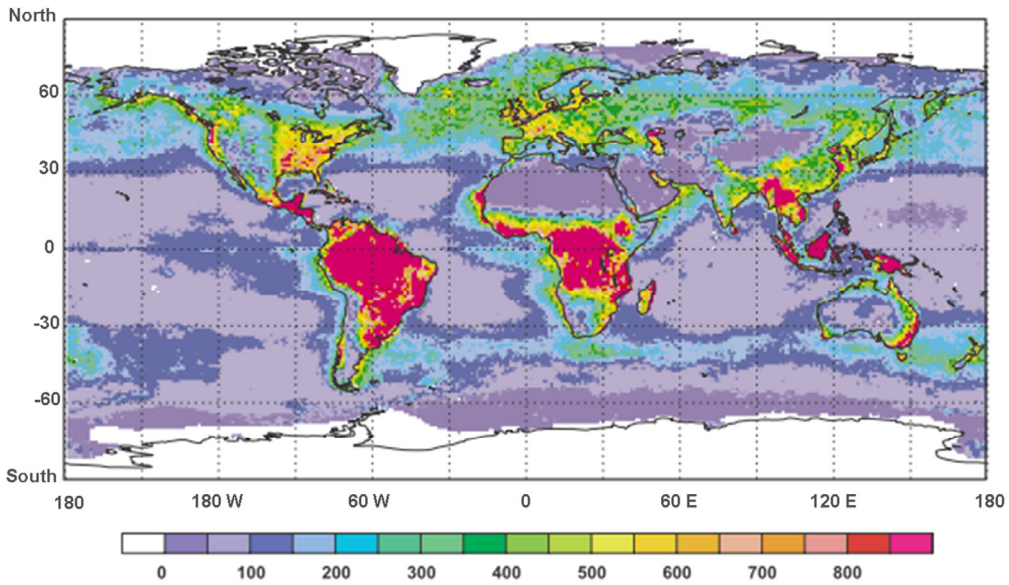


Figure 1.3 – Annual net primary production [g C m^{-2} yr^{-1}] of the Earth's Biosphere. Estimates are derived from satellite data averaged from 1978 - 1983 and from 1982 - 1990 for the oceans and the terrestrial realm which contribute 46% and 54% to an estimated global amount of 104.9 Gt (10^9 tons) C yr^{-1}. Half of the global oceanic net primary production comes from the tropical region between 23°N – 23°S while, despite a larger southern ocean area, the rest of the Northern and Southern oceanic hemispheres contribute approximately equally to net primary production (modified after Field et al. 1998).

Generally, oceanic net primary production can be found in the range of 30-65 Gt C yr^{-1} (Duarte & Cebrián 1996 and references therein, Buitenhuis et al. 2013) with most recent estimates of ~56 Gt C yr^{-1} (Buitenhuis et al. 2013). Gross primary production amounts to roughly twice the net primary production and is in the order of 100-120 Gt C yr^{-1} both on land and in the ocean (Chavez et al. 2010, Buitenhuis et al. 2013).

The similar production estimates are remarkable, given the distinct differences between the oceanic and terrestrial realm, as heterotrophic activity related to primary production varies greatly between the realms. On land,

heterotrophic activity is dominated by detritovorous organisms (feeding on and breaking down dead plant material) whereas in the oceans it is dominated by herbivorous zooplankton. This leads to large differences in the standing stocks of photoautotrophic organisms: approximately 500 Gt C are estimated for plants on land whereas only 1 Gt C is estimated in marine phototrophic protists (Buitenhuis et al. 2013). The turnover times of the organisms also vary on a similar ratio: 10 yrs on land in comparison to 1 week in the oceans (Buitenhuis et al. 2013). However, whereas the major components of the terrestrial plant biomass like stems and roots do generally not photosynthesize but rather respire, nearly all oceanic plant biomass is photosynthetically active (Field et al. 1998) leading to the similar rates of primary production.

1.1.2 *Pelagic vs. benthic production*

Primary production in the oceans is governed by the contribution of various phototrophs from several ecosystems. They reach from pelagic oceanic and coastal phytoplankton (i.e. passively drifting phototrophic organisms, Greek: *plankton = the straying/drifting*) over coral reef algae, seagrasses and mangroves to microphytobenthos (i.e. the substrate associated phototrophic organisms, Greek: *benthos = sum of all creatures existing at the bottom zone (benthal) of the oceans*). The bulk of marine primary production is governed by phytoplankton, which have a share of approximately 50% in the total net primary production of the oceans (Raymont 1966, Duarte & Cebrián 1996). Nevertheless, in some coastal coral-reef and macroalgae dominated ecosystems, benthic primary production can contribute ≥ 90% to carbon fixation (e.g. Delesalle et al. 1993, Borum & Sand-Jensen 1996).

Compared to phytoplankton, and related to a less obvious appearance and quantitatively smaller global representation, much less is known about microphytobenthos. Global estimates are difficult as there is only few data limited to certain geographical zones. Many areas, especially polar and tropical locations and deeper regions (> 20 m) are under-represented in studies (Cahoon 1999). Based on the available data, microphytobenthos contribution is estimated to amount to ≤ 5% (~0.34 - 0.50 Gt C yr^{-1}) of global oceanic primary production. Thus, microphytobenthos represent the smallest fraction in a holistic comparison of primary production contributors (Valiela 1995, Duarte &

Cebrián 1996, Cahoon 1999). Nonetheless, locally, microphytobenthos production can be of high importance and in many shallow ecosystems, their biomass can equal or exceed that of the phytoplankton in overlaying waters on an areal basis (Mac Intyre et al. 1996). Thus, their contribution to primary production can be significant - in estuaries for example, microphytobenthos primary production can account for up to 50% of the total primary production (Underwood & Kromkamp 1999). Interestingly, by resuspension into the overlaying water, microphytobenthos can also contribute noticeably to pelagic production (Guarini et al. 1998, Brito et al. 2012).

Due to their somewhat cryptic nature, in the past, microphytobenthos had been given much less attention than phytoplankton, marsh grasses, and macroalgae which have been more extensively studied in the context of benthic primary production (Cahoon 1999). Correspondingly, it has often been recognized that in shallow coastal waters the important contribution of the benthic microflora has probably been underestimated for a long time (Raymont 1966, Cahoon 1999, McGee et al. 2008, Glud et al. 2009). Upon recognizing this gap in our knowledge, the body of studies on microphytobenthos in shallow marine ecosystems has increased in the last decades (Cahoon 2006, Glud et al. 2009) (Fig. 1.4).

There are two important aspects of microphytobenthos that distinguish them from phytoplankton which are crucial in their ecological function. Firstly, their high biomass concentration at the sediment-water interface (benthic microalgae are often 10^4-10^5 times as concentrated in sediments as microalgae in the overlaying waters) which export on average a much higher proportion of their net primary production than other microalgal communities (Cahoon 1999, Duarte & Cebrián 1996), and secondly, their influence on their microhabitat. In the context of this thesis, the focus in the following will be given to these benthic primary producers in the shallow subtidal, which also have been referred to as the “Secret Garden” in the context of unvegetated, shallow-water marine habitats in the past (MacIntyre et al. 1996).

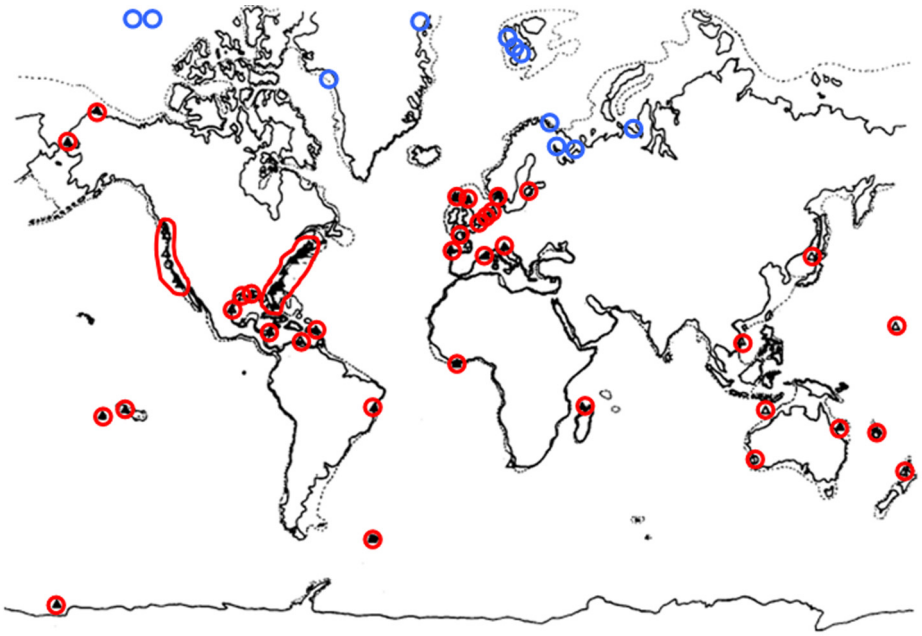


Figure 1.4 – Global map of MPB studies. Shown are intertidal and subtidal locations of 85 studies reporting on MPB production and/or biomass as compiled by Cahoon (1999) (red contours), and (later) studies in Arctic locations as reported by (Glud et al. 2009) (blue contours) (modified after Cahoon 1999).

1.2 WHAT IS MICROPHYTOBENTHOS AND WHY IS IT IMPORTANT?

Microphytobenthos (MPB) describes microscopic photosynthetic eukaryotic algae and cyanobacteria which live, grow and are consumed in the top few mm of illuminated shallow water ecosystems and can significantly influence oxygen and nutrient fluxes across the sediment-water interface (Sundbäck et al. 1991, MacIntyre et al. 1996, Bartoli et al. 2003) (Fig.1.5).

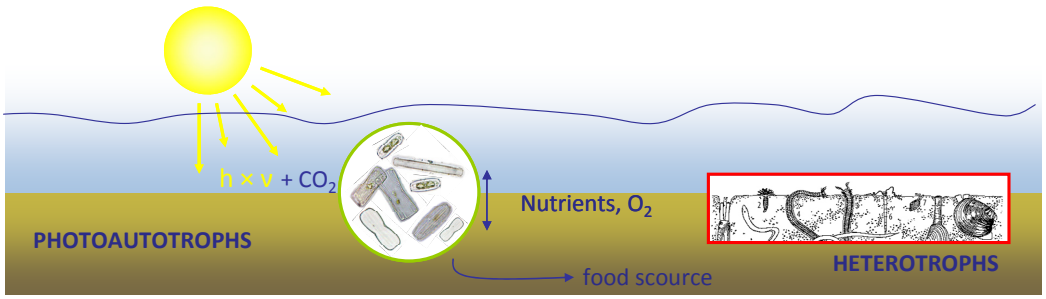


Figure 1.5 – Simplified schematic overview of MPB function.

MPB commonly includes representatives of several algal classes with motile pennate diatoms (*Bacillariophyceae*) usually dominating sand- and mudflats (MacIntyre et al. 1996, Cahoon 1999). Additionally, cyanobacteria, chlorophytes, dinoflagellates and euglenoids can all make up MPB communities, where cyanobacteria and flagellates are abundant in less exposed habitats while dinoflagellates are more common in tropical sediments (MacIntyre et al. 1996).

MPB organisms grow and can be found in nearly all aquatic ecosystems around the globe. Their habitats range from polar lakes (Whalen et al. 2013) to intertidal and subtidal soft-bottoms, rocky shores, brackwater lagoons and tropical coral-reef systems (e.g. MacIntyre et al. 1996, Uthike & Klumpp 1998, Werner et al. 2008, Jackson et al. 2013). One of the most important habitats is represented by soft-bottom areas, such as the sandy sediments of the continental shelves. Approximately 33% of the continental shelf receives enough light to potentially sustain a positive net community production (Gattuso et al. 2006). Sandy sediments cover approximately 70% of the world's shelf regions ≤ 200 m (Emery 1968) which in turn comprise approximately 7.5% of the total ocean area representing about 27.122×10^6 km^2 which can potentially accommodate benthic primary production (Menard & Smith 1966).

In addition to their key role as major producers in shallow subtidal habitats and as a high quality food source for benthic heterotrophs, MPB play an important role in oxygen and nutrient cycling, and in stabilization of the sediments (MacIntyre et al. 1996, Cahoon 1999, Smith et al. 1998, Yallop et al. 2000). Due to chain formation and the excretion of extracellular polymeric substances, MPB can form stable biofilms (visible as a brownish/greenish shimmer on the sediment surface), which function as a soft barrier between the underlying sediment and overlaying water. This biofilm formation helps the MPB to resist displacement by water movement, reduces damage by minimizing the resuspension of small sediment particles, and reduces their own resuspension, which can prevent transport to less favorable sites and thus helps decrease grazing by suspension feeders (Cahoon 1999).

1.2.1 *Factors controlling microphytobenthos biomass and productivity*

MPB assemblages exhibit high degrees of heterogeneity in biomass and species composition and show a patchy distribution over spatial (depending on the

habitat, from micrometers to kilometers) and temporal scales (Underwood & Kromkamp 1999, Fenchel & Glud 2000, Jesus et al. 2005). Their abundance, species composition and photosynthetic performance are influenced by the interplay of various abiotic and biotic parameters. Bottom-up factors such as available light-energy, temperature and nutrients and top-down regulation by grazers play a major role in determining the success of MPB biomass growth and productivity (e.g. Cadée & Hegemann 1974, Colijn & Vanbuurt 1975, Admiraal & Peletier 1980, Colijn & de Jonge 1983, MacIntyre et al. 1996, Underwood & Kromkamp 1999, Montani et al. 2003). In addition, environmental parameters such as substrate stability and disturbances from dynamic wave action, iceberg scouring in Polar Regions, or river discharges (which introduce sediment load and can significantly change salinity and turbidity) can play a determining role (Stevenson et al. 1996, Laudien et al. 2007). The main factors influencing MPB shall be shortly addressed in more detail in the following.

1.2.1.1 *Light*

Besides being the most obvious parameter, sunlight is the major energy source for primary production and thus represents a critical limiting factor for seafloor-associated phototrophs. It varies from short-term (e.g. clouds; minutes to hours) over daily (day/night) to long-term, seasonal (day-length) scales. Most commonly, the term "photosynthetically active radiation" (PAR) is used when light is mentioned in the context of photoautotrophs. Although some organisms can use wavelength other than those defined by the range of PAR (e.g. cyanobacteria; Jorgensen et al. 1987), it describes the wave band of the light-spectrum of solar radiation which is mainly used in photosynthesis, i.e. 400-700 nm. Depending on the habitat, light intensity can vary greatly and may range from 1700 µmol photons m^{-2} s^{-1} in intertidal flats (Barranguet et al. 1998) to < 1% of surface intensity in less than 1 m depth in highly turbid or productive waters (Stevenson et al. 1996).

The water column defines the amount of light and spectral quality that reaches the seafloor by its depth and clarity. The latter depends on the scattering and absorption characteristics of the water which is related to dissolved organic matter, suspended inorganic particles and pigments

(Stevenson et al. 1996, Krause-Jensen & Sand-Jensen 1998). This light attenuation in the water column is often rapid and results in a euphotic zone depth (classically defined as 1% of incident surface irradiances) that can reach beyond 50 m in clear waters (Stevenson et al. 1996). In sediments, it is usually confined to the upper 1-3 mm (Fenchel 1971, Underwood & Kromkamp 1999, Ichimi et al. 2008). The sediment itself also influences light quantity and quality by its absorption and scattering characteristics. These are tightly linked to sediment type and particle size as well as pigment content and amorphous organic matter (Kühl et al. 1994, Kühl & Jorgensen 1994, Lassen & Jorgensen 1994).

Overall, the available light at the surface of and in the seafloor defines a compensation limit for the distribution of MPB (Cahoon 1999). This limit is made up by the compensation irradiances a) for photosynthesis (where gross photosynthesis equals autotrophic respiration, thus net photosynthesis is zero), b) for growth (gross primary production compensates carbon losses by respiration, herbivory, reproduction and exudation of dissolved organic carbon) and c) for community metabolism (where gross community primary production equals respiration demands of the entire community) (Gattuso et al. 2006).

Photosynthesis responds quantitatively to changes in light, therefore environmental variations in quality and quantity of irradiance are assumed to account for much of the variation in MPB physiology, population growth and community structure (Stevenson et al. 1996). There are major spatial and temporal gradients in light availability in MPB habitats and the organisms have to be able to adapt to a widely fluctuating light climate. Besides immediate responses like their migration capabilities to meet light variations (as well as e.g. tide-related temperature variations in intertidal flats), MPB can physiologically adapt to the light regime in their natural habitats by the variation of their pigment composition (Jordan et al. 2010). There are some indications e.g. that MPB in Polar Regions are particularly well adapted to low light regimes (Cahoon 1999). Even under very low light intensities MPB appear to be capable of sustaining photosynthesis and growth. In many cases values for growth lie well below the average of 5-10 µmol photons m^{-2} s^{-1} and 1% surface incident irradiance (Cahoon 1999). Theoretical minimum light intensities to sustain growth were estimated to be 0.1% of surface incident light flux (Falkowski 1988).

Regarding photosynthesis, light is generally, due to its photochemical function, more relevant in the light-reaction than in the dark-reaction which is mainly enzymatically controlled (see BOX 1). Generally, to establish a relationship of photosynthesis and light, so called photosynthesis versus irradiance curves (*PI*-curves) are generated (BOX 2). They enable to evaluations of ecophysiological responses to light, and predictions of *in situ* photosynthesis when *in situ* light intensities are recorded continuously (Stevenson et al. 1996, Sevilgen et al. 2013). Low light adaptations can be reflected in *PI*-curves by increased photosynthetic efficiency (α) at low light levels, lower P_{max}, and lower I_k or I_{max} (see BOX 2) which reflect increased quantities of antennae pigments and decreased quantities of dark-reaction enzymes (Stevenson et al. 1996).

PI-curves of natural assemblages represent the integration of many responses from the different community members which are exposed differently to light within the sediment. Thus, community *PI*-curves are often heterogeneous and the transition areas (low-light to saturation, and saturation to inhibition) may be broader in natural communities than in single species responses.

BOX 2 – PHOTOSYNTHESIS-IRRADIANCE (*PI*) RELATIONS

Photosynthesis is a non-linear function of light intensity (Fig. 1.6). Generally, at low light intensities, photosynthesis increases linearly with increasing light and is primarily limited by the number of photons that are captured by the photosynthetic pigments in the reaction centers (Fig. 1.1). With increasing light intensity, photosynthesis slowly starts to level off towards saturation where it is rather limited by dark-reaction processes like the activity of the carbon fixing enzyme RuBisCO (Fig. 1.2). When light intensity increases more, photosynthesis either reaches an asymptotic maximum or declines due to photoinhibition.

The parameters of a *PI*-curve can be fit using a hyperbolic tangent model which enables us to make photosynthesis estimates for known light intensities.

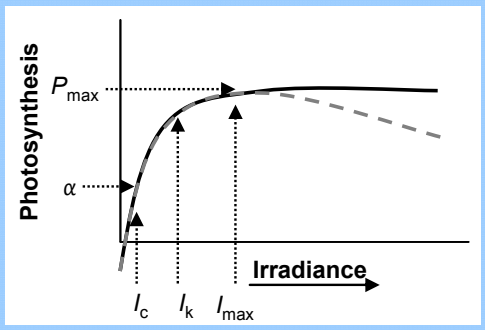


Figure 1.6 – Photosynthesis versus irradiance curves with no photoinhibition (black solid line) and photoinhibition (grey dashed line). P_{max}:maximum rate of photosynthesis, α: initial, linear slope, I_{max}: irradiance of P_{max}, I_k: irradiance at onset of photosaturation (half-saturation constant), I_c: compensation irradiance where photosynthesis equals respiration.

1.2.1.2 *Nutrients*

The nutrient environment is an important determinant for the abundance and species composition of MPB as certain taxa exhibit preferences for particular levels of specific nutrients (Underwood & Kromkamp 1999). Nutrients in this context mainly refer to nitrogen and phosphorus as the most important and commonly investigated nutrients, as well as silicate which plays an important role for diatom growth. Located at the sediment-water interface, MPB receive nutrients from both the overlaying water and the sediment pore-waters. Because they can form stable and dense biofilms at sediment surfaces (see section 1.2), they remain stationary and thereby expose themselves to a steady overlaying water-flow which continuously supplies nutrients, which however are often at low concentrations. Alongside this nutrient supply, sediment pore-waters often provide high nutrient concentrations to MPB, ensuring that nutrient-limitation does not occur. Due to high rates of remineralization within sediments, sediment pore-waters can be substantially enriched in dissolved organic matter and inorganic nutrients, creating favorable conditions for MPB to thrive (MacIntyre et al. 1996, Cahoon 1999). By absorbing substantial parts of sediment originated nutrients, MPB form an active filter at the sediment-water interface, reducing the nutrient flux towards the overlaying water (Sundbäck et al. 1991, Bourgeois et al. 2010). In some cases this effect has been shown to be sufficient enough to create limiting nutrient conditions for phytoplankton (e.g. Sigmon & Cahoon 1997).

In relation to ecosystem-internal nutrient recycling, nutrient-input changes seasonally and can locally be altered by river run-offs, glacier melts in Polar Regions and, increasingly by eutrophication (land erosion, sewage effluents, aquacultures etc.) (Welker et al. 2002, Scholz & Liebezeit 2012, Tantanasarit et al. 2013 and references therein).

Alterations in nutrient supply can influence MPB community growth and composition. Experimental enrichments with nitrogen and phosphate could demonstrate that both photosynthesis and biomass development could be stimulated in subtidal MPB (Underwood & Kromkamp 1999). Furthermore, nitrate and phosphate additions altered the taxonomic composition of subtidal MPB assemblages, causing a shift from a nutrient-limited cyanobacteria-

dominated community towards a community with increased diatom numbers (Underwood & Kromkamp 1999, Larson & Passy 2012).

1.2.1.3 *Grazing*

MPB are the base of the benthic marine food-web and are fed upon by many heterotrophic levels. Besides bacteria that consume organic matter which is excreted by MPB, there is a large diversity in micro-, meio- and macrofauna that indirectly or directly feed on benthic diatoms (Miller et al. 1996, Cahoon 1999). The heterotrophic community ranges from microfaunal protozoans like ciliates (Epstein et al. 1992) to copepods (Decho & Flegger 1988) and nematodes, as well as macrofaunal snails, bivalves and polychaetes (Miller et al. 1996). Among these, the macrofauna plays the most important role as grazers of the MPB with mostly surface and sub-surface deposit feeders and, subsequent to resuspension, obligate or facultative suspension feeders. Although grazers in subtidal areas can reduce MPB abundance, in many cases the biomass of the phototrophic microbial community does not decline below 50% (Miller et al. 1996). Aside from feeding, by their bioirrigating activity and reworking of the sediment, infaunal organisms can increase nutrient fluxes (Retraubun et al. 1996) which locally can have a beneficial effect for MPB (Chennu et al. 2014).

1.2.1.4 *Temperature*

Temperature has a major influence on rates of MPB photosynthesis and growth. It is assumed to set the upper limit of the growth rate which should be most apparent when other factors are optimized (Epply 1972, Li 1980). Temperature can be tightly linked to light intensities and varies greatly (on average, seasonally and daily) depending on the geographical location and habitat. Whereas in intertidal mudflats and rocky shores temperature can vary drastically and is directly influenced by the sunlight, subtidal areas are mainly influenced by seasonal changes in the temperatures of the water masses and local factors (currents, river run-off, glacier melt).

The role of temperature for MPB can be examined on different levels and can be either direct (affecting the physiology) or indirect (affecting environmental factors important for MPB). On an autoecological level it can be

reflected in alterations of fatty acids and heat shock proteins. On the population level temperature influences potential maximum growth rates as different populations and species exhibit different minimum, maximum and optimum temperatures for growth. Within a community, the influence of temperature will be reflected in the dominance of certain algal classes, species composition and diversity, geographical distributions and species interactions. And finally at the ecosystem level, the responses of biomasses and potential maximum areal primary production rates will be influenced by temperature (Stevenson et al. 1996, Cibic et al. 2012, Vieira et al. 2013).

With respect to photosynthesis, temperature primarily affects the metabolism through its control of enzyme reaction rates. Thus, with increasing temperatures, the kinetic energy increases until a point is reached when denaturation (of the enzymes) rates exceed this kinetic effect. Under light saturating conditions, the photosynthetic performance will be primarily controlled by the dark-reaction enzymes (RuBisCO) and the enzymes controlling ATP regeneration (Stevenson et al. 1996). Many algae are capable of acclimatizing to changing temperatures. They can accomplish this by increasing their concentrations of RuBisCO and other enzymes to compensate e.g. for low temperatures. Additionally they can increase their light harvesting efficiency (by increase of chlorophyll *a*) at higher temperatures to compensate for the CO_2 fixation (Stevenson et al. 1996). Thus, within their physiological limitations, MPB are capable to adapt to different temperatures.

1.2.2 *Measuring microphytobenthos productivity and biomass*

Mainly due to physical constraints, most of the marine, benthic primary production studies have been conducted in the shallow water habitats of which approximately 50% come from the intertidal zone (Cahoon 2006). Net primary production is generally defined as gross production minus the autotrophic respiration. However, in studies of MPB communities, autotrophic and heterotrophic respiration cannot be easily separated, thus respiration also includes the respiration of the heterotrophic organisms. Accordingly, hereafter, net primary production refers to the integrated community response.

One difficulty with assessing MPB production is that there are no adapted standard methods for measuring benthic microalgal biomass and

production (Cahoon 2006). Important measures to estimate MPB productivity are biomass growth and photosynthetic carbon uptake or oxygen production.

Most of the earlier studies quantified benthic microalgal production by the radiotracer method (^{14}C incorporation) (e.g. Colijn & de Jonge 1983) or by measuring dissolved oxygen fluxes (e.g. Billerbeck et al. 2007) using light-dark core- or chamber-incubations. The radiotracer method relies on the incorporation of labeled inorganic carbon and in principle, depending on the length of incubation, quantifies gross primary production. However, controlling uniform introduction and specific labeling experienced by the active MPB community is difficult and thus this method is rarely used nowadays (Glud et al. 2009). Alternatively, chamber incubations targeting oxygen fluxes are carried out to estimate community respiration in the dark and net production in the light which are added up to gross production estimates. The method is widely applied and when larger sediment areas are enclosed, the small-scale patchiness of the MPB communities can to some extend be accounted for. Despite their simple application, chamber incubations usually change local hydrodynamics which highly influence sedimentary processes, are usually only carried out in small numbers, and represent the bulk sedimentary response.

For more detailed insights, fine-scale measurements are realized by direct measurement of oxygen in the MPB community (this thesis). Oxygen microsensors are minimally invasive and allow for high resolution characterization of the distribution, production and consumption of O_2. They enable punctual and direct measurements of gross production (by the light-dark shift method, Revsbech et al. 1981, Revsbech & Jorgensen 1983) and assessment of net photosynthetic oxygen production and respiration as derived from steady state oxygen profiles in the light and in the dark (Revsbech & Jorgensen 1983, Kühl et al. 1996). Thus, although their horizontal resolution is very poor and does not account for MPB patchiness, they enable high resolution on a temporal and vertical scale.

Only a few studies have estimated benthic MPB production by measuring the light flux and using *PI*-relationships to determine the production (Cahoon 1999 and references therein), as the results are often heterogeneous due to large variations on temporal and spatial scales. In 2006, about 50% of all reported estuarine and coastal production estimates came from oxygen

exchange measurements, 40% were constituted by ^{14}C-studies and 10% resulted from microsensor based investigations (Cahoon 2006).

MPB biomass can be used as a proxy for the distribution of production and can offer a rather easy and quick alternative when production measurements are not possible. MPB biomass is almost always measured and expressed as the amount of chlorophyll *a*, the major photosynthetic pigment. This can be assessed by several techniques: Spectrophotometry allows for a rapid determination of bulk chlorophyll *a* concentration and is a routine method often used when vast numbers of samples need to be analyzed (Brotas et al. 2007). Similarly, high performance liquid chromatography (HPLC) quantifies pigment concentrations but simultaneously allows for the differentiation of multiple pigments and their degradation products (Brotas & Plante-Cuny. 2003, Cibic et al. 2007). Although this method is costly, it has been shown to be the most accurate one (Brotas et al. 2007). In addition to the direct measurements of pigment concentrations optical methods can be applied which allow non-destructive estimations of photopigment quantity and quality. An increasing number of studies apply fluorometric methods such as pulse-amplitude-modulated (PAM) fluorometry (e.g. Barranguet & Kromkamp 2000, Glud et al. 2002, Vieira et al. 2013). The technique is based on the fluorescence response of the light harvesting complexes to saturating light pulses, applied to dark-adapted samples. Based on the ratio of minimal fluorescence and variable fluorescence after light-saturation, it enables assessment of the photosynthetic efficiency. Additionally, as the minimum fluorescence correlates with chlorophyll *a* content, it is used as a proxy for MPB biomass and has been applied to follow MPB biomass changes at the sediment surface (Serôdio et al. 1997, Jesus et al. 2005). Hyperspectral imaging can be used similarly and records the light that is back-scattered from the MPB community which contains the spectral imprints of the existing photopigments. It allows to draw conclusions on the pigment biomass and composition of MPB assemblages and enables to monitor MPB dynamics on the sediment surface with high spatial and temporal resolution (Chennu et al. 2013).

1.3 MICROPHYTOBENTHOS IN A CHANGING WORLD

1.3.1 *Why should we study MPB in cold-water regions?*

Compared to temperate study sites the number of MPB studies from cold water areas, especially from subtidal Polar Regions are very limited. On a global scale it is very difficult to estimate the distribution of benthic photosynthetic organisms and their potential contribution to primary production in the Arctic and Antarctic, as e.g. satellite-derived data on light distribution in the marine realm is often poor in high latitudes (Gattuso et al. 2006). While the importance of MPB in shallow water food-webs is recognized by now (Middelburg et al. 2000), their role in ecosystem functioning in Polar Regions has only just started to be tackled (Gilbert 1991, Glud et al. 2002, Glud et al. 2009). Accordingly, subtidal MPB *in situ* data and studies characterizing their function in cold-water regions are very rare. Only five years ago, in 2009, was it pointed out, that regarding benthic primary production, the entire Arctic region is grossly under-sampled, especially in subtidal habitats such as rocky shores and sandy sediments (Glud et al. 2009). This scarcity of data gains particular importance considering that the Arctic continental shelf area < 200 m comprises about 25% of the global coastal region, receiving > 1% of surface downwelling irradiance, thus potentially accommodating primary production (Glud et al. 2009). Investigations of MPB in cold-water habitats like the Arctic are not only relevant to understand the fundamental role of MPB in polar benthic ecology but also relevant in the context of changes observed in these regions resulting from global climate change. High latitudinal ecosystems are characterized by prevailing extreme conditions, and it can be assumed that they will be affected when basal environmental parameters alter and that it is possible to detect changes in these systems first.

1.3.2 *Global climate change and consequences for MPB*

The climate of the Earth is changing and has undergone drastic anthropogenic influence since the industrialization by increased emissions of CO_2 which, as an efficient green-house gas in our atmosphere, results in increasing amounts of heat retained by the Earth's biosphere and thus temperature changes on the planet (BOX 3). Impacts are evident as increases in average global atmospheric

and ocean temperatures, increased frequency of heavy precipitation, widespread melting of snow and ice, and rise of the sea-level. Especially in high latitudes (north of 65°N) this temperature increase is of high importance as in these latitudes it has increased about twice the global average (between 1965 and 2005) (IPCC, 2007).

BOX 3 – GLOBAL TEMPERATURE CHANGES

Temperature increase is widespread over the globe and is greater at higher northern latitudes. Average Northern Hemisphere temperatures during the second half of the 20th century were very likely higher than during any other 50-year period in the last 500 years and showed distinct increases in the Arctic realm.
The oceans have been taking up over 80% of the heat added to the climate system and observations since 1961 show that the average temperature of the global ocean has increased to depths of at least 3000m (IPCC, 2007).

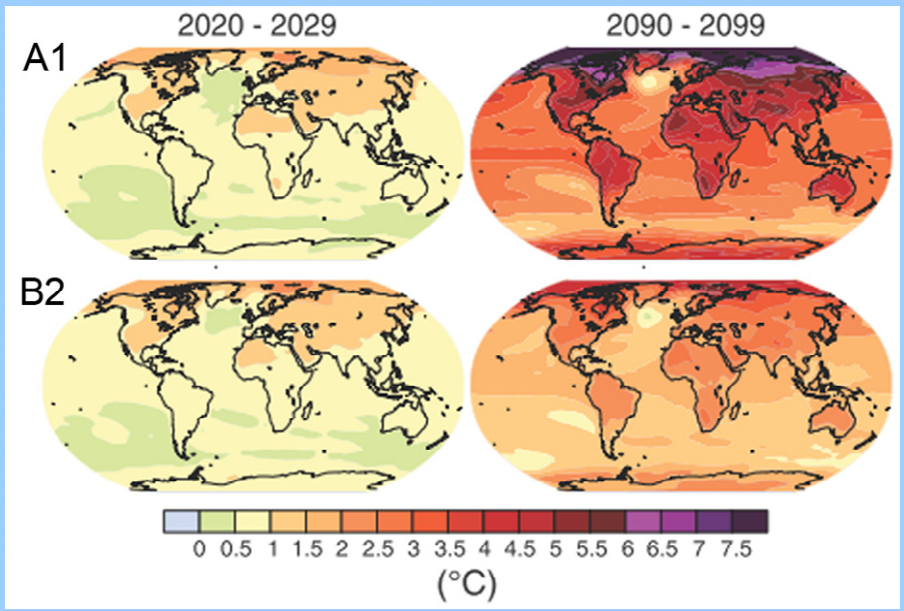


Figure 1.7 – Global warming. Projected surface temperature changes for the early and late 21st century relative to the years 1980-1999. The panels show Atmosphere-Ocean- General-Circulation-Model average projections for the "worst-case" (A1) and "best-case" (B2) IPCC Special Report Emission Scenarios 2000, averaged over the decades 2020-2029 and 2090-2099 (modified after Bernstein et al. 2007, IPCC Synthesis Report 2007, Fig. 3.2).

Consequently, the marine biota will face changes in environmental factors and will have to cope with altering *in situ* conditions. Besides changes in local water temperatures, temperature increases will entail increased freshwater input from melt-waters (in Polar Regions) and increased precipitation and run offs (in temperate regions). This has consequences for water quality, e.g. salinity, turbidity, sediment and nutrient load as well as for the light regime due to input of erosion and glacier material. In addition, the drastic change of sea-ice cover

will have effects on the light regime influencing onset of potential primary production throughout the year in the Arctic (Serreze et al. 2007, Laxon et al. 2012, Zhang et al. 2012). All together this means, that MPB will have to react and adapt within the constraints of their biological capabilities.

1.4 TWO SUBLITORAL COLD-WATER REGIONS – SAME BUT DIFFERENT?

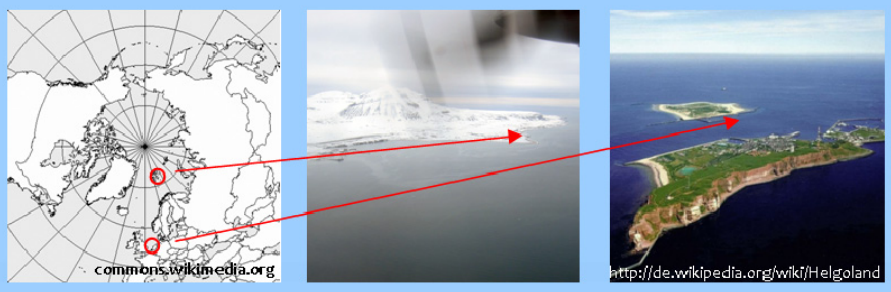
In this thesis I compare subtidal sediments from an Arctic (Spitsbergen, Svalbard Archipelago, Arctic Sea) and a temperate region (Helgoland, North Sea) (Manuscript I). The location of these sites offers characteristics which motivate a closer look and comparison of their biota. Despite their geographical separation of around 2800 km, 25° latitudinal distance and the respective climatic differences, especially in their temperature and light regime (Manuscript II), they are oceanographically tightly interlinked by North Atlantic water masses (BOX 4). Thus, west Spitsbergen with its adjacent fjords along the west coast is highly influenced by the extension of the North Atlantic currents. One of the smaller fjords along Spitsbergen's west coast is the Kongsfjorden. The marine ecosystem of Kongsfjorden was assigned as a model system for many ecological studies in the last decades and its detailed characterization was summarized in the reviews of Hop et al. (2002) and Svendsen et al. (2002). Many studies have revealed that the biota in Kongsfjorden can be categorized as sub-Arctic (boreal) rather than Arctic.

Kongsfjorden is an open fjord of approximately 26 km length, 3 to 8 km width and maximum water depth of 400 m at the west coast of Svalbard at 79°N and 12°E (Svendsen et al. 2002). As it has no sill at its entry, the exchange of water masses at the fjords opening highly influences the marine physical and biological aspects of the fjord. While the outer fjord is highly influenced by oceanographic conditions, the inner fjord is mainly influenced by three tidal glaciers of which the smaller one (Blomstrandbreen) is located at the middle of its northern coast, and the two main glaciers (Kongsbreen and Kronebreen) at the end of the fjord (Svendsen et al. 2002). Based on this, the outer fjord is denominated as the Atlantic part whereas the inner fjord (under glacial influence with substantial discharges of terrestrial and glacial freshwater, especially during summer) is characterized as the Arctic part (Svendsen et al.

2002). In the course of future climate change, it is predicted that the fjord will be influenced from both directions and thus is qualified as a suitable site and sensitive indicator for climate change phenomena (Svendsen et al. 2002).

BOX 4 – THE STUDY SITES AND HOW THEY ARE LINKED

The two study sites Helgoland and Svalbard are located in the temperate and Polar Region of the Northern hemisphere. The specific sampling sites in approximately 5 m depth are characterized by sediment flats of fine sands.



Svalbard and Helgoland are tightly interlinked by the same water masses (Fig. 1.8). The North Atlantic Current transports warm water masses from the Gulf Stream towards north-western Europe. Whereas one of its side arms defines the North Sea, it progresses northward as the Norwegian Atlantic Current and continues as the West Spitsbergen Current along the West coast of Spitsbergen.

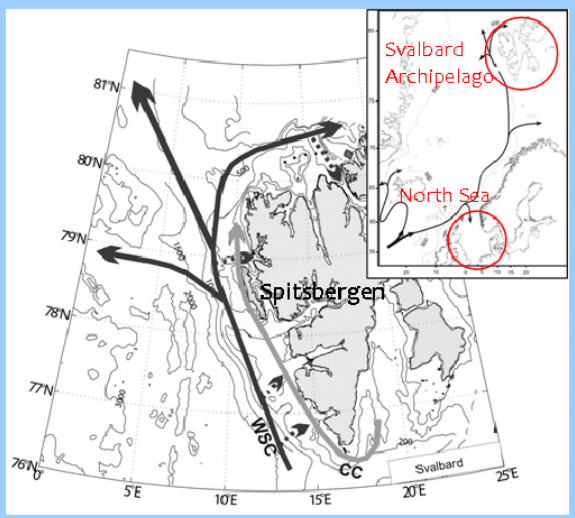


Figure 1.8 – Ocean currents with prevailing surface currents along Spitsbergen. The West Spitsbergen Current (WSC) transports warm water masses of the North Atlantic along the West Coast of Spitsbergen whereas colder waters on the shelf are transported by Arctic Type Water masses of the Coastal Current (CC).
(modified after http://sp.lyellcollection.org)

The temperate site (Düne Süd) is located approximately 100 m south-west of the Helgoländer Düne, a small island adjacent to the main island of Helgoland, located in the south-eastern part of the North Sea. Numerous studies on phytoplankton ecology have been carried out at Helgoland and since 1873 the surface water temperature of the North Sea at Helgoland has been recorded continuously (Wiltshire & Manly 2004). This gives valuable insights

into warming trends and possible implication for the aquatic food web. However, data for subtidal MPB from the German North Sea, including Helgoland, is missing.

In both study sites, Kongsfjorden and Helgoland, subtidal sandy sediment flats were chosen for the presented investigations, as the distribution and importance of these habitats for benthic primary production is evident but still under-investigated (see section 1.1.2). Field investigations and laboratory studies at Kongsfjorden were carried out in Ny-Ålesund and on Helgoland at the Biologische Anstalt Helgoland. Both locations enable easy, diver supported accessibility and excellent on-site research facilities, which were essential for the conducted *in situ* and laboratory studies.

1.5 OBJECTIVE OF THE THESIS

The aim of this thesis is to study natural MPB communities and their habitats from two subtidal cold-water sites and to compare their responses to alterations in parameters associated with global climate change. Temperature has been selected as a main experimental parameter because it is expected to be most severely and rapidly altered. In addition, as a result of melting glaciers, disturbed precipitation dynamics and river input, changes in nutrients were also applied. Thus, the approach in this thesis is split into two main objectives: (i) the basic habitat and MPB community characterization and (ii) the response of MPB to changes in environmental parameters (BOX 5). Based on these, the following specific research questions were established.

1.5.1 *Research questions*

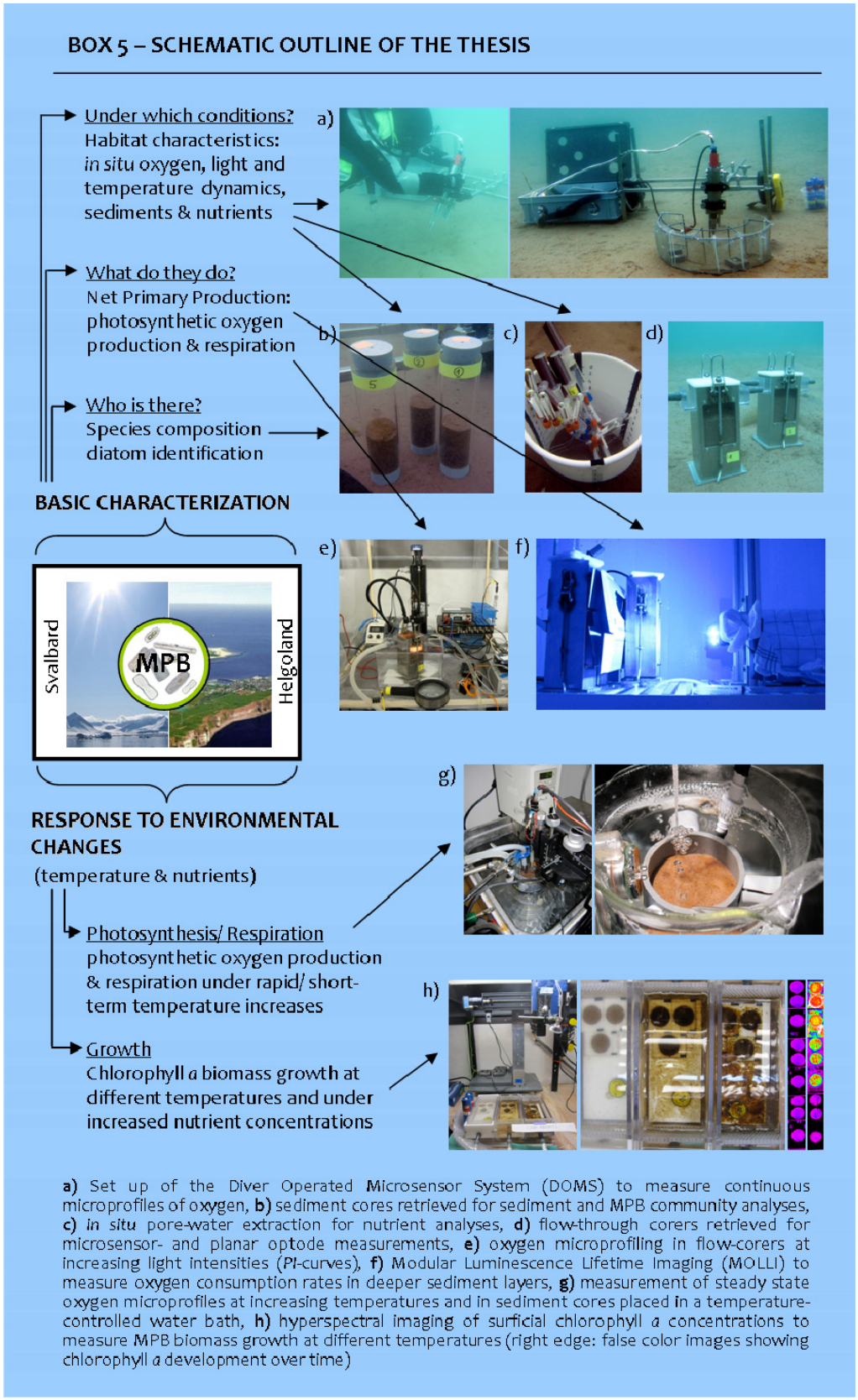
The specific questions in this study were:

(i) How do MPB net photosynthesis and respiration (in terms of oxygen production and consumption) compare in Helgoland and Svalbard? Who are the main players of the MPB community, and what is the daily net oxygen budget under natural (*in situ*) conditions? Will the communities and their photosynthetic performances (light adaptation, maximum photosynthesis) be site-specific, or will they be similar? *see Manuscript I*

(ii) Will the photosynthesis and respiration response of the temperate and sub-Arctic MPB community to short-term temperature increases be different? Will the sub-Arctic site be more adapted as it experiences higher temperature fluctuations *in situ* on a short term scale (daily/ hourly) than the temperate site? Will the MPB communities develop towards a net-heterotrophy as previously shown for benthic communities? *see Manuscript II*

(iii) Will an upward shift of the temperature baseline and increase of nutrients have consequences for the growth of MPB communities from Helgoland and Svalbard? Will the MPB community grow best under lowest, intermediate or highest temperatures from within the *in situ* temperature range? Will their growth be dependent on the addition of nutrients? Will they grow with the same speed and reach similar biomasses, or will there be differences because of differences in their natural light and temperature regimes? *see Manuscript III*

The approaches to these research questions and the respective results are presented in the following manuscripts.



a) Set up of the Diver Operated Microsensor System (DOMS) to measure continuous microprofiles of oxygen, **b)** sediment cores retrieved for sediment and MPB community analyses, **c)** *in situ* pore-water extraction for nutrient analyses, **d)** flow-through corers retrieved for microsensor- and planar optode measurements, **e)** oxygen microprofiling in flow-corers at increasing light intensities (*PI*-curves), **f)** Modular Luminescence Lifetime Imaging (MOLLI) to measure oxygen consumption rates in deeper sediment layers, **g)** measurement of steady state oxygen microprofiles at increasing temperatures and in sediment cores placed in a temperature-controlled water bath, **h)** hyperspectral imaging of surficial chlorophyll *a* concentrations to measure MPB biomass growth at different temperatures (right edge: false color images showing chlorophyll *a* development over time)

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ii. MANUSCRIPTS

OVERVIEW OF MANUSCRIPTS

This thesis includes three manuscripts which were prepared for publication in peer-reviewed journals - one is accepted, two are pending submission. The contributions of authors on the study design, data acquisition, data analyses and writing of the manuscript are outlined for each manuscript.

MANUSCRIPT I:

Oxygen budgets in subtidal arctic (Kongsfjorden, Svalbard) and temperate (Helgoland, North Sea) microphytobenthos communities
Sevilgen D.S., de Beer D., Al-Handal A.Y., Brey T., Polerecky L.

Contributions: Conceived and designed the study: DS. Performed the study: DS, LP. Analyzed the data: DS, LP, AH. Contributed reagents/material/analytics: DS, LP, DB, TB, AH. Wrote the paper: DS, LP, TB, DB.

(accepted for publication in Marine Ecology Progress Series, MEPS)

MANUSCRIPT II:

Rapid temperature increases stimulate net heterotrophy in subtidal microphytobenthos communities similarly in a sub-arctic and temperate site.
Sevilgen D.S., Brey T., deBeer D., Polerecky L.

Contributions: Conceived and designed the study: DS, LP. Performed the study and aquired data for the study: DS, LP. Analyzed the data: DS. Contributed reagents/material/analytics: DS, TB, DB, LP. Wrote the paper: DS, TB, DB, LP.

(to be submitted to Marine Biology)

MANUSCRIPT III:

Will an upward shift of the temperature baseline change biomass growth of sub-arctic and temperate microphytobenthos communities? A quantitative pilot-study
Sevilgen D.S., Chennu A., Brey T. Polerecky L.

Contributions: Conceived and designed the study: DS. Performed the study and aquired data for the study: DS, AC, LP. Analyzed the data: DS, AC. Contributed reagents/material/analytics: DS, AC, LP, TB. Wrote the paper: DS, AC, TB.

(to be submitted to Polar Biology)

2. MANUSCRIPT I

OXYGEN BUDGETS IN SUBTIDAL ARCTIC (KONGSFJORDEN, SVALBARD) AND TEMPERATE (HELGOLAND, NORTH SEA) MICROPHYTOBENTHOS COMMUNITIES

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accepted for publication in MEPS (Dec 2013)

2.1 ABSTRACT

We compared primary production and respiration of a temperate (Helgoland, North Sea) and subtidal Arctic (Svalbard, Kongsfjorden) microphytobenthos community during summer. The diatom communities were generally characterized as cosmopolitan, displayed no site specificity, and had similar chlorophyll *a* and fucoxanthine concentrations. Their net and gross photosynthesis rates and light adaptation intensities, derived from laboratory microsensor measurements, were also similar, despite differences in water temperature. Daily oxygen fluxes across the sediment-water interface were estimated by combining laboratory microprofile- and planar optode-measurements with *in situ* data on oxygen penetration and light dynamics. During the study period, the Svalbard sediments were on average net heterotrophic while the Helgoland sediments were net autotrophic (-22.4 vs. 9.2 mmol O_2 m^{-2} d^{-1}). This was due to high infaunal abundance in the Svalbard sediments that caused high oxygen uptake rates in the sediments and consumption below the euphotic zone. Additionally, bioirrigation of the sediment due to infaunal burrow ventilation was reduced by light, thus the sedimentary oxygen inventory was reduced with increasing light. Conversely, light enhanced the oxygen inventory in the Helgoland sediments. Oxygen dynamics in the Svalbard sediments were therefore dominated by bioirrigation whereas in the Helgoland sediments they were dominated by photosynthetic oxygen production.

2.2 INTRODUCTION

Microphytobenthos (MPB) are communities of photosynthetic microorganisms (diatoms, dinoflagellates and cyanobacteria) that live in the uppermost surface layers of the seafloor (MacIntyre et al. 1996). They constitute a food source for heterotrophic organisms and can significantly affect solute exchange across the sediment-water interface. In sandy and muddy habitats they are mostly dominated by diatoms (Sundbäck et al. 1991, MacIntyre et al. 1996). In coastal areas, MPB production can be similar to or even exceed phytoplankton production and thus contribute significantly to ecosystem primary production (MacIntyre et al. 1996, Cahoon 1999, Underwood & Kromkamp 1999, Glud et al.

2002, Cahoon 2006). A recent review for shallow waters (< 30m) in the Artic concluded that also there benthic microalgae on average exceed pelagic productivity (by ~50%; Glud et al. 2009).

MPB communities are well studied from temperate to tropical regions and in various habitats such as brackwater lagoons, salt marshes, intertidal flats and subtidal sediments (MacIntyre et al. 1996, Cahoon 1999, Underwood & Kromkamp 1999, Cahoon 2006, Glud et al. 2009). MPB are the most important primary producers in intertidal flats (Scholz & Liebezeit 2012) and are well studied accordingly (e.g. MacIntyre et al. 1996, Denis et al. 2012, Scholz & Liebezeit 2012). Except for a few studies dealing with MPB and benthic food webs from the North Sea (Riauxgobin et al. 1987, Reiss et al. 2007, Evrard et al. 2010), subtidal studies from the German North Sea area are lacking.

As pointed out by Glud et al. (2009), subtidal MPB, particularly in colder waters and high latitudinal regions like the Arctic, are grossly under-sampled. This is a significant gap of knowledge, given that the Arctic continental shelf area accounts for ~1/4 of the global shelf area (areas with less than 200 m water depth; Menard & Smith 1966, Jakobsson 2002) of which about 1/5 receives enough light to harbor MPB (Gattuso et al. 2006). It is likely that these systems are sensitive and will respond rapidly to global change effects such as ocean warming. The drastic decline of sea ice cover in the Arctic (Serreze et al. 2007, Laxon et al. 2012, Zhang et al. 2012) will change underwater light regimes, with more light becoming available earlier in the year and for longer time periods. As MPB can quickly adapt to ambient light conditions (Kühl et al. 2001, Glud et al. 2002), these changes can potentially stimulate pelagic and benthic primary production. Additionally, stimulation of benthic productivity and remineralization will occur as a result of increasing temperature. For example, the study of Hancke & Glud (2004), which examined the short-term temperature response of photosynthesis and respiration in a high Arctic and two temperate MPB communities, showed that both processes were stimulated, although respiration was stimulated more than photosynthesis. The MPB response was similar at all sites despite their latitudinal difference, indicating no geographically related temperature adaptation. Similarly, in the study of Wieland and Kühl (2000), which examined oxygen and sulfide cycling in hypersaline microbial mats from Solar Lake, Egypt, a short-term temperature

increase stimulated respiration more than photosynthesis. Thus, it appears that at elevated temperatures benthic systems will gradually become more heterotrophic, and this trend will hold across large geographical distances.

In this study, we aimed to add to the body of available literature that compares the activity of microphytobenthic communities from geographical regions characterized by largely different prevailing temperatures. Of particular interest were subtidal communities, which are largely under-sampled. As study sites, we chose subtidal sediments from the Arctic (Svalbard) and from a temperate site in the North Sea (Helgoland). Despite the north-south distance of about 2800 km (25° latitude) between these two sites, they are oceanographically closely connected by the same North Atlantic water masses. The West Spitsbergen Current transports warm waters of the North Atlantic Current along the western coast of Svalbard, influencing the fjord systems along the coastline and their ecology (Svendsen et al. 2002, Hop et al. 2012). Recent studies of soft sediments in Kongsfjorden report high amounts of MPB and a net heterotrophic state at most sites (Glud et al. 2009, Woelfel et al. 2009, Woelfel et al. 2010).

Specifically, our aim was to describe and compare habitat characteristics, MPB communities, primary production and oxygen dynamics in the two sites. We hypothesized that (i) oxygen dynamics in the sediments are controlled by MPB activity, and that (ii) the two study sites show differences in temperature, light and MPB community composition, but the photosynthetic performance of the MPB communities are similar owing to optimal adaptation to local conditions. We conducted laboratory experiments using oxygen microsensors to determine net photosynthesis and respiration rates as a function of light, and planar optodes to measure deeper sediment respiration. Additionally, we monitored oxygen distributions in the sediments and incident light intensities *in situ* over a period of at least 24 hours, and combined these data with the laboratory results to estimate daily oxygen budgets.

2.3 MATERIALS AND METHODS

2.3.1 *Study area and sampling*

This study was carried out in shallow subtidal sediments from Svalbard (Arctic, Norway, 78°45' N 16°00' E) and Helgoland (North Sea, Germany, 54°10' N 7°53' E). The Svalbard site, called Brandal, is located in the glacial fjord Kongsfjorden (Fig. 2.1a). The fjord, located at the western coast of Svalbard, is influenced by water masses of the West Spitsbergen Current, coastal Arctic water and, in the inner part, by four large tidal glaciers terminating at the east and north sides of the fjord (Svendsen et al. 2002). The estimated annual average water temperature is slightly above 0°C and the diurnal tidal range is approximately 2 m (Ito & Kudoh 1997, Hop et al. 2002, Svendsen et al. 2002). The Helgoland site, Düne Süd, is located about 100 m south-west from the Helgoländer Düne, which is an island located in the south-eastern part of the North Sea (Fig. 2.1b). Average annual water temperature is around 10°C and the tidal range is about 2 m (Wiltshire et al. 2008).

Sampling and measurements were carried out at water depths of about 5 m through summer months of 2009-2012. They included sediment characterization, *in situ* measurements of oxygen and light, sampling for pore-water nutrients and MPB biomass, and laboratory measurements of photosynthesis and respiration in freshly collected sediment samples (Table 2.1).

2.3.2 *Sediment characterization*

Sediments were collected by Scuba divers using cylindrical acrylic cores (inner diameter 50 mm, length 200 mm). The cores were sliced in 2 cm sections and each section was desalinated and dried until constant weight. The grain size distribution, sorting and skewness were determined as previously described (Wentworth 1922, Friedman & Sanders 1978) using a sieve-column with mesh sizes of 63, 125, 250, 500, 1000 and 2000 µm. Porosity was determined from weight loss on drying until constant weight at 80°C. Sediment permeability was measured in two cores per site with a constant head permeameter (Klute & Dirksen 1986).

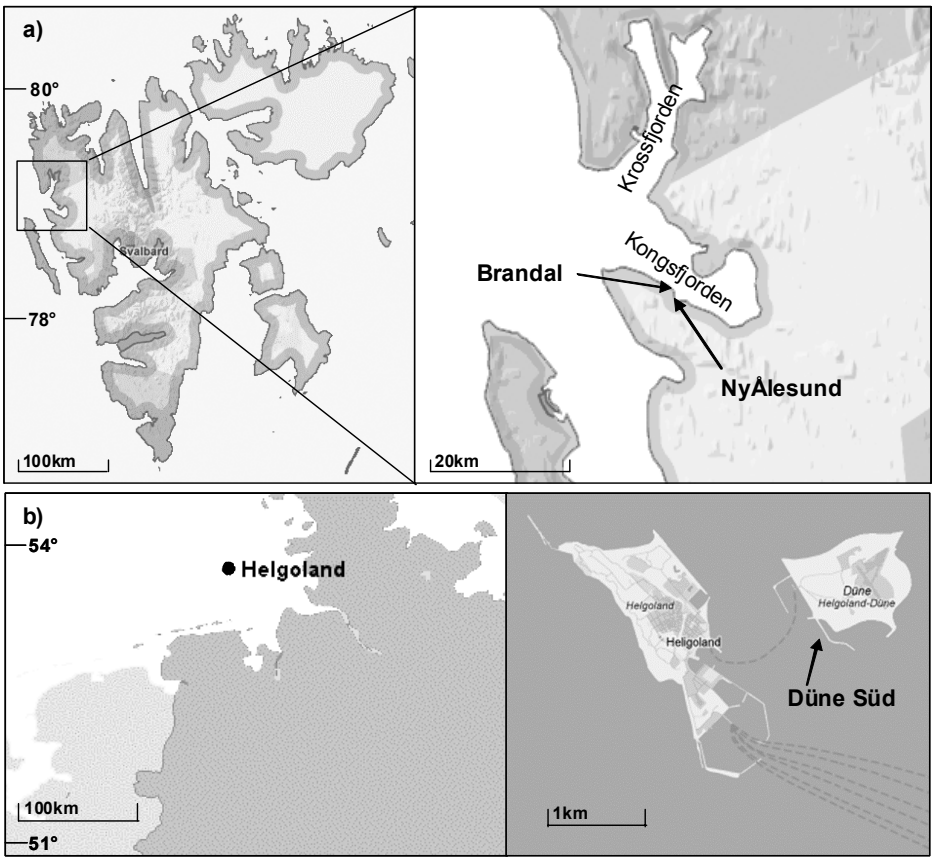

Figure 2.1 - Maps showing the locations of the studied sites Brandal (Svalbard, Arctic, Norway) and Düne Süd (Helgoland, North Sea, Germany). Modified from maps.google.com.

Table 2.1 - Overview of the measurements conducted at the study sites in Svalbard and Helgoland.

	Brandal **Kongsfjorden, Svalbard, Arctic**	**Düne Süd** **Helgoland, Germany, North Sea**
MEASUREMENTS/ SAMPLING	date	date
oxygen profiles/ incident light, *in-situ*	June 2010	August 2009
sediment	June 2010	August 2009
pore-water nutrients	June 2010	-
photosynthesis and respiration rates, laboratory	June-July 2011	June-August 2009
MPB pigments	June 2012	May 2012

2.3.3 *Biogeochemical characterization of sediments and pore-waters*

Total carbon (TC), nitrogen and sulfur contents in freeze-dried and ground samples of the sediment (depth intervals 0-1, 1-2, 2-4, 4-6, 6-8, 8-10 cm) were determined by high temperature combustion (Carlo Erba NA-1500 CNS analyzer). Total inorganic carbon (TIC) was measured in a CO_2 Coulometer (CM 5012, UIC) after acidification with 20% (w/v) phosphoric acid (CM 5130

Acidification module UIC). Total organic carbon was calculated as TOC = TC - TIC.

Pore-water for the analysis of nutrients (NO_2^-, NO_3^{2-}, NH_4^+, PO_4^{2-}, Si^{2+}) was sampled *in situ* by Scuba divers using soil moisture samplers (10 cm Rhizon samplers, pore size 0.1 µm). For vertical profiles, a bottomless plastic bucket (30 cm diameter) was pushed 20-25 cm into the sediment, emptied and left to rest for about 24-72 hours. Subsequently, rhizones were inserted horizontally from the inside outwards into the sediment through holes made in 2 cm vertical intervals and down to a maximum depth of 12 cm. Pore-water was extracted either with a syringe or into vacuum vials. Samples were immediately transported to the laboratory and analyzed for nutrients using a 5 channel QuAAtro nutrient analyzer (Seal) equipped with a Jasco fluorometer for NH_4^+ analyses. NH_4^+ was analyzed as previously described (Kerouel & Aminot 1997), and specific analytical protocols for the remaining nutrients were applied (Seal Analytical).

2.3.4 *Photopigment analyses*

Concentrations of chlorophyll *a* and fucoxanthine were used as a measure of MPB biomass. The upper 3 mm of the sediment were sampled, freeze dried and weighted. To extract the pigments, 99.8% acetone was added, the samples were vortexed for 5 s, left in an ultrasound bath with iced-water for 3 min, and stored afterwards at -28°C for 24 h in the dark. The extracts were filtered (Acro disc CR 4 mm syringe filters with a 0.45 µm PTFE Membrane) and analyzed by high performance liquid chromatography (HPLC) as previously described (Wright et al. 1991). The HPLC system consisted of a Waters 2695 separation module and a Waters 996 photodiode array detector (Waters, MA). Pigment standards supplied by DHI, Denmark, were used for calibration. The MPB samples were originally taken from the same cores where photosynthesis and respiration rates were measured by microsensors (see below). However, because these samples were lost, pigments were analyzed from a later measurement campaign (Table 2.1).

2.3.5 *Diatom identification*

Sub-samples of the upper 5 mm of sediment cores (2 per site) were taken and fixed in Lugol's solution for identification and quantification of diatoms. Diatom samples were first washed with deionized water to remove salts, and then cleaned by boiling in 30% hydrogen peroxide. Few drops of 50% hydrochloric acid were added to the diatom suspension to ensure the removal of the hydrogen peroxide, which was followed by several rinses with distilled water. Cleaned diatoms were allowed to settle on cover slips, which were placed on a metal tray that was kept stable away from any disturbance. After air drying at room temperature, permanent diatom slides were made using Naphrax diatom mountant medium. Diatoms were identified and photographed using a Zeiss Axioplan 2 light microscope (LM; Carl Zeiss AB, Gotheneburg, Sweden) with differential interference contrast. Their quantification was done in three levels: very rare (observed only once per slide), rare (few specimens per slide) and frequent (may constitute up to 10% of frustules on a slide). The main taxonomic literature based on which diatoms were identified included Hustedt (1958, 1961–1966), Hendey (1964), Germain (1981), Krammer & Lange-Bertalot (1986, 1988), Krammer & Lange-Bertalot (2000), Witkowski et al. (2000) and Scott & Thomas (2005).

2.3.6 In situ *light and temperature measurements*

In situ intensities of downwelling photosynthetically active radiation (PAR) were measured in 30 s or 60 s intervals using submersible light-loggers (Odyssey Dataflow Systems, Christchurch/ New Zealand) positioned at the sediment surface close to the points were the microsensor measurements were done. *In situ* temperature in Brandal was measured at 5 m depth in 20 min intervals during one year (June 2011- June 2012) using HOBO Pendant and TidbiT loggers (ONSET, Massachusetts/ USA). Because similar measurements could not be done in Helgoland, temperature data for this site were taken from the Helgoland Roads data series (Wiltshire et al. 2008).

2.3.7 In situ *oxygen microprofile measurements*

Vertical oxygen microprofiles in the sediment were measured *in situ* with amperometric Clark-type oxygen microelectrodes (Revsbech 1989) connected to a diver-operated microsensor profiler (DOMS; Weber et al. 2007). Sensors had a tip diameter of 10-50 µm and a stirring sensitivity of < 1.5%. Linear calibration of the sensors was derived from *in situ* measurements in the anoxic parts of the sediment and from laboratory measurements in air-bubbled sea-water at *in situ* temperature. Subsequently, *in situ* O_2 concentrations were calculated based on the measured salinity and temperature (Li & Gregory 1974, Garcia & Gordon 1992). Profiles were continuously measured during at least 24 hours, with two replicate time-series in Svalbard and one in Helgoland. Measurements were done on sand plains with a visible cover of diatoms, as identified by the light red-brown color of the sediment surface. A semi-circular steel grid (10 cm height) was set up approximately 15 cm in front of the sensor to prevent possible entanglement with floating macroalgae.

2.3.8 Ex situ *oxygen microprofile measurements and rate calculations*

Laboratory microsensor measurements were carried out in the Marine Lab in Ny-Ålesund, Svalbard, and at the Biologische Anstalt Helgoland. Sediment samples were randomly collected from close vicinity to the *in situ* microsensor measurements and transported to a climate room, where they were stored for a maximum of 48 h in a flow-through system with natural seawater. During storage, temperature and light conditions mimicked those determined *in situ*, the latter achieved by a programmable illumination-system (GHL Profilux PLUS II). The Helgoland samples were illuminated with a stepless light:dark cycle of 15:9 h with a maximum light intensity of 100 µmol photons m^{-2} s^{-1} (higher maximum light intensities could not be reached due to set-up limitations). In contrast, continuous illumination was applied for the Svalbard samples, with intensities varying between 30 and 130 µmol photons m^{-2} s^{-1} over a 24 h period.

Vertical profiles of oxygen were measured in 3 replicate sediment cores for each site. During the measurements, the samples were kept at *in situ* temperature using a thermostat (Julabo F32) or by using water that was continuously pumped from a water tank in the climate room. Illumination was

provided by a halogen lamp (Schott KL 1500), and the measurements were done at downwelling light intensities increasing from 0 to 600 µmol photons m^{-2} s^{-1}. At each intensity level, oxygen profiles were measured at 3 to 5 randomly selected positions after a delay of 30-60 min to ensure steady state. The Svalbard sediments contained large numbers of bioirrigating infauna, which disturbed the microsensor measurements. To minimize this disturbance, the overlaying water was made anoxic (by purging with N_2) before the measurement, which temporarily stopped animal activity. Alternatively, the upper 5 mm of the sediment were removed, and the rest of the sediment was sieved to remove the larger animals. After reestablishing the sediment layers, the core was let to rest for 12 h in the climate room at *in situ* light and temperature conditions before the measurement. This treatment was not required for the Helgoland samples.

Areal rates of net photosynthesis, P_{net}, and respiration, R_{phot}, in the euphotic layer of the sediment were calculated from the measured oxygen gradients as previously described (Kühl et al. 1996). The molecular diffusion coefficient of oxygen, D_0, corrected for temperature and salinity according to the Unisense gas tables (Li & Gregory 1974, Garcia & Gordon 1992), was used to calculate the O_2 fluxes in the diffusive boundary layer, whereas the effective diffusion coefficient, D_e, calculated as $D_e = D_0\Phi$, where Φ is sediment porosity (Kühl et al. 1996), was used for flux calculations in the sediment. The measured rates of P_{net} were plotted against the incident light intensities, I, and fitted with the function $P_{net} = P_{gross,\,sat}\,[1\text{-exp}\,(-\ln 2 \times I/I_{1/2})] + R$ (Webb et al. 1974). During the fit, the value of the parameter R was forced to be equal to the measured rate R_{phot}. Assuming that the rates of respiration in the dark and in the light were equal, the parameters $P_{gross,sat}$ and $I_{1/2}$ obtained from the fit represent the areal rate of gross photosynthesis at light saturation and the half-saturation light intensity, respectively. This fitting was done separately for *P-I* curves measured in each core, and the fitted values were averaged.

2.3.9 *Sedimentary oxygen consumption rates*

Potential sedimentary oxygen consumption rates (OCR) were measured as previously described (Polerecky et al. 2005, Volkenborn et al. 2010). Freshly

collected rectangular sediment cores (width 70 mm, length 200 mm) equipped with a planar optode (Precht et al. 2004) were set up in a climate room, and the measurements were carried out at *in situ* temperature and in the dark. Oxygenated water was carefully injected next to the optode into the anoxic sediment regions at depths from 3.0 to 8.5 cm below sediment surface, and depletion in pore-water O_2 concentration was monitored in 30 s intervals. This was done simultaneously in 2 to 3 spots in each core. In three of the four cores measured, this was repeated twice, leading to overall 3-6 measurements per core. The average size of the measured spots (as seen by the optode) was 0.33 ± 0.14 cm^2, with one area exceptionally large (0.68 cm^2) and one exceptionally small (0.07 cm^2). The values of OCR, expressed per volume of sediment, were calculated as rates of the pore-water O_2 depletion multiplied by sediment porosity (Polerecky et al. 2005). These measurements were done only for Svalbard sediments because of the evidence of deeper subsurface sediment oxygenation (see Results), and not for the Helgoland sediments.

2.4 RESULTS

2.4.1 *General settings*

The Svalbard and Helgoland sites are generally characterized as cold and temperate, with annual average water temperatures during the studied years of 2.4°C and 10.1°C, respectively. During the *in situ* study period (June 2010 for Svalbard and August 2009 for Helgoland), average water temperatures in Svalbard and Helgoland were 4.8°C and 18.3°C, respectively. Over a 24 h day period, light was present continuously in Svalbard, whereas there were 9 h of darkness in Helgoland (Table 2.2).

2.4.2 *Sediment characteristics*

Sediments at the sites were similar and generally characterized as well sorted, fine, porous, permeable sands (Table 2.2). Permeability did not significantly differ with depth down to 10 cm between the two sites (supplementary Table S2.2). Porosity decreased with depth in Svalbard and was significantly higher than in the Helgoland sediment (supplementary Table S2.2). The most striking

difference was a large abundance of macrofauna in the Svalbard sediments (mainly visible as polychaetes, crustaceans and sedimentary anemones) which were largely absent in Helgoland sediments (Fig. 2.2).

Total carbon and nitrogen contents were about 2-fold higher in Svalbard than in Helgoland. Nitrogen contents were very low. Total organic carbon content was high at both sites, representing about 95% of the total carbon (Table 2.2). Bulk sediment C:N ratios ranged from 76 to 198 in Svalbard and from 63 to 145 in Helgoland, and were not significantly different between the sites (supplementary Table S2.2).

Pore-water nutrient concentrations in the upper 12 cm in Svalbard sediments were generally low (see supplementary Fig. S2.2). Silicate and nitrate concentrations were mostly ≤ 10 µM, whereas ammonium and phosphate usually ranged between 1 and 5 µM. Nitrite concentrations were low, varying between 0.1 and 0.2 µM. Especially in the upper 5-6 cm of the sediment, nitrogen compounds displayed notably low concentrations. Below these depths, only a single profile showed an increase, and overall no significant trends with depth were detected. This suggests high bioventilation activity of infauna (see below). With the exception of silicate, nutrient concentrations in the overlaying water were approximately 10-fold lower than in the top centimeter of the sediment. For silicate the difference was about 4-fold. Thus, pore-water nutrients could not be considered as limiting for microphytobenthos. Due to sample loss during transport, pore-water nutrient profiles are not available for the Helgoland site.

Table 2.2 - Characteristics of the studied sites in Svalbard and Helgoland. Shown are mean ± SD values for N replicate measurements. The carbon, nitrogen, sulfur and pigment data are normalized to sediment dry weight.
[1] data taken from the Helgoland Roads Series (Wiltshire et al. 2008).
[2] downwelling PAR intensities are calculated for the 24 h time frame starting from 14:00 local time. Values after '±' represent measurement uncertainty related to unidentical vertical alignment of the two light loggers used.

	SVALBARD	HELGOLAND	
GENERAL SETTINGS			N
sampling site	Brandal	Düne Süd	
geographical coordinates	N 78°56.816', E 011°51.068'	N 54° 11.594', E 07° 52.802'	
sampling depth [m]	5 ± 0.2	4.5 ± 0.2	
water temperatures [°C], annual	2.4 ± 2.1 (06.2011 - 06.2012)	10.1 ± 5.7 (06.2009 - 06.2010) [1]	26375, 242
water temperatures [°C], during study period	4.8 ± 1.6 (22.6.-12.7.2010)	18.3 ± 0.3 (August 2009) [1]	28080, 20
salinity	31 ± 2	34	
light/dark duration [h]	24/0	15/9	
in-situ PAR [µmol photons m^{-2} s^{-}	22.-23.06 (23.-24.06.2010)	11.-12.08.2009	
min	4 (12)	0	2, 2
max	230 (73)	339	2, 2
average ± SD [2]	49 ± 46 (33 ± 21)	62 ± 78	2, 2
SEDIMENT			
median grain size [µm]	147 ± 3	224 ±12	6, 3
sorting	0.50 ± 0.01	0.49 ± 0.06	6, 3
skewness	0.061 ± 0.004	-0.09 ± 0.02	6, 3
porosity [vol%], 0 - 10 cm	0.44 ± 0.02	0.41 ± 0.02	5, 2
permeability k [10^{-11} m^{-2}]	0.95 ± 0.17	1.37 ± 0.06	2, 2
total carbon (TC) [%wt]	2.56 ± 0.18	1.12 ± 0.30	6, 5
total nitrogen [%wt]	0.019 ± 0.005	0.012 ± 0.003	6, 5
total sulfur [%wt]	0.033 ± 0.008	< LOD (0.001)	6, 5
total inorganic carbon (TIC)	0.10 ± 0.02	0.06 ± 0.02	6, 5
total organic carbon (TOC = TC-TIC) [%wt]	2.46 ± 0.20	1.06 ± 0.32	6, 5
C/N	137 ± 50	95 ± 31	6,5
MPB BIOMASS			
Chlorophyll-a [µg g^{-1} dw]	13.9 ± 5.6	12.9 ± 6.0	5, 6
Fucoxanthine [µg g^{-1} dw]	7.6 ± 2.2	8.7 ± 3.5	5, 6

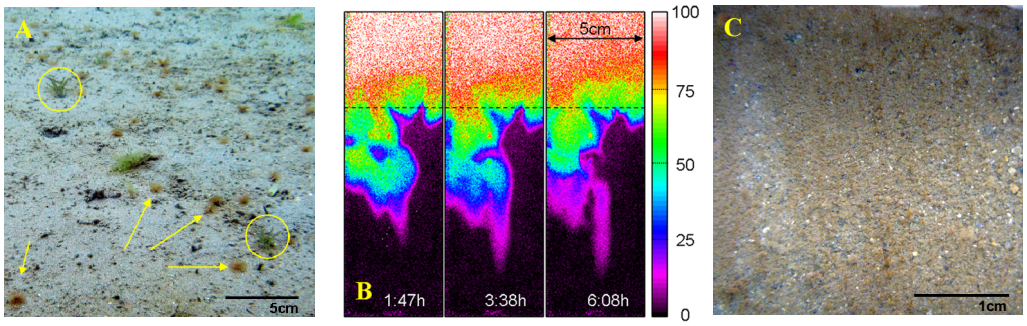

Figure 2.2 - A) Image of the sublittoral Arctic sediment from Svalbard/Brandal (5 m depth, June 2010), showing dense infaunal population. Arrows indicate the visible extended feather-like tentacle crowns of sedimentary polychaetes, circles highlight tentacles of sedimentary anemones. B) Examples of oxygen distributions in the highly bioirrigated Svalbard sediment at specific times during a 24 hour long measurement. Dashed lines indicate approximate locations of the sediment surface, the color bar gives oxygen concentrations in per-cent of air saturation. C) Close up image of the sediment surface in a sediment core from Helgoland/Düne Süd (4.5 m depth, August 2009). (Photographs by D.S.Sevilgen)

2.4.3 *MPB community*

MPB biomass was highly variable within both sites, and there was no significant difference between the Svalbard and Helgoland site with respect to chlorophyll *a* and fucoxanthine concentrations in the top 3 mm of the sediment (supplementary Table S2.2). With respect to the MPB community composition, both sites were dominated by diatoms. The diatom inventory of both sites showed several common species, but also a number of species present at either site exclusively. Interestingly, these species were not specific for polar or temperate habitats (see below). Instead, both sites were dominated by species that are cosmopolitan in marine and brackish waters and commonly found at the sediment-water interface in coastal areas.

Overall 40 diatom species were identified, of which 18 were exclusively found in Svalbard, 11 in Helgoland, and 11 at both sites (see supplementary Table S2.1 and Fig. S2.1). All species belonged to the classes Bacillaryophyceae (39 species) and Coscinodiscophyceae (1 species). They were distributed amongst 19 genera, of which 11 are known to have species commonly found in Polar Regions, including *Amphora, Diploneis* and *Navicula*, which cover 50% of all identified species in the studied sediments. Frequently found species exclusively from the Svalbard sediments were *Plagiotropos lepidoptera, Donkina carinata, Planothidium delicatulum, Amphora sulcata, Pleurosigma normanii* and

Navicula directa. These species have been reported from Polar Regions previously but are also common in marine and brackish waters of the North Sea, the western Baltic and the NW Atlantic. Frequently observed species exclusive for the Helgoland sediments included *Navicula cancellata* and *Petroneis humerosa*, which occur widespread in temperate marine and brackish waters, in coastal areas and on sandy shores but also in Polar Regions. Likewise, the species frequently found at both sites, *Diploneis smithii, Petroneis marina* and *Amphora marina,* are described as ubiquitous and were also identified in polar habitats before.

2.4.4 In situ *light and oxygen measurements*

In Svalbard, ambient light was present 24 h per day (June 2010). Light intensities generally followed a day-night cycle, with higher intensities during the day hours and lower intensities during the night, but sometimes weather conditions (cloud cover) caused intensities during the day to be lower than during the night (Fig. 2.3C). *In situ* oxygen microprofiles showed a small but detectable peak close to the sediment surface (Fig. 2.3A), indicative of photosynthetic activity by MPB in the euphotic zone of the sediment. However, the most striking feature of the profiles were elevated oxygen concentrations at depths of several centimeters below the sediment surface (Fig. 2.3A), demonstrating intensive bioirrigation of the sediment due to the ventilation activity of the present infauna. The average thickness of the oxygenated sediment layer was 2.25 cm over 24 h. However, the complete set of measured profiles (data not shown) suggested that the bioventilation-induced oxygen penetration likely reached down to 6 cm. Depth-integration of the *in situ* O_2 profiles showed that the O_2 inventory in the euphotic zone of the sediment (top 2.5 mm) varied from 0.1 to 0.3 mmol O_2 m^{-2}, whereas the inventory below the euphotic zone was up to 10-fold larger (Fig. 2.3C). The latter inventory values were likely underestimated, since the O_2 concentrations were measured away from infaunal burrows, where they are known to be lower, and they often did not reach zero at deepest points of the profiles. Unexpectedly, the inventory in the euphotic zone did not correlate with light ($p = 0.49$; Fig. 2.3E), suggesting another mechanism to control oxygen dynamics, most likely bioventilation.

Interestingly, the inventory below the euphotic zone was significantly negatively correlated with light (R=-0.43, $p < 0.001$; Fig. 2.3E), suggesting increased infaunal activity at low light, which is similar to results obtained previously (Wenzhöfer & Glud 2004). When integrated over a 24 h period, oxygen inventory below the euphotic zone constituted between 77% (first measuring day) and 92% (second measuring day) of the total sedimentary oxygen inventory, suggesting highly significant impact of the infauna on sediment biogeochemistry at this site.

In Helgoland, *in situ* light intensities followed a 15:9 h day-night cycle (Fig. 2.3D). In the light, oxygen showed a clear peak slightly below the sediment surface and penetrated down to 5 mm (Fig. 2.3B), indicative of photosynthetic activity by MPB in the euphotic zone and respiration below. In contrast, oxygen concentrations in the dark steeply declined with depth due to respiration, penetrating at most 2 mm. During the day, oxygen inventory in the euphotic zone of the sediment was in the range of 0.2-0.6 mmol O_2 m^{-2} and about 2 to 10-fold higher than during the night. As expected, the inventories in and below the euphotic zone were significantly positively correlated with ambient light ($p < 0.0001$; Fig. 2.3F). When integrated over a 24 h interval, oxygen inventory in the euphotic zone constituted about 92% of the total sedimentary oxygen inventory, suggesting dominant role of light for sedimentary oxygen dynamics at this site.

2.4.5 *Laboratory rate measurements*

Because the oxygen profiles measured *in situ* were not in steady state and, due to the highly significant animal activity in Svalbard, not diffusively controlled, the rates of photosynthesis and respiration in the studied sediments were determined by laboratory microsensor measurements in treated cores. Similar to other benthic microbial communities, the net photosynthesis rates increased with incident intensity of PAR and reached saturation at high PAR intensities (Fig. 2.4). Notably high variability between measurements at each light intensity was most likely due to pronounced heterogeneity in surficial chlorophyll *a* concentrations within the samples. Fitting of the *P-I* curves revealed that photosynthetic parameters (net and gross photosynthesis and the rates of

respiration in the euphotic zone) were not significantly different between the two studied sites (Table 2.3, supplementary Table S2.2).

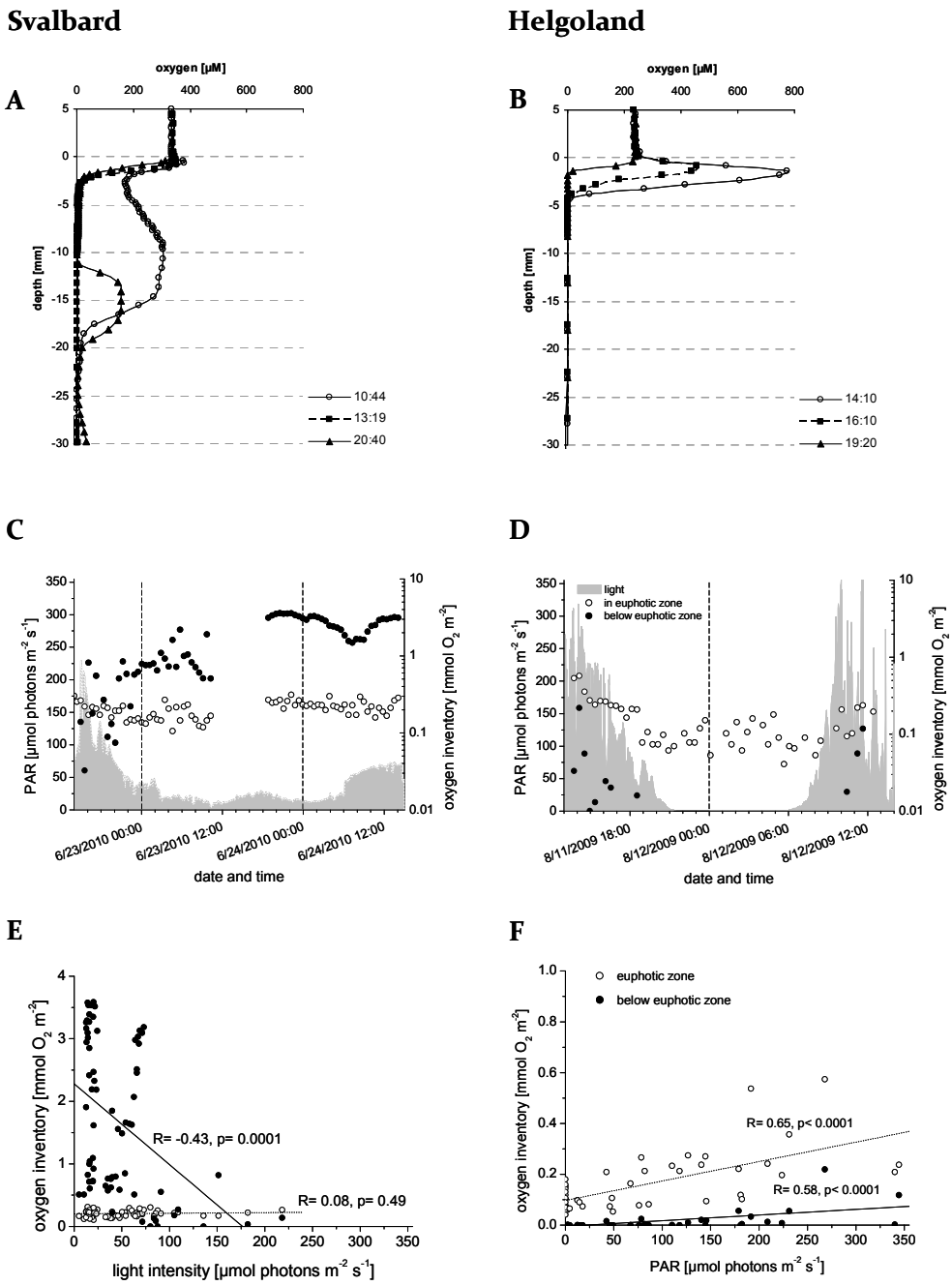

Figure 2.3 - (A-B) Selected *in situ* oxygen profiles in the studied sediments, measured at times indicated in legend. Zero depth corresponds to sediment-water interface. (C-D) *In situ* downwelling PAR intensity, overlaid with the oxygen inventories in and below the euphotic zone, as a function of time. Measurements in panel C and D were done in June 2010 and August 2009, respectively. Vertical dashed lines indicate midnight. (E-F) Correlations between the oxygen inventories and downwelling PAR intensities, derived from the time-series measurements shown in panels C-D.

The average half-saturation PAR intensities, $I_{1/2}$, which characterize the adaptation of the community to light, as well as the average compensation PAR intensities, I_c, i.e., the intensities at which the community photosynthesis equals respiration, were comparatively low and also similar for both sites (Table 2.3). Thus, the MPB from both sites were low-light adapted and showed similar photosynthetic performance.

Volumetric rates of oxygen consumption in subsurface sediments from Svalbard were highly variable within and between the measured sediment cores, and ranged from about 8 to 44 µmol O_2 m^{-3} s^{-1}. No significant trends with depth in the top 8 cm of sediment were observed. The areal respiration rates, obtained by integration of the volumetric rates over the average O_2 penetration depth determined *in situ* (2.25 cm), were about 6-fold higher than the areal respiration rates in the euphotic zone and similar to the areal gross photosynthesis rates at light saturation (Table 2.3). Because oxygen is most likely present also below 3 cm depth, areal respiration rates below the euphotic zone are probably underestimated.

2.4.6 *Estimates of daily oxygen budgets*

Using the values of gross photosynthesis, respiration and light adaptation intensity estimated from laboratory measurements, and the values of available downwelling PAR intensity and depths of sediment oxygenation determined *in situ*, we estimated daily light and oxygen budgets in the studied Svalbard and Helgoland sediments. Because each parameter was estimated with some uncertainty, we combined the values so as to estimate the average as well as the minimum and maximum values for the daily budgets.

For the Svalbard site, the PAR dose during the 24 h period was about 3.5 mol photons m^{-2} d^{-1}. In comparison, the daily PAR dose at the Helgoland site was by about 50% higher, despite the extended period (9 h) of darkness (Table 2.4). The estimated daily gross primary production was very similar for the Svalbard and Helgoland sites (Table 2.4). This is somewhat counterintuitive considering the roughly 50% higher daily PAR dose and about 15% higher rates of gross photosynthesis at light saturation in Helgoland (Table 2.3), and is related to the combined effects of the generally low-light adaptation of the two

communities and of the continuous illumination at Svalbard, which led to an additional 9 h of primary productivity per day.

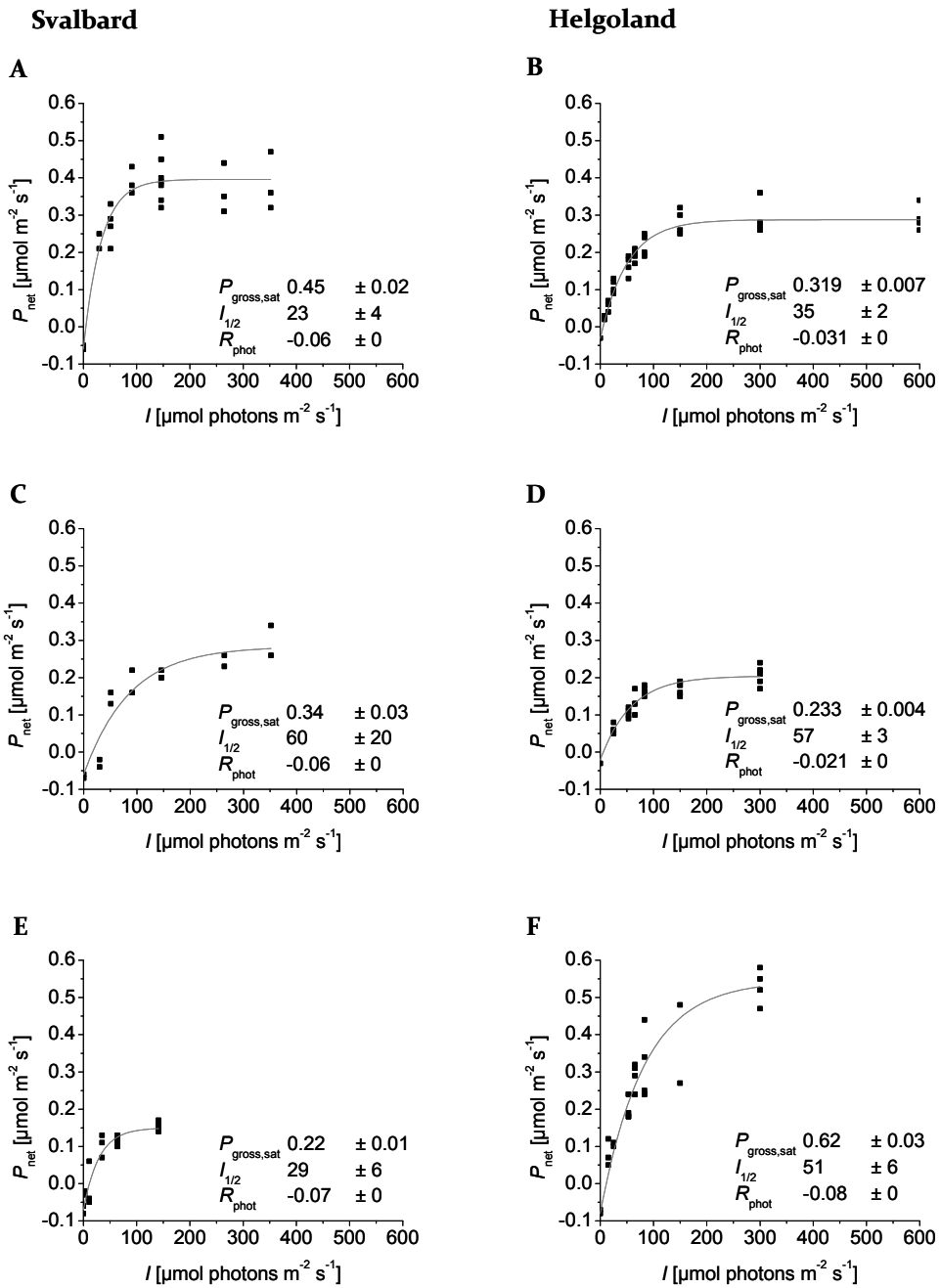

Figure 2.4 - Net photosynthesis rates as a function of downwelling irradiance (P-I curves), as obtained from laboratory microsensor measurements in replicate sediment cores from Svalbard (A, C, E; June 2010) and Helgoland (B, D, F; August 2009). Symbols in each graph show experimental values obtained in random locations within one core, lines show the least-square fit by the model $P_{net} = P_{gross,\,sat}\,[1\text{-}\exp(\text{-}\ln 2 \times I/I_{1/2})]$ + Rphot, with the values (means ± SE) of the corresponding fitting parameters given in the graph.

Table 2.3 - Photosynthesis (*P*) and respiration (*R*) rates derived from laboratory measurements by oxygen microsensors and planar optodes. P_{net} and P_{gross}: net and gross photosynthesis, R_{phot}: respiration in the euphotic zone, $I_{1/2}$: half saturation downwelling PAR irradiance, I_c: compensation irradiance, R_{below_phot}: respiration below the euphotic zone. Shown are averages ± SD as well as minimum and maximum values of N replicate measurements. n.d.: not determined.
***** values calculated for the average depth of oxygen penetration of 2.25 cm, as derived from *in situ* oxygen profiles during a 24 h interval (N = 43 profiles).

	SVALBARD			HELGOLAND			
							N
MICROPROFILES	[µmol O_2 m^{-2} s^{-1}]						
	Average ± SD	*min*	max	*Average ± SD*	*min*	max	
P_{net}	0.28 ± 0.12	0.15	0.39	0.34 ± 0.17	0.2	0.54	3, 3
R_{phot}	-0.062 ± 0.007	-0.055	-0.07	-0.048 ± 0.032	-0.029	-0.085	3, 3
P_{gross}	0.34 ±0.11	0.22	0.45	0.39 ± 0.21	0.23	0.62	3, 3
	[µmol photons m^{-2} s^{-1}]						
	Average ± SD	*min*	max	*Average ± SD*	*min*	max	
$I_{1/2}$	34 ± 22	23	60	43 ± 11	35	55	3, 3
I_c	7 ± 2	5	8	14 ± 9	6	23	3, 3
PLANAR OPTODES = R_{below_phot}	[µmol O_2 m^{-3} s^{-1}]						
	Average ± SD	*min*	max	*Average ± SD*	*min*	max	
R_{below_phot}, volumetric	-16.4 ± 15.8	-8.3	-44.3	n.d.			5
	[µmol O_2 m^{-2} s^{-1}]						
	Average ± SD	*min*	max	*Average ± SD*	*min*	max	
R_{below_phot}, areal*	-0.37 ± 0.36	-0.19	-1	n.d.			3

Considering only the euphotic zone, about 40% of the daily primary production at Svalbard would be remineralized by aerobic respiration. However, due to the much deeper O_2 penetration linked to sediment bioirrigation, the total estimated aerobic remineralization at this site exceeded production at least 2-fold, possibly up to about 5-fold, leading to a grossly negative daily O_2 budget (Table 2.4). In contrast, remineralization by aerobic processes occurred only within the upper 5 mm of sediment at the Helgoland site, and the estimated net daily O_2 budget was positive (about 9.17 mmol O_2 m^{-2} d^{-1}; Table 2.4).

Table 2.4 - Daily light dose and estimated daily oxygen budgets for the two studied sites. Shown are averages ± SD as well as minimum and maximum values of N replicate measurements. The Svalbard budgets were estimated using the average *in situ* oxygen penetration depth of 2.25 cm (part A) and values ranging from 2 to 6 cm (part B).

	SVALBARD			HELGOLAND			
24h light dose	[mol photons m^{-2} d^{-1}]						n
	3.5 ± 1.0			5.3			2, 1

A) ESTIMATED *in situ* OXYGEN BUDGETS

	[mmol O_2 m^{-2} d^{-1}]						
	Average ± SD	*min*	*max*	*Average ± SD*	*min*	*max*	
P_{gross}	14.8 ± 7.0	9.9	22.9	13.4 ± 5.7	8.5	19.7	6, 3
R_{phot}	-5.4 ± 0.6	-4.8	-6.1	-4.2 ± 2.7	-2.5	-7.3	3, 3
R_{below_phot}	-31.8 ± 30.8	-16.2	-86.2	-			3, -
P_{net}	-22.4 ± 31.6	-82.4	1.9	9.2 ± 6.3	1.1	17.2	3, 3

B) ESTIMATED *in situ* OXYGEN BUDGETS for different depths of oxygen penetration

	[mmol O_2 m^{-2} d^{-1}]				
P_{net}	*Average ± SD*	*min*	*max*		
depth [cm]					
2	-18 ± 13	-73	4	-	3, -
4	-47 ± 23	-149	-11	-	3, -
6	-75 ± 34	-226	-25	-	3, -

2.5 DISCUSSION

2.5.1 *Microphytobenthos communities in Svalbard and Helgoland*

During the study period both MPB communities were dominated by diatoms. The observed large overlap (28%) of identified species at both study sites and the dominance of cosmopolites can be linked to the specific oceanographic setting of Kongsfjorden. Distinct Atlantic influence renders Kongsfjorden rather in the sub-Arctic than in the Arctic realm. Correspondingly, endemic species are rare and North Atlantic species are present at all trophic levels of the fjord ecosystem (Hop et al. 2002, Hop et al. 2012). Some of the identified diatom species occur bipolar (e.g. *Navicula directa* and *Actinocyclus actinochilus*). Nonetheless, one should consider that morphological species analyses alone are sometimes not sufficient to decide on species identity (Mann 2010). Molecular biological tools are required to check for the existence of cryptic species, which

are likely to have developed due to the large geographical distance between the sites.

2.5.2 *Microphytobenthos photosynthesis in Svalbard and Helgoland*

Our data shows that the concentrations of chlorophyll *a* and fucoxanthine, the half-saturation and compensation light intensities and the productivity at light saturation are similar for the studied MPB communities from Svalbard and Helgoland. Thus, despite the differences in ambient water temperatures, the two communities have similar photosynthetic potential and performance and thus seem to be adapted to the prevailing local conditions.

With respect to light adaptation, MPB communities from both of our studied sites showed low-light adaptation, similar to results obtained previously for comparable communities. For example, the half-saturation irradiance values, $I_{1/2}$, for the Svalbard community (34 ± 22 µmol photons m^{-2} s^{-1}) include the average value of 19 µmol photons m^{-2} s^{-1} obtained by Glud et al. (2009) for another Arctic MPB community (from Greenland) during summer, while the slightly larger $I_{1/2}$ values determined for the Helgoland community (43 ± 11 µmol photons m^{-2} s^{-1}) are comparable to those found previously for a temperate subtidal site in Brest, France (40-58 µmol photons m^{-2} s^{-1}; Longphuirt et al. 2007). The compensation irradiances were also generally low (7 and 14 µmol photons m^{-2} s^{-1} for Svalbard and Helgoland, respectively), although somewhat larger than those reported previously for an Arctic fjord in Greenland (4.7 µmol photons m^{-2} s^{-1}; Glud et al. 2009) or for diverse coastal MPB communities (2.8 µmol photons m^{-2} s^{-1}; Gattuso et al. 2006).

With respect to MPB photosynthesis, our data are also similar to those available in the literature for comparable communities (Table 2.5). For example, daily benthic primary production estimated for our Svalbard site (10-23 mmol O_2 m^{-2} d^{-1}) lies within the range determined for other sites in Kongsfjorden (2-48 mmol O_2 m^{-2} d^{-1}; Woelfel et al. 2010), although it is smaller than previous estimates for the same site (Brandal, 37 to 47 mmol O_2 m^{-2} d^{-1}; Woelfel et al. 2010). This discrepancy is likely due to differences in light conditions used to estimate the daily O_2 budgets. While we used the naturally variable light intensities measured *in situ* to estimate daily oxygen budgets in this study, our

calculations for budgets of the study of Woelfel et al. (2010) were based on a fixed light intensity they used (100 µmol photons m^{-2} s^{-1}) and thus on a higher daily light dose (8.6 mol photons m^{-2} d^{-1}) than that measured by us (3.5 mol photons m^{-2} d^{-1}, Table 2.4). It is known that when *in situ* conditions are not taken into account, extrapolations can lead to overestimated budgets (Denis et al. 2012). Regarding the temperate site, our estimated daily O_2 budget (9 to 20 mmol O_2 m^{-2} d^{-1}) was similar to but somewhat lower than that which we determined for the study by Hancke and Glud (2004) for a comparable site in Nivå Bay, Denmark (28.8 mmol O_2 m^{-2} d^{-1}) (Table 2.5).

Table 2.5 - Comparison of daily oxygen budgets in sublitoral MPB communities from different arctic and temperate sites.
***** Values estimated based on the data in the respective study. Daily gross production rates from our study are calculated using 24 h *in situ* light intensities, whereas calculations for the other studies are extrapolated from production rates at fixed experimental light intensities (see discussion for details).
[a] integrated sediment O_2 consumption (euphotic & below euphotic zone)
[b] O_2 consumption within the euphotic zone

	depth [m]	Gross O_2 production [mmol O_2 m^{-2} d^{-1}]	O_2 consumption [mmol O_2 m^{-2} d^{-1}]	Date	Method	Study
All sites Kongsfjorden	≤ 5 to 30	2 to 48*	-2 to -35[a]	Summer 2007	planar O_2 optode	Woelfel et al.
Brandal, Kongsfjorden*	5m	~ 38 to 47*	~ -35[a]		sensor spots	2010
Nivå Bay, Denmark	0.4 ± 0.3	28.8*	~ -8 to -9[b]	February 2001	O_2 microsensors	Hancke & Glud
Adventfjord, Svalbard/Arctic	1.3 ± 0.6		~ -8 to -9[b]	May 2000	O_2 microsensors	2004
Brandal, Kongsfjorden	5	10 to 23	-5 to -6[b]	Summer 2010/2011	O_2 microsensors	this study
			-16 to -86[a]		planar O_2 optodes	
Düne Süd, Helgoland	5	9 to 20	-3 to -7[b]		O_2 microsensors	

Although the difference could be due to local conditions, also here it is possible that it is related to the differences in light conditions applied when calculating the budget (i.e., constant incident light intensity of 140 µmol photons m^{-2} s^{-1} resulting in a daily light dose of 12 mol photons m^{-2} d^{-1} vs. variable light intensity measured in situ with a daily light dose of 5.3 mol photons m^{-2} d^{-1} for the study of Hancke and Glud (2004) and our study respectively).

2.5.3 *Benthic respiration in Svalbard and Helgoland*

Our respiration rates, integrated for the whole sediment community of Brandal are in the same range as determined previously for intact sediment cores of the same site, although our maximum estimate (-86 mmol O_2 m^{-2} d^{-1}) exceeds previously determined maximum rates by Woelfel et al. (2010) by a factor of

about 2.5. These differences may be related to heterogeneity of the studied system. Our sediment dark respiration rates of the euphotic zone determined from microsensors measurements are slightly lower but well comparable with rates measured by Hancke & Glud (2004) using the same method (Table 2.5) and show no differences between the arctic and temperate site.

While respiration in the euphotic zone was similar in the Svalbard and Helgoland sediments, total respiration in the Svalbard sediment grossly exceeded respiration in Helgoland sediments due to high infaunal activity. Laudien et al (2007) reported high infaunal biomass in Brandal/Kongsfjorden (2,260 infauna individuals m^{-2} at 5 m water depth), with macrozoobenthos composed of annelids (79%), molluscs (11%) and crustaceans (8%), and the shallow (5 m) soft sediment fauna dominated by suspension-feeding or surface and sub-surface detritovorous polychaetes and deposit-feeding amphipods. It has been suggested that macrozoobenthos communities in the intertidal and shallow subtidal in the Arctic develop seasonally, and that in the shallow subtidal community development (species richness, diversity and biomass) is mainly affected by ice-scouring (Bick & Arlt 2005, Laudien et al. 2007). Therefore the macrozoobenthos community found here will likely not persist throughout all seasons but may be present only during summer months. Owing to irrigation and oxygenation of deeper sediment layers by this fauna, biotic oxygen respiration and the abiotic oxidation of reduced inorganic compounds are stimulated. This results in elevated oxygen consumption, which in our study was roughly 10-fold higher as compared to the Helgoland sediments. Macrozoobenthos as present in the Svalbard sediment was not observed in Helgoland sediments and our measurements indicated no bioirrigation at the studied site. No infauna studies on shallow subtidal sandy sediments around Helgoland are published.

To enable measurements of diffusion controlled microsensor profiles, the infaunal activity in the Svalbard sediments had to be stopped. The purging with N_2 and sieving the sediment to eliminate the fauna may have affected our measurements. Regarding the microphytobenthos, cores were illuminated to enable ongoing photosynthesis during N_2 purging and the upper 5 mm were left intact for the sieved sediments. Regarding the infauna, the exclusion and reduction of infauna activity was not selective and likely included meiofauna

activity as well. We have to consider that microsensor derived respiration rates for Svalbard thus may be underestimated, and the net photosynthesis rates may be overestimated accordingly.

2.5.4 *Drivers of high infauna abundance and respiration in Svalbard sediments*

Total organic carbon content and C:N ratios were higher in the Svalbard than in the Helgoland sediments but not significantly different. The TOC content detected in Svalbard is in line with previous descriptions of marine surface sediments in the Kongsfjorden-Krossfjorden system (Kim et al. 2011). Organic matter in marine sediments can originate from benthic, pelagic and also sea-ice production or from terrestrial input. The high C:N ratios indicate terrestrial organic matter input (e.g., coal), representing refractory carbon. Recently, Kim et al (2011) analyzed single organic matter contributors to sediments of the Kongsfjorden-Krossfjorden system. They documented high coal-derived organic matter content in marine surface sediments in close vicinity to Ny Ålesund, which is a former coal mining site. Ancient organic matter is much less or not degradable compared to fresh organic matter. Thus, it can elevate carbon concentrations, supporting very high C:N ratios, but will not be available as a food source for the infauna.

Spring blooms in Kongsfjorden produce a great amount of bio-available organic matter (e.g., 27-35 g C m^{-2} during the spring bloom from April 18th to May 13th 2002; Hodal et al. 2012), which can be either grazed in the water column or sink to the sea floor where it is grazed or buried. Hodal and colleagues showed that the spring bloom production in April/May was highest in shallow water depths (0-10 m), usually showing maxima at 5 m. The high pelagic organic matter input represents an increased food supply. This, in turn, can support high abundance of infauna, which bioirrigate the sediment through ventilation of their burrows and thus increase extent and depth of penetration of oxygen into the sediment. As the interfacial oxygen uptake increased, sedimentary oxygen consumption rates increase. Additionally, sediment reworking, which is another effect of bioturbating animals, returns reduced compounds from anoxic layers to the oxic part of the sediment which then contribute to oxygen consumption through biotic and abiotic oxidation.

2.6 CONCLUSION

For the studied Arctic (Svalbard, Norway) and temperate (Helgoland, Germany) sites, microphytobenthos communities and their photosynthetic potential showed no site specificity and were similar during the studied summer months. A dense infauna population was observed in the Svalbard sediments that we speculate to be fueled by organic matter input from previous pelagic (spring) blooms. Oxygen budgets of the Svalbard sediments are therefore highly controlled by infauna activity and result in net heterotrophy, whereas the net positive oxygen budget in the Helgoland sediments is primarily governed by the photosynthetic oxygen production and thus controlled by light. Thus, we decline the first hypothesis that oxygen dynamics are controlled by MPB activity at both sites, and confirm the second hypothesis that both MPB communities show similar photosynthesis performance.

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2.9 SUPPLEMENT

2.9.1 *Supplementary tables*

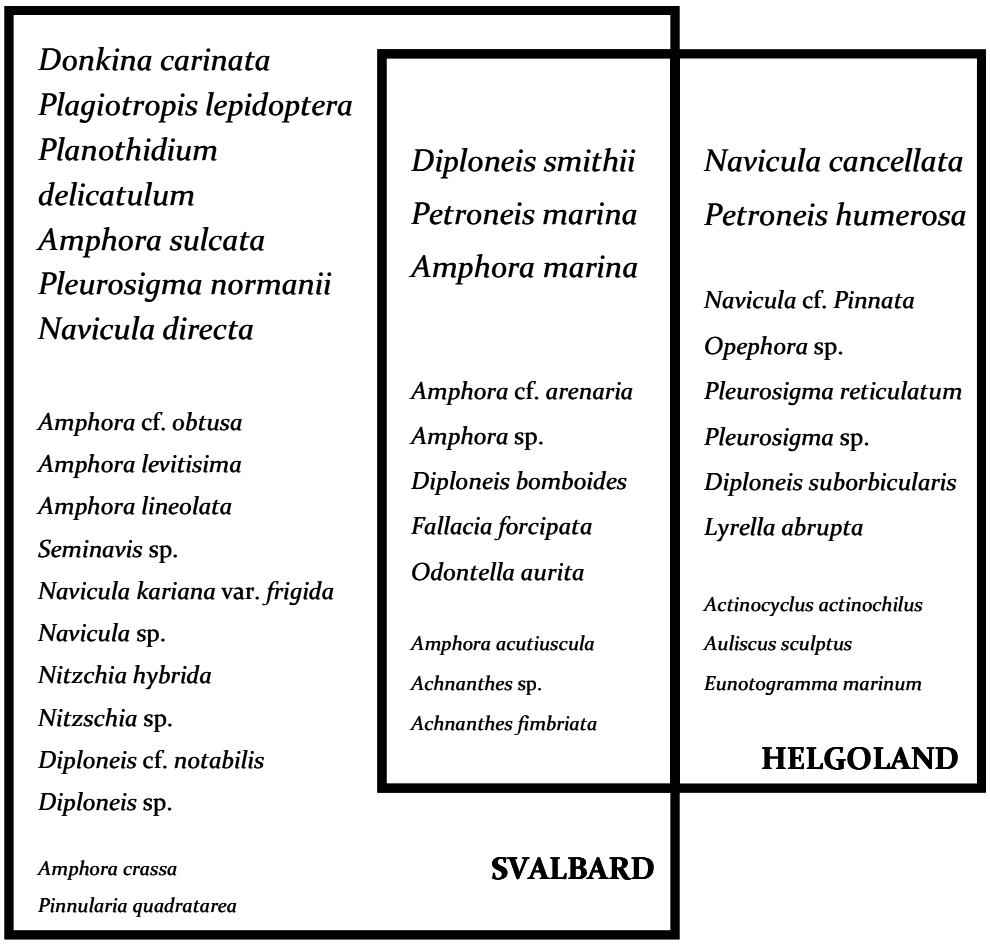
Supplementary Table S2.1: Taxonomy and abundances of the identified diatoms from the studied sites in Helgoland and Svalbard. Abundances are quantified as frequent (+++), rare (++), very rare (+) and absent (-).

CLASS	ORDER	FAMILY	*GENUS*	*SPECIES*	frequency Hel / Sval
Coscinodiscophyceae	Coscinodiscales	Hemidiscaeae	*Actinocyclus*	*actinochilus*	+ / -
Bacillariophyceae	Naviculales	Sellaphoraceae	*Fallacia*	*forcipata*	++ / ++
		Diploneidaceae	*Diploneis*	*smithii*	+++ / +++
				bomboides	++ / ++
				cf. *notabilis*	- / ++
				cf. *sp.*	- / ++
				suborbicularis	++ / -
		Naviculaceae	*Seminavis*	*sp.*	- / ++
			Navicula	*directa*	- / +++
				karianria var. Frigida	- / ++
				sp.	- / ++
				cancellata	+++ / -
				cf. *pinnata*	++ / -
		Pinnulariaceae	*Pinnularia*	*quadratarea*	- / +
		Plagiotropidaceae	*Plagiotropis*	*lepidoptera*	- / +++
		Pleurosigmataceae	*Pleurosigma*	*normanii*	- / ++
				sp.	++ / -
				reticulatum	++ / -
			Donkinia	*carinata*	- / +++
	Achanthales	Achanthidiaceae	*Planothidium*	*delicatulum*	- / +++
		Achnanthaceae	*Achnanthes*	*sp.*	+ / ++
				fimbriata	+ / +
	Thalassiophysales	Catenulaceae	*Amphora*	*marina*	++ / +++
				cf. *arenaria*	++ / ++
				sp.	++ / ++
				acutiuscula	+ / ++
				cf. *obtusa*	- / ++
				levitisima	- / ++
				lineolata	- / ++
				crassa	- / +
				sulcata	- / +++
	Bacillariales	Bacillariaceae	*Nitzschia*	*hybrida*	- / ++
				sp.	- / ++
	Lyrellales	Lyrellaceae	*Petroneis*	*marina*	+++ / ++
				humerosa	+++ / -
			Lyrella	*abrupta*	++ / -
	Centrales	Eupodiscaceae	*Odontella*	*aurita*	++ / ++
	Fragilariales	Fragilariaceae	*Opehora*	*sp.*	++ / -
	Anaulales	Anaulaceae	*Eunotogramma*	*marinum*	+ / -
	Triceratiales	Triceratiaceae	*Auliscus*	*sculptus*	+ / -

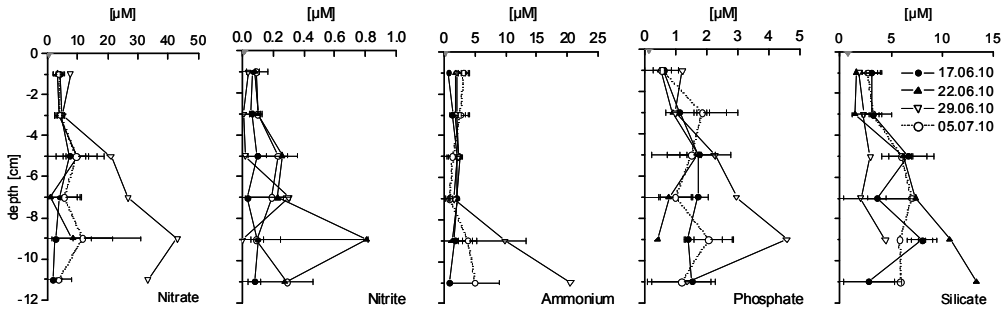
Supplementary Table S2.2: One-way ANOVA results of comparisons between the two studied sites (Helgoland and Svalbard) with respect to various parameters describing the site or its MPB community.

Parameter	df	F-value	p-value
Permeability	1	11,83	0,08
Porosity	4	6,03	0,04
C:N	5	3,42	0,09
chlorophyll *a*	5	0,08	0,78
fucoxanthine	5	0,06	0,81
P_{net}	2	0,29	0,62
P_{gross}	2	0,13	0,73
R_{phot}	2	0,06	0,05

2.9.2 *Supplementary figures*



Supplementary Figure S2.1: Diatom species distribution between the two studied sites in Helgoland and Svalbard. The font sizes from largest to smallest indicate relative abundance, given as frequent, rare, and very rare.



Supplementary Figure S2.2: Vertical profiles of pore-water nutrients in the Svalbard sediment extracted *in situ* with rhizones (open symbols). Filled symbols show nutrient concentrations in the overlaying water. Data represent measurements done on different dates (see legend). When available, error-bars indicate SD of 2 replicate measurements. Comparable data for the Helgoland site is not available due to sample loss during transport.

3. MANUSCRIPT II

RAPID TEMPERATURE INCREASES STIMULATE NET HETEROTROPHY IN SUBTIDAL MICROPHYTOBENTHOS COMMUNITIES SIMILARLY IN A SUB-ARCTIC AND TEMPERATE SITE

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3.1 ABSTRACT

We used microsensors and planar optodes to investigate the effect of short-term temperature increases on photosynthesis and respiration rates of microphytobenthos communities from two subtidal sites with different yearly averages and diurnal temperature fluctuations (Helgoland, Germany vs. Svalbard, Norway).

For both sites, areal rates of net photosynthesis did not vary significantly with temperature, whereas areal rates of dark respiration increased significantly with increasing temperature for temperatures below an optimum. Temperature response of the volumetric rates of net photosynthesis and dark respiration was stronger for the Helgoland community but not significantly different from the Svalbard community. Thus, despite distinctly different local temperature dynamics, the overall temperature response was similar for both studied communities.

Generally temperature response of respiration was stronger than the response of net photosynthesis in the temperate and sub-Arctic sediments, but at close to saturating light intensities net autotrophy was sustained over all experimental temperatures at both sites. However, due to the increasing light demand needed to sustain this net autotrophy *in situ*, in combination with the stronger temperature response of the community respiration in the dark, we conclude that on a short time scale both systems will gradually turn heterotrophic with increasing temperatures. This will become apparent particularly at the Svalbard site due to a significant contribution of macrozoobenthos to total sediment respiration. On longer time scales we expect that this net heterotrophy will likely result in a reduction of the abundance and activity of heterotrophic organisms leading to a gradual return towards net autotrophy.

3.2 INTRODUCTION

Microphytobenthos (MPB) can contribute significantly to shallow-water primary production (Cahoon 2006, Gattuso et al. 2006). In MPB communities, irradiance and temperature are the main factors controlling photosynthesis, respiration and thus the overall productivity (Grant 1986, Davison 1991).

Temperature can have direct and indirect effects on primary productivity. Direct effects are due to the temperature dependence of biological processes, such as the metabolic activity of the organisms involved. For example, the activity of enzymes involved in photosynthetic carbon assimilation is temperature dependent, which will be mirrored in the photosynthetic activity, growth rate or pigment content of phototrophic cells (Davison 1991, Jodlowska 2013). Indirect effects concern the physico-chemical processes involved in mass-transfer within the sediment and across the sediment water interface (SWI). For example, solute diffusivity increases with increasing temperature, while solute solubility and the thickness of the diffusive boundary layer (DBL) above the sediment surface, both of which influence solute diffusion across the SWI, decrease with increasing temperature (Jorgensen & Revsbech 1985, Jorgensen & Marais 1990, Wieland & Kühl 2006).

Temperature response of a community is typically described with the Q_{10} value, a factor by which the rate of a process changes under a temperature increase of 10°C (Falkowski 2007). Short-term temperature responses of photosynthesis under light-saturating conditions typically exhibit an increase with increasing temperatures to an optimum with a $Q_{10} \approx 2$ (Raven 1988, Davison 1991), after which they decline again. This response can differ depending on the temperature acclimation of the phototrophic community (Davison 1991), which involves a variety of physiological responses to short-term temperature fluctuations. It may include changes in the cellular activity of enzymes associated with photosynthesis such as the CO_2 fixing enzyme Rubisco, associated with the Calvin cycle, or changes of the photosynthetic electron transport rate (Raven 1988, Davison 1991). Related to these acclimations, temperature responses may vary amongst communities grown at different temperatures, and it has been shown for macroalgae that specimen grown at lower temperatures exhibit reduced sensitivity to temperature changes, thus lower Q_{10} values, than those grown at higher temperatures (Davison 1991).

Respiration rates exhibit similar responses to temperature increases as photosynthesis rates, increasing to an optimum followed by a decline. Typically Q_{10} values of 2-3 (Thamdrup & Fleischer 1998, Thamdrup et al. 1998, Hancke & Glud 2004) are reported for sediment micro-communities, although values sometimes can be higher (Hancke & Glud 2004).

In MPB communities, photosynthesis and heterotrophic processes occur in close association with each other (Kühl et al. 1996, Wieland & Kühl 2000a). Photosynthesis results in the production of oxygen and organic compounds, of which both are utilizable by heterotrophs. An increase in photosynthates increases the food supply for heterotrophic organisms and therefore their activity, being represented by their respiration. This, in return, will increase the supply of inorganic compounds utilizable by the phototrophs. Simultaneously, increased heterotrophic activity demands higher amounts of oxygen, which has to be served from photosynthetic oxygen production or from the overlaying water. Thus the response of photosynthesis to changes in environmental parameters such as light and temperature will likely affect heterotrophic processes and vice versa (Wieland 2000a).

Wieland and Kühl (2000b) conducted a comprehensive microsensor study of short-term temperature effects on oxygen and sulfide cycling in hypersaline cyanobacterial microbial mats. They showed that temperature affected both reaction rates and depth distribution of processes involved in oxygen and sulfide cycling. At increasing temperatures, photosynthesis and respiration increased differently, overall driving the system to a net heterotrophy. Hancke and Glud (2004) presented a similar study of short-term temperature effects but in diatom dominated MPB communities from three subtidal sites. They also showed that higher temperatures stimulated heterotrophic activity more than photosynthesis, and suggested that an increase in temperature will gradually lead to net heterotrophic systems. Additionally, they showed that there were no differences in the temperature responses between the investigated temperate and high Arctic sites. A similar conclusion was reached in a study that compared temperature dependence of oxygen respiration in Arctic and temperate coastal sediments (Thamdrup & Fleischer 1998). In 2011, Alsterberg et al. reported on the autotrophic and heterotrophic temperature response in subtidal sediments from Sweden. They showed that heterotrophic variables like bacterial production, meiofauna biomass and dark fluxes of oxygen clearly responded stronger to warming than autotrophic variables such as oxygen production, biomass and species composition of benthic microalgae, but could not demonstrate a complete shift to a heterotrophic state upon a temperature increase of 4°C in their experiment.

Besides differences in yearly temperature averages, MPB communities from different habitats and biogeographic zones may experience highly different short-term temperature dynamics (Davison 1991).For example, besides seasonal temperature variations, dial temperature dynamics were observed and mentioned previously (Hancke & Glud 2004). These dynamics should be considered in temperature response studies but have so far not received much attention.

The available data on temperature responses of oxygen production and consumption in subtidal MPB communities is limited to the above mentioned publications. Given that this data is crucial to assess the effect of future temperature changes within benthic systems, this is a considerable gap of knowledge.

Pronounced short-term temperature differences are found at the sites presented in this study. The temperate site in the North Sea (Helgoland) and the sub-Arctic site in the Kongsfjorden (Svalbard) differ in two aspects: annual temperature maxima differ by 10°C and the average yearly temperature in Svalbard is ~8°C lower than in Helgoland (this study). Additionally, the temperature fluctuations are distinctly stronger in Svalbard: summer temperatures at the Svalbard site fluctuate greatly (> 3°C) within hours and minutes (this study) whereas in Helgoland the temperature fluctuates < 1°C per day (Wiltshire et al. 2008).

This study aimed to evaluate the effects of short-term temperature variations on photosynthesis and respiration in MPB communities from a temperate (Helgoland) and sub-Arctic (Svalbard) site, and assess whether the responses differ in relation to the long-term averages and short-term temperature dynamics experienced by the communities *in situ*. In line with previous studies, we hypothesized that the activities are significantly affected by short-term temperature variations, and that the effect of temperature on respiration is larger than that on photosynthesis. Additionally, due to the fact that the Svalbard community is exposed to rapid and comparably large *in situ* temperature fluctuations, we expected that its activity will be less sensitive to rapid temperature increases than that of the Helgoland community. To test these hypotheses, we used oxygen microsensors and planar optodes to measure photosynthesis and respiration rates in freshly collected sediment cores after

acclimation of about 60 minutes at temperatures that were varied in the range similar to that experienced by the communities *in situ*.

3.3 MATERIALS AND METHODS

3.3.1 *Study sites and sampling*

Sediment samples were collected from the shallow subtidal temperate site "Düne Süd" (North Sea, Helgoland) and the shallow subtidal Arctic site "Brandal" (Kongsfjorden, Svalbard) (Table 3.1), hereafter referred to as Helgoland and Svalbard. Both sites are oceanographically closely interlinked by the same water masses of the North Atlantic Current, which influence the fjord systems and their ecology along the western coastline of Svalbard (Svendsen et al. 2002, Hop et al. 2012), rendering the Kongsfjorden rather in the sub-Arctic than Arctic realm.

All sediment samples were collected by SCUBA divers from an area of 50–100 cm in size, using opaque PVC cores (length 200 mm, inner diameter 48 mm). After retrieval the sediment cores were put in a cooling box filled with seawater from the sampling site. Samples from Kongsfjorden were transported within one hour to the Marine laboratory in Ny-Ålesund. Samples from Helgoland were transported to the field-laboratory within one hour and kept at *in situ* temperatures in seawater flow-through basins until transport with cooling boxes to the mainland laboratory in Bremen (Germany) within maximum 4 hours. Until the measurements, all sediment cores were kept in aquaria in climate rooms at corresponding *in situ* temperature and light conditions of the respective sites (Table 3.1).

3.3.2 *Temperature measurements*

Temperature at the sampling site in Svalbard was recorded every 20 minutes throughout one year from June 2010 to July 2011 in 5 m depth using submersible TidbiT temperature loggers (ONSET, Massachusetts, USA). In addition, high-resolution temperature dynamics during the sampling period (June/July 2011) were measured in 2-minute intervals in Svalbard and hourly running averages were derived. Daily temperatures over one year and hourly temperature

recordings throughout the study period for Helgoland were obtained from the Helgoland Roads data series (Wiltshire et al. 2008).

Table 3.1 - Basic description of the temperate (Helgoland) and sub-Arctic (Svalbard) study sites, as well as of the conditions during the short-term temperature increase experiments and sediment pigment content. Shown are averages ± SD of n replicate measurements.
[a] Average *in situ* downwelling irradiance at the sediment surface during light hours from a previous study (11.-12.08.2009 and 22.-23.6-2010 for Helgoland and Svalbard respectively) (Sevilgen et al. 2013)
[b] Half saturation downwelling irradiance as determined from laboratory measurements (Sevilgen et al. 2013)
* Data is taken from the Helgoland Roads series (Wiltshire et al. 2008)

	Helgoland Düne Süd (North Sea, Germany)	Svalbard Brandal (Kongsfjorden, Arctic)	n
SITE CHARACTERISTICS			
geographical coordinates	N 54° 11.594, E 07° 52.802	N 78°56.816', E 011°51.068'	
sampling time	March/April 2012	June/July 2011	
sampling depth	2.4 ± 0.2	5 ± 0.2	2, 3
temperature during sampling [°C]	4.5 ± 2.1	5.0 ± 1.6	2, 3
salinity [PSU]	32-34	29-34	
porosity [Φ]	0.51 ± 0.08	0.65 ± 0.07	5, 5
irradiance [µmol photons m^{-2} s^{-1}] [a]	103 ± 115	49 ± 46	2, 2
$I_{1/2}$ [µmol photons m^{-2} s^{-1}] [b]	43 ± 11	34 ± 22	3, 3
annual temperature range [°C]	2.7 - 18.0 *	-10.7	
EXPERIMENTAL CONDITIONS			
experimental temperatures [°C]			
microsensor measurements	4, 8, 12, 16, 20, 24	0, 3, 6, 9, 12, 15	
climate room	4	4	
light intensity [µmol photons m^{-2} s^{-1}]	85	62	
PIGMENTS			
photopigment biomass [µg g^{-1}] ± SD			
chlorophyll *a*			
light-setup	13.2 ± 5.5	13.1 ± 3.4	6, 4
dark-setup	12.5 ± 7.2	11.7 ± 4.7	6, 4
fucoxanthin			
light-setup	8.3 ± 3.6	12.7 ± 13.9	6, 4
dark-setup	7.7 ± 3.7	6.0 ± 1.9	6, 4
chlorophyll *a* + fucoxanthin			
light-setup	21.6 ± 9.0	25.8 ± 17.1	6, 4
dark-setup	20.2 ± 10.8	17.7 ± 6.5	6, 4
fucoxanthin: chlorophyll *a* ratio	0.7 ± 0.2	0.5 ± 0.1	12, 7

3.3.3 *Experimental protocol*

Setup - For temperature manipulations, the sediment cores were submersed in a glass beaker filled with natural seawater. The beaker was placed in a cooling bath with a temperature control unit (Thermo Haake DC10 and Haake D8, USA). To document temperature changes, a submersible temperature logger (tidbit v2) was put into each beaker that recorded temperature dynamics throughout the measuring time in one minute intervals. Two of these manipulation systems were set up in parallel: one in the dark for respiration

measurements and one in the light to measure photosynthetic oxygen production (hereafter named dark- and light-setup, respectively). To assure light-saturating conditions, downwelling irradiance in the light-setup was chosen according to *in situ* values determined previously for the two studied sites (Sevilgen et al. 2013), namely 85 and 62 µmol photons m^{-2} s^{-1} for the Helgoland and Svalbard samples, respectively (Table 3.1). Illumination was provided by a fiber optic halogen lamp (SCHOTT KL-1500 electronics, Germany), and the downwelling irradiance of the photosynthetically active radiation (PAR) was measured with a light meter (LiCor LI-250 Light Meter, USA). Laminar flow (~1 cm s^{-1}) above the sediment surface was maintained by an air-jet from a bent Pasteur pipette connected to an air pump. Gentle bubbling of the water with an aquarium air pump maintained atmospheric aeration in the seawater throughout measurements.

In the Svalbard sediments, a dense infaunal population complicated the microsensor measurements on MPB and thus had to be removed. This was done by first removing approximately the upper 5 mm of the sediment and then sieving the next 5 cm of the sediment through a 1 mm mesh. After reassembling the sediment layers back in the original order, the cores were allowed to rest for at least 24 h in a climate room under mimicked *in situ* light and temperature conditions before the measurements. Cores from Helgoland were left untreated because no macrozoobenthos infauna was present.

Microsensor measurements - Oxygen microprofiles were measured using Clark-type oxygen microsensors with a guard cathode (Revsbech 1989b), which had a tip diameter of 50-100 µm, response time < 1 s, and stirring sensitivity of < 1.5%. A two-point sensor calibration was done separately for each experimental temperature, based on signals in the aerated overlying seawater (100% air saturation) and in the anoxic parts of the sediment (0% oxygen). The microsensor was mounted on a motorized micromanipulator (VT-80 with a Faulhaber motor) connected to a computer, which allowed automatic measurements. Initial positioning of the microsensor tip at the sediment surface was aided by a magnifying glass. In each replicate core and at each experimental temperature (Table 3.1) three to six steady state microprofiles were measured randomly in different spots. The first set of profiles was measured at the lowest

experimental temperature, and each subsequent set of profiles was measured after a new steady state at the higher temperature was reached, which took about an hour. The microsensor tip was kept within the production zone (at a depth showing net photosynthesis production in light) during the temperature transition to follow the temperature response and to enable quantitative assessment that the steady state has been reached. Steady state of the system was confirmed by an invariant signal of the sensor and by measuring and comparing repetitive microprofiles within the same location. For each microsensor profile the oxygen penetration depth (O_2-pd), the depth of the net production zone (light profiles only) were determined and averaged.

3.3.4 *Rate calculations*

Generally, profile analyses and rate calculations were done as described previously (supplementary Fig. S3.1; Kühl et al. 1996, Hancke & Glud 2004). Despite previously determined bulk sediment porosity (Table 3.1) (Sevilgen et al. 2013), for the calculation of the oxygen exchange rates measured in the present study, the porosity of the top sediment layer was estimated from the ratio of the oxygen concentration gradient in the water directly above and below the SWI of biologically inactivated sediments (Glud et al. 1995, Kühl et al. 1996, Revsbech 1989a). The technique is based on Fick's first law of diffusion (equation 1, supplementary Fig. S3.1) and the assumption that if a steady state diffusive flux (J) is a continuous function of depth, and no production or consumption is taking place in the sediment, a change in the concentration gradient across the sediment-water interface must be due to different diffusion coefficients in the overlying water (D_o, corrected for temperature and salinity (Li & Gregory 1974, Garcia & Gordon 1992)) and in the sediment (D_e). Thus, the ratio between the two diffusion coefficients was estimated as $D_o/D_e = [\delta C(z)/\delta z]_w/[\delta C(z)/\delta z]_s$, where subscripts w and s refer to the gradients measured in the overlying water and sediment, respectively.

Areal rates – Areal rates of net photosynthesis (P_{net}) integrate the gross oxygen production in the production zone and the oxygen consumption of the entire oxic zone of the sediment in the light. The net photosynthesis production zone

was defined as the zone within the oxygenated sediment having net O_2 production in light with the lower boundary of the production zone being defines by the inflection point of the O_2 profile, i.e. where the downward oxygen gradient was maximal (supplementary Fig. S3.1a). This is the depth where gross photosynthesis equals light respiration and below which respiration exceeds photosynthesis. P_{net} was estimated as the diffusive O_2 flux into the overlaying water (J_{up}) from microsensor profiles measured in the light (equation 2, supplementary Fig. S3.1a). Areal dark respiration, R_{dark}, corresponded to the flux of O_2 into the sediment (J_{down}) calculated from the oxygen gradient at the sediment surface (equation 3, supplementary Fig. S3.1b). Since the diffusive boundary layer at the sediment water interface was not always clearly discernible, the effective diffusion coefficient, D_e, was used for all flux calculations.

Volumetric rates – In addition to areal rates, volumetric rates were calculated. Volumetric net photosynthesis rates of the production zone ($P_{net, vol}$) integrate the gross production and oxygen consumption in the light in the production zone only. They reveal solely the biological response as the sum of the activity of phototrophic and heterotrophic organisms in the production zone. $P_{net, vol}$ were calculated by dividing the total flux of oxygen out of the production zone ($|J_{up}+J_{down}|$) by the thickness of the production zone (equation 4, supplementary Fig. S3.1a). Volumetric rates of dark sediment respiration integrated for the oxic sediment zone ($R_{dark, vol}$) were calculated by dividing the areal rates by the oxygen penetration depth (O_2-pd) (equation 5, supplementary Fig. S3.1b).

Since the thickness of the production zone generally exceeded the oxygen penetration depth of the dark profiles (see supplementary Fig. S3.2), a trustworthy differentiation into volumetric respiration of the production zone and below the production zone in the dark profiles was not possible. Therefore a further differentiation into respiration of the autotrophic vs. the heterotrophic micro-community was not done.

3.3.5 *Sub-surface oxygen consumption in Svalbard sediments*

High numbers of infaunal organisms frequently oxygenate deeper sediment regions down to several cm depth in the Svalbard sediment (Sevilgen et al. 2013). Thus in addition to the MPB community response in the upper mm of the sediment, the potential respiration rates of sub surface sediment regions ($R_{deep, vol}$) in the Svalbard sediments were also measured. This was done using planar optodes as previously described (Polerecky et al. 2005, Volkenborn et al. 2010). Sediment cores were set up in a climate room and sedimentary oxygen consumption rates were measured by artificially injecting aerated water into anoxic sediment regions and following the subsequent decline of oxygen over time. The experimental setup was described in detail previously (Sevilgen et al. 2013). After the measuring set of oxygen consumption rates at one temperature, the temperature of the climate room was increased to the next temperature step and left for acclimatization for at least 12-24 h before a next measuring set was started.

3.3.6 *Quantification of activation energy E_a and the Q_{10} factor*

Temperature dependence of a reaction is quantifiable using the activation energy (E_a) which is derived from Arrhenius plots, based on the Arrhenius equation, formulated as

$\ln k = -E_a/RT + \ln A$.

It describes the temperature dependence between the rate of a reaction (k; in our case the photosynthesis and respiration rates) and the absolute temperature T (K = t [°C] + 273.15), with R being the universal gas constant (8.3144 J K^{-1} mol^{-1}) and A the Arrhenius constant. When ln (k) is plotted against $1/T$, $-E_aR$ equates to the slope of the linear fit of data points in the linear part of the Arrhenius graph. From this, E_a (kJ mol^{-1}) were derived and expressed as the average $E_a \pm$ SE. The E_a were then used to calculate Q_{10} values, to compare these with literature data, as

$Q_{10} = \exp\,(10E_a\,(RT(T+10))^{-1})$.

For all data, Q_{10} (commonly expressed for a temperature increase from 0 to 10°C) were calculated from measurements at 2 and 12°C (T and T + 10°C), as 2°C is the

lowest common experimental temperature both MPB communities experience naturally. Stronger temperature dependences are reflected in higher E_a and Q_{10}.

3.3.7 *Statistical analysis*

The effect of temperature on the rates of photosynthesis and respiration (ln-transformed) were tested statistically using the software JMP 9.0 (SAS Institute Inc., North Carolina, USA). First, the temperature effect on rates measured within and between replicate cores from each site was tested (ANCOVA model 1, supplementary Table S3.1). For both sites, the variation between replicate cores within one site was found to be significant ($p < 0.05$). However, to determine the effect of temperature in the between-site comparison this effect had to be minimized. Therefore, in a second step we analyzed the residuals (RES_lnRATE) of the within-site ANCOVAs instead of the original RATE data, using the ANCOVA model 2 (supplementary Table S3.1). The residuals obtained represent the variance that is left after the effect of cores has been removed (Grafen & Hails 2002). Analyses of variance (ANOVA) of the E_a values derived from Arrhenius plots were used to test for significant differences in the temperature response between sites.

3.3.8 *Photopigment analyses*

After microsensor measurements, the top 5 mm of the sediment cores were sampled to determine chlorophyll *a* and fucoxanthin content as a measure of microphytobenthos biomass. The core surface area was slightly frozen with liquid nitrogen vapor (modified after Wiltshire et al. 1997), pushed out of the core, and divided into three equally sized sub-samples which were then entirely frozen in liquid nitrogen before all samples were freeze-dried (Christ Alpha 1-4 Loc-1m) for 48 hours in the dark.

Throughout the preparation for subsequent pigment analyses with high performance liquid chromatography (HPLC), the freeze-dried sediments were kept on ice and treated in the dark or under dimmed light. Samples of approximately 1 g were used for extraction with 99.8% cooled acetone. They were left for 3 min in an ice-cooled ultrasound bath, vortexed and stored for 24 h at -28°C in the dark. For the analyses, the extract was vortexed, filtered

(Acro disc CR 4 mm syringe filters with 0.45 µm PTFE Membrane, PALL Life Sciences) and measured using HPLC as described by Wright et al. (1991). Pigments were identified and quantified by comparing with pigment standards (DHI, Denmark).

3.4 RESULTS

3.4.1 In situ *temperature dynamics*

In the Svalbard site, *in situ* temperatures varied annually between -1.9°C and 8.8°C (Fig. 3.1a), representing a yearly average of 2.5°C and a dynamic range of about 11°C. A large part of this variation occurred on short time scales, with temperatures changing by 4°C within a few minutes to hours, and by 5°C during several days (Fig. 3.1c). During the study period temperature varied from about 3°C at the beginning of June 2011 to 7°C at the end of July 2011, with similar short-term fluctuations. In Helgoland, the annual dynamic range was higher (16°C), with temperatures varying between 2.7 and 18°C (Fig. 3.1a). Maximal daily fluctuations were below 1°C (Fig. 3.1b), and during the study period (March to April 2012) *in situ* temperature increased from 4°C to 6°C. Thus, annually, the Svalbard site is on average by about 8°C colder, has a smaller dynamic temperature range (by about 5°C), and experiences distinctly larger short-term temperature fluctuations than the Helgoland site.

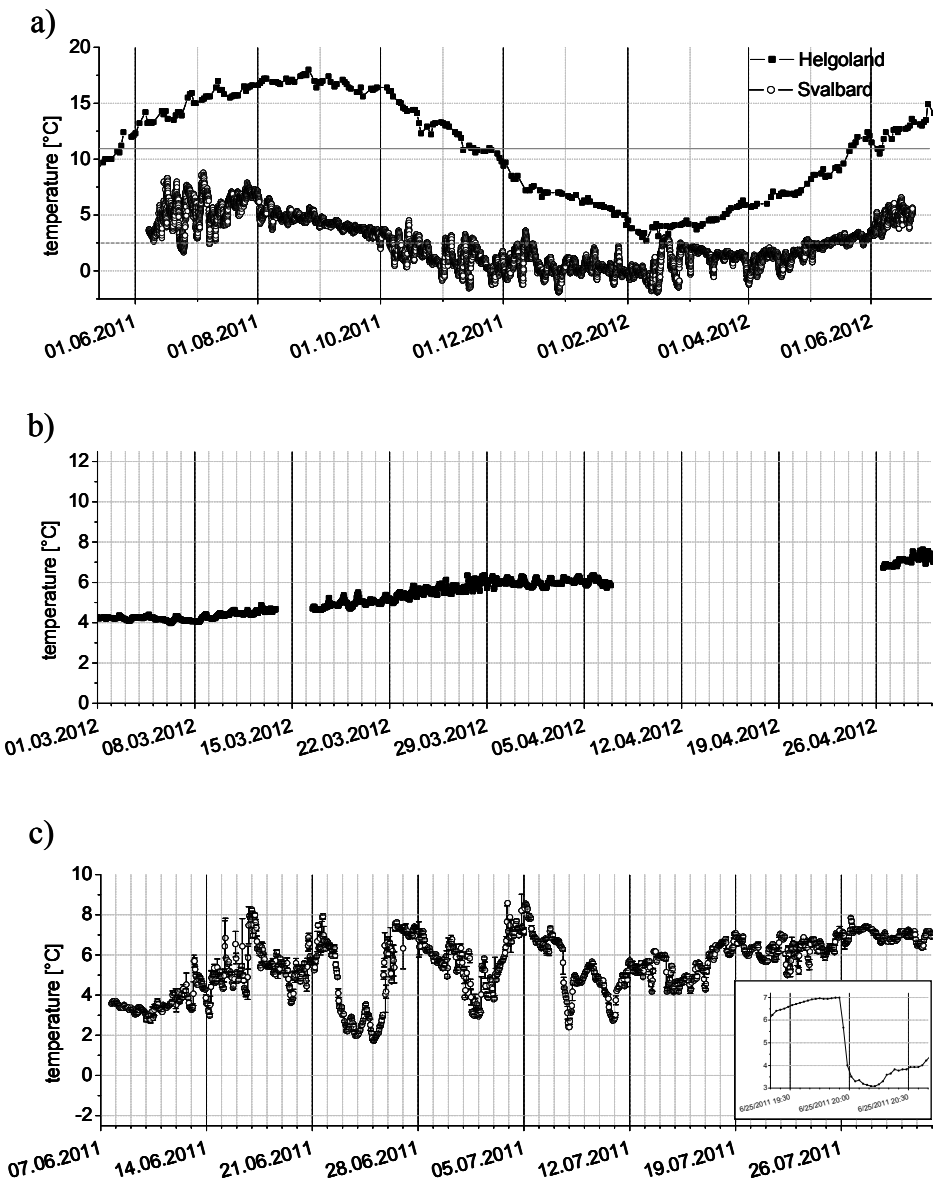


Figure 3.1 - Temperature dynamics measured at the temperate site (Helgoland ■) and the sub-Arctic site Brandal (Svalbard ○) in approximately 5 m water depth. Shown are a) temperature measured in intervals of 24 hrs (Helgoland) and 20 min (Svalbard) throughout one year (vertical lines indicate months, horizontal grey lines (— & - - -) the annual mean temperature for Helgoland and Svalbard respectively), b) one hour resolution temperatures during the study period in Helgoland (March/April 2012) and c) hourly averages from 20-minute resolution temperature dynamics during the study period in Svalbard (June/July 2011) (grids in b & c are one-day intervals). The box in c) displays and example of the short-term temperature change within a few minutes in Svalbard (vertical lines indicate 30 minutes). Temperature data for Helgoland is taken from the Helgoland Roads series (Wiltshire et al. 2008).

3.4.2 *Oxygen penetration depth (O_2-pd) and depth of the production zone*

The general trends of oxygen penetration depth (O_2-pd) and depth of the production zone are summarized in Fig. 3.2 and supplementary Fig. S3.2. A decrease in O_2-pd with temperature was not very pronounced in Svalbard sediments and showed high variability between cores, both in the light and in the dark. In contrast, O_2-pd in Helgoland sediments clearly decreased with increasing temperature, specifically in the light profiles (Fig. 3.2a). This suggests that both light-enhanced respiration and dark respiration are affected more strongly by temperature in Helgoland than in Svalbard sediments. Generally O_2-pd in Helgoland sediments was slightly larger in the light and smaller in the dark than in Svalbard sediments suggesting an overall stronger biological temperature response of the temperate community.

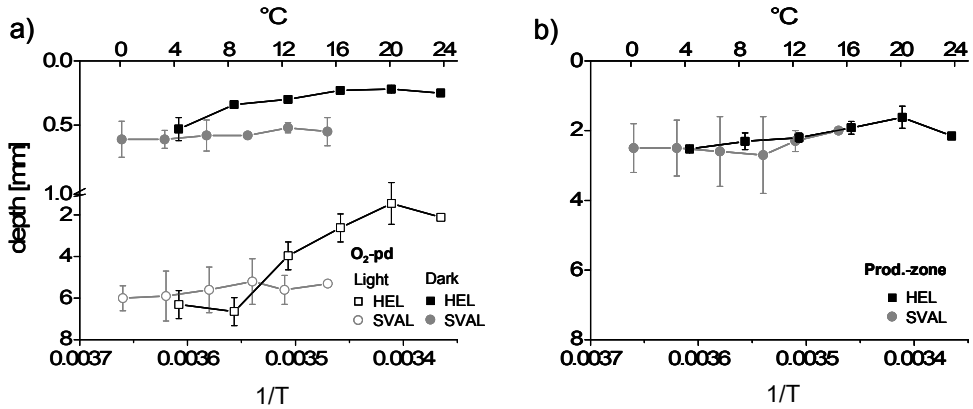


Figure 3.2 - a) Averaged depth of the net oxygen production zone (Prod.-zone, ■ HEL & ● SVAL) and b) oxygen penetration depth (O_2-pd) in the light (open symbols) and in the dark (filled symbols) in sediments as derived from oxygen microprofiles measured in the light (85 and 90 µmol photons m^{-2} s^{-1} for HEL and SVAL) and in the dark. Shown are averages ± SE of n replicate cores measured at various temperatures (HEL: n = 6 and SVAL: n = 2-4 per temperature) with a minimum of three measurements that were averaged for each core and temperature.

3.4.3 *Thickness of the production zone*

In Svalbard sediments, the production zone was highly variable within and between replicate cores, and remained on average at about 2.5 mm at all experimental temperatures (Fig. 3.2b). In contrast, in Helgoland sediments it varied less, and a clear decrease from about 2.5 to 1.5 mm could be detected in the temperature range from 4°C to 20°C. This indicated that in Svalbard

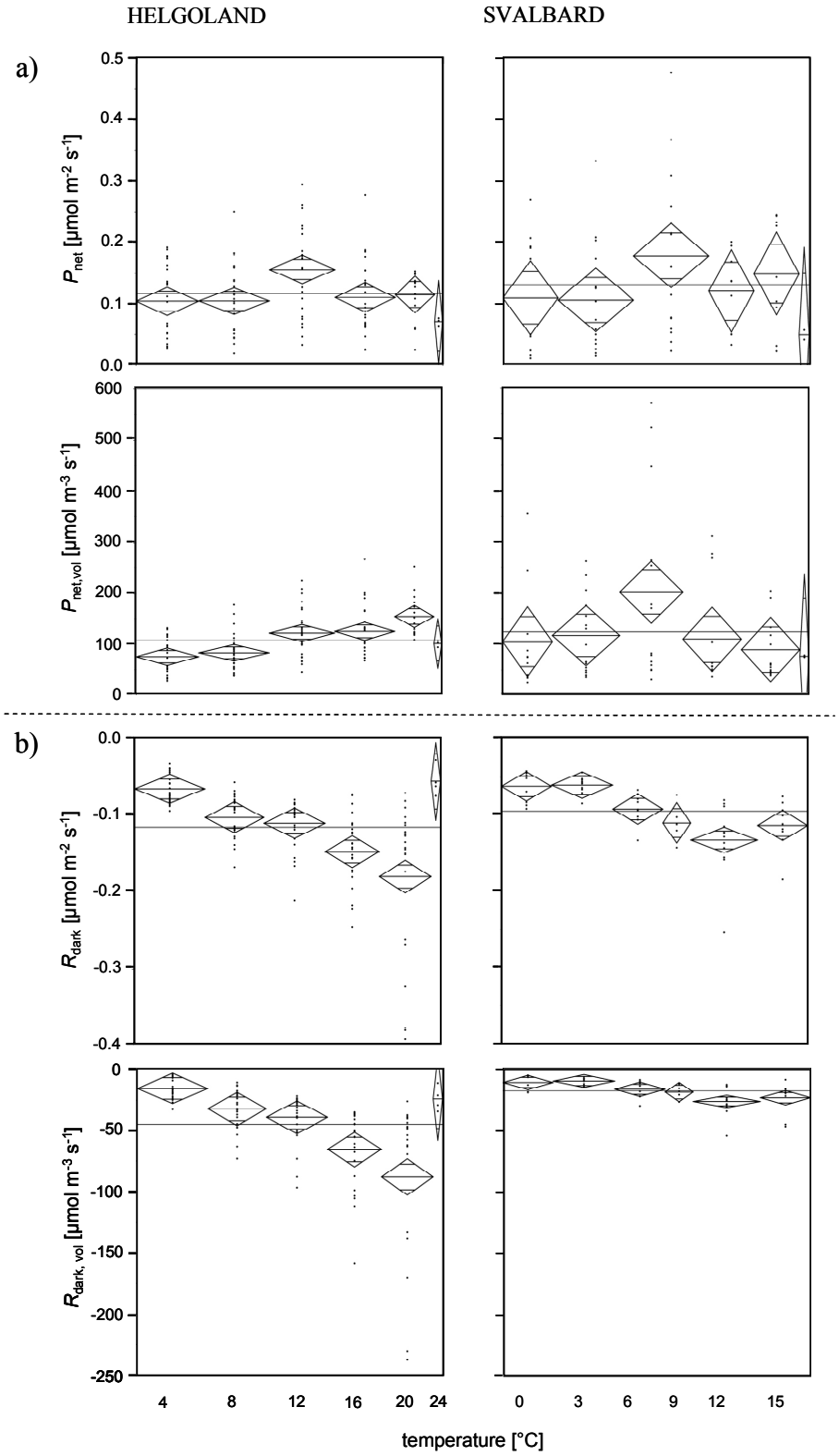
sediments both areal photosynthesis and respiration changed with temperature in the same way, whereas in Helgoland sediments the response to temperature was stronger for respiration than for photosynthesis.

3.4.4 *Photosynthesis and respiration rates*

Rates of net photosynthesis (P_{net}) and respiration (R_{dark}) were derived from steady state O_2 microsensor profiles measured at light-saturating conditions and in the dark, respectively. Generally, at a given temperature the rates were highly variable within and between the replicate cores, which was true for both Helgoland and Svalbard samples. This complicated our aim to see significant effects of temperature on net photosynthesis and respiration.

Areal rates of P_{net} were similar in Svalbard and Helgoland sediments (Fig. 3.3a, upper panels). They were hardly affected by temperature, except for a marked increase at an intermediate temperature (12°C for Helgoland, 6°C for Svalbard), which was significant for the Helgoland ($p = 0.03$) but not for the Svalbard sediments ($p = 0.29$). Additionally, the rates appeared to decrease at the highest experimental temperature, but due to the high variability this decrease was not significant for any of the sites ($p > 0.36$). Overall, in the temperature range from 4-20°C in Helgoland and 0-12°C in Svalbard, the areal rates of P_{net} had no significant trend with temperature, implying a Q_{10} of 1. As the rate response over the applied temperature range did not show a typical metabolic temperature response as expected for biological systems, Q_{10} were not calculated for the areal rates of P_{net}.

→ **Figure 3.3** - Oxygen fluxes at the sediment water interface representing a) areal net photosynthesis oxygen production and volumetric rates of net photosynthesis oxygen production in the light (P_{net}, $P_{net, vol}$; upper four panels) and b) respiration in the dark (R_{dark}, $R_{dark, vol}$; lower four panels), as derived from microsensor measurements in surface sediments from Helgoland (left side) and Svalbard (right side). Shown are single measurements (•) and mean-diamonds from one-way ANOVA. Horizontal lines in the middle of the diamonds represent the group means, the vertical span represents the 95% confidence interval for means of each temperature group and the horizontal extend of each group is proportional to the sample size. The horizontal line over all temperatures represents the overall mean of the whole temperature series.



In contrast to areal rates, volumetric rates of photosynthesis $P_{net, vol}$ showed clearer trends in the Helgoland sediments with temperature (Fig. 3.3a, lower panels). The rates in the temperate sediments continuously increased in the temperature range from 4°C to 20°C, with the corresponding Q_{10} value of

1.6 ± 0.03 (Fig. 3.4a). At the highest experimental temperature, the rates significantly decreased. For Svalbard samples, the trend with temperature was not significant (p = 0.0592) (Fig. 3.4a). For a comparison, Q_{10} values were derived, resulting in a number closer to 1 (1.2 ± 0.05). Nonetheless statistical analysis showed that when sites were tested against each other for temperature responses, the interaction term was nearly, but overall not significant (p = 0.0511) (Table 3.2).

Areal rates of dark respiration, R_{dark} were higher in Svalbard than in Helgoland and increased significantly with increasing temperature for both sites (Table 3.2), reaching a maximum at 20°C and 12°C in Helgoland and Svalbard sediments, respectively (Fig. 3.3b, upper panels). After this maximum the rates dropped, but the decrease was significant only for the Helgoland samples (p < 0.0005). Q_{10} values were similar for both sites and around 1.9 (Table 3.2).

Similar trends were detected for the volumetric rates of R_{dark}, which clearly increased in the temperature range of 4 to 20°C and 0 to 12°C for the Helgoland and Svalbard samples, respectively, before decreasing at the highest experimental temperature (Fig. 3.3b, lower panels). The corresponding Q_{10} values for these temperature ranges (Fig. 3.4b) were slightly higher in Helgoland (2.8 ± 0.07) than in Svalbard (2.3 ± 0.11), but the difference was not significant (Table 3.2). Thus the heterotrophic temperature response from the temperate and sub-Arctic MPB communities was essentially the same.

Table 3.2 - Activation energies (E_a, kJ mol^{-1}) and $Q_{10(2\text{-}12°C)}$ values of areal and volumetric rates of net photosynthesis and respiration from microphytobenthos communities in sandy sediments of Helgoland and Svalbard. Shown are mean values ± SE derived from Arrhenius plots of rates from n replicate measurements of net photosynthesis ($P_{net, vol}$) and dark respiration (R_{dark} & $R_{dark, vol}$). Additionally, E_a and Q_{10} values were determined for potential deep sub-surface sediment respiration ($R_{deep, vol}$) with planar optodes in Svalbard sediments. p-values indicate the significance level of differences found by ANOVA.

		$P_{net, vol}$	R_{dark}	$R_{dark, vol}$	$R_{deep, vol}$
HELGOLAND	E_a	30.3 ± 2.1	37.8 ± 1.8	70.4 ± 2.9	n.d.
	Q_{10}	1.6 ± 0.03	1.8 ± 0.03	2.8 ± 0.07	n.d.
	n	5	6	2	-
	p-value	<.0001	<.0001	<.0001	-
SVALBARD	E_a	14.7 ± 4.4	45.2 ± 3.7	55.3 ± 5.4	103.7 ± 12.3
	Q_{10}	1.2 ± 0.05	2.0 ± 0.06	2.3 ± 0.11	4.8 ± 0.5
	n	3	6	2	3
	p-value	0.06	<.0001	<.0001	< .0001
HEL vs. SVAL	p-value	0.05	0.32	0.2	-

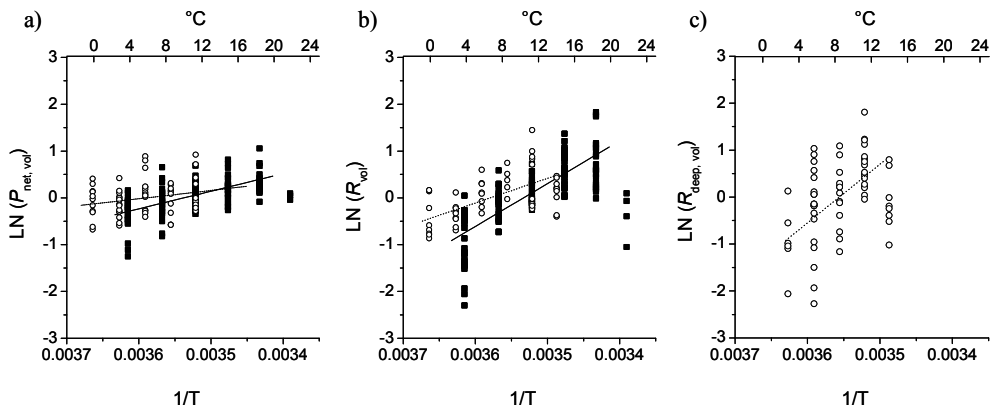


Figure 3.4 - Arrhenius plots of the volumetric rates used to derive E_a and Q_{10} of a) net photosynthesis ($P_{net, vol}$) and b) dark respiration (R_{vol}) in surface sediments of Helgoland (■) and Svalbard (○) and c) dark respiration in deeper sub surface sediments of Svalbard ($R_{deep, vol}$) as a function of temperature. Shown are linear regressions of the residuals of the ln-transformed rates (see Material and Methods) calculated from oxygen microsensor measurements (a & b) and planar optodes (c).

3.4.5 *Pigment biomass*

Chlorophyll *a* and fucoxanthin concentrations in the top 5 mm of the sediments were determined subsequently to the temperature experiments in the same cores that were measured with microsensors. Due to high variation between the sampled cores within both study sites (Supplementary Fig. S3.3), no significant differences in pigment concentrations were detected between the light- and dark-incubated cores from each site ($p > 0.37$, Table 3.1), or between the cores from the two studied sites ($p > 0.44$, Table 3.1). Also chlorophyll *a* to fucoxanthin ratios were similar at both sites (about 0.6; Table 3.1). Since this ratio for diatom monocultures ranges between 0.6-0.8 (Jeffrey et al. 1997), whereas communities dominated by organisms that lack fucoxanthin but predominantly contain chlorophyll a such as green algae and cyanobacteria have similar raios (Lucas & Holligan 1999), this indicated that the studied MPB communities were to a similar extent dominated by diatoms.

3.4.6 *Oxygen consumption in deeper sediment layers in Svalbard*

Due to a significantly increased oxygen penetration depth associated with the bioirrigating activity of infauna in Svalbard sediments, total oxygen uptake rates in these sediments are considerably larger than those determined for the

surficial sediment layer by microsensors (Sevilgen et al. 2013). The contribution of these deeper sediment horizons, and especially its temperature response, was quantified using planar oxygen optodes (Fig. 3.5). Volumetric rates of oxygen consumption, $R_{deep, vol}$, measured in sediment depths between 1.2 and 9.1 cm, were generally independent of depth. Similarly to the respiration rates at the sediment surface, $R_{deep, vol}$ increased in the temperature range from 3°C to 12°C and dropped at the highest experimental temperature (Fig. 3.4c). The corresponding Q_{10} value was about 4.8, i.e., significantly larger than the Q_{10} values for the surficial sediments (Table 3.2). Thus, the temperature response of the deeper sediments in Svalbard, which is a combination of responses of the microbial and chemical oxygen-consuming processes, is about 2-fold stronger than the response of the near-surface sediments.

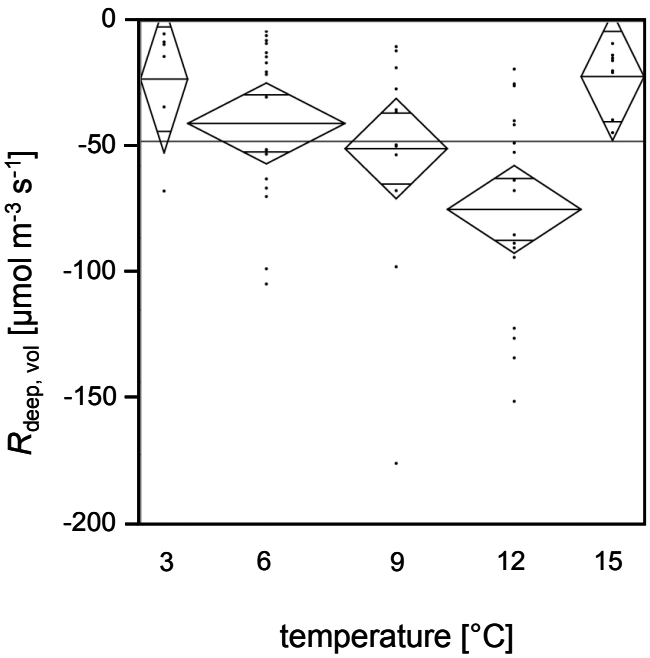


Figure 3.5 - Volumetric dark respiration rates measured in deeper sub-surface sediments in Svalbard ($R_{deep, vol}$) by planar optodes. Shown are single measurements (•) and mean-diamonds from one-way ANOVA. Horizontal lines in the middle of the diamonds represent the group means, the vertical span represents the 95% confidence interval for means of each temperature group and the horizontal extend of each group is proportional to the sample size. The horizontal line over all temperatures represents the overall mean of the whole temperature series.

3.5 DISCUSSION

3.5.1 *Net photosynthesis*

The light-saturated rate of photosynthesis is mainly controlled by the carbon metabolism and therefore strongly affected by temperature (Wieland & Kühl 2006, Falkowski & Raven 2007). If an overall increase in gross photosynthesis production was accompanied by an equal increase in light-respiration, no change would have been visible in the areal net photosynthesis rates. Indeed, areal net photosynthesis rates (P_{net}) did not increase with increasing temperature, and the sediments from the studied temperate (Helgoland) and sub-Arctic (Svalbard) site showed no difference in their temperature response. Generally the temperature effect in areal rates is biased by mass transfer effects and the decrease of oxygen penetration depth. Therefore the physiological temperature effects were derived from volumetric rates.

Volumetric rates integrate the gross photosynthesis production and light-respiration within the production zone only. Whereas temperature significantly affected volumetric net photosynthesis rates in Helgoland, this was not the case for Svalbard sediments. This was mirrored in Q_{10} values that were higher in Helgoland than in Svalbard sediments (1.6 vs. 1.2). At the same time oxygen consumption below the production zone in the light seemed to have increased stronger in the temperate than in the sub-Arctic site, as the thickness of the production zone decreased with increasing temperature. As the data showed high variability and the averages were similar, the sites overall did not differ in their temperature response of net photosynthesis ($p = 0.0511$). However, it should be pointed out that although the sites did not differ significantly, the p-value was close to the lower boundary of the significance level (0.05) and there is strong evidence that temperature affects MPB net photosynthesis differently depending on the site. A power analysis showed that increasing the sample size only marginally by $n = 2$ would have been enough to drop the value below the significance level.

Our data from the temperate site are comparable to observations from previous studies and Q_{10} values for $P_{net, vol}$ in Helgoland are within the range of what has been described for temperate MPB before (Table 3.3). Related to the small temperature response of the sub-Arctic MPB community, the Q_{10} for

$P_{net,\ vol}$ from the presented Svalbard sediments are smaller than values determined for MPB from another Arctic fjord (Table 3.3).

Table 3.3 - Comparison of Q_{10} values (± SE) for photosynthesis and respiration of microphytobenthos communities from different studies. Temperature intervals used to determine Q_{10} were 2-12°C for this study and 0-10°C for the other studies.

Study	Site	Climate	$P_{net, vol}$	R_{vol}
			Q_{10}- values	
Hancke & Glud, 2004	Niva Bay, Denmark	temperate	1.5	5.2
	Trondheimsfjord, Norway	temperate	2.1	2.6
	Adventfjord, Svalbard	arctic	1.6	3.2
Thamdrup & Fleischer, 1998	Weser Estuary sediments	temperate	-	3.3
	Svalbard sediments	arctic	-	1.8
this study	Helgoland, Germany	temperate	1.6 ± 0.03	2.8 ± 0.07
	Kongsfjord, Svalbard	arctic	1.2 ± 0.05	2.3 ± 0.11

3.5.2 *Respiration*

Temperature had a significant effect on areal sediment dark respiration (R_{dark}) in both sites. Overall, areal respiration rates were higher in Svalbard than in Helgoland (Fig. 3.3) but revealed a similar temperature response at both sites (Table 3.2). The oxygen penetration depth in the dark decreased significantly with increasing temperature in Helgoland and almost stayed the same in Svalbard (Fig. 3.2). This indicates that specifically in Helgoland the increased demand for oxygen could not be balanced and seemed to be limited by mass transfer processes. Volumetric respiration rates, solely representing the biological activity, were higher in Helgoland than in Svalbard although the temperature response between the two sites was statistically not different. In both sites $R_{dark,\ vol}$ were significantly influenced by temperature, being represented in Q_{10} values that were 1.8 and 1.9 times higher for Helgoland and Svalbard as compared to their Q_{10} of $P_{net,\ vol}$.

Although indications of a stronger temperature response of the temperate than the sub-Arctic MPB were given (as represented by the Q_{10} of volumetric rates), the large heterogeneity of our results suggest that the sample size was too small to derive distinct statistical differences.

In order to evaluate the additional impact of the dense macrofauna on respiration rates in Svalbard sediments, potential oxygen consumption rates were measured with planar optodes ($R_{deep, vol}$). Derived Q_{10} were distinctly higher than those of volumetric respiration rates measured with microsensors (Q_{10} values 4.8 ± 0.5 vs. 2.3 ± 0.11). We presume that this elevated Q_{10} value is mediated by meio- and macrozoobenthos organisms. Besides enhanced microbial respiration due to availability of oxygen by bioirrigation, we captured infaunal respiration in this experiment. Macrozoobenthos respiration typically displays larger Q_{10} values than micro-communities in sediments (Thamdrup & Fleischer 1998, Hancke & Glud 2004, Peck et al. 2002, Doyle et al. 2011). We speculate that this leads to the distinctly elevated values. Additionally, as the infauna was separated from the sediment cores prior to measurements, this response was not captured by the microsensor approach.

3.5.3 *Phototrophy vs. heterotrophy*

Ecosystems are predicted to shift towards heterotrophy with an increase in temperature owing to different metabolic demands of photosynthesis and respiration (Yvon-Durocher et al. 2010). Stronger temperature response of respiration compared to that of photosynthesis has also been shown previously both in sediment-associated algae, plankton communities and microbial mats (Davis & MacIntyre 1983, Grant 1986, Lefèvre et al. 1994, Robinson 2000, Hancke & Glud 2004, Alsterberg et al. 2011). Besides the increase of dark respiration with increasing temperatures, also light respiration will add to increased oxygen demands as there is an increased supply in photosynthates during light conditions.

Our findings confirm the main trend, i.e., respiration showed a stronger response to short-term temperature increases than net photosynthesis, thus a gradual shift towards heterotrophy. This is especially the case for Svalbard sediments where the phototrophic/heterotrophic equilibrium is strongly controlled by the activity of a dense macro-infauna that greatly increases total sediment respiration and strongly reacts to rapid temperature changes. Sub-surface sediment respiration in Svalbard was shown to be 6 times higher than respiration within the euphotic zone (Sevilgen et al. 2013). The Q_{10} of sub-

surface sediments as determined in this study was on average 4.8, thus > 2-fold than the Q_{10} of MPB respiration. Inferring from this, the heterotrophic oxygen consumption of the sub-Arctic benthic community will be governed by > 90% by the sub-surface sediment community upon rapid temperature increases. This example gives indications of the importance to study and incorporate single sub-community responses to to derive comprehensive conclusions and predict holistic ecosystem responses better.

3.5.4 *Short-term temperature responses and long-term temperature changes*

The present study shows that the studied MPB communities respond rapidly to temperature changes. The profiles from which the rates are derived represent a steady state that reflects a combination of the physiological temperature response of the community as well as mass transfer processes, which also depend on temperature. Whereas upon temperature increase the equilibration of the system into a steady state took some time (about 60 min), the MPB communities responded within minutes. Thus, when temperature has an effect on photosynthesis or respiration rates, changes will evoke an immediate physiological response. This is important to consider with regard to two aspects:

Firstly, in combination with high *in situ* temperature fluctuations as seen in the present study it means that estimates for primary productivity can not only be derived based on average temperatures but that extrapolations need to incorporate the local short term variability. This becomes especially evident when taking into account that the local short-term temperature variability is highly dynamic. Depending on the geographic settings, rapid temperature changes *in situ* may change seasonally and be more pronounced in some months than in others as seen in the case of Kongsfjorden (see Fig.1a). This can be speculated to be related to currents or e.g. glacier melts that influence the temperature variability and also other abiotic factors such as e.g. salinity and turbidity. Thus, as physiological responses react rapidly to such changes, it means the productivity is short term specific and likely varies greatly over a day, week or month.

Secondly, the yearly global temperature increases represent only a fraction of the *in situ* short-term variations. As the communities adapt and react

instantly to short term temperature variations, we can speculate that related to temperature in the context of global climate change the communities gradually adapt to the changing conditions and will not change in their temperature sensitivity.

3.5.5 *Comparing with the past*

Putting our findings in relation to similar studies such as the one of Hancke and Glud (2004) who studied the Adventfjord on Svalbard as an Arctic site and compared it to two temperate sites in Norway and Denmark, offers the unique opportunity to directly compare and look at possible changes in temperature responses of MPB communities during the last decade. Although due to a large variability in our data a comparison of our findings with their study needs to be done with caution, it reveals that Q_{10} values of net photosynthesis are slightly lower for the Arctic sites (1.6 and 1.2 for $P_{net,\ vol}$ from their and our study respectively) and Q_{10} values for the temperate site in our study are the same as what has been described for one of the temperate sites in their study (Table 3.3). The same accounts for the respiration responses. Values for the Arctic site determined in our study lie between the values determined in the study of Hancke and Glud (2004) and in another study by Thamdrup & Fleischer (1998) (Table 3.3) suggesting that temperature responses can be variable and may be related to prevailing local conditions.

It is not possible to state if there has been a shift in the MPB community composition but it is likely that over time and under steady temperature increases the dominances of species that are adapted to different optimum temperatures within a community change. The Q_{10} values for the temperature response of photosynthesis and dark respiration between our study and the previous study (Hancke & Glud 2004) however lead to the assumption that overall there was no change in the temperature response between the communities studied in this study and a decade ago.

Temperature is only one important factor that comes along with multiple other variables related to global environmental change. Despite studies on individual species and functional groups throughout many trophic levels (Hillebrand et al.

2010, Hicks et al. 2011) many species and community responses to climate change are still uncertain (Drinkwater et al. 2010). MPB communities usually represent an assembly of various species from different functional groups (diatoms, cyanobacteria, dinoflagellates) that commonly exhibit specific temperature optima for growth and photosynthesis performance (Davison 1991). Previous findings have shown that changing environmental conditions such as temperature increases alter benthic diatom communities (Cibic et al. 2012). The authors showed that certain diatoms, in this case of the genera *Nitschia* and *Navicula*, of which both have species in the Svalbard MPB community (Sevilgen et al. 2013) showed a positive stepped trend in their abundance with increasing temperature whereas others (*Pleurosigma*) showed negative trends. Putting our findings in the context of global temperature increases, we suggest that related to the rather small annual temperature increases that usually lie within the seasonal or even daily temperature fluctuations, the MPB community compositions are likely to alter on a long term (decades). As the two study sites are closely interlinked by the same water masses, this could result in a gradual adjustment of the Svalbard MPB community towards the community found in Helgoland. At the same time, if short-term fluctuations persist, the temperature responses will likely remain similar within and between the study sites throughout the coming decade.

3.6 ACKNOWLEDGEMENTS

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3.8 SUPPLEMENT

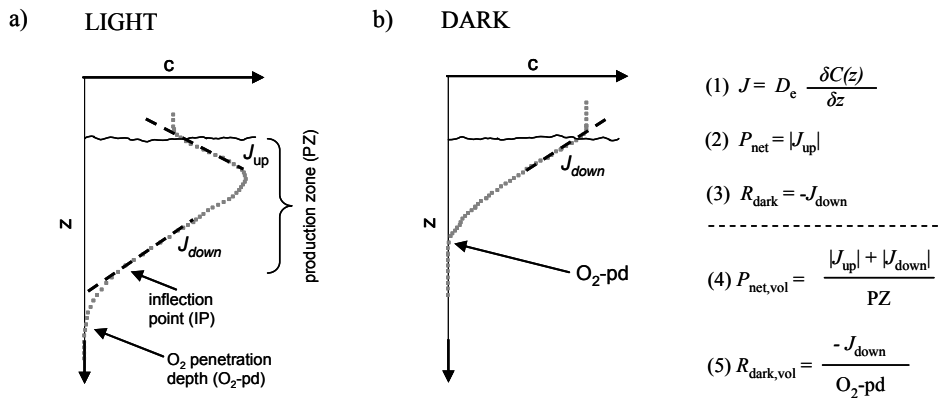
3.8.1 *Supplementary tables*

Supplementary Table S3.1 - Analyses of covariance (ANCOVA) models used to test the within site (replicate cores, CORE) and between site differences of the effect of temperature (1/T) on photosynthesis and respiration rates (lnRATE, RES_lnRate) under short term temperature increases in the two study sites (SITE) Helgoland and Svalbard. See Methods section for further description.

Model	variable		factors
1	ln(RATE)	vs.	CORE & 1/T & CORE × 1/T
2	RES_lnRate	vs.	SITE & 1/T & SITE × 1/T

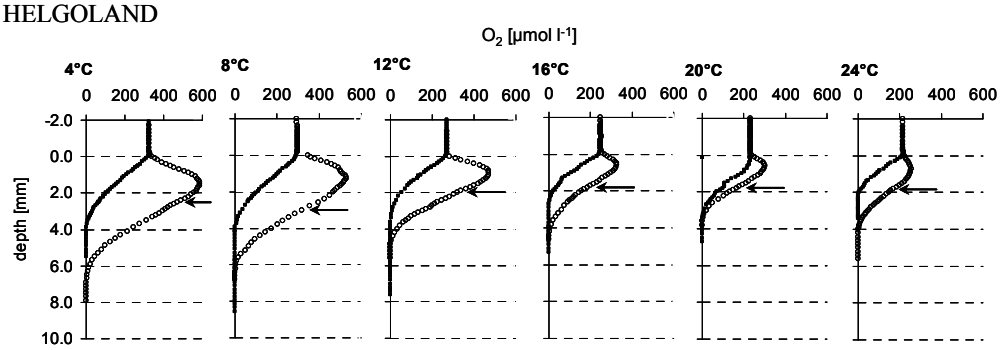
where			
	ln(RATE)	=	ln of photosynthesis or respiration rates
	RES_lnRate	=	residuals of the ln of photosynthesis or respiration rates
	CORE	=	replicate core per within a study site
	1/T	=	temperature with T = t + 273.15°C
	SITE	=	study site (Helgoland and Svalbard)

3.8.2 *Supplementary figures*

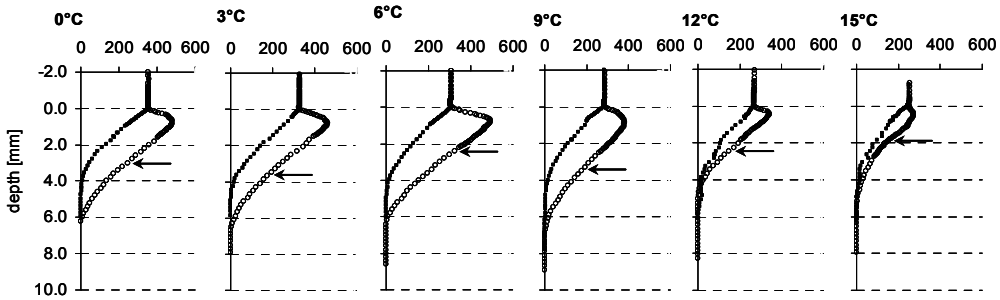


Supplementary Figure S3.1 - Schematic overview of diffusive oxygen microprofiles measured under steady state conditions in the light (a) and in the dark (b) with the corresponding equations for the calculations of areal (equation 2 & 3) and volumetric (equation 4 & 5) net photosynthesis and dark sediment respiration rates based on Fick's first law of diffusion (equation 1).

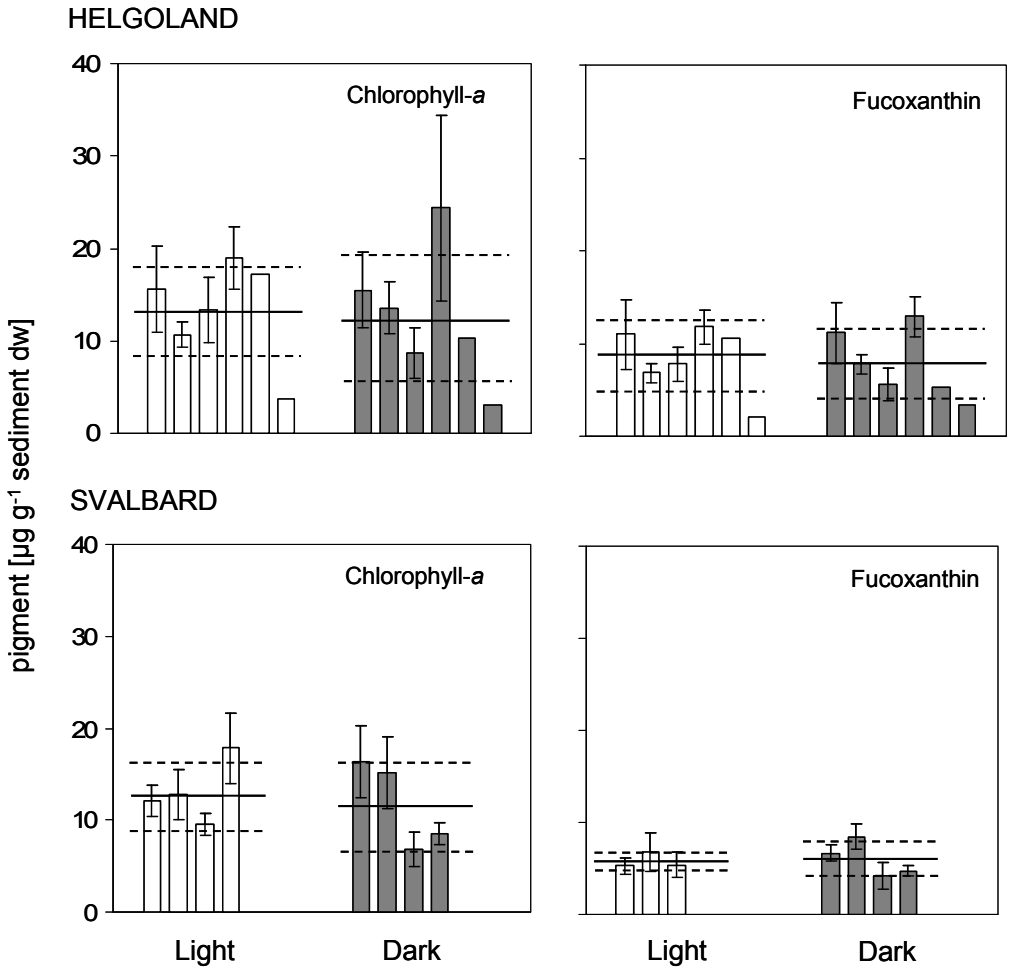
HELGOLAND



SVALBARD



Supplementary Figure S3.2 - Example profiles of oxygen in the shallow subtidal Helgoland (a) and Svalbard (b) sediments as a function of temperature. Shown are steady state profiles measured in the light (○) (85 and 90 µmol photons m^{-2} s^{-1} for Helgoland and Svalbard respectively) and in the dark (■). Arrows indicate the inflection point delimiting the depth of the production zone. The steady state light profiles where reached within approximately 60 min after the establishment of the respective temperature.



Supplementary Figure S3.3 - Photopigment concentration as a measure of MPB biomass represented by chlorophyll *a* and fucoxanthin concentrations in replicate cores. Shown are averages ± SD of three sub-samples from each replicate core previously used for oxygen microprofile measurements in the light (white bars) and in the dark (grey bars). Averages ± SD for light and dark cores are represented by solid and dashed horizontal lines in each panel separately.

4. MANUSCRIPT III

WILL AN UPWARD SHIFT OF THE TEMPERATURE BASELINE CHANGE BIOMASS GROWTH OF SUB-ARCTIC AND TEMPERATE MICROPHYTOBENTHOS COMMUNITIES? A QUANTITATIVE PILOT STUDY

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4.1 ABSTRACT

Subtidal microphytobenthos (MPB) communities from a temperate (Helgoland, Germany) and a sub-Arctic site (Svalbard, Norway) were incubated in the laboratory at different temperatures, under mimicked *in situ* light cycles and nutrient-enriched conditions. Our aim was to study the response of natural MPB community growth to an increase in the baseline temperature, which is expected in the course of global climate change. We applied temperatures that spanned the extreme range of *in situ* temperatures (5, 12 & 20°C and 0, 5, & 10°C for Helgoland and Svalbard, respectively), coupled with non-limiting nutrient supply. We monitored the dynamics of chlorophyll *a* content by hyperspectral imaging to calculate growth-rates and doubling times. Additionally we used high-performance liquid chromatography to study changes in the composition of major MPB pigments.

Growth rates of the communities were low compared to values reported by previous studies, and resultant biomass maxima occurred at the intermediate temperatures at both sites. Growth rates did not differ significantly between sites nor between intermediate and high temperatures (0.34 ± 0.16/0.33 d^{-1} and 0.24 ± 0.05/0.22 ± 0.07 d^{-1} for intermediate/high temperatures for Helgoland and Svalbard respectively). No growth was detectable at 5°C in Helgoland and growth at 0°C in Svalbard was very low. Analyses of MPB pigments indicated a heterogeneous community, dominated by diatoms in all treatments.

Our observations indicate that a future rise of the temperature baseline will not induce a net change in growth rates. Overall MPB biomass may decrease due to a shift in community composition and cell size structure. However, further long-term community level studies under natural conditions are required to strengthen the so far quite limited data base.

4.2 INTRODUCTION

In recent years, microphytobenthos (MPB) has been found to play a significant ecological role in shallow coastal regions (MacIntyre et al. 1996, Cahoon 1999, 2006, Glud et al. 2009). MPB of soft marine substrates are communities of phototrophs (mainly diatoms, cyanobacteria and dinoflagellates) that live on and in the very top layers of the sediments within the euphotic zone. They

constitute the base of shallow water benthic food webs (besides import from the pelagic zone and macroalgal debris), and mediate solute exchanges at the sediment-water interface. Thus they are of high importance regarding the nutrient- and carbon fluxes in these systems (MacIntyre et al. 1996, Cahoon 2006).

MPB biomass is controlled by various factors. While the growth of MPB (i.e. the net biomass increase) is governed by temperature as well as by light- and nutrient availability (Admiraal 1977 and references therein), the depletion of MPB is caused mainly by grazing and environmental perturbations. Nutrient limitation appears to be of minor significance for benthic microalgal growth, since pore-water nutrient concentrations are generally high (Cadée and Hegemann 1974, Admiraal et al. 1982). Besides light as an obvious parameter, temperature plays a major role in the physiology of MPB and benthic communities. It affects autotrophic and heterotrophic metabolism and thus individual performance of species and communities (Admiraal 1977, Davison 1987, 1991, Karsten et al. 2006, Jodlowska and Latala 2013). By this, temperature influences the dynamics of each population, determined by its temperature adaptation window: within their genetic limitations and environmental constraints, species of an MPB assemblage will acclimate to the imposed conditions. If the conditions are towards the limits of the species adaptation capabilities, light and temperature may act as a selective force, consequently altering the assemblage composition towards more tolerant or better-adapted species that outcompete less adapted ones (Geider 1987, Defew et al. 2004).

MPB communities are found almost everywhere, from arctic lakes to tropical coral reefs (Whalen et al. 2013, Uthicke and Klumpp 1998), from stable-temperature habitats to habitats with extreme seasonal and diurnal temperature oscillations (e.g. temperate intertidal mudflats & rocky shores), reflecting their strong adaptation capabilities that have evolved over long time scales. A recent carbon uptake study on phytoplankton, representing the same main functional groups as present in MPB (Tortell et al. 2008), reported that optimum temperatures for growth is strongly related to mean environmental temperature, indicating that the temperature history and potential past adaptations (Boyd et al. 2013) play an important role in the growth response. Thus, besides the local nutrient and light supplies, the temperature history and

prevailing temperature dynamics are major factors determining the present MPB communities, their activity and adaptation potentials. This in turn has implications for growth and recovery responses to ecosystem perturbations and raises the question: how will MPB communities cope with the ongoing long term trend of ocean warming?

The upper ocean is warming on a global scale (Sarmiento et al. 1998, Levitus et al. 2009) and this trend is likely to continue in the foreseeable future (IPCC 2013). It is also visible at our study sites in the temperate North Sea (Helgoland, +1.13°C during 1962-2002, (Wiltshire and Manly 2004)) and the sub-Arctic Kongsfjorden at Svalbard where temperatures have increased at almost twice the average global rate in the last century (no local long term data but see Anisimov et al. (2007)). Following these trends we expect overall prolonged times of warmer periods and that the temperature baseline will gradually shift upwards. The longer these trends continue, the closer the local species and populations may approach their limits of temperature tolerance. Together with an increase in water temperatures, we may locally expect nutrient increases such as from thawing permafrost in the Arctic realm (Anisimov et al. 2007). According to these changes in environmental parameters, we expect changes in MPB growth and photosynthetic performance with consequences for the whole benthic community. Pursuant to these expected changes in important environmental parameters, the resultant response of MPB assemblages in terms of growth and photosynthetic performance hold significant consequences for the whole benthic community.

To date, studies of the changes in growth responses to changes in environmental factors, such as temperature, nutrients, light and salinity, have focused on isolated strains or single-species cultures of phytoplankton or benthic diatoms and cyanobacteria (Admiraal 1977, Longhi et al. 2003. Karsten et al. 2006, Scholz and Liebezeit 2012, Boyd et al. 2013, Jodlowska and Latala 2013). Few reports exist of *in situ* studies or on natural assemblages (Admiraal and Peletier 1980, Sundbäck and Snoeijs 1991) and represent a considerable gap in our knowledge about the physiological response of these MPB communities. The specific growth rates of single strains and species provide insights to speculate on trends in the potential distribution of species-dominances under changing environmental conditions. However, they do not provide any reliable

means of predicting the response of entire complex communities due to multifarious interactions. Therefore, we believe that the study of natural assemblages is relevant for understanding ecosystem function.

The two sites presented in this study are in the temperate and Polar Regions of the Northern hemisphere. Although they face very different meteorological conditions, they can be considered comparable due to some specific aspects. Their differences are marked in the distinctly different temperature and light regimes: temperatures in Helgoland vary between 2°C and 18°C during one year (Wiltshire and Manly 2004) whereas the annual range in Svalbard is much smaller, -2°C to 9°C (Sevilgen et al. 2014). Thus, the annual mean temperature in Helgoland is about 8°C higher than in Svalbard (~10°C vs. 2.5°C, respectively). Additionally, short term temperature fluctuations are much stronger in Svalbard than in Helgoland (4-5°C vs. < 1°C d^{-1} in Svalbard and Helgoland respectively, Sevilgen et al. 2014). Furthermore, while the temperate site is governed by a day:night cycle of 16:8 h during summer, the Arctic site receives light throughout 24 h. However, the two sites are in fact tightly interlinked by the same water masses: the North Atlantic Current, which transports warm waters from the Gulfstream, influences the climate of north-west Europe and continues northwards as the Norwegian Atlantic Current. Further on, it progresses as the West Spitsbergen Current along the western coast of Spitsbergen, where Kongsfjorden is located (Svendsen et al. 2002). This tight coupling is evident in biotic similarities - in Kongsfjorden North-Atlantic species are common at all trophic levels (Hop et al. 2002, Hop et al. 2012). Hence, from a biological point of view, despite its geographical location, Kongsfjorden is rather to be categorized as sub-Arctic than Arctic and better classified as a transition zone between Arctic and Atlantic boreal regions (Wlodarska-Kowalczuk and Pearson 2004). This view is also supported by the MPB communities present at Helgoland and Kongsfjorden. Sevilgen et al. (2013) found no endemic diatom species in Kongsfjorden and just one site-specific algae at all. The two sites shared 27.5% of all species and most diatom species were categorized as cosmopolitan. This setting allows for investigation of the temperature response of taxonomically similar MPB associations that, however, live under distinctly different environmental conditions.

With respect to the chosen study sites, we posed the following questions regarding MPB community growth:
(i) How will different temperatures within the natural range of annual temperatures affect the MPB biomass growth at each site?
ii) Will the MPB communities from the sites exhibit different temperature optima for growth as they experience distinctly different annual temperature means and dynamics (or will their response be similar as the communities are similar)?
(iii) What is the role of temperature, nutrients and their coupled role for microphytobenthos growth?

To address these questions, we incubated natural sediment with MPB communities from the sites in temperature-controlled flow-through chambers of nutrient-enriched seawater for 1-4 weeks. We eliminated potential grazers and mimicked summer light cycles of the two sites. We monitored surficial chlorophyll *a* (Chl *a*) concentrations using hyperspectral imaging to assess MPB growth and derived specific growth rates and doubling times of the communities. Additionally we analyzed the concentration of selected pigments, representative for important functional groups (diatoms) to identify potential shifts in pigment composition due to incubation at different temperatures.

4.3 METHODS

4.3.1 *Sediment sampling*

Sediment samples were collected in the spring and summer months of two consecutive years (2011 & 2012) from two shallow subtidal sites: the temperate site of "Düne Süd" near Helgoland (North Sea) and the Arctic site called "Brandal" in Kongsfjorden (Svalbard) (Table 4.1). Both sediments were characterized as well sorted, fine, porous, permeable sands. Detailed site and sediment characteristics have been described in a previous study (Sevilgen et al. 2013).

Table 4.1 - Overview of sampling parameters at the two study sites Helgoland and Svalbard during the collection of subtidal sandy surface sediments for subsequent MPB growth experiments.

Parameter	Helgoland, Germany, North Sea Düne Süd	Kongsfjorden, Svalbard, Arctic Brandal
geographical coordinates	N 54° 11.594, E 07° 52.802	N 78°56.816', E 011°51.068'
sampling date [MM/YYYY], (*in situ* temperature [°C])	03/2011, (4°C) 02/2012, (3°C) 04/2012, (6°C)	06/2011, (5°C) 07/2011, (6°C) 06/2012, (6°C)
sampling depth [m]	2.8 ± 0.9	5 ± 0.2

All sediment samples were collected by SCUBA divers. When possible, sediment areas with an obvious MPB cover, visible as a brown-olive tint on the sediment surface, were sampled by carefully collecting approximately 100 ml of the upper 5 mm of sediment into 200 ml sample bottles (Kautex Textron GmbH, Germany). After retrieval the sediment samples were placed in a cooling box filled with seawater from the sampling site. Samples for the first growth series (2011) (for terminology and experimental set-up see Fig. 4.1) from Kongsfjorden were transported within one hour to the Marine laboratory in Ny-Ålesund and subsequent sampling sets were shipped at *in situ* temperatures to Germany (Bremen). Sample bottles from Helgoland were transported to the field-laboratory within one hour. They were kept outdoors in seawater flow-through basins at *in situ* temperature and natural illumination, until transport with cooling boxes to the mainland laboratory within maximum 4 hours. At their final destination (Bremen, Germany) all sediment samples were kept in natural seawater aquaria in climate rooms at corresponding *in situ* temperatures of the respective sites until further use. Sediment samples were adjusted from their *in situ* temperatures to experimental temperatures stepwise (2°C d^{-1}) in climate rooms or with the aid of temperature-controlled water-baths within 24 h after arrival. Before the start of the growth experiments the samples were kept in aerated seawater and under illumination (70-80 µmol photons m^{-2} s^{-1}) with 15:9 h and 24:0 h of light:dark conditions for Helgoland and Svalbard samples respectively.

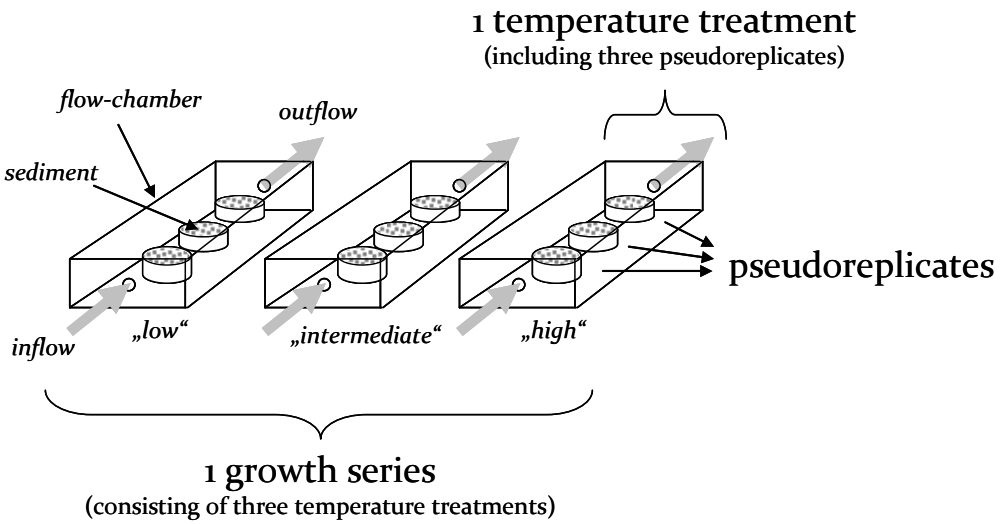

Figure 4.1 - Schematic overview of the growth experiments of MPB in temperate (Helgoland) and sub-Arctic (Svalbard) subtidal sediments. Experiments included three replicate growth series per site (N = 3 per site). One growth series included three temperature treatments (individual flow-through chambers with 5, 12 & 20°C for Helgoland and 0, 5 & 10°C for Svalbard, denoted as "low", "intermediate" and "high" temperatures). Each temperature treatment contained three pseudo replicates.

4.3.2 *Temperature-controlled incubations*

For each site, three temperature-controlled flow-through chambers with incubation temperatures of 5, 12 and 20°C (± 1°C) for Helgoland and 0, 5 and 10°C (± 1°C) for Svalbard were set up, representing the low, intermediate and close to high temperatures which the communities experience naturally during the course of one year. For both sites these temperatures were denominated as "low", "intermediate" and "high" (Fig. 4.1), and will be referred to as such hereafter. The flow-through chambers were connected to aquarium water pumps (Hagen Elite aquarium aeration membrane pump) which were placed in temperature-controlled water reservoirs (10 l) and pumped natural seawater from Kongsfjorden (first of three growth series for Svalbard sediments) or the North Sea (all other growth series). Temperature in the water baths was maintained by the use of cooling coils connected to temperature control units (Thermo Haake DC10 or Haake D8). Submersible temperature loggers (tidbit v2, Onset Computer Corporation, USA) were placed in each flow-through chamber to continuously record the temperature of the water throughout the experiment. Additionally, temperature was recorded during each hyperspectral scan (see below) using a thermometer. Into each flow-through chamber three plastic

basins (each 40 × 50 × 10 mm with cylindrical cavities of 30 mm diameter and 5 mm depth), were placed and filled with approximately 3.5 cm^{-3} of pre-adapted homogenized sediment from the sites, representing pseudoreplicates within one temperature treatment (Fig. 4.1).

Light – During the incubations, sediments were exposed to illumination that mimicked average natural light intensities and dynamics within a dial cycle. The continually modulated light cycles were administered by a programmable illumination-system (GHL Profilux PLUS II) equipped with full-spectrum daylight-lamps. The light intensity in LUX at the sediment surface and photosynthetically active radiation (PAR, µmol photons m^{-2} s^{-1}) at the sediment basins were monitored throughout the experiment using submersible light and PAR-loggers (HOBO pendant, ONSET Computer Corp., USA; ODYSSEY Dataflow Systems PTY Limited, New Zealand). Helgoland sediments were illuminated with a light:dark cycle of 15:9 h with maximum light intensities of 65 µmol photons m^{-2} s^{-1}. In contrast, Svalbard sediments were illuminated with a light:dark cycle of 24:0 h and intensities from 10 to 65 µmol photons m^{-2} s^{-1} (Fig. 4.2). Maximum light intensities on clear summer days can temporarily reach values of > 200 µmol photons m^{-2} s^{-1} at the sampling sites but due to frequent cloud cover light intensities are generally much lower (~ 20 to 70 µmol photons m^{-2} s^{-1}) (Sevilgen et al. 2013). As summarized by Hanelt et al. (2004) and Karsten et al. (2006), rapid changes in weather conditions render the underwater light climate generally unstable, and together with increasing turbidity in the water during summer months, very low light conditions are frequently observed at the sediment surface.

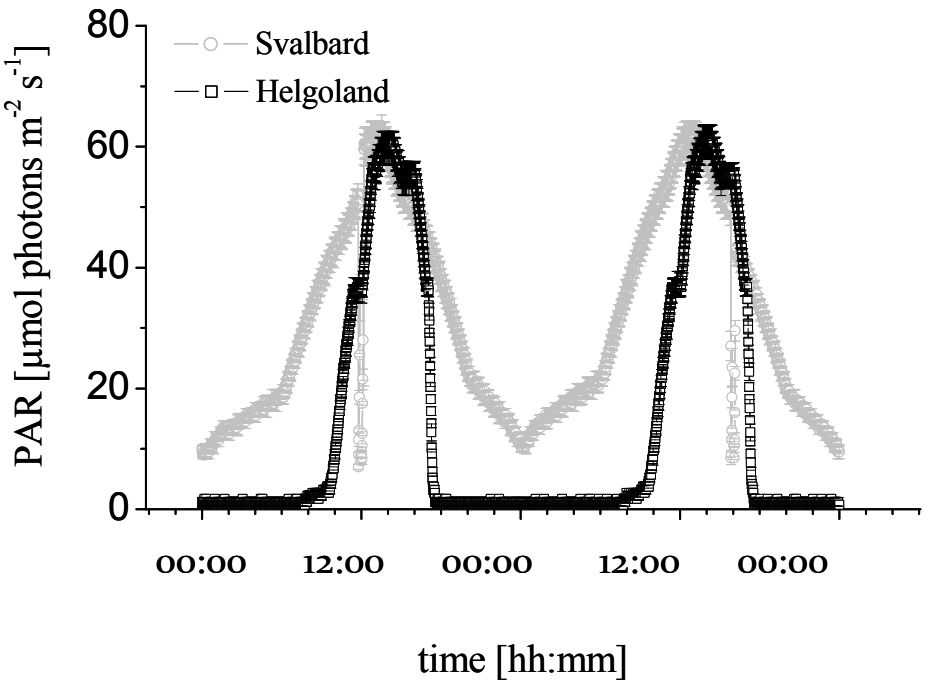

Figure 4.2 - The average intensity of photosynthetic active radiation (PAR) applied throughout the temperature incubation of subtidal MPB communities in sandy sediments from a temperate (Helgoland) and sub-arctic (Svalbard) site as measured by (N = 2) light loggers in the experimental setup per site. Light-intensity drops around noon in the Svalbard series are due to temporary shading of the illumination system during hyperspectral scans (see *Methods*). Error bars represent SD.

Nutrients - In the initial phase of each incubation (except one growth series without an initial phase), sediments in the flow-through chambers were flushed with natural seawater to see if MPB growth would be initiated under a natural seawater nutrient load. After the initial phase, the circulating sea-water was spiked with nitrate ($NaNO_3$), phosphate ($NaH_2PO_4 \times H_2o$) and silicate ($Na_2O_3Si \times 5H_2O$) to assure saturating nutrient conditions for the initiation of growth, and to avoid growth inhibition due to nutrient limitation. Final nutrient concentrations of the recirculating seawater were adjusted to that of f/2 medium, i.e. approximately 882 µmol l^{-1} of nitrate, 36 µmol l^{-1} of phosphate and 106 µmol l^{-1} of silicate (Guillard and Ryther 1962, Guillard 1975). Every second day, 50% of the water in the reservoirs was exchanged with similarly prepared seawater medium after water samples were collected to monitor the nutrient concentration in the flow-through chambers.

4.3.3 *Monitoring biomass growth*

We considered the chlorophyll *a* concentration in the surficial sediment as a proxy for the active MPB biomass. We recorded the surficial chlorophyll *a* signal using a hyperspectral imager and protocols described in Chennu et al. (2013). The hyperspectral imaging system recorded back-scattered light (400-900 nm range; resolution ~ 1 nm) from the sediment, and the detected signal was converted into reflectance spectra using a spectral reference board (a diffusely-reflecting white plastic board placed within the flow-through chamber that was regularly cleaned during the incubations). These spectra were used to calculate a microphytobenthos index (MPBI), which was converted into chlorophyll *a* concentrations (µg chl *a* ml^{-1} PW) in the top millimeter of the sediment using a linear calibration for the recorded grain-size distribution of 125-250 µm for the sediment (Chennu et al. 2013). The time taken to scan across all flow-through chambers was approximately 2 minutes, during which the illumination from the daylight-spectrum lamps was replaced by that from a broadband halogen lamp.

4.3.4 *Growth measurements*

Growth curves were represented as the increase in chlorophyll *a* concentration over time, whereas one growth curve represents the average of the pseudoreplicates per temperature treatment (Fig. 4.1). Growth rates (µ), expressed as µg chl *a* ml^{-1} PW d^{-1} were estimated during the exponential growth phase (when present), according to the following equation:

$$\mu = [\log(B_{t2}) - \log(B_{t1})] / [t2-t1], \quad (1)$$

where B_{t1} and B_{t2} represent the chlorophyll *a* biomass at times t1 and t2 respectively. The doubling-time (DT) was calculated from the growth rate as $DT = \log(2)/\mu$.

4.3.5 *Pigment analyses*

To follow biomass development and possible changes in pigment composition, sediment samples collected from the start (representing inoculated biomass) and end of the incubations were frozen with liquid nitrogen and subsequently analyzed for chlorophyll *a*, fucoxanthin, diatoxanthine, diadinoxanthine and zeaxanthin content using high-performance liquid chromatography (HPLC).

While chlorophyll *a* is present in all oxygenic phototrophs, fucoxanthin and diatoxanthin are major diagnostic pigments of diatoms. The frozen samples were freeze-dried for 48 hours in the dark and sub-samples of approximately 1 g were used for extraction with 99.8% cooled acetone. Throughout pigment extraction, samples were kept on ice and treated under dimmed light. After acetone addition, samples were placed for 5 min in an ice-cooled ultrasound bath, vortexed and stored for 24 h at -20°C in the dark. For the pigment analyses, the extract was filtered (Acrodisc 4 mm syringe filters with 0.45 µm PTFE membrane, Pall Corp., USA) and measured using a Waters 2695 separation module (Waters Corp., USA) as described by Wright et al. (1991). Pigments were identified and quantified by comparing with pigment standards (DHI, Denmark).

4.3.6 *Statistics*

Statistical analyses in this study were performed using JMP 9.0 (SAS Institute Inc., USA) with a significance criterion α set to 0.05. To assess the contributions of the individual parameters (site, replicate, temperature) on the variability of the recorded MPB growth, analysis of variance (ANOVA) was performed on the logarithm-transformed data (for normality) with the growth rate µ considered as the dependent variable. Through this, we determined whether the growth rates were significantly different between the treatments with different temperatures across replicate series within one site. Additionally we tested if growth rates of treatments with the same temperature between the replicate series within one site differed significantly. Finally, average and standard deviations of the growth rates were calculated from the three independent replicates per temperature treatment per site and the difference between the average values of the sites was tested for statistical significance.

4.4 RESULTS

4.4.1 *MPB biomass growth*

Generally MPB growth was detected at both sites. Exceptions at the temperate site Helgoland were the low temperature treatments and two of the high temperature treatments which did not show any change in MPB biomass

throughout the course of the incubations (Fig. 4.3a-c). In Svalbard sediments, growth was recorded at all temperatures, albeit with small biomass increases and zero growth in one replicate of the low temperature treatments compared to the intermediate and high temperature treatments (Fig. 4.3d-f).

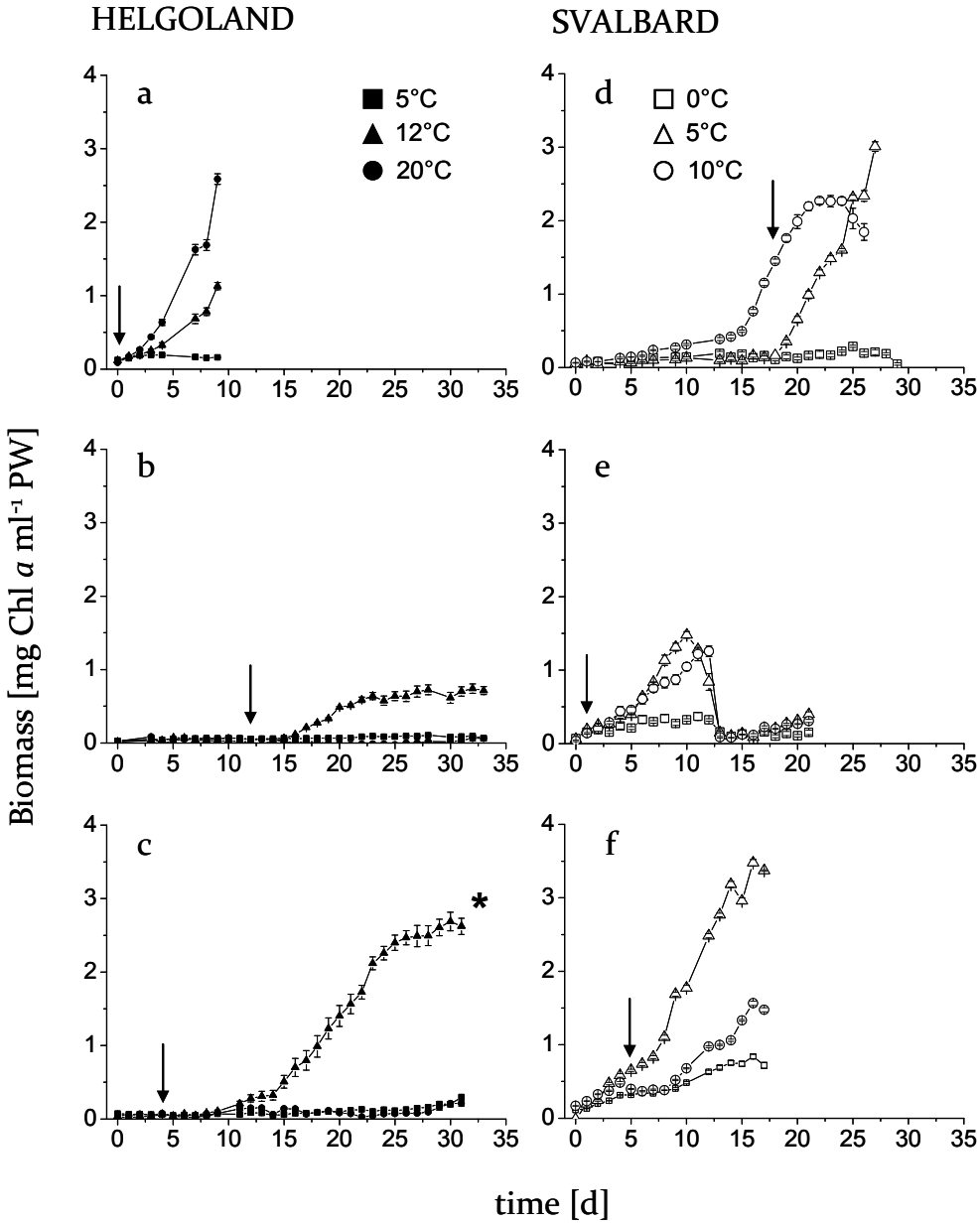

Figure 4.3 - Time-series of MPB biomass measured as chlorophyll *a* concentration in the top 1 mm of temperate (Helgoland) and sub-Arctic (Svalbard) subtidal sandy sediments. Three replicate temperature incubations (see legend) are shown for each site. Each data point in the growth series represents the average ± SD of (N = 3, and * indicates N = 2) pseudoreplicate temperature incubations. Arrows indicate the day of nutrient addition (see *Methods* for further information).

When growth was measurable, almost all growth curves showed an initial lag phase followed by an exponential growth phase which was used for growth-rate determination. The exponential growth phase lasted four to seven days in Svalbard and nine days in all Helgoland series (Table 4.2). In three of all growth series biomass did not reach a saturation state before the incubations had to be terminated due to time constraints or temperature control failure (Fig. 4.3a, e & f). In one case, some biomass was lost due to the development of oxygen bubbles that caused the MPB mat to lift and float away (Fig. 4.3d, 5°C).

Between-replicate variability of growth-rates in the same temperature treatments within each site was significant (Table 4.3). This was not the case for average growth rates from intermediate and high temperatures within one site: In Helgoland, rates at 12°C and 20°C did not differ significantly (μ = 0.3 d^{-1}, $p > 0.05$), (Table 4.3, two no-growth replicates excluded). The same was observed at Svalbard for growth rates at 5°C and 10°C (μ = 0.2 d^{-1}) which did not differ significantly ($p > 0.05$) but were 3-fold higher than growth rates at 0°C (Table 4.3).

Table 4.2 - Overview of replicate growth series of MPB communities in sandy sediments from a temperate (Helgoland) and sub-Arctic study sites (Svalbard).

Site	replicate	dates of incubation	duration [d]	tempearture [°C]	growth	duration of the exponential growth phase [d]
Helgoland	a	14.03.-23.03.11	9	5	no	-
				12	yes	9
				20	yes	9
	b	18.02.-22.03.12	33	5	no	-
				12	yes	5
				20	no	-
	c	26.03.-26.04.12	31	5	no	-
				12	yes	9
				20	no	-
Svalbard	a	06.06.-05.07.11	29	0	no	-
				5	yes	6
				10	yes	7
	b	06.07.-27.07.11	21	0	yes	6
				5	yes	4
				10	yes	6
	c	30.06.-17.07.11	17	0	yes	6
				5	yes	5
				10	yes	4

Maximum biomasses within the duration of the incubations varied widely between temperature treatments and replicates, and significant differences were found amongst the growth series at intermediate temperatures within Helgoland. Thus, although the growth rate at 12°C of the second growth series was higher than the growth rate of the third growth series (data not shown), the maximum biomass at intermediate temperatures in the third Helgoland series was > 4 times higher in the third than in the second series (Fig. 4.2b & c). Due to slow growth rates and a related lack of data from the biomass saturation state, analogous evaluations for Svalbard sediments were not possible. Nonetheless, although the biomass did not always saturate in sediments from this site, at the end of each growth series, the biomass was always higher for the intermediate than for the high temperature treatment ($p < 0.001$, $p = 0.027$ & $p < 0.001$ for the first, second and third Svalbard growth series respectively) (Fig. 4.2d-f). Thus, irrespective of the corresponding growth rates, the highest biomass was achieved at intermediate temperatures at both sites.

Table 4.3 - Summary of average growth rates (μ) and doubling times (DT) determined from the growth-series of MPB communities of sandy sediments from a temperate (Helgoland) and sub-Arctic (Svalbard) study site showing exponential growth. Shown are averages ± SD from n-replicate measurements. p-value indicates the inter-site differences amongst incubations of the same temperature within one site.

Site	experimental temperature [°C]	specific growth rate [μ]	p-value	doubling time (DT)	n
Helgoland	5	-	-	-	0
	12	0.34 ± 0.16	0.040	2.34 ± 0.77	3
	20	0.33	-	2.10	1
Svalbard	0	0.07 ± 0.07	<0.0001	8.06 ± 3.89	2
	5	0.24 ± 0.05	0.002	2.94 ± 0.60	3
	10	0.22 ± 0.07	0.0001	3.40 ± 1.09	3

4.4.2 *Nutrient induced growth*

In Helgoland sediments none of the series showed growth in the initial phase before the addition of nutrients. While in the first series (Fig. 4.2a) growth started one day after nutrient addition, the onset of growth only started after four to five days in the following series (Fig. 4.2b & c). Interestingly, in Svalbard

sediments, in two of the three growth series biomass started to increase before the addition of nutrients (Fig. 4.2d & f). In those series which did not show growth in the initial phase, growth was stimulated within one to four days after nutrient addition.

4.4.3 *Pigment content and composition*

Due to thermal damage of samples, pigment analyses could only be carried out for one growth series in Svalbard and two in Helgoland (Fig. 4.3b, c & f). Diatoxanthin, diadinoxanthine and zeaxanthin were almost not detectable in all samples analyzed (data not shown). Chlorophyll *a* and fucoxanthin were identified as the major pigments in the sediment samples and their concentrations generally mirrored the results from hyperspectral imaging (Fig. 4.4). Thus highest pigment contents were detected in the 12°C incubations in Helgoland and in the 5°C incubations in Svalbard (Fig. 4.4a). Pigment concentrations varied considerably between pseudoreplicates within and between temperature treatments at both sites.

Only considering the three major pigments chlorophyll *a* (chl *a*), fucoxanthin (fuco) and diatoxanthine, the balance between these pigments changed during the course of the incubations (Fig. 4.4b). In all series analyzed, the relative chlorophyll *a* content was lower and fucoxanthin content was higher at the end of the experiment than in the inoculum, whereas diatoxanthine was not detectable or did not change (Fig. 4.4).

Pure diatom samples exhibit a fuco:chl *a* ratio of 0.6-0.8 (Barranguet et al. 1997, Jeffrey et al. 1997). In Svalbard sediments, the fuco:chl *a* ratio of the inoculum was 0.21 ± 0.03 and increased significantly ($p < 0.02$) to 0.44 ± 0.05, 1.53 ± 0.40 and 0.92 ± 0.04 at 0°C, 5°C and 10°C respectively. The differences in the ratios suggest differences in diatoms, or pigment quality of the present organisms. Overall, in the Helgoland sediments fuco:chl *a* ratios were significantly higher than in Svalbard ($p = 0.04$). Compared to the inoculum, which had a ratio of 4.4 ± 4.9, the fuco:chl *a* ratio at the 12°C incubations in Helgoland did not change significantly ($p > 0.3$) and were 2.8 ± 3.3 and 0.5 ± 0.2 in the second and third growth series respectively.

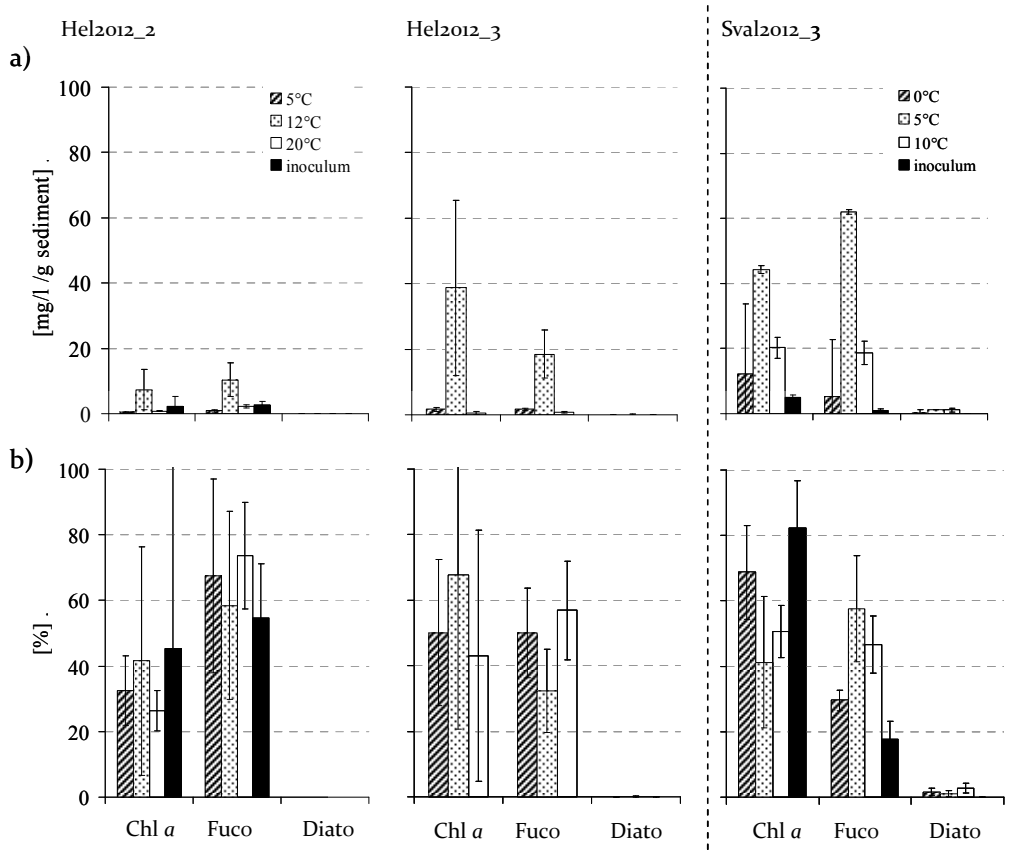

Figure 4.4 - Concentration (panel a) and percental distribution (panel b) of chlorophyll *a* (Chl *a*), fucoxanthin (Fuco) and diatoxanthin (Diato) from sediments of the second and third growth-series from Helgoland (Fig. 4.3b, c), and the third growth-series from Svalbard (Fig. 4.3f). Shown are averages ± SD of N = 3 replicate measurements for each experimental temperature from the beginning (inoculated sediment) and the end of each growth series.

4.5 DISCUSSION

4.5.1 *Community growth versus single species growth*

The focus of this study was the growth response of natural MPB communities at different temperatures in nutrient enriched seawater under mimicked *in situ* (thus variable) light conditions. Commonly, growth experiments are carried out under constant and saturating light intensities on single strains or species which are picked as representatives for important functional groups (cyanobacteria, diatoms, coccolithophores, dinoflagellates) of phytoplankton or MPB communities (Admiraal et al. 1977, Longhi et al. 2003, Karsten et al. 2006, Boyd et al. 2013). These studies enable to evaluate in detail species-specific thermal optima and growth responses. However, the drawback of such

investigations is that growth responses for selected strains or species do not suffice to explain or to predict community response to changing environmental conditions because (i) usually there are no models for each strain or species of a community and single strain or species response can be very variable, (ii) these models do not take into account potential interactions between species such as competition for light and nutrients and (iii) they usually do not include dynamic conditions such as natural day/night cycles. Phytoplankton diatoms, e.g., exhibit significant inter- and intra-specific variation in temperature-related growth (Boyd et al. 2013). Boyd et al. (2013) also pointed out difficulties in linking growth responses of certain strains to their provenance e.g. to claim region-specific responses, as even strains from the same region may react differently to temperature changes. On the level of functional groups, very little is known about whether certain functional groups can slowly acclimate or adapt on larger timescales to temperature (Boyd et al. 2013) which would imply that certain groups could merge and eventually outcompete and replace others. Furthermore, given that the dominance of functional groups varies seasonally (Pinckney et al. 1995), based on studies of isolates it is almost impossible to predict with accuracy community responses under natural, thus variable conditions. This recommends much caution in extrapolating the conclusions drawn from growth-rate measurements of isolates to assemblages of similar organisms in nature.

4.5.2 *Growth rates of the natural assemblage of microphytobenthic communities*

The present pilot study showed no significant differences in growth rates between the studied temperate (Helgoland) and sub-Arctic (Svalbard) MPB communities which were highest at intermediate temperature treatments at both sites. Initially this similar response of MPB growth at both sites may be surprising, given the large difference between the annual average and daily ranges of temperatures between Helgoland and Svalbard. Nonetheless, the intermediate temperature treatments for both represent the temperatures that are close to the respective annual average temperatures sites (12 vs. 10°C for Helgoland and 5 vs. 2.5°C for Svalbard).

The presented growth series represent a mixture of all present species and functional groups in the MPB community. Thus they do not follow a classical growth curve as seen in isolated cultures of microorganisms. Nonetheless, the growth series of the MPB communities show a sigmoid pattern with an exponential growth phase similar to the standard growth patterns of isolated species. The growth rates (μ) derived from this exponential growth phase ranged between 0.07 and 0.34 d^{-1} (doubling times (DT) of 8.1 and 2.3 days). These values are in the lower range of reported μ and DT for single species of benthic marine diatoms ranging from subtidal polar to temperate intertidal species (Admiraal 1977, Admiraal and Peletier 1980, Longhi et al. 2003, Karsten et al. 2006, Scholz and Liebezeit 2012) and phytoplankton organisms (Boyd et al. 2013). Generally, the exponential growth phase in our incubations lasted ~50% longer than previous observations of intertidal temperate diatoms (Scholz and Liebezeit 2012) which, together with the determined μ, reflects slow community growth in the temperate and Arctic MPB. Given that the growth curves determined in our study represent the cumulative growth of all present species with each possibly displaying different temperature and growth optima, a protracted exponential growth phase is not surprising. An additional reason for the comparatively slower growth is likely methodological, i.e. the applied light conditions: in this study the mimicked *in situ* dial light cycle provided high intensities of photosynthetically active radiation only within a narrow time frame (see Fig. 4.2). This is in contrast to previous growth studies which used constant light conditions (e.g. Admiraal 1977, Longhi et al. 2003, Scholz and Liebezeit 2012). Results from growth experiments under constant illumination however can not be transferred onto growth responses of natural communities by implication, especially the more the daylight dynamics deviate from constant illumination. Thus, a reduction of maximum illumination and continuous changes in light intensity, as represented by natural daylight dynamics, will result in slower community growth responses. This assumption is supported by a previous study which described sub-optimal light conditions and temperature as limiting factors for the cell division rates of diatom cultures (Admiraal and Peletier 1980). So although in our study a light:dark cycle of 15:9 h was applied for the temperate site, which is close to preferred cycles for maximum growth of benthic diatoms (16:8 h) (Admiraal and Peletier 1980, Scholz and Liebezeit 2012)

and probably sufficient for growth of some species (Longhi et al. 2003, Karsten et al. 2006), the applied light dynamics overall have contributed towards the low growth rates of the communities.

Despite low rates, MPB growth was initiated faster in Svalbard than in Helgoland sediments. This happened both before and after the addition of nutrients. We speculate that this could be due to a larger proportion of smaller MPB organisms in Svalbard sediments. Growth rate is an inverse function of cell- size; due to their larger surface to volume ratio, small cells are biologically more active, assimilate nutrients at higher rates and are able to divide faster (Admiraal 1977, Williams 1964). Accordingly, high growth rates could be observed in small-sized diatoms before (as, e.g., *Navicula* in intertidal flats) (Scholz and Liebezeit 2012). Nevertheless, whether the growth of small-sized species initiated an earlier onset of community growth remains a hypothesis which would need further investigations since the overall community growth rate in Svalbard was not higher compared to the Helgoland community.

At low temperatures little or no growth was observed at both sites. This is somewhat surprising as both treatments with low temperature were not limited in nutrients and we expected that growth of the communities would also occur at the natural low temperature level. As found in a growth study of two Antarctic diatom species, although growth rates were generally low (~0.25-0.4 d^{-1}), the optimal temperature for growth was the lowest applied (0°C) and the authors concluded that the investigated microalgae were very well adapted to the prevailing temperatures at the locations where the microalgae were found (Longhi et al. 2003). Contrarily, in another study on two epiphytic Arctic diatoms from Kongsfjorden (Karsten et al. 2006) optimum growth was detected between 12-14°C, very well matching the outcome of the present study. Possible reasons for no growth at low temperatures in Helgoland could be damage of the MPB community during thermal acclimation to experimental temperatures, or a disease. The latter cause seems unlikely, as two out of three independent growth series showed zero growth. Although previous studies suggest even slower temperature acclimation for MPB species (Longhi et al. 2003), in our study the highest increase in MPB biomass from Helgoland occurred at 12°C, i.e. far above the initial *in situ* temperatures of 3-6°C

(Table 4.1). Thus we also exclude too rapid acclimation as a potential cause of damage and the no-growth phenomenon still lacks a reasonable explanation.

Generally our data suggest slow growth of the MPB communities at both sites. Although mainly addressing cyanobacteria, previous studies on growth rates of marine microbial communities (Paerl et al. 1993, Pinckney et al. 1995) indicate that short-term incubation periods (5 days) failed to show nutrient-related growth enhancement. In the latter study, significant changes in biomass were only evident after 22 days of exposure to enhanced nutrient levels. Although four out of six of our growth series extended three full weeks, nutrient enriched conditions were almost always of a shorter duration than the full experimental duration. In our growth series where nutrient-enriched conditions exceed these three weeks, indeed biomass sometimes also began to increase at lower temperatures (Fig. 4.2c). Thus it is possible that the incubations did not always last long enough to span the full potential growth response of the community.

4.5.3 *Community shifts at different growth temperatures*

The use of HPLC pigment analysis is widespread in phytoplankton ecology to study the taxonomic composition of communities (Brotas and Plante-Cuny 2003 and references therein). Although more investigations of the pigment diversity and distribution of subtidal MPB communities are required, photosynthetic pigments can be used as indicators of the relative abundance of major taxonomic groups and thereby provide a method to assess changes in the composition of phototrophic functional groups (Pinckney et al. 1995 and references therein). Thus fucoxanthin can be used as the marker pigment for diatoms, and zeaxanthin can be indicative for cyanobacterial biomass. From this the ratios of fuco:chl *a* and zea:fuco can be used to infer relative abundances of diatoms and cyanobacteria in MPB communities. Zeaxanthin was not detectable in all sediment samples indicative of the absence of cyanobacteria. The fuco:chl *a* ratio of communities solely consisting of diatoms ranges from 0.6-0.8 (Barranguet et al. 1997, Jeffrey et al. 1997). In previous studies, values for MPB communities in the upper sediment layers displayed lower values of 0.3 to 0.5 (Brotas and Plante-Cuny 2003). Fuco:chl *a* ratio in the inoculum from Svalbard

was 0.4. Contrarily, the ratio for Helgoland in the inoculum of the second growth series was much higher (4.4 ± 4.9) and strongly exceeded the ratio generally found for pure diatom cultures reported so far. However, fuco:chl *a* ratios as high as 2.34 and 2.04 for single benthic diatom species have been reported before (Josefson and Hansen 2003, Cibic et al. 2008). These higher ratios were suggested to result from adaptations to low PAR (Cibic et al. 2008). This possibility is in line with the absence of diatoxanthine and diadinoxanthin which indicates that the MPB communities in this study were low-light adapted (Cibic et al. 2008). Low-light adaptation is consistent with a previous study (Sevilgen et al. 2013) that reported low half-saturation and compensation irradiances for the MPB communities from the two study sites. Although the relative pigment composition changed in the temperature incubations as compared to the inoculum, generally our sample size is too small and the data too heterogeneous to draw reliable conclusions regarding changes in community composition.

In conclusion, this pilot study on subtidal MPB communities from a temperate and sub-Arctic site showed that communities from both regions are similar in their growth response, showing optimum growth at intermediate temperatures of their natural annual range, despite distinct and large differences in temperature and light regimes between the two sites. Linked to the dynamic light intensities applied that mimicked *in situ* daylight cycles, and together with a holistic community response that integrates the growth response of each species present, the net growth rates of the communities were generally low with little or no growth detected at low temperatures. Average growth rates at intermediate and high temperatures showed no significant differences within or between sites. Although the final biomass differed between the temperature treatments, the maximum biomasses occurred at the intermediate temperature at both sites, indicative for optimum growth conditions for both MPB communities at intermediate temperatures and suggestive of a large portion of community members adapted to average temperature levels within the annual temperature range. Thus, while an upward shift of the temperature baseline due to global warming will likely cause an eventual reduction in the total MPB biomass, the growth rate in the community will likely not change.

Studies of growth of natural MPB communities under different environmental conditions are ecologically highly relevant since they allow us to integrate the physiological responses of a variety of co-existing community members. A deeper understanding of natural assemblage responses to environmental perturbations is needed to allow more accurate prediction of large-scale ecosystem-level responses that long-term climate change will entail. As community responses highly differ from single species responses and are largely missing, further studies of intact MPB communities under changing environmental parameters are inevitable to enhance our understanding of such complex relationships.

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iii. DISCUSSION

5. DISCUSSION

The work within this thesis addresses microphytobenthos (MPB) communities from a subtidal temperate (Helgoland) and a sub-Arctic site (Spitsbergen). It contributes valuable information about the photosynthetic potential, *in situ* production and community respiration – thus their primary production. Furthermore, it highlights MPB community responses of photosynthesis, respiration and growth to environmental changes, with a focus on temperature-increases as a major parameter that is expected to alter in the course of global climate change.

The presented results comprise snap-shots of the studied sites, which were investigated during the summer months. The following section will discuss the main findings of the thesis (BOX 6) and put them in a broader context. Using the carbon production by MPB at the two study sites, I will present global carbon production estimates. Furthermore, moving from a global

BOX 6 – MAIN FINDINGS

Question 1 – Do MPB communities from the two study sites differ?
→ NO

- similar diatom dominated communities
- similar rates of net photosynthesis, gross photosynthesis and respiration, and similar light adaptation intensities

Question 2 – Does the short-term temperature response of the MPB communities differ?
→ NO

- similar temperature response (the response in the temperate MPB community was higher but not significantly different)
- no effect of temperature increases on net photosynthesis at both sites
- Increase of respiration with increasing temperature at both sites
- at both sites the temperature response of respiration was stronger than that of photosyntehsis

Question 3 – Does MPB community growth differ between sites and does it change with an upward shift of the temperature baseline?
→ NO

- both communities showed similar growth rates at intermediate and high temperatures
- MPB biomasses were highest at intermediate (i.e. close to average annual) temperatures

perspective, I will progress to small-scale observations and discuss the differences between the study sites as well as the potential impact of global climate change on the systems. Additionally, I will outline suggestions for future approaches that can support a better understanding of subtidal MPB communities.

5.1 UPSCALING PRODUCTION - GLOBAL CARBON ESTIMATES

Generally, gross oxygen production estimates from the two investigated study sites were similar and are in accordance with previous studies (see Manuscript I). Following the calculations by Cahoon (1999) and Glud et al. (2009), global carbon estimates were derived for the two study sites (Table 5.1). The calculations are based on the estimation of a global shelf area of 27.122×10^6 km² (Menard & Smith 1966) and the assumption that the depth range of 0–50 m (1/4 of total area) receives enough light to support MPB production. Furthermore I assumed that the *in situ* gross production rates based on measurements in 5 m depth in this thesis (see Table 2.4) stay the same over the depth range of 0–50 m, thus overestimating potential production values. Additionally, production estimates in the Arctic are based on 90-120 days of the year that receive enough light for MPB production (Glud et al. 2009), and on 365 days of light in the temperate region (Table 5.1).

Table 5.1 – Carbon estimates for MPB global primary production (GPP). Shown are estimates ± SD for N replicates. Calculations are based on [1] 90 light-days, [2] 120 light-days, [3] 365 light-days

GPP [Gt C yr^{-1}]	Study	N
0.34	Cahoon (1999)	
0.11 ± 0.05 [1]	Sevilgen et al. (2013), Arctic	3
0.15 ± 0.07 [2]	Sevilgen et al. (2013), Arctic	3
0.44 ± 0.21 [3]	Sevilgen et al. (2013), Arctic	3
0.40 ± 0.21 [3]	Sevilgen et al. (2013), temperate	3

Interestingly, these rough estimates show that on average, values from this study are within the range of global estimates that have been calculated and measured before (Cahoon 1999 and references therein). However, the variability

is very high (50%), likely due to the small scale heterogeneity. This highlights the caution that should be taken when extrapolating data to a global scale.

Similarly, comparisons can be made on a smaller geographic scale. A comparison with previous MPB production measurements in Kongsfjorden (assuming an area of 3.7 km^2 covering the depth range of 0–30 m, Woelfel et al. 2010) reveal that the mean summer gross primary production rates measured in my thesis are lower but comparable with average estimates for the entire Fjord (Table 5.2).

Table 5.2 – Carbon estimates for MPB gross primary production (GPP) in Kongsfjorden. Estimates are calculated based on 90 light-days.

GPP [kg C yr^{-1}]	Site	Study
70.6	Kongsfjorden	Woelfel et al. (2010)
165.0	Brandal	Woelfel et al. (2010)
49.2	Brandal	Sevilgen et al. (2013)

However, when comparing production estimates of the specific study site only (Brandal), the values represent only one third of previously determined estimates (Table 5.2). This gives another example of the high variability within MPB systems, once more emphasizing the significance of their spatial and temporal heterogeneity.

5.1.2 MICROPHYTOBENTHOS –A PART OF THE WHOLE

Similar photosynthetic potential of the MPB communities and gross production estimates from the two study sites may be misleading with respect to the particular local *in situ* status. Significant differences in *in situ* net primary production rates were found between the sites and resulted from differences of infauna assemblages (Manuscript I, Table 2.4). During our study period infaunal organisms were highly abundant at the Spitsbergen site, resulting in markedly elevated oxygen demands and a quicker overall shift towards net heterotrophy under *in situ* light conditions, which are dynamic and frequently at sub-saturating intensities.

This highlights the importance of integrated approaches which include measurements of dynamic *in situ* parameters when studying MPB community

production and deriving estimates. Infauna abundance and diversity for example are highly variable on a spatial and temporal scale (seasons) as has been suggested for the study site in Kongsfjorden (Bick & Arlt 2005, Laudien et al. 2007). Woelfel et al. (2010) reported that 30-80% of all sediment surfaces from several stations in Kongsfjorden (0-30 m) were densely populated by infauna organisms. The same studies (and references therein) also suggest that instead of being an exception, it is likely that infaunal organisms are frequently present throughout soft sediments at different depths in the shallow Arctic. Thus, observation of additional key players in the benthic community and the inclusion of their activity are vital for solid estimates of *in situ* production.

Besides biotic parameters, abiotic parameters also vary. Light plays a crucial role in primary production but estimates often do no consider *in situ* dynamics. Thus, many calculations are based on constant light conditions, often of saturating intensities, and it is known, that these calculations can lead to overestimated budgets (Denis et al. 2012).

My findings show that *in situ* circumstances can be distinctly different between sites and seasons and that the MPB community response alone as derived from laboratory measurements does not suffice to enable accurate estimations and extrapolations of *in situ* net production.

5.2 HELGOLAND VS. SVALBARD - DIFFERENT BUT THE SAME

Based on the presented chapters within this thesis, I deduce that there are generally no site-specific differences in the MPB communities between the temperate and sub-Arctic site and their photosynthetic capacities, i.e. the present diatom species and their potential for primary production (BOX 6). Furthermore, my research demonstrated that despite distinctly different local temperature dynamics, temperature increases provoke similar effects in the two biogeographically different MPB communities. This regards (i) photosynthesis and respiration (Manuscript II), and (ii) biomass growth of the MPB communities (Manuscript III).

Despite the different climates and temperature dynamics throughout the course of a year at the two sites, the commonality of the MPB communities and their activity is not very surprising. The North Sea and the West Spitsbergen Current have the same “water-mass-history” of the North Atlantic.

The classification of the outer part of Kongsfjorden as a transition zone between the North Atlantic and Arctic marine realm is generally accepted (Hop et al. 2002). Thus, my results are in accordance with previous studies that identified many organisms in Kongsfjorden as Atlantic (Hop et al. 2002, Hop et al. 2012). This is even more evident considering that the investigated study site in Kongsfjorden is located at the outer, "Atlantic" part of the fjord (see section 1.4).

Interestingly, the temperature responses between the two study sites were similar with experimental temperatures in the manipulation experiments that were selected according to the site-specific temperature regime. This shows that the MPB communities of each site are adapted to the local temperature regime. This accounts for both photosynthesis and respiration responses and is furthermore underlined by the findings of the growth study. Highest final biomasses and growth rates at both sites were measured at intermediate temperatures. These resembled the same temperatures as those of highest net photosynthesis rates during the experiments of short-term temperature responses (Manuscript II, Fig. 3.3a). Interestingly, these temperatures are the ones closest to the particular annual average temperatures of each site (Manuscript II, Fig. 3.1a). This suggests that MPB communities are adapted to average local temperature conditions. This phenomenon is known from phytoplankton communities, where a) optimum temperatures for growth were strongly related to mean environmental temperatures and b) it was shown that the temperature history with potential past adaptations play an important role in the temperature responses (Tortell et al. 2008, Boyd et al. 2013).

5.3 SMALL CHANGES, SMALL DIFFERENCES - THE ROLE OF GLOBAL CLIMATE CHANGE

Environmental conditions in the Earth's biosphere have been rapidly changing in the last decades. However, with respect to the MPB communities from the two presented study sites, the annual temperature changes resulting from global climate change are below natural temperature fluctuations. Thus I suggest that due to their adaptation potential, the relatively slow *in situ* temperature changes are not expected to significantly affect the activity of MPB communities in the near future. It has been argued before that the consequences of global climate change in Kongsfjorden will be more visible in

the pelagic ecosystem compared to the benthos (Hop et al. 2002). Due to the influence from the Arctic and Atlantic, the pelagic system is likely to be more sensitive, whereas the benthic ecosystem is more likely to be affected by long-term changes in hydrography as well as secondary effects of temperature increase, such as glacier melt and sedimentation (Hop et al. 2002). In comparison to direct temperature changes within the habitat of MPB, these secondary changes may have a larger impact. Earlier reduction of sea ice cover in the Arctic may result in earlier pelagic production, subsequently influencing the benthos by a) increased light attenuation in the water column and b) increased organic matter flux to the sediment surface, which in return can fuel the heterotrophic activity of the benthos.

The direction in which MPB communities may develop remains an unanswered question, as changes may also depend on additional factors such as increased runoff from glaciers, snowmelt and land, or increased precipitation and river-runoff in temperate regions, which may alter nutrient conditions. However, changes are likely to occur in small steps. To resolve and follow potential differences over time or between communities, which can be very little (Manuscript II), a high number of replicates and a sagacious combination of methods is necessary to derive statistical significances from potential trends. These can for example be reflected in community composition.

Although species composition analyses as done in this thesis are very useful for a first overview of the predominant organisms, further studies that make use of molecular tools is encouraged. Compared to classical morphologically based methods such as microscopy, molecular approaches identify organisms on a deeper level and thus can enable identification of cryptic species within and also among the different communities. With respect to the presented study sites, between-community differences within the same species are likely to have developed due to the large geographical distance and the related differences in environmental conditions. Due to a finer resolution, molecular approaches may help to reveal differences in communities that are not visible under the microscope but which are potentially present and could be linked to environmental differences.

5.4 COMMUNITY VS. SINGLE SPECIES RESPONSE

The growth study (Manuscript III) revealed that the community growth response did not necessarily reflect what is known from single-species studies. Large heterogeneity was also reflected in the *PI* curves generated for the two communities (Manuscript I, Fig. 2.4). This is not surprising but clearly underlines the importance for studies of entire communities as opposed to single species. Because of their complexity, community response studies are carried out only rarely and studies are rather performed on isolates. Although they give valuable insights and enable the prediction of tendencies, they make realistic estimates difficult as the individual responses of species may not be the same when they are part of a complex community.

The challenge that underlies community studies is obvious- they require many parameters to be taken into account (seasonality, abiotic factors). Therefore, to enable the acquisition of sound data, community studies will need substantially more replicates and increased expenditure of time. This often makes implementation highly problematic and thus related studies are so far missing. Nevertheless, these approaches are highly important to allow for realistic *in situ* estimates and reliable predictions regarding future scenarios of changes in environmental parameters.

5.4 METHODOLOGICAL LIMITATIONS AND FUTURE PROSPECTS

The potential and importance of MPB as a primary producer in the marine realm and the challenge of obtaining sound datasets that enable transferability of their production onto larger scales and future scenarios are equally important.

To study MPB activity different techniques have proven to be suitable (see section 1.2.2). However, each method has its strength and drawbacks. For detailed activity studies such as the ones presented in this thesis, microsensors offer large benefits as they allow the resolution of small-scale dynamics while being minimally invasive. According to their function they provide a micro-scale insight and there is often a significant amount of variation seen due to the patchy nature of MPB communities. Thus, extrapolations need to be interpreted with caution. As most of the studies are limited either in their scope of time, space or methodology, a combination of methods should be highly encouraged

for a comprehensive understanding of bulk community responses and the underlying mechanisms. Specifically regarding environmental change scenarios, I believe that for a sound understanding and comparability of data, the combination of methods and expertise is inevitable. In fact, MPB ecologists face the same problems as phytoplankton ecologists. In a recent scientific-community wide study (Boyd et al. 2013), it was pointed out that datasets that are built upon a common protocol are fundamental to make projections of how ocean biota and ecosystems function and will respond to changes. Based on the fact that global change is occurring and likely to continue in the coming decades, these datasets are necessary to parameterize models that can help predict future scenarios (Boyd et al. 2013). To successfully study entire communities, a community-wide approach is thus suggested. While the authors bring many arguments which clearly show the need of such data, simultaneously they describe the difficulties within the scientific community that leads to limitations within these approaches. Amongst these is e.g. the acquisition of basic data which is often categorized as repetitive or unnecessarily simplistic.

Long-term data sets such as those already existing for phytoplankton in the North Sea (Wiltshire & Manly 2004) have proven to be essential to understand long-term trends with respect to global changes. However, comparable studies for MPB and studies on the community level from different seasons at the same study sites are largely missing. Long-term data, supported by *in situ* monitoring technologies (Chennu et al. 2013) offer a good approach to help filling this gap. In combination with multi-tool approaches that target different levels of MPB ecology, and by combining single-species studies with studies on the community level, it will help to gain crucial information to further assess the relevance of MPB in marine subtidal systems.

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DECLARATION/ERKLÄRUNG

Gemäß §6 (5) der Promotionsordnung der Universität Bremen für die mathematischen, natur- und ingenieurwissenschaftlichen Fachbereiche, erkläre ich hiermit, dass ich die vorliegende Arbeit mit dem Titel

Microphytobenthos in cold-water sublittoral systems - their ecological role and response to changing environmental conditions

1. selbständig verfasst und geschrieben habe,

2. außer den angegebenen Quellen und Hilfsmitteln keine weiteren verwendet habe, und

3. die den benutzten Werken inhaltlich oder schriftlich entnommenen Stellen als solche kenntlich gemacht habe.

Ebenfalls erkläre ich hiermit, dass es sich bei den von mir abgegebenen Arbeiten um drei identische Exemplare handelt.

Bremen, 07.02.2014

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Duygu Sevgi Sevilgen

“Twenty years from now you will be more disappointed by the things you didn't do than by the ones you did do. So throw off the bowlines. Sail away from the safe harbor. Catch the trade winds in your sails. Explore. Dream. Discover.”
Mark Twain