The effect of climate change on the carbon balance between photosynthesis and respiration in Antarctic microalgae

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Dissertation

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The biological process of the carbon cycle in the Antarctic Ocean is controlled by the photosynthetic activity of the primary producers. The amount of fixed carbon does not only depend on the photosynthetic activity but also on the carbon losses due to respiration. Thus, the ratio photosynthesis to respiration (rP/R) is an important parameter to predict the effect of climate change on the Antarctic ecosystem. Indeed, the ongoing changes in climate change are influencing the dynamics of environmental conditions, which has tremendous effects on the phytoplankton community. Therefore, two ecologically relevant species from the Southern Ocean were here investigated: the diatom *Chaetoceros* sp. and the prymnesiophyte *Phaeocystis antarctica*, studying the changes in the rP/R under global climate change conditions. Three main parameters were examined i.e temperature, salinity and iron limitation. The P/R ratio was significantly affected by temperature, while salinity had only a secondary importance, although with species-specific differences. More specifically, the values were ranging from 12.3 to 7.5 for *Chaetoceros* sp. and from 12.4 to 2.5 for *P. antarctica*. The changes in this ratio were principally due to variations in respiration, rather than in photosynthesis. *Chaetoceros* sp. appears to be less flexible in the regulation of the extent of photoprotective mechanisms (non-photochemical quenching and alternative electrons), but its photoprotective level was generally higher than in *P. antarctica*. Regarding iron limitation, data were successfully collected only for *Chaetoceros* sp.. The P/R ratio, equal to 2.8, did not change under iron limitation, with iron limited cells showing a very efficient acclimation to the lowered assimilatory metabolism by decreasing their respiratory losses.

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Summary

Anthropogenically increased CO_2 emissions and climate change are believed to strongly influence the ecological status of the marine ecosystem. Particularly, the Southern Ocean (SO) plays an important role for Earth's climate by controlling the amount of dissolved inorganic carbon stored in the ocean. This can be done through two important carbon pumps, one physical and the other biological. Although they are clearly interconnected, the focus of this thesis was on the biologic pump and, specifically, on the crucial contribution of the so-called primary producers. These organisms constitute the basis of the food web and while few studies have investigated the photosynthetic activity of phytoplankton, there is only very scarce knowledge about the carbon losses due to respiration. The reason for this lack of information is principally attributable to methodological limitations. Photosynthesis can be assessed by ^{14}C uptake, oxygen evolution or by fluorometric devices; nevertheless, a quantitative estimate of the respiration from phototrophs is still difficult. The few data present in literature report a great variability among different species in the ratio of photosynthesis to respiration (rP/R). Additionally, other factors such as temperature, nutrient availability (e.g. iron) and light are expected to influence the rP/R and consequently, the primary productivity.

To fill this information gap, more measurements are required. Therefore, in this thesis, P/R ratios for ecologically relevant Antarctic species were measured in response to environmental conditions. For this purpose, two key species were investigated: the diatom *Chaetoceros* sp. and the prymnesiophyte *Phaeocystis antarctica*, both of which are ubiquitous in the SO. The former is able to thrive in the sea ice with cold and highly saline water, but also in meltwater; the latter has been observed in both deep and shallow mixed layers.

To test the temperature and salinity dependence of rP/R, *Chaetoceros* sp. and two isolates of *P. antarctica* were grown at three different temperatures (i.e. -1 °C, 1°C and 4°C) and four different salt concentrations (i.e. 20, 35, 50 and 70 PSU). The photosynthesis (P) and respiration (R) rates were determined by oxygen evolution and consumption rates (Clark electrode), respectively. In addition, PAM-fluorescence analysis was used for estimation of the relative electron transport rate (rETR) and the potential of non-photochemical quenching (NPQ).

No clear trend in temperature or salinity-dependent changes in photosynthetic values in *Chaetoceros* sp. and *P. antarctica* were measured. In contrast to P, a general trend of increasing R, with the increase of growth temperature from 1 to 4°C at 35 PSU, was observed in both strains, in *P. antarctica* also at 50 PSU. These changes in R significantly influenced the temperature-dependent changes of the P/R ratio. Consequently, two major conclusions could be drawn: firstly, the changes in rP/R were primarily due to variations in R but not in P, and secondly, rP/R is primarily temperature-dependent, whereas the impact of the salinity is of minor importance. Additionally, this study also provides taxon-specific values of respiratory losses in SO phytoplankton.

Accordingly, for all investigated experimental conditions, the respiratory losses in relation to gross primary production (GPP) were in the range of 8 – 14% in *Chaetoceros* sp., 8 – 25% and 8 – 33% in *P. antarctica* strain 764 and 109, respectively. The species-specific differences of rP/R were also evident under different seasonal conditions where *P. antarctica* showed significantly higher rP/R values in autumn/winter compared to spring/summer, whereas the season-specific rP/R values did not vary significantly in *Chaetoceros* sp..
The present data set was used to calculate net primary production (NPP) for specific irradiance, temperature, and salinity combinations, which represent different seasonal conditions. The comparison of species-specific NPP for the different seasons showed a comparable pattern. The highest NPP was calculated for the ‘Summer’ condition (high irradiance, short dark period, and high water temperatures). Despite the large season-specific differences in rP/R, the comparison of the calculated NPP with season-specific rP/R and P values revealed that the NPP is clearly correlated with the P, but not with R, except for the ‘Winter’ condition and this could be due to the very short light period of this condition.
A positive NPP was calculated only for *Chaetoceros* sp. and *P. antarctica* strain 109. Possibly these algal strains are able to keep R at a minimum and to maintain the cells energetic balance during ‘Winter’ condition.

A distinctive species-specific difference in the acclimation to different temperature and salinity combinations is based on the observation of lower variations of physiological parameters in *Chaetoceros* sp. than in *P. antarctica*. This could be a species-specific acclimation strategy, and the differences observed in the present study might reflect the specific adaptation of Antarctic phytoplankton to different environmental conditions,

e.g. to sea ice or highly stratified water conditions for *Chaetoceros* sp., in contrast to deeply mixed waters in the pelagic zone in *P. antarctica*. It was additionally intended to evaluate the effect of changes in rP/R on NPP, showing that there is no correlation between rP/R and NPP for different seasonal conditions. This was due to the finding that phytoplankton cells were able to keep respiratory losses relatively low, according to our data, R in the range of 10 – 15% should represent realistic values to convert measured GPP in NPP under field conditions. With respect to the low level of respiratory losses, an accurate determination of photosynthesis rates becomes even more important. Additionally, the observation of a very different extent of alternative electron pathways in the comparison of *Chaetoceros* sp. and *P. antarctica* is a remarkable result. Thus, the estimation of NPP by the measurement of, for example, variable chlorophyll (Chl) *a* fluorescence in populations with different species composition and at different seasonal conditions could be significantly influenced by the activity of these alternative electron pathways. Nevertheless, it should be emphasized that changes of other environmental factors (e.g. nutrient availability, grazing pressure) may induce stronger variation of rP/R. In this case, the impact on NPP needs to be re-evaluated.

The SO is also well known as being HNLC (high nutrients, low chlorophyll), where iron is recognised to be a major factor limiting primary production. Few studies are present in literature about the effect of iron limitation in SO phytoplankton, and well known is how this parameter negatively affect the physiology of the photosynthetic cells, e.g. decreasing the yield of photosynthetic efficiency (Fv/Fm) and reducing the Chl content per cell. Nevertheless, important information about respiration have not been reported yet. Therefore, in this thesis, the effect of iron availability on rP/R was investigated as a third parameter.

Remarkably, working in an iron free environment is a tricky task: the risk of iron contamination is very high as iron is everywhere, algae with limited iron supply are difficult to cultivate and grow much slower than in rich media. For this reason, rP/R in *Chaetoceros* sp. was measured, but no data were successfully collected for the same parameter in *P. antarctica*.

A novel approach was used for the first time in this study, combining chlorophyll *a* fluorescence, O_2 evolution and particulate organic carbon production measurements to understand the effect of low iron availability on the usage of photosynthetic electrons in cell metabolism and, finally, in carbon production.

Chaetoceros sp. cells showed a very efficient acclimation to iron limitation by decreasing their respiratory losses. This compensates for the inevitable limitations of photosynthesis and the ratio of photosynthesis to respiration did not change in response to iron-deplete conditions. Further experiments with other important SO species, like *P. antarctica*, are necessary to understand the species-specificity of these effects, and more studies are needed as well to infer the influence of P/R ratio on NPP in dependence of iron availability. Nonetheless, a complete dataset on rP/R variability was successfully collected with 3 important parameters in the SO (i.e. temperature, salinity and iron limitation), which are strongly affecting primary production under climate change conditions.

Zusammenfassung

Es wird angenommen, dass der menschlich bedingte Anstieg von CO_2 Emissionen und der Klimawandel das marine Ökosystem stark beeinflussen. Besonders der südliche Ozean spielt eine wichtige Rolle für das Klima der Erde durch die Kontrolle der Menge der aufgelösten anorganischen Kohlenstoffe, welche im Ozean eingespeichert sind. Das ist dank zweier wichtiger CO_2 Pumpen möglich: einer physikalischen, einer biologischen. Obwohl sie offensichtlich gekoppelt sind, bezieht der Fokus dieser Doktorarbeit auf der biologischen Pumpe und, besonders auf dem entscheidenden Beitrag von sogenannten Primärproduzenten. Diese Organismen konstituieren die Grundlage für die Nahrungskette und, während einigen Studien die photosyntetische Aktivität von Phytoplankton untersucht haben, gibt es nur eine mangelnde Kenntnis von Kohlenstoffverluste durch Respiration. Der Grund für diese mangelhafte Information ist hauptsächlich bedingt durch methodologische Einschränkungen. Photosynthese ist schätzbar durch ^{14}C Verbrauch, Sauerstoffentwicklung oder fluorometrische Apparate; nichtsdestotrotz ist eine quantitative Bestimmung der Respiration von Phototrophen noch schwierig. Die wenigen aktuellen Daten in der Literatur berichten von einer bedeutenden Variabilität zwischen unterschiedlichen Spezies in der Ratio von Photosynthese durch Respiration (rP/R). Zusätzlich wird erwartet, dass andere Faktoren wie Temperatur, Verfügbarkeit der Nährstoffe (z.B. Eisen) und Licht die rP/R und somit die Primärproduktion beeinflussen. Um diese Informationslücke zu füllen, sind weitere Messungen wichtig. Deshalb wurde in dieser Doktorarbeit die P/R Ratio von ökologisch relevanten Antarktischen Spezies, als Antwort auf umweltbedingte Bedingungen, gemessen. Zum diesem Zweck, wurden zwei wichtige Spezies untersucht: die Diatomee *Chaetoceros* sp. und die prymnesiophyte *Phaeocystis antarctica*, beide sind ubiquitäre im südlichen Ozean. Die erste prosperiert im Meereis mit kaltem und hoch salzigem Wasser, aber auch im Schmelzwasser; die zweite ist sowohl in tiefen als auch in flachen gemischten Schichtungen zu finden.

Um die Abhängigkeit von Temperatur und Salzgehalt von rP/R zu testen, *Chaetoceros* sp. und zwei Stämme von *P. antarctica* wurden bei drei unterschiedlichen Temperaturen (d.h. -1 °C, 1°C und 4°C) und vier unterschiedlichen Salzkonzentrationen (i.e. 20, 35, 50 und 70 PSU) herangezüchtet. Die Photosynthese (P) und Respiration (R) Raten wurden durch die Menge von Sauerstoffentwicklung bzw. Sauerstoffkonsum bestimmt (Clark Elektrode).

Zusätzlich wurden PAM-Fluoreszenz Analysen zur Bestimmung der relativen elektronischen Transportmenge (rETR) und dem Potenzial von non-photochemische *quenching* (NPQ) benutzt. Es wurden keine durch bestimmte Temperatur oder Salzgehalt bedingten Änderungen von photosynthetischen Werten bei *Chaetoceros* sp. und *P. antarctica* gemessen. Im Gegensatz zu P, ein genereller Trend von ansteigendem R, mit dem Anstieg der Wachstumstemperatur von 1 bis 4°C bei 35 PSU, wurde bei beiden Stämmen beobachtet, bei *P. antarctica* auch bei 50 PSU. Diese Änderungen von R beeinflussten die Temperatur bedingt Änderungen von P/R Ratio besonders. Daraus können zwei wichtige Fazite gezogen werden: zunächst hauptsächlich kamen die Änderungen von rP/R durch Änderungen von R nicht durch P zustande, und zweitens, rP/R ist vor allem temperaturbedingt, während die Wirkung des Salzgehalt von geringer Bedeutung ist. Nenneswert ist, dass diese Studie auch Taxon-spezifische Werte von respiratorischen Verlusten in SO Phytoplankton bereitstellt.

Dementsprechend lagen für alle erforschten experimentellen Bedingungen, die respiratorischen Verluste bezüglich der Bruttoprimärproduktion (GPP) zwischen 8 – 14% bei *Chaetoceros* sp., 8 – 25% und 8 – 33% beim *P. antarctica* Stamm 764 bzw. 109. Die speziesspezifischen Unterschiede von rP/R waren auch unter unterschiedlichen saisonalen Bedingungen evident, wo *P. antarctica* zeigte bedeutsame höhere rP/R Werte im Herbst/Winter verglichen mit Frühling/Sommer zeigte, während sich die saisonabhängigen rP/R Werte nicht bedeutsam bei *Chaetoceros* sp. änderten.
Die aktuellen Daten wurden zur Kalkulation der Nettoprimärproduktion (NPP) für spezifische Bestrahlungsstärke, Temperatur, und Salzgehalt- Kombinationen, welche unterschiedliche saisonale Bedingungen repräsentieren. Der Vergleich zwischen der speziesspezifische NPP zu unterschiedlichen Jahreszeiten zeigte ein vergleichbares Muster. Die höchste NPP wurde zu ‘Sommer’-Bedingungen errechnet (hohe Bestrahlungsstärke, kurze dunkle Periode, und hohe Wassertemperaturen). Trotz der großen saisonabhängigen Unterschiede in rP/R, zeigte der Vergleich zwischen dem kalkulierten NPP mit saisonbedingten rP/R und P-Werte, dass NPP eindeutig mit P korrelierte, jedoch nicht mit R, außer zu ‘Winter’ Bedingungen, was aus der sehr kurzen Lichtperiode bei diesen Bedingungen resultieren könnte.
Eine positive NPP wurde einzig für *Chaetoceros* sp. und *P. antarctica* Stamm 109 kalkuliert. Möglicherweise können diese Algenstämme R auf einem Minimum halten und die Zellen somit die Energiebalance während der ‘Winter’-Bedingungen aufrechterhalten.

Ein distinktiver speziesspezifischer Unterschied in der Akklimatisierung von unterschiedlichen Temperaturen und Salzgehalt Kombinationen basiert auf der Beachtung von niederen Variationen von physiologischen Parametern bei *Chaetoceros* sp. statt bei *P. antarctica*. Dies könnte eine speziesspezifische Akklimatisierungsstrategie sein, und die Unterschiede, die in der aktuellen Studie beobachtet wurden, könnten die spezifische Anpassung von Antarktischem Phytoplankton an unterschiedliche Umweltbedingungen wiederspiegeln, z.B. an Meereis oder hoch geschichtete Wasserbedingungen für *Chaetoceros* sp., im Gegensatz zu tiefem gemischtem Wasser in der pelagischen Zone in *P. antarctica*. Zusätzlich war es beabsichtig, die Wirkung von Änderungen in rP/R auf NPP zu beurteilen, um zu zeigen, dass keine Korrelation zwischen rP/R und NPP zu verschiedenen saisonalen Bedingungen besteht. Dies war bezüglich der Erkenntnis, dass Phytoplanktonzellen die respiratorischen Verluste relativ niedrig halten können, in Übereinstimmung mit unseren Daten, R zwischen 10 – 15% sollte realistische Werte repräsentieren, um gemessene GPP in NPP unter Feldbedingungen umzuwandeln. In Bezug auf das niedrige Niveau der respiratorischen Verluste, wird die genaue Bestimmung von Photosynthese Raten wird sogar wichtiger. Zudem ist die Beachtung von einer sehr unterschiedlichen Masse von alternativen Elektronenwegen im Vergleich zwischen *Chaetoceros* sp. und *P. antarctica* eine bedeutsames Ergebnis. Daher könnte die Bestimmung von NPP durch die Messung von, z.B. variabler Chlorophyll (Chl) *a* Fluoreszenz in Populationen mit unterschiedlicher Spezieskomposition und zu unterschiedlichen saisonalen Bedingungen bedeutend durch die Aktivität von diesen alternativen Elektronenwegen beeinflusst sein. Nichtsdestotrotz sollte hervorgehoben werden, dass die Änderungen von anderen Umweltfaktoren (e.g. Verfügbarkeit von Nährstoffen, streifender Druck) starker Änderungen von rP/R herbeiführen könnte. In diesen Fall müsste die Auswirkung auf NPP neu bewertet werden.

Der Südliche Ozean (SO) ist auch bekannt als HNLC (= *high nutrients, low chlorophyll*), wo Eisen als Hauptfaktor für die Begrenzung der Primärproduction anerkannt ist. Einige Studien zum Effekt von Eisenmangel in SO Phytoplankton sind in der Literatur zu finden, und sehr bekannt ist wie dieser Parameter die Physiologie der photosynthetischen Zellen negativ beeinflusst, z.B. sinkender Ertrag von photosynthetischer Effizienz (Fv/Fm) und die Reduzierung des Chl-Gehalts pro Zelle. Dennoch sind wichtige Informationen bezüglich der Respiration noch nicht bekannt. Deswegen wurde in dieser Doktorarbeit die Wirkung von Eisenverfügbarkeit auf rP/R als dritter Parameter erforscht.

Die Arbeit in eisenfreien Umwelt ist eine komplizierte Aufgabe: das Risiko einer Eisenkontamination ist sehr hoch (Eisen ist überall), Algen sind mit einem limitiertem Eisenangebot schwierig zu kultivieren und wachsen viel langsamer als im eisenreichen Medium. Aus diesen Grund, wurde rP/R bei *Chaetoceros* sp. gemessen, aber keine Daten wurden für den gleichen Parameter in *P. antarctica* gesammelt.
Es wurde eine neue Vorgehensweise zum ersten Mal in dieser Studie eingesetzt, kombinierende Messungen aus Chlorophyll *a* Fluoreszenz, O_2 Evolution und partikulärer organischer Kohlenstoffproduktion, um die Wirkung von niedriger Eisenverfügbarkeit in Bezug auf den Gebrauch von photosynthetischen Elektronen im Zellstoffwechsel und letztlich in der Kohlenstoffproduktion zum verstehen.

Chaetoceros sp. Zellen zeigten eine sehr effiziente Akklimatisierung bei Eisenmangel durch die Reduzierung von respiratorischen Verlusten. Das kompensiert die unvermeidlichen Einschränkungen von Photosynthese und der Anteil von Photosynthese durch Respiration änderte sich nicht als Antwort auf die eisenärmeren Bedingungen. Zusätzliche Experimente mit anderen wichtigen SO Spezies, wie *P. antarctica*, sind erforderlich, um die Spezies-Spezifität auf diese Wirkungen zu verstehen. Weitere Studien sind notwendig, um die Wirkung der P/R Ratio auf NPP in Abhängigkeit der Eisenverfügbarkeit abzuleiten.
Trotzdem ist es hier gelungen einen kompletten Databestand von rP/R-Variabilitat mit den drei wichtigen Parametern im SO (d.h. Temperatur, Salzgehalt und Eisenmangel), welche die Primärproduktion unter klimatsch veränderten Bedingungen stark beeinflussen, zu sammeln.

1. Introduction

1.1. The carbon cycle and climate change

In the Earth system, carbon is continuously recycled through natural processes (**Figure 1**). Different processes take place at various rates: from short-term fluctuations, which occur daily and seasonally, to very long-term cycles, which occur over hundreds of millions of years. Four main reservoirs are responsible for interchanging carbon, i.e. the atmosphere, the terrestrial biosphere, oceans and fossil fuels. The different carbon reservoirs are closely inter-connected and therefore, strongly influence each other. The recent effects of climate change are currently modifying important characteristics of the carbon cycle. Due to global warming, atmospheric and water temperatures are rising, influencing both the abiotic and biotic parameters of the carbon cycle. The increasing atmospheric CO_2 concentration is lowering the pH of sea water, causing ocean acidification, with dramatic consequences for the living organisms (e.g. bleaching of coral reefs, (Hughes et al. 2018)). The effects of such changes are still not completely understood, fueling more questions about the future adaptability and survival chances of not only marine organisms, but also the entire environment.

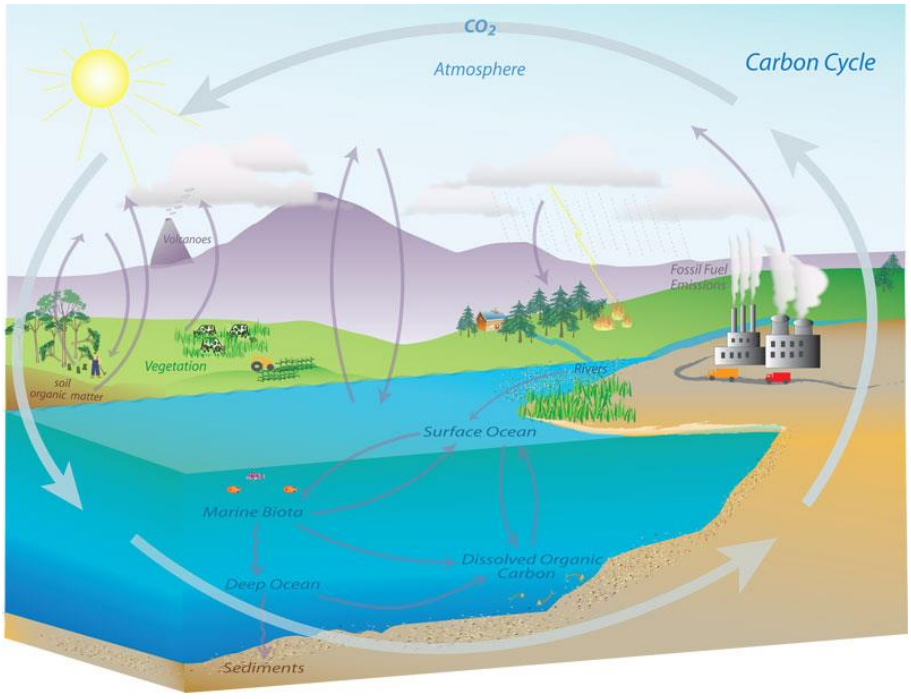

Figure 1 - The global C cycle from NOAA (National Oceanic and Atmospheric Administration 2019). The four carbon reservoirs are depicted. The dark arrows represent the interchange between reservoirs, the light arrows the all carbon cycle.

1.1.1. The increase in atmospheric CO_2

Humankind has been living on this planet for more than 2 million years, taking advantage of the resources present here. Since the beginning of the industrial revolution, a huge increase in greenhouse gas (GHG) emissions has occurred (Intergovernmental Panel on Climate Change 2014). Nowadays, the presence of climate change is an important topic not only for scientists, but also for political and economic decision makers (Intergovernmental Panel on Climate Change 2018). Nevertheless, the estimation of the precise contribution of humans to climate change is not an easily solvable issue. Among the hazardous gases emitted methane (CH_4), nitrite (NO_2^-) and carbonic anhydride (CO_2) can be enumerated.

Long-term monitoring of trends in atmospheric carbon dioxide are available from NOAA Earth System Research Laboratory, where the atmospheric CO_2 at Mauna Loa Observatory is regularly updated. While in 1959 the concentration of atmospheric CO_2 was about 316 ppm (Keeling et al. 1976), the present data report a concentration above 415 ppm (Earth System Research Laboratory 2019).

The atmospheric CO_2 concentration is one of the key drivers of climate change. Therefore, several ambitious projects have been started to face challenging questions about climate change research. An example is the 'Global carbon budget project', with its first report in 2003, which has been yearly updated since then (e.g. Le Quéré et al. 2018). To understand how the increase of CO_2 is affecting the global climate, not only estimations of carbon emitters (sources), but also carbon absorbers (sinks) are important (**Figure 2**). Flows of carbon between the above mentioned four reservoirs make the carbon cycle possible. For instance, plant biomass and photosynthesis take up CO_2 on land, while respiration and fossil emission release CO_2 in the atmosphere. Analogous processes take place also in the oceans, where phytplankton is responsible both for photosynthesis and respiration. On the one hand, part of the here produced biomass sinks in ocean sediments; on the other hand, bacterial respiration represents another source of carbon. Microbial respiration (source) is found also in soil, whereas fossil carbon (sink) can be here stored for ages. Any change in the cycle that shifts carbon out of one reservoir puts more carbon in the other reservoirs. Thus, the carbon cycle functions as long as carbon emissions (sources) and sequestrations (sinks) are in balance. Dramatic effect on climate change are registered, once fluxes are imbalanced. For example, changes that put carbon gases into the atmosphere result in warmer temperatures on Earth (Riebeek 2011).

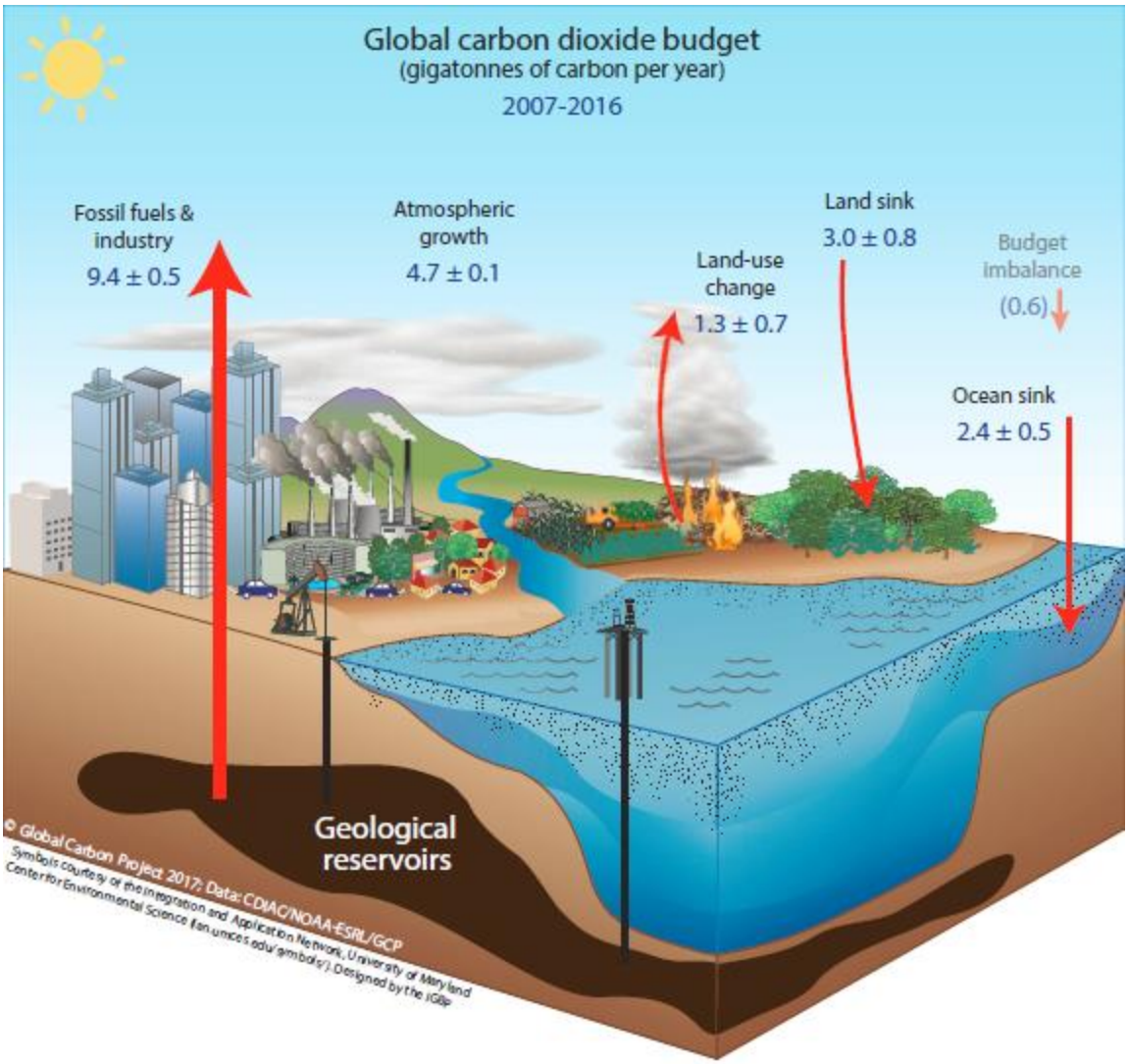

Figure 2 - Schematic representation of the overall perturbation of the global carbon cycle caused by anthropogenic activities, averaged globally for the decade 2007–2016. In the figure, the values represent the imbalance inherent the emission from fossil fuels and industry, emissions from deforestation and other land-use change, the growth rate in atmospheric CO_2 concentration and the uptake of carbon by the sinks in the ocean and land reservoirs (Le Quéré et al. 2018).

1.1.2. The terrestrial and marine ecosystems as carbon sinks

As represented in **Figure 2** the two largest sinks on Earth are the vegetation on land masses and the oceans.

It is well known that vegetation on Earth is an important reservoir to absorb CO_2, through photosynthesis. During this process, plants use energy from sunlight to fix CO_2 from the atmosphere to create biomass. In so doing, carbon is sequestered from the atmosphere and stored in the plant structures. Plants are also able to perform the inverted reaction: namely, release CO_2 back to the atmosphere through respiration. The energy produced by photosynthesis can be stored in carbohydrate and used to gain energy thanks to respiration. Thus, carbon can be transferred between CO_2 and organic material through a very basic left right arrow reaction (shown in a simplified version in equation 1). Respectively, photosynthesis occurs when the reaction proceeds to the right, while respiration occurs when the reaction proceeds to the left.

$$6CO_2 + 6H_2O + energy \leftrightarrow C_6H_{12}O_6 + 6O_2 \quad (1)$$

Terrestrial plants are, however, not the only photosynthetic organisms present on Earth. Indeed, a significant percentage of the net global photosynthesis, almost half of it, is made up from marine phytoplankton (Bowler et al. 2009). Despite the small size of these microorganisms, phytoplankton affect the carbon cycle significantly. These organisms, also called primary producers, are key players in the marine ecosystem. They constitute the basis of the food web, with not only direct effects on feeding of marine predators, but indirectly also affecting humans on Earth. Indeed, marine phytoplankton is contributing to the absorbance of anthropogenically produced CO_2 through photosynthesis. The carbon sink of this organisms contributes to the deporting of carbon into the deep ocean, where it can be stored for a long time.

Oceans are the major sink of carbon on Earth (Raven and Falkowski 1999), but the efficiency of the ‘biological pump’ is very much dependent on the relative proportion of organic and inorganic carbon leaving the surface mixed layer, which is controlled by ecological, biogeochemical and physical factors. Specifically, the ‘physical pump’ (see paragraph 1.2.1), contributes to maintain an equilibrium between CO_2 in atmosphere and oceans.

However, with increasing anthropogenic CO_2 emissions, the atmospheric CO_2 invades the oceans causing a decrease of seawater pH, a process called "ocean acidification." The lowered pH, but also the concomitant changes in other properties of the carbonate system, affects marine life and the cycling of carbon in the ocean (Wolf-Gladrow and Rost 2014).

The importance of the topic led to massive effort to quantify the strength of the oceanic sink for anthropogenic CO_2 (Sabine et al. 2004). Interestingly, comparing the beginning of this century with the 90s, even an increase in the ocean CO_2 uptake was shown in a recent publication (Gruber et al. 2019).

1.2. The Southern Ocean

1.2.1. Carbon pumps in the Southern Ocean

70% of the Earth is covered by water, and the Southern Ocean (SO) alone accounts for 10% of the entire global ocean, while it is responsible for about 40% of the entire anthropogenic carbon uptake (Landschützer et al. 2015). This uptake is possible thanks to the 'ocean carbon pump', defined for the first time in 1985 by Volk and Hoffert. Two main components of this pump are described: a physical-chemical and a biological one. The former, also called solubility pump, is a response to solubility differences of CO_2 in warm and cold water (Levy et al. 2013). The latter enables the transfer of organic carbon from the surface to the deep ocean (Honjo et al. 2014). Phytoplankton constitute the engine of the 'biological pump' (Chisholm 2000, **Figure 3**).

The efficiency of the carbon pump and the consequent carbon sequestration to the deep ocean depends on the physical characteristics of the SO such as water temperature, extent of sea ice cover, wind speed, stratification, changes in nutrient dynamics, pH, light conditions, and salinity of surface waters. Climate-induced changes in the physical characteristics of the SO and the responses by phytoplankton differ substantially among environments (Turner et al. 2014; Deppeler and Davidson 2017).

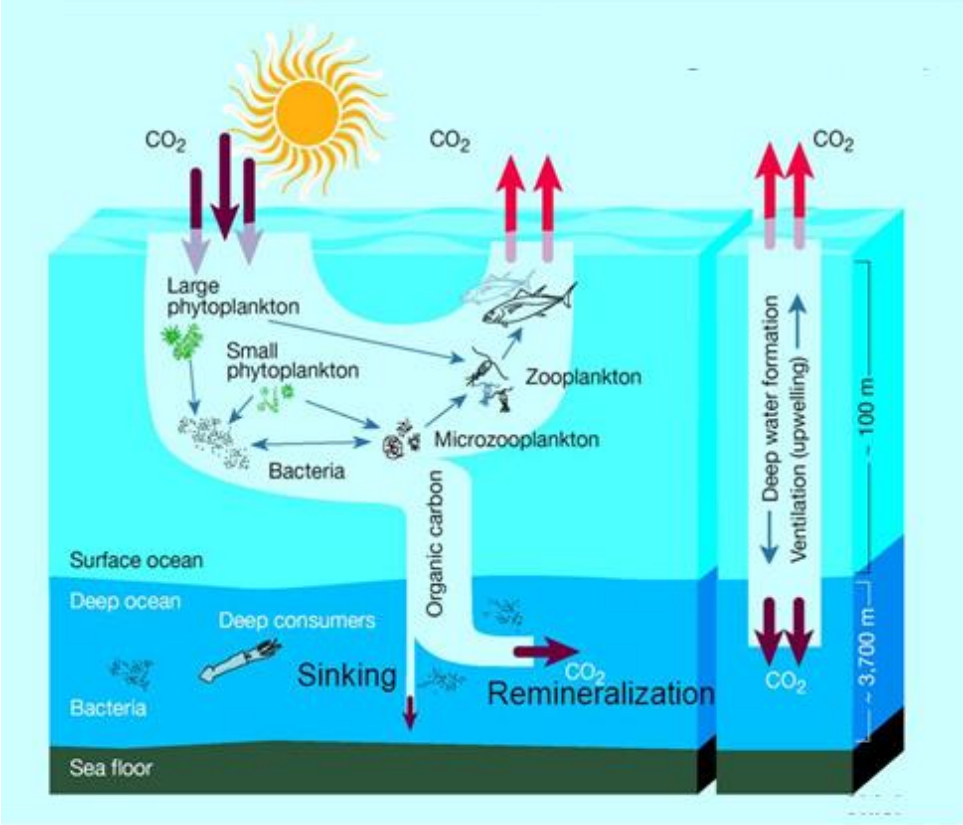

Figure 3 - The biological (on the left) and solubility (on the right) pump from Chisholm (2000).

1.2.2. Seasonal variations in the Southern Ocean

In the Southern Ocean, apart from climate change, seasonality plays an important role in affecting physical, chemical and biological factors. Light radiation is not constant during the year, but depends critically on the solar cycle. Seasonal snow and ice-cover periodically block sunlight from reaching polar ecosystems (Clark et al. 2013). Since photosynthesis is a light driven reaction, the irradiance represents a crucial factor in primary production. Sea ice itself constitutes one of the major factors controlling primary productivity (Smith and Comiso 2008). Not all organisms can survive in such a harsh environment, but some can exceptionally thrive here, as confirmed by the presence of ice algae (Thomas and Dieckmann 2002). The extent of Antarctic sea ice is extremely variable (Cavalieri and Parkinson 2008) and this is not only affecting light levels, which are extremely low in the ice matrix, but also temperatures and salinities, as depicted in **Figure 4**.

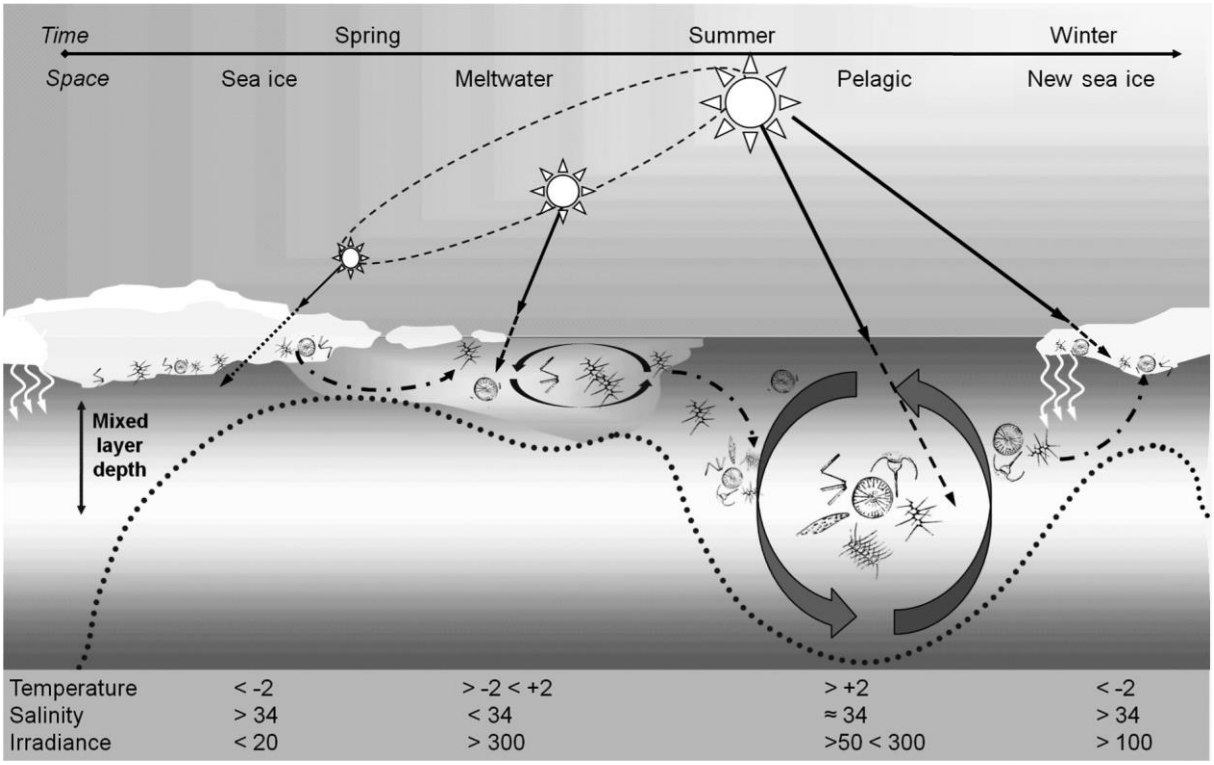

Figure 4 - Spatial and temporal evolution and decay of sea ice in the Antarctic marine ecosystem from Petrou and Ralph (2011). Temperature (°C), salinity (PSU) and irradiance (in µmol photons m^{-2} s^{-1}) properties for each environment are tabulated.

The lowest temperatures are found in brine channels ranging from -2 to below -20 °C. Sea surface temperatures in the SO, instead, range from 5 to -1.86 °C (Gäbler-Schwarz 2009). The increasing temperature influences the natural Antarctic characteristics, causing reduced ice cover and a rapid freshening of Antarctic bottom water (Rintoul 2007). Organisms able to thrive in a certain range of temperatures and salinities might not survive severe changes, driven by climate change.

On the one hand, ocean surface salinities are relatively stable, varying from 33.5 to 34.9 Practical Salinity Unit (PSU) in the open water (Smith et al. 2005). On the other hand, high salinity fluctuations are measured inside the ice column, reaching up to 200 PSU (Cox and Weeks 1983).

Moreover, seasonal changes in open water area affect also the nutrient concentration in the SO. With the retreat of the sea ice, a rapid nutrient drawdown is observed, driven by the summer blooming of Antarctic phytoplankton (Arrigo et al. 1999).

Undoubtedly, SO phytoplankton is a major player in the global biogeochemical cycle of nutrients (Falkowski 1994; Litchman et al. 2015). The strong heterogeneity in the distribution and concentration of nutrients in the SO boosts the growth of multifaceted communities, which exhibits a plethora of diversified mechanisms to cope successfully with the changing conditions. In order to resolve the impact of these changes on the SO community, more studies about the SO phytoplankton assemblage, its key species, and the factors affect its formation, distribution and processes are required (Rizkallah Issak 2014; Boyd 2002).

Such an interesting and variable ecological niche deserves more attention, although the complexity of this ecosystem represents a challenge for the researchers. The remote position of the SO and methodological restrictions have impeded more experiments. Nevertheless, with modern techniques and international efforts, the limitations of working in the Antarctic continent can be overcome.

1.2.3. Southern Ocean and phytoplankton adaptation to iron limitation

The Southern Ocean is well known as the world's largest HNLC (=high nutrients, low chlorophyll) region. Although macronutrients like N and P are abundant there, the primary production is severely limited (Moore et al. 2013). The principal cause can be ascribed to iron limitation, controlling the structure of phytoplankton communities and the efficiency of the biological carbon pump (Petrou et al. 2016).

Iron is essential for redox-based reactions, which include photosynthesis, respiration, and nitrate and sulfur utilization by phytoplankton (Raven 2013). These reactions are indispensable for the vitality of photosynthetic cells. Therefore, algae have developed alternative strategies to efficiently use the available iron. Morrissey and Bowler reviewed the iron utilization in marine cyanobacteria and eukaryotic algae, stressing peculiarities of the two domains and the existence of species-specific variances (Morrissey and Bowler 2012). Diatoms, for example, developed different strategies. One option is constituted by the decrease of the concentration of iron-rich photosystem I and cytochrome b6f complex, but not of photosystem II, as described for the oceanic diatom *Thalassiosira oceanica*. In so doing, the cellular iron requirement of the oceanic diatom is markedly decreased, but not its photosynthetic rates (Strzepek and Harrison 2004).

The marine diatom *Phaeodactylum tricornutum*, instead, appears to use metabolic reconfigurations to acclimate to low iron levels (Allen et al. 2008). Processes carried out by components rich in iron, such as photosynthesis, mitochondrial electron transport, and nitrate assimilation, are down-regulated; while gene clusters encoding for components of iron capture and uptake mechanisms are upregulated. Another strategy to decrease iron requirements involves the cell size reduction, significantly reducing the functionality of the two photosystems and electronic transport rates between them, rather than affecting pigment production (Morrissey and Bowler 2012).

Antarctic species have to cope with dissolved iron concentration below 1 nM (Smetacek et al. 1997), reason why their iron requirements are generally low. Strzepek and colleagues were the first, who focused on the adaptive strategies specifically adopted by Southern Ocean phytoplankton (Strzepek et al. 2011). They identified two distinct adaptations to reduce iron limitation in Antarctic diatoms: firstly, increasing the flux of bioavailable iron, e.g. through siderophores; secondly, reducing the biochemical requirements for iron. Other algal classes were investigated, such as the representative haptophyte *Phaeocystis antarctica*. In this case, growth and bloom forming of *Phaeocystis* ability stopped under limiting iron levels. Following iron supplementation, a fast recovery in growth and productivity is recorded (Marchetti et al. 2012).

For over 30 years, the important role of iron in Southern Ocean phytoplankton has been discussed. "The iron hypothesis" suggested that iron deficiency is limiting the productivity in Southern Ocean. Hence, phytoplankton is unable to take advantage of the excess surface nitrate and phosphate that, if used, could result in total Southern Ocean increase in production (Martin 1990). To prove this hypothesis, iron fertilization experiments were conducted by adding iron compounds to the SO waters (Boyd et al. 2007; Baar et al. 2005). This artificial fertilization induced diatom-dominated phytoplankton blooms, accompanied by considerable carbon dioxide drawdown in the ocean surface layer. Although it was not possible to adequately resolve the fate of bloom biomass in these experiments, the authors postulated an increase of carbon fixation and export to the ocean bottom (Smetacek et al. 2012). Field studies have shown how iron concentration naturally affects the photophysiology of phytoplankton communities (Trimborn et al. 2015). *Ex situ* experiments have also investigated the photophysiology of natural phytoplankton assemblage, together with Fe and C uptake under iron limitation (Hoppe et al. 2013). The positive impact of iron repletion was observed also in single organisms during laboratory studies, both in diatoms (Petrou et al. 2014) and *P. antarctica* (Koch et al. 2019).

Nonetheless, due to their phylogenetic diversity, different phytoplankton taxa will likely respond in different ways (Collins et al. 2014). For instance, a decrease in the abundance of diatom is expected due to increased stratification in the future ocean (Bopp et al. 2005). Thus, it appears that some species are more sensitive than others, although more investigations are necessary. For ocean fertilization to become a viable option to sequester CO_2, more extensive and targeted fieldwork are required. Better mathematical models of ocean biogeochemical processes are needed, both to interpret field observations and to make reliable predictions about the side effects of large-scale fertilization (Lampitt et al. 2008).

Modifications in the marine carbon cycle are expected, due to rising temperatures, changes in nutrient availability and ocean acidification. The interplay betweeen these changing factors, as well as the extrapolation of their effects in the sequestration fluxes, represents one of the great challanges. A taxa-specific understanding of environmental controls of phytoplankton (Boyd et al. 2010), combined with a geographically and seasonally explicit understanding of food web structure and environmental changes, will be needed to gain a predictive understanding of the biological pump (Passow and Carlson 2012).

1.2.4. Key players in the Southern Ocean phytoplankton

Among the variety of species present in the SO, two taxa are extremely important for the biogeochemical cycle, namely diatoms and haptophytes. The former are primarily regulating the carbon and silicate cycle (Tréguer et al. 2018; Smetacek 1999), the latter the marine sulfur cycle (Schoemann et al. 2005). Significant taxon-specific differences exist in the photophysiology of the two clades. Studies reported differences in response to changes in irradiance (Arrigo et al. 2010; Kropuenske et al. 2010; Mills et al. 2010; van de Poll et al. 2011), iron limitation (Alderkamp et al. 2012) and ocean acidification (Trimborn et al. 2017; Beszteri et al. 2018). According to these studies, the haptophyta *P. antarctica* efficiently used light under low irradiance levels, while the diatoms *Fragilariopsis cylindrus* and *Chaetoceros brevis*, were better protected from photoinhibition under high light levels. The ecological niche occupancy might explain the characteristic photoacclimation strategies of the two taxonomic groups (Petrou et al. 2016). While *P. antarctica* thrives in the deeply mixed water column, *F. cylindrus* prospers in the shallow mixed layer depth.

The productivity and species composition of Antarctic phytoplankton community was investigated also by Heiden et al. (2019). In this case, the impact of ocean acidification and high solar radiation was examined, showing that *P. antarctica* might increase its competitiveness toward diatoms under ocean acidification, OA, irrespective of light availability.

Not only spatial variances, but also seasonality (reviewed in 1.2.21.2.2) are playing a role in the success of one taxa over the other. As depicted in **Figure 5**, during the austral spring (September-November), the ice melts and the nutrients are used up. With the beginning of the summer (December) open water rises, together with primary productivity, to decrease again at the end of the season. Following those changes, more adaptable species can outcompete the others, taking advantage of more suitable characteristics, e.g. in nutrient utilization, light and temperature acclimations.

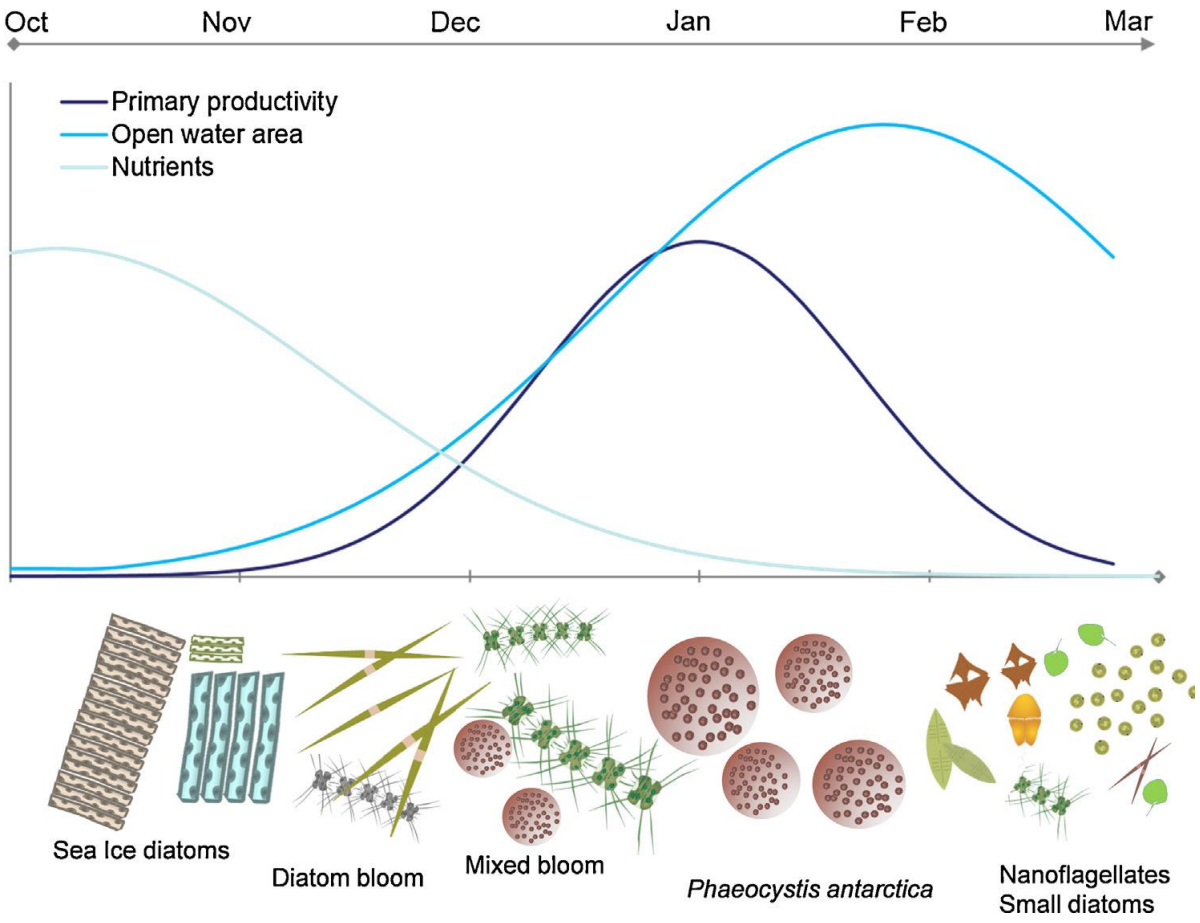

Figure 5 - Seasonal variations in phytoplankton primary productivity, open water area and nutrient concentration from Petrou et al. (2016).

The whole picture becomes more complicated when the concomitant effects of other parameters, such as iron and CO_2, are taken in consideration. Shifts in the phytoplankton community composition are detected (Feng et al. 2010), affecting not only the community structure itself, but also important biogeochemical parameters of this region. More experiments are needed to unravel the mechanisms driving such changes. Furthermore, the plasticity of the autochthonous species should not be taken for granted, while climate change threatens the equilibrium of the ecosystem.

1.2.5. Climate change effects on Southern Ocean phytoplankton

In recent decades, the oceans have undergone large physical and biogeochemical modifications in response to human induced global change, as revealed by a variety of in situ and remote sensing observations (Bopp et al. 2013). These changes encompass ocean surface warming, changes in ocean salinity, modifications of water density structure and stratification, as well as an increase in dissolved inorganic carbon concentrations and a decrease in seawater pH in response to ocean uptake of anthropogenic carbon (Doney 2010). Due to global warming and increased sea ice melt (**Figure 6a** and **Figure 7**), the SO surface waters are becoming warmer and fresh (Hauck et al. 2015). Globally, sea surface temperature has increased by about +0.7 °C over the last 100 years and sea surface pH has declined by about 0.1 pH unit since pre-industrial times (Bindoff et al. 2007).

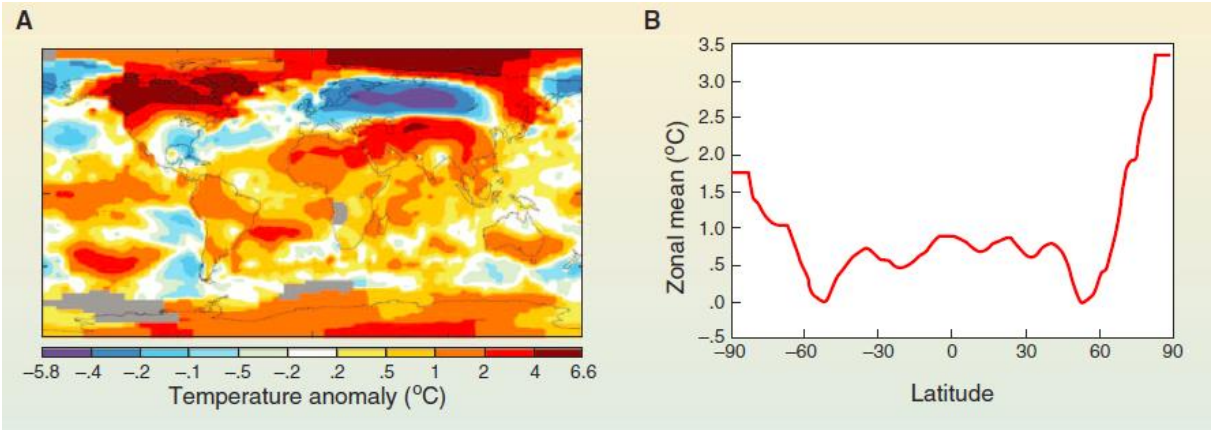

Figure 6 - Recent changes in ocean temperature. (A) Surface temperature anomaly for January 2010 relative to the mean for 1951–1980. (B) The same data presented in (A) as a function of latitude, marking the dramatic increase of temperatures at the poles [Credits: NASA Goddard Institute for Space Studies].

Some of the most striking impacts of global climate change have appeared in polar oceans, where temperatures and acidities are changing at more than twice the global average (Hoegh-Guldberg and Bruno 2010, **Figure 6b**). The physical and chemical modifications that are occurring have the potential to affect marine organisms and ecosystems; large shifts in species size, spatial range, and seasonality of primary production have been registered (Doney et al. 2012). Life cycle and physiological requirements of many polar organisms are closely tied to the annual cycles of sea ice and available sunlight (see paragraph 1.2.2). Model projections reveal that greater light availability caused by a reduction in sea ice may increase open-water phytoplankton primary production (Arrigo et al. 2008; Steinacher et al. 2010).

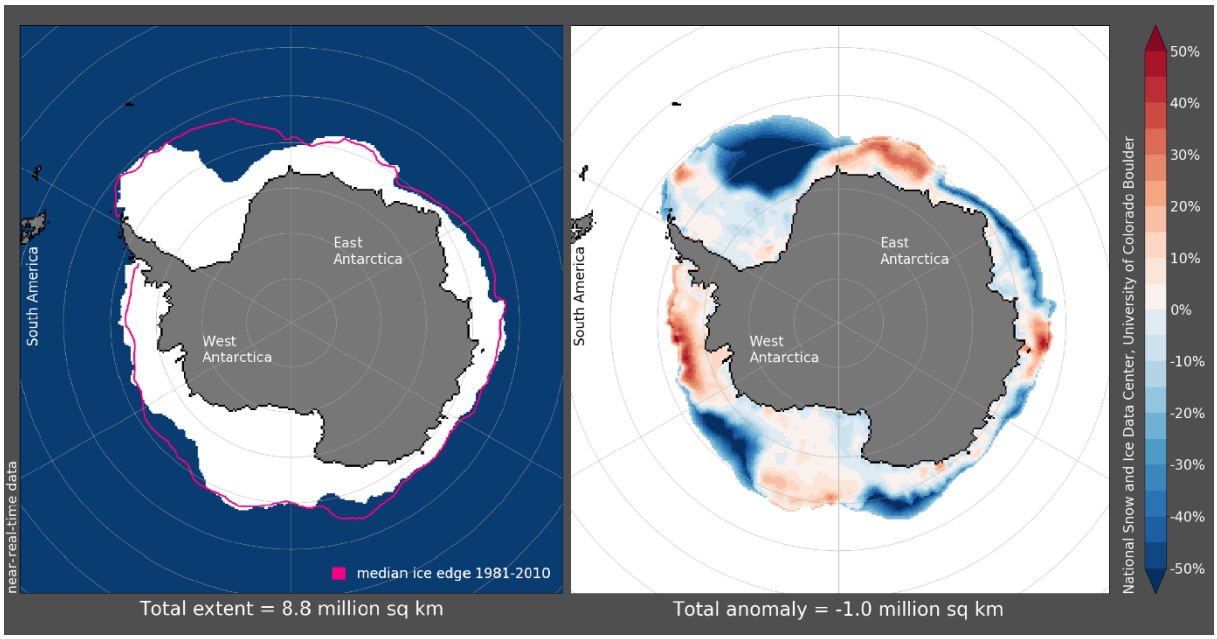

Figure 7 - Sea Ice Index and Sea Ice Concentration anomalies in May 2019. The Total anomaly in the right figure represents the lessening of the Sea Ice Concentration. The monthly Sea Ice Index provides a quick look at Antarctic-wide changes in sea ice. It is a source for consistently processed ice extent and concentration images and data values since 1979. Monthly images show sea ice extent with an outline of the 30-year (1981-2010) median extent for that month (magenta line).

Polar marine ecosystems are intimately tied to sea-ice extent and seawater temperatures, which together influence food sources, organism growth and reproduction, and biogeochemical cycles. Some organisms may be able to acclimate to temperature changes, others might perish due to physiological intolerance to new environments. A southward retreat of winter sea ice (**Figure 7**) will diminish the areal extent of dense, shallow phytoplankton blooms and increase that of deep blooms with ramifying effects on biogeochemical cycles and grazer populations (Smetacek and Nicol 2005).

Apart temperature and salinity, nutrients plays an important role in primary production. SO is severely limited by iron (see paragraph 1.2.3), therefore productive regions tend to be restricted to the Antarctic continental margin and only extend offshore where water enriched with iron from land contact or from upwelling along shelves and continental slopes mixes with oceanic water impoverished in iron (Smetacek and Nicol 2005). Though, not only presence or absence of iron is critical for phytoplanktonic growth, but also the iron bioavailability. Trimborn et al. (2017) investigated the impact of ocean acidification on iron bioavailability in Antarctic phytoplankton species, demonstrating that different iron-species affect species composition. The altered response of phytoplankton has important implications for future biological CO_2 sequestration by the SO.

Moreover, it is not only the autochthon Antarctic community where changes are occurring, but also in non-native species, which are colonizing the remote continent. Climate-driven biological invasion in Antarctica has been extensively reviewed (Hughes et al. 2015; McCarthy et al. 2019). This phenomenon alters further the community structure, changing the species interactions and causing a decline in diversity.

1.3. Net primary production

The carbon cycle (see paragraph 1.1) is critically dependent on net primary production. Net primary productivity (NPP) is defined as the difference between photosynthesis, P (also GPP for gross primary production) and community respiration, R (equation 2).

$$NPP = GPP - R \quad (2)$$

While GPP relies only on phototrophs organisms, which are able to fix organic carbon through photosynthesis, the R contribution could be autotrophic or heterotrophic. The exact contribution of phototroph organism in NPP represents a critical question. The so-called primary producers are fundamental for the functioning of the biological carbon pump (reviewed in 1.2.1), leading ultimately to fish production and sustained oxygen levels in the atmosphere (Quay et al. 2010). Among the many species here present, phytoplankton represents the bottom of the food chain in the Antarctic food web, as depicted in **Figure 8**. Therefore, phytoplankton contribution is crucial for the estimation of primary production.

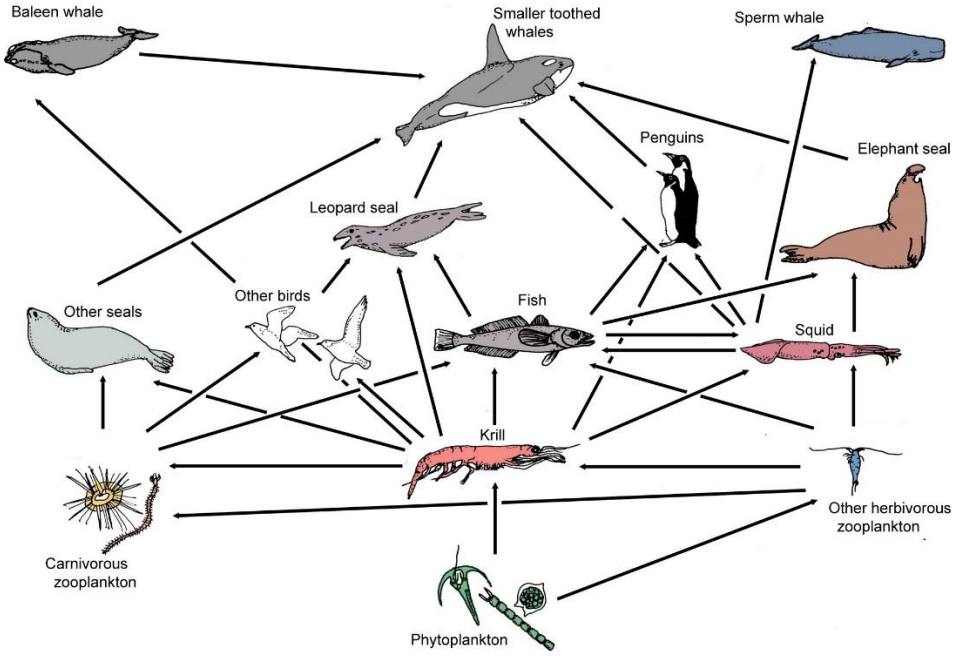

Figure 8 - The Antarctic food web from Cool Antarctica website (Antarctic Ocean Food Web).

1.3.1. Measuring primary production

Over the last 50 years, ^{14}C bottle incubations (Stemann Nielsen 1952) have been the benchmark for aquatic primary production estimation. This method employs the incorporation of the radiotracer ^{14}C, to determine the carbon fixation in the field. The main advantage is represented by the sensitivity of this technique. In addition, extensive positive results for primary production are always obtained, allowing the collection of large data sets. Shortly after its introduction, the validity of this method was questioned (Peterson 1980). The main problem remains the ambiguity about the measurement of net or gross production. Despite the fact that methodological uncertainties were acknowledged, ^{14}C incubations still dominate our understanding of primary production in the ocean (Quay et al. 2010).

Meanwhile, other means to measure oceanic production have been developed, such as measurements of chlorophyll fluorescence variables or the isotopic composition of surface water (Marra 2009). The big drawback of chlorophyll-based model is that they require knowledge of the ratio carbon to chlorophyll *a* (C/Chla), but this ratio is under stringent physiological and thus environmental control (Geider et al. 1997).

Fluorescence can be induced by both solar radiation and artificial illumination. On the one hand, its measurement is a non-invasive method, permitting high temporal sampling rates, closely matched to sampling rates for physical variables (e.g., temperature, salinity, oxygen, etc.). On the other hand, fluorescence methods are not able to measure alternative electron transports and are dependent on variable nutrient conditions and irradiance levels (Kolber and Falkowski 1993), influencing the quantum yield of fluorescence and the absorption cross section especially in fieldwork.

Moreover, advanced optics and remote sense techniques are now available to estimate ocean color. In this way, no sampling is required for the calculations of primary production. More specifically, the selective absorption of the photosynthetic pigment chlorophyll *a* can be used as a proxy for the quantification of phytoplanktonic biomass, based on satellites-derived measurements (Falkowski et al. 1998). However, these estimations still rely on ^{14}C uptake for the validating algorithms to convert algal biomass into productivity estimates (Carr et al. 2006). Certainly, without meaningful comparisons of different methods, precise information about phytoplankton variability, both in physiological parameters and species composition, cannot be deduced.

Despite the importance of the topic, measuring rates of marine primary production (PP) remain elusive since there is no absolute PP standard against which methodological accuracy can be tested (Quay et al. 2010).

1.3.2. Respiration: an unknown factor

As mentioned above, ^{14}C assimilation would always give positive results, although in natural marine communities the net production can be either positive or negative, due to the effects of different factors. Firstly, both autotrophic and heterotrophic organisms are present in the oceans. Generally, the first are photosynthesizing, converting CO_2 into biomass, while the second are respiring. This process is light dependent, so it takes place only during the daytime. During the night, no carbon assimilation, through photosynthesis, but a respiratory carbon release occurs. If primary production is measured only considering positive values, respiration is not necessarily taken in account (negative values are not measured). Indeed, one point of criticism of the ^{14}C method consist in its doubtful capability to distinguish between net and gross productivity (see paragraph 1.3.1).

Secondly, respiration can be affected by stressful conditions and the equilibrium between photosynthesis and respiration varies not only with changing light regimes and nutrients availability (Geider and Osborne 1989), but also with temperature (Regaudie-de-Gioux and Duarte 2012).

Even before ^{14}C was used, the light-dark oxygen method constituted a standard approach for measuring photosynthesis in aquatic systems. The accumulation of oxygen in clear container (light bottle) represents NPP by the enclosed community, and the consumption of oxygen in a dark bottle is a measure of respiration. GPP is estimated by subtracting the dark bottle result from the light bottle result (Cullen 2001). Thus, respiration in the light and in the dark are assumed to be equal. Nonetheless, this assumption does not generally hold, so errors in estimation of the respiratory component of GPP occurred (Geider and Osborne 1992). Furthermore, some of the respiration attributed to phytoplankton may be bacterial, or the phytoplankton population may increase in the light bottle during the experimental time, but not in dark bottle. Additionally, one recurring concern using ^{14}C method is the effect of the incubation container on metabolic processes, the so called 'bottle effect.' Bottle effects may be apparent over long incubation times and are thought to arise from contamination from the vessel walls, loss of turbulence, or damage to organisms (Marra 2009). In this way, the vessels are affecting the measurements, giving erroneous results.

Methods based on direct oxygen measurements are less sensitive than techniques using the isotopic tracer ^{14}C. However, careful implementation of procedures using automated titration or pulsed oxygen electrodes can yield useful and reliable data, even from oligotrophic waters of the open ocean (Cullen 2001).

There are relatively few data sets in the literature for phytoplankton respiration over a range of growth conditions, for example mostly limited to a modest range of intensity from about 50–200 μmol quanta m^{-2} s^{-1}, so the fidelity of respiration models may not be robust over the full range of light intensity that is relevant to phytoplankton communities. Progress has been made in the last decades, but respiration remains the biggest unknown factor in our understanding of the C budget of the ocean (Moisan and Mitchell 2018).

1.3.3. State of the art about Photosynthesis to Respiration ratio (rP/R)

The photosynthesis to respiration ratio is defined as the ratio of the net oxygen produced by photosynthesis (P), to oxygen release in total ecosystem respiration (R). Since P and R are both sensitive to various types of stress (as described above, paragraph 1.3.2), this ratio varies not only according to the day : night rhythm, but also in response to variations in the abiotic and biotic conditions.

In 1954, Ryther was the first to indicate the importance of the photosynthesis to respiration ratio in evaluating the significance of productivity measurements (Ryther 1955). In his paper, some important points about this ratio were underlined. Firstly, the loss of activity through respiration is extremely variable and, hence, a constant correction factor cannot be applied. Secondly, the respiration : photosynthesis ratio is dependent not only upon light, but equally as well upon any other factors which have a different effect upon the two processes, for example nutrient depletion. Additionally, he recognized that in measuring photosynthesis by ^{14}C uptake, a considerable loss of photosynthetic activity may incur through respiration. The magnitude of this error, introduced by the respiratory loss of ^{14}C, is proportional to the ratio of respiration to photosynthesis. In this paper, rP/R was investigated over days, reporting values between 1 and 12 in a nutrient-deficient pure culture of marine *Chlamydomonas*. Ryther underlined that, during exponential growth of *Chlamydomonas*, respiration is 5-10% of photosynthesis, but in nutrient starved, non-growing, cultures respiration may equal photosynthesis.

In the 70s, systematic work was done to measure photosynthesis, respiration and growth rates, searching for a relationship valid for all algal species. However, this approach was soon recognized as unsuccessful, since the rP/R differs between taxa and species. In 1975, Humphrey reported an example of the species-variability of this ratio, culturing eleven unicellular algae (Humphrey 1975). The lowest rP/R observed was equal to 3.5, the highest 18.1, for *Amphidinium carterae* and *Monochrysis lutheri*, respectively. Similar results were obtained by Burris, who measured ratios of photosynthesis to respiration ranging from 1.3 to 10.3 in eight different species of algae (Burris 1977).

The importance of the topic lead to studies also at the beginning of this century, when Vona and colleagues compared photosynthesis and respiration of three different species of algae, i.e. *Koliella antarctica*, *Chorella saccarophila* and *Chorella sorokiniana* (Vona et al. 2004). The first two are cryophilic algae, while the third is a mesophilic alga. Once more, a great variability in rP/R was measured, as showed in **Table 1**. Specifically, P/R ratio ranged between 3 and 7 in *K. antarctica* and *C. saccarophila* (precisely, 3.4 to 7.1 the former and 3.1 to 4.7 the latter), whereas *C. sorokiniana* reached, surprisingly, values above 50. This huge diversity in rP/R was not only species-specific, but also temperature dependent, confirming the impossibility of considering this value constant, as mentioned already 50 years before.

	rP/R		
T (°C)	*Koliella antarctica*	*Chorella saccarophila*	*Chorella sorokiniana*
5	5.2	3.7	n.d.
10	5.9	4.3	57.3
15	7.1	3.6	53.5
20	5.9	3.1	38.9
25	6.6	3.5	32.1
30	5.6	3.2	35.2
35	3.4	4.7	21.3

Table 1 - Temperature dependence of photosynthesis : respiration ratio (rP/R) in *Koliella antarctica*, *Chorella saccarophila* and *Chorella sorokiniana* (n.d. = not determined). P/R ratios were calculated from gross photosynthesis and respiration data reported in Vona et al. (2004).

The temperature dependency of P and R is surely an important parameter, together with other important factors that need to be taken in account, such as seasonality. Water temperatures change in different seasons, having a strong effect on metabolic rates. Furthermore, warm ocean waters are typically prevalent in oligotrophic regions of the oceans, whereas cold waters are generally more productive, supporting relative higher plankton biomass, as is evident from the examination of the correspondence between global maps of chlorophyll *a* and temperature in the ocean (Regaudie-de-Gioux and Duarte 2012). Hence, rP/R depend also on the site or region chosen for experiments.

Due to the remote position and the harsh environment, polar regions present more methodological limitations than temperate regions. During Austral winter, temperatures decrease dramatically in the Antarctic and ice formation prevents the feasibility of some experiments. Therefore, it is not surprising than only few studies focus specifically on PP and R in the Southern Ocean and predominantly during Austral summer (Arístegui et al. 1996).

It might be argued that there is no primary production in winter anyway, but as long as *in situ* measurements prove the opposite, we cannot exclude it.

In addition, it was observed that the comparison of estimates of marine phytoplankton primary production derived from different methods reveals very large variations (Regaudie-de-Gioux et al. 2014). Generally, comparisons among the methods are limited and only very few studies compared also *in situ* and *in vitro* methods (Corno et al. 2006; Robinson et al. 2009).

1.4. Aim of the Thesis

Global climate change is believed to influence tremendously the dynamics ruling the environmental conditions, affecting important parameters, e.g. water temperature, salinity, pH value, and nutrient supply. The acclimation of the phytoplankton communities to these changes will lead to significant modifications in important metabolic processes, such as photosynthesis and respiration. Therefore, understanding how climate change influences the ratio of photosynthesis to respiration (rP/R) has become highly important for the estimation of the global carbon balance. Measurements of photosynthetic and respiration rates are spatially and temporally constrained, especially in harsh environments like Antarctica. While some studies investigated the photosynthetic activity of Antarctic phytoplankton (Palmisano et al. 1987; Petrou and Ralph 2011), knowledge about the carbon losses due to respiration is very scarce, principally due to methodological limitations (Marra 2009; Moisan and Mitchell 2018). Data presented in the literature show a high variability in the ratio between photosynthesis and respiration, proving the ratio is species–specific and temperature dependent (Vona et al. 2004). Additionally, other factors such as nutrient availability (e.g. iron) are expected to influence the rP/R and consequently, the primary productivity.

Therefore, the aim of this thesis was firstly to retrieve experimental data on the variability of rP/R in ecological Antarctic phytoplankton species under different conditions; secondly, to estimate the quantitative effect of rP/R variations on integrated net primary production rate. The range of variability of rP/R in response to different growth conditions was investigated, testing this basic hypothesis: the P/R ratio varies as a function of temperature and nutrient availability in an ecotype-dependent manner.

In this respect, two key species from the Southern Ocean were investigated: the diatom *Chaetoceros* sp. and the prymnesiophyte *Phaeocystis antarctica*. Both diatoms and *P. antarctica* are ubiquitous in the Southern Ocean, often forming large blooms around much of the Antarctic continent (Kropuenske et al. 2009). The former is a typical psychrophilic species, able to thrive in the sea ice with cold and high saline water but also in meltwater (Thomas et al. 1992; Thomas and Dieckmann 2002). The latter has been observed in both deep and shallow mixed layers in the Southern Ocean (Arrigo et al. 2010).

Laboratory-based methods were employed to measure photosynthetic and respiration rates, fluorescence and cell parameters, from two ecologically relevant species of the Southern Ocean.

More specifically, the effect of temperature, salt concentration and iron limitation were examined, analysing the following hypotheses:

1. P/R ratios are temperature dependent in a way that the daily carbon assimilation rate is significantly influenced.

Photosynthetic and respiration rates were derived from P-I curves measured by O_2 gas exchange methods (Clark electrode/oxygen-sensitive optode). In addition, PAM-fluorescence and FRRf were used to measure electron transport rates and to estimate the possible contribution of alternative electron transport under different growth conditions. In this way, information about the physiological status of the cells in dependence on three different temperatures (i.e. -1, +1, +4 °C) were collected.

2. Temperature dependency of rP/R persists under different salinity conditions.

This was tested by using 4 different salinity concentrations from 20, 35, 50 and 70 PSU in *Chaetoceros* sp. and two *P. antarctica* strains.

Salinity tolerance in different isolates of *P. antarctica* was previously tested, verifying that no genetically, but geographical-based response occurred (Gäbler-Schwarz 2009; Gäbler-Schwarz et al. 2015).

3. rP/R remains constant under iron-limiting conditions

For the first time, a combination of chlorophyll *a* fluorescence, O_2 evolution and particulate organic carbon production measurements were used to understand the effect of low iron availability on the usage of photosynthetic electrons in cell metabolism and finally in carbon production in *Chaetoceros* sp.

Key physiological parameters under three different temperatures in combination with four different salinities were coupled with estimation of the quantitative effect of rP/R variations on integrated net primary production rates.

2. Material and Methods

The experimental work of this thesis was mainly conducted in the Plant physiology laboratory of the University of Leipzig, under the supervision of Prof. Dr. Christian Wilhelm and Dr. Torsten Jakob. Here, the effect of temperature and salinity on the rP/R variability were investigated, whereas the influence of iron limitation on this ratio was examined at the Alfred Wegener Institute (AWI, Bremerhaven) in the 'EcoTrace' group, under the supervision of Prof. Dr. Scarlett Trimborn.

2.1. Temperature and salinity

2.1.1. Culture condition

Cultures of the Antarctic diatom *Chaetoceros* sp. and two strains of the Haptophyte *Phaeocystis antarctica* were obtained from Dr. Steffi Gäbler-Schwarz (AWI Bremerhaven, Germany). The *Phaeocystis* strains were sampled and isolated on RV Polarstern cruises and at an Antarctic research station between 2005 and 2007 (Gäbler-Schwarz et al. 2015), whereby strains 109_27 and 764_48 were isolated from the Lazarev Sea (ANT XXIII-2) and from Prydz Bay (ANT XXIII-9), respectively. All cultures were grown in GP5 Medium (Loeblich and Smith 1968), modified in this study with respect to the use of marine salt instead of seawater. The cultures were maintained in polystyrene culture flasks with filter screw caps (Carl Roth) in a climate chamber (Economic Lux Chamber, Snijders Labs) under low-light conditions (10 µmol photons m^{-2} s^{-1}; 16:8 hours light-dark cycle). The cultures were used for experiments in their exponential growth period between 6 and 10 days post inoculation. The number of replicates (n) given in the results section is equivalent to the number of biological replicates (a detailed listing of the number of replicates is presented in **Table 2**). Since the measurements of oxygen evolution rates were characterized by a relatively low signal-to-noise ratio, the number of biological replicates for this type of measurements was expanded up to n = 11 to increase the statistical relevance. The number of replicates (n) given in the results section is equivalent to the number of biological replicates.

		Temperature								
		-1 °C			+ 1 °C			+ 4 °C		
Parameters	Salinity	*C. sp.*	*P.a. 109*	*P.a. 764*	*C. sp.*	*P.a. 109*	*P.a. 764*	*C. sp.*	*P.a. 109*	*P.a. 764*
P_{max}	20	X	X	X	8	11	7	10	6	4
	35	7	5	5	8	7	8	9	5	4
	50	8	5	5	7	5	5	12	6	5
	70	7	n.d.	n.d.	X	X	X	X	X	X
R	20	X	X	X	8	11	7	10	6	4
	35	7	5	5	8	7	8	9	5	4
	50	8	5	5	7	5	5	12	6	5
	70	7	n.d.	n.d.	X	X	X	X	X	X
rP/R	20	X	X	X	8	11	7	10	6	4
	35	7	5	5	8	7	8	9	5	4
	50	8	5	5	5	5	5	11	6	5
	70	7	n.d.	n.d.	X	X	X	X	X	X
NPQ	20	X	X	X	8	11	7	10	6	4
	35	7	5	5	8	7	8	9	5	4
	50	8	5	5	7	5	5	10	6	5
	70	7	n.d.	n.d.	X	X	X	X	X	X
P_F / P_O	20	X	X	X	8	10	7	10	6	4
	35	7	4	5	8	7	7	9	5	4
	50	6	5	5	7	5	6	10	6	5
	70	n.d.	n.d.	n.d.	X	X	X	X	X	X
a^*_{phy}	20	X	X	X	3	3	3	3	3	3
	35	3	3	3	3	3	3	3	3	3
	50	3	3	3	3	3	3	3	3	3
	70	n.d.	n.d.	n.d.	X	X	X	X	X	X

Table 2 - Temperature and salinity dependence of gross Photosynthesis (P_{max}), Respiration (R), Photosynthesis to Respiration ratio (rP/R), Non Photoyhemical Quenching (NPQ_{max}), fluorescence-based to oxygen-based Photosynthetic rates (P_f/P_o) and Chla-specific *in vivo*-absorption coefficient (*a*phy*) in *Chaetoceros* sp., *Phaeocystis antarctica* strain 109 and 764, (nd = not determined).

Three different temperature treatments were applied, namely -1, +1, and +4 °C (± 0.5 °C), in combination with different salinities of the growth medium: 20, 35, 50, and 70 practical salinity units (PSU; **Table 2**). More precisely, growth temperature of -1 °C was combined with salinities of 35, 50, and 70 PSU whereas growth temperatures of 1 and 4 °C were combined with salinities of 20, 35, and 50 PSU, respectively. The combinations of 20 PSU at -1 °C and 70 PSU at 1 or 4 °C were omitted since they are practically impossible to realize. A salinity well below 35 PSU can be found only in regions with melting sea ice (T > 0 °C), whereas salinities as high as 70 PSU can be reached only in the brine channels of sea ice (T < 0 °C). The salinity of the medium was adjusted by the addition of the respective amount of marine salt. Depending on the growth rates of the cultures under the different experimental conditions, the cultures were acclimated for a period of at least two weeks (usually four weeks) to the new condition before starting physiological measurements.

2.1.2. Chlorophyll *a* determination

Chlorophyll *a* (Chla) concentrations were determined spectrophotometrically by extraction with 90% acetone according to the protocol from Jeffrey and Humphrey (1975). Algal samples (5 mL) were collected on glass-fiber filters, 2.5 mL acetone was added, and cells were broken in a cell homogenizer (Precellys Evolution, Bertin Technology, France). After centrifugation (2 min, 12.500 x g, Sigma 1-14, Sigma, Germany), absorbance of the pigment extract was measured with a spectrophotometer (Hitachi U2000, Tokyo, Japan) at 664 and 630 nm.

2.1.3. Measurements of photosynthesis rates and variable chlorophyll fluorescence

Oxygen-based (P_O) and fluorescence-based (P_F) photosynthesis rates were measured and calculated as described in detail in Wagner et al. (2006). Essentially, oxygen evolution and variable Chlorophyll (Chl) fluorescence were measured by light-irradiance curves (P-E curves) in a so-called Light pipette equipped with a special cuvette (Topgallant LLC, Salt Lake City, UT, USA). A 3-ml aliquot of cells (equals a Chla concentration of 4 – 6 µg mL^{-1}) from each experimental condition was transferred into the cuvette and maintained at the respective growth temperature under continuous stirring in darkness for 5 min. For P-E curves, six actinic light levels (21, 50, 107, 207, 415, 713 µmol photons m^{-2} s^{-1}) were applied for 4 min each. These light periods alternated with dark periods of 4 min length each. Measurements of P-E curves always started with an initial 4-min dark period. Oxygen evolution was measured using a Clark-type electrode (MI 730, Microelectrodes Inc., NH, USA). For the calculation of PO (µmol O_2 [mg Chla]$^{-1}$ h^{-1}) the oxygen solubility in dependence of the medium salinity and the measuring temperature (Benson and Krause 1984) was taken into account. Net oxygen evolution and dark respiration rates were derived from the average oxygen evolution rates measured during the last minute of each light and dark period, respectively. Gross oxygen production was derived by correcting net oxygen evolution rates for the corresponding dark respiration (R; µmol O_2 [mg Chla]$^{-1}$ h^{-1}) measured after the respective light periods. It should be noted that no enhanced post-illumination respiration (Beardall et al. 1994) was observed in the measurements.

Moreover, the respiration rates showed very little variability with respect to the preceding irradiance levels.

The ratio photosynthesis/respiration (rP/R) was derived from the maximum value of photosynthesis (Pmax) divided by the mean value of all respiration rates measured within a specific P-E curve.

In parallel with oxygen evolution, the variable Chl fluorescence parameters were determined, whereby F_o and F_m are the minimum and maximum fluorescence in darkness, respectively, and F and F_m' are the steady-state minimum and maximum fluorescence under actinic illumination, respectively. Fluorescence-based photosynthesis rates (µmol O_2 [mg Chla]$^{-1}$ h^{-1}) were estimated as:

$$P_F = \Phi_{PSII} \times Q_{phar} \times 0.5 \times 0.25/(d \times Chl) \quad (3)$$

where Φ_{PSII} is the effective quantum yield of PSII (Genty et al. 1989), Q_{phar} is the amount of absorbed radiation (Wagner et al. 2006), *d* is the optical path length of the measuring cuvette, and Chl is the Chla concentration of the algal suspension. The factors 0.5 and 0.25 are based on the assumption that the linear transport of one electron requires two quanta and that four electrons are required for the evolution of one molecule of oxygen, respectively. It is thus assumed that PF represents the maximum amount of electrons (expressed as oxygen equivalents) transported through the electron transport chain, whereas P_O is the oxygen evolution rate of PSII biased by alternative electron pathways, such as the Mehler-reaction or cyclic electron transport (Schreiber and Neubauer 1990). Therefore, the ratio P_F/P_O describes the activity of alternative electron-consuming reactions (STREB et al. 2005; Bailey et al. 2008).

The oxygen-based and fluorescence-based P-E curves were fitted according to Eilers and Peeters (Eilers and Peeters 1988). The derived fitting parameters (a, b, and c) were used to calculate P_{max} and the light saturation index (E_k value) according to Eilers and Peeters (1988):

$$P_{\max} = 1/\left(b + 2\sqrt{a \times c}\right) \quad (4)$$

$$E_k = c/\left(b + 2\sqrt{a \times c}\right) \quad (5)$$

In addition to the estimation of P_F, the variable fluorescence parameters were used to calculate the extent of non-photochemical quenching (NPQ) according to Schreiber et al. (1995):

$$\mathrm{NPQ} = (F\mathrm{m} - F\mathrm{m}')/F\mathrm{m}' \quad (6)$$

where F_m is the maximum fluorescence measured at the end of the initial dark period of P-E curve measurements. The maximum NPQ values (NPQ_{max}) and the half-saturation irradiance of NPQ_{max} (E_{50}) were derived from fitting of the light-response curves of NPQ using the Hill equation (Serôdio and Lavaud 2011). A representative example of the fitted light-dependent NPQ measured in *C.* sp. and in *P. antarctica* is shown in **Figure 9**.

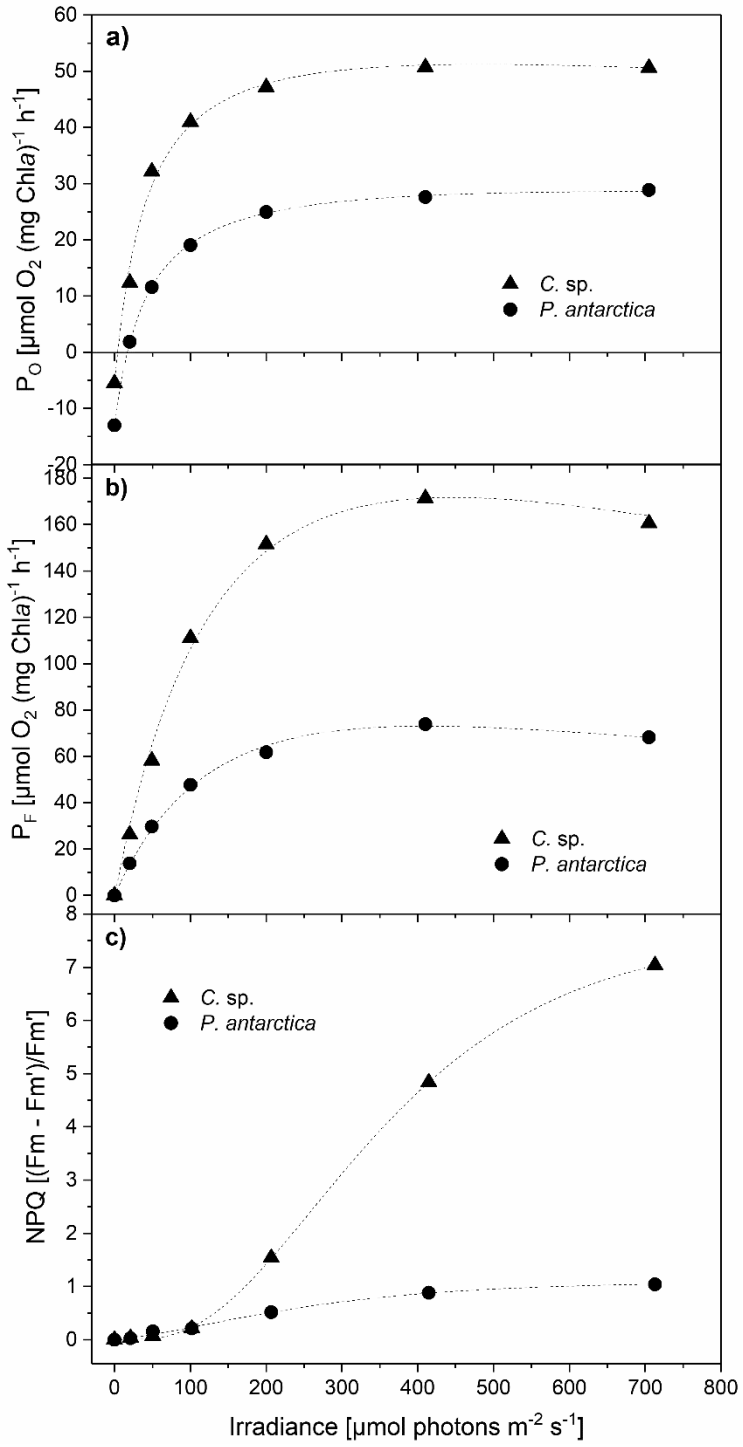

Figure 9 - Representative example of measurements of photosynthesis rates and non-photochemical quenching (NPQ). In a) oxygen-based net photosynthesis rates (P_O; µmol O_2 [mg Chla]$^{-1}$ h^{-1}) as function of irradiance in *Chaetoceros* sp. (filled triangles) and *Phaeocystis antarctica* (strain 109; filled circles) grown at 4 °C and 35 PSU are depicted. Dotted lines show the fitted photosynthesis-irradiance curves of *Chaetoceros* sp. and *P. antarctica*, respectively. In b) the fluorescence-based gross photosynthesis rates (P_F; µmol O_2 [mg Chla]$^{-1}$ h^{-1}) as function of irradiance in *Chaetoceros* sp. and *P. antarctica* are depicted. c) Light-dependent increase of non-photochemical quenching (NPQ; [Fm-Fm']/Fm') in *Chaetoceros* sp. and *P. antarctica.*

2.1.4. Cellular optical properties

The *in vivo*-absorption spectra of algal cells were measured in a dual-beam spectrophotometer (M500, Zeiss, Jena, Germany). The photometer was equipped with an adapter for dispersive samples (Zeiss) to allow a very close placement of the sample to the detector and to correct for light scattering. The Chla-specific *in vivo*-absorption coefficient, a^*_{phy} (cm^2 [mg Chla]$^{-1}$) was calculated as:

$$a^*_{phy}(\lambda) = 2.3 \times A(\lambda)/d \times \text{Chl} \quad (7)$$

where 2.3 is the conversion factor from log10 to ln, A is the absorption of the sample (400 – 700 nm), d is the path length of the cuvette (0.01 m), and Chl is the Chla concentration of the sample (mg m^{-3}). In the results section, the mean values of the Chl-specific absorption ($\bar{a}^*_{phy}$) are given.

The knowledge of the emission spectra of the light source and of a^*_{phy} allows the estimation of the amount of photosynthetically active radiation absorbed by the algal cultures, Q_{phar}. The estimation is based on the following equation according to Gilbert et al. (2000):

$$Q_{phar} = \int_{400\,nm}^{700\,nm} Q(\lambda) - Q(\lambda) \times e^{-\left(a^*_{phy}(\lambda) \times Chl \times d\right)} \quad (8)$$

where Q_{phar} is the photosynthetically absorbed radiation (µmol m^{-2} s^{-1}), Q is the photosynthetically available (incident) radiation (µmol m^{-2} s^{-1}), and d is the optical path length (m).

2.1.5. Estimation of net primary production

To describe the potential effect of different assumptions for rP/R on NPP estimations (e.g. for field samples) the expected daily NPP (NPP_F, NPP_O) was modeled from measured fluorescence- and oxygen-based P-E curves (P_F and P_O, respectively; see above) and considering either measured respiration rates or the assumption of different respiratory loss rates (10, 20, and 30% of GP).

For the respective experimental conditions, the mean values of light-dependent GP (derived from measured P-E curves, see above) were fitted according to Eilers and Peeters (1988). An example of a fitted P-E curve is presented in **Figure 9**. The derived fitting parameters (a, b, c) were used to estimate daily NPP_F and NPP_O (µmol O_2 [mg Chla]$^{-1}$ d^{-1}) as:

$$NPP = \int_{0h}^{24h} (E/[(a \times E^2) + (b \times E) + c]) - R \quad (9)$$

where E is the amount of incident irradiance (µmol photons m^{-2} s^{-1}; see below) and R is the respiration rate. The respiration rates were derived from the mean value of all respiration rates measured within a specific P-E curve. The incident irradiance was based on four daily light climates (**Figure 10**) representing model estimates of different seasonal *in situ*-light conditions (Petrou and Ralph 2011): winter sea ice, spring melt water, summer pelagic water, and autumn new sea ice. These light climates were combined with the fitting parameters derived from specific temperature and salinity conditions that reasonably represent the seasonal conditions during spring, summer, autumn, and winter (**Table 3**). To take into account the dynamics of light conditions, NPP was estimated for 10-min time intervals and integrated over 24 h.

Season	Temperature	Salinity	Light condition
Spring	1 °C	20 – 35 PSU	Meltwater
Summer	1 – 4 °C	35 PSU	Pelagic
Autumn	-1 – 1 °C	35 PSU	New sea ice
Winter	-1 °C	35 – 50 PSU	Sea ice

Table 3 - Experimental conditions and assumed light conditions used for the estimation of daily net primary production (NPP) under different seasonal conditions from measured photosynthesis and respiration rates. In case of a given range of temperature or salinity values, NPP was calculated as mean value of the respective NPP at the specific conditions. Light conditions were adopted from Petrou and Ralph (2011).

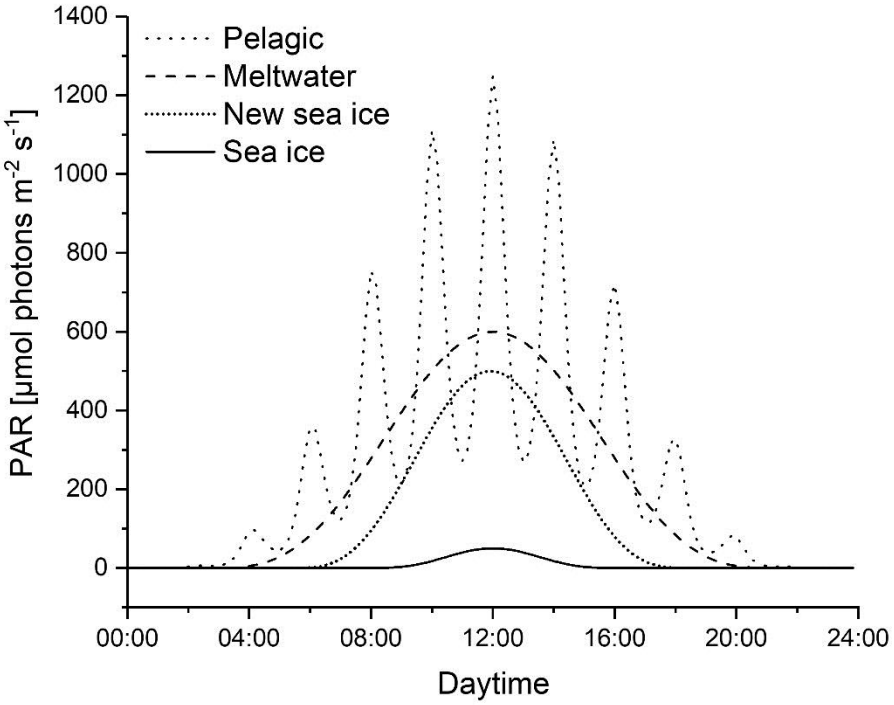

Figure 10 - Light conditions used for the estimation of daily net primary production from measured photosynthesis and respiration rates. Light conditions were adopted from Petrou and Ralph (2011) and represent in situ irradiance (PAR, photosynthetically available radiation) for phytoplankton in summer (Pelagic), autumn (New sea ice), winter (Sea ice), and spring (Meltwater).

2.2. Iron limitation

2.2.1. Experimental conditions

The Antarctic diatom *Chaetoceros* sp. (obtained from Dr. Steffi Gäbler-Schwarz, AWI Bremerhaven, Germany) was kept for more than six months in stock cultures with Fe-deplete and –replete natural Antarctic seawater medium. For the preacclimation phase (over 2 weeks) and the main experiment, the diatom was grown at 2 °C in semi-continuous dilute cultures at 100 μmol photons m^{-2} s^{-1} with a 16:8 h light : dark cycle, using light-emitting diodes (LED) lamps (SolarStinger LED SunStrip Marine Daylight, Econlux). Light intensities were adjusted using a LI-1400 datalogger (Li-Cor, Lincoln, NE, United States) with a 4p-sensor (Walz, Effeltrich, Germany). The $f/2_R$ medium (Guillard and Ryther 1962) was prepared from sterile- and acid-cleaned-filtered (0.2 µm) natural low-iron Antarctic seawater, supplemented with chelexed (ChelexR 100, Sigma-Aldrich, Merck) macronutrients (100 µmol L^{-1} Si, 100 µmol L^{-1} NO_3, 6.25 µmol L^{-1} PO_4) and vitamins (30 nmol L^{-1} B_1, 23 nmol L^{-1} B_7, and 0.228 nmol L^{-1} B_{12}). To this seawater water, either a trace metal mix containing no iron (Control treatment) or to which an addition of 4 nM $FeCl_3$ (+Fe treatment) was made, was given. The trace metal mixture contained zinc (0.16 nmol L^{-1}), copper (0.08 nmol L^{-1}), cobalt (0.09 nmol L^{-1} Co), molybdenum (0.05 nmol L^{-1}), and manganese (1.9 nmol L^{-1}). These trace metal additions were adjusted to maintain the ratio of the original F/2 recipe and represent trace metal concentrations typical for Antarctic HNLC waters. The main experiment lasted 8 days for the Control treatment and 7 days for the +Fe treatment. 4L polycarbonate incubation bottles were used and triplicates of each treatment were run in parallel. Final sampling of all treatments took place when the cells were in exponential growth phase, ensuring stable carbonate chemistry (**Table 4**).

	Control	**+Fe**
Alkalinity (µmol kg^{-1})	2248 ± 5	2242 ± 6
DIC (µmol kg^{-1})	2180 ± 8	2192 ± 5

Table 4 - Concentrations of total alkalinity (TA) and dissolved inorganic carbon (DIC) determined at the end of the experiment for *Chaetoceros* sp. grown under iron replete (+Fe) and deplete (Control) conditions. All values are mean ± standard deviation (n=3).

In order to minimize iron contamination, all sampling and handling of the incubation bottles were conducted under a laminar flow hood (Class 140 100, Opta, Bensheim, Germany) using trace metal clean techniques. Briefly, culture work was conducted in 4 L polycarbonate (PC) bottles (Nalgene, Thermo Fisher Scientific, Waltham, MA USA), which had all been soaked for 1 week in 1% Citranox solution (Sigma-Aldrich, St. Louis, MO, USA) and successively for 1 week in 1 M hydrochloric acid (high-performance liquid chromatography [HPLC] grade, Merck Millipore Corporation). Between each soaking step, the bottles were rinsed seven times with ultrapure water (Merck Millipore Corporation). Finally, the trace metal-cleaned equipment/bottles were air dried under a clean bench (U.S. class 100) and stored in three polyethylene (PE) bags until usage.

2.2.2. Fluorescence measurements

Chlorophyll *a* (Chla) fluorescence was measured with a fast repetition rate fluorometer (FRRf, FastOcean PTX sensor, Chelsea Technologies Group Ltd, West Molesey, UK) connected with a FastAct Laboratory system (Chelsea Technologies Group Ltd). All measurements were performed at 2 °C after a 10 min dark acclimation period. The excitation wavelength of the fluorometer's LED was 450 nm, with an automated adjustment of the light intensity to 1.2×10^{22} µmol photons m^{-2} s^{-1}. A single turnover mode was set with a saturation phase consisting of 100 flashlets on a 2 µs pitch followed by a relaxing phase of 40 flashlets on a 50 µs pitch. According to Kolber et al. (1998), photosynthetic efficiency was determined by measuring the minimum (Fo) and maximum fluorescence (Fm) to calculate the maximum quantum yield of photochemistry in PSII (Fv/Fm) using the following equation:

$$Fv/Fm = (Fm - Fo)/Fm \tag{10}$$

For the measurement of photosynthesis versus irradiance curves (P-E curves), five actinic light levels (21, 50, 107, 207, 415 µmol photons m^{-2} s^{-1}) alternating with dark periods were applied for 5 min each. The effective (see below) and the maximum quantum yield were measured 6 times at the end of each light and dark period, respectively. The FRRf device supplied actinic irradiance and the irradiance level was previously checked with a light sensor (ULM-500 Universal Light Meter equipped with a Spherical Micro Quantum Sensor US-SQS, Walz

GmbH, Effeltrich, Germany). Absolute electron transport rates (aETR, e^{-} $PSII^{-1}$ s^{-1}) at each light level were calculated according to the equation (2) following Suggett et al. (2004; 2009):

$$aETR = \sigma PSII\ x\ ((Fv'/Fm')/(Fv/Fm))\ x\ E \quad (11)$$

where σPSII is the functional absorption cross section of PSII photochemistry (nm^2 $PSII^{-1}$).

Fv'/Fm' denotes the effective PSII quantum yield under ambient light and E represents the respective irradiance level (photons m^{-2} s^{-1}). The maximum absolute electron transport rates ($aETR_{max}$) was measured at 492 µmol photons m^{-2} s^{-1}.

Using the Stern–Volmer equation, non-photochemical quenching (NPQ) of chlorophyll *a* fluorescence was calculated as

$$NPQ = Fm/Fm' - 1 \quad (12)$$

The photosynthesis-irradiance (P-E) curves based on fluorescence measurements were fitted according to Eilers and Peeters (1988). From the derived fitting parameters (a, b, and c) the light-saturating index (E_k) was calculated:

$$E_k = \frac{c}{b+2\sqrt{a\times c}} \quad (13)$$

In addition to the parameters named above, the analysis software of the FRR fluorometer FastPro8 (Chelsea Technologies Group Ltd, West Molesey, UK) provides a measure of the number of functional PSII reaction centers per volume ($RCII_{vol}$, nmol m^{-3}). Together with the cell number and the cellular Chla content ($Chla_{cell}$, pg $cell^{-1}$) of the actual sample, it allows the conversion of aETR (e^{-} $PSII^{-1}$ s^{-1}) into an equivalent oxygen production rate (fluorescence-based photosynthesis rate P_F, µmol O_2 [mg Chla] $^{-1}$ h^{-1}):

$$Pf = (aETR \times RCII_{cell} \times 3600)/(4 \times Chla_{cell}) \quad (14)$$

where $RCII_{cell}$ is the number of PSII reaction centers per cell (amol $cell^{-1}$), 3600 is the conversion factor from s to h and the factor 4 considers that water splitting releases four electrons per O_2.

After the completion of the fluorescence light curve (FLC) curve, an additional dark-adaptation period of 10 min was applied, followed by a single turnover flashlet to check for recovery of PSII. Using the Fv/Fm measured before and after the FLC-curve, the yield recovery was calculated and given as % of the initial Fv/Fm (before the FLC-curve). All measurements (n = 3) were conducted at the growth temperature of 2 °C.

2.2.3. Oxygen-based photosynthesis and respiration rates

Photosynthesis and respiration rates were measured using an oxygen microsensor system (PreSens, Regensburg, Germany). The fundamental principle of optode technology is based on the ability of selected substances to act as dynamic luminescence quenchers. A representation of an optode-based oxygen sensor is reported in **Figure 11**. Specifically, the PreSens sensor is constituted by an oxygen-sensitive luminophore, based on a platinum porphyrine complex (Tengberg et al. 2006; Bittig et al. 2018).

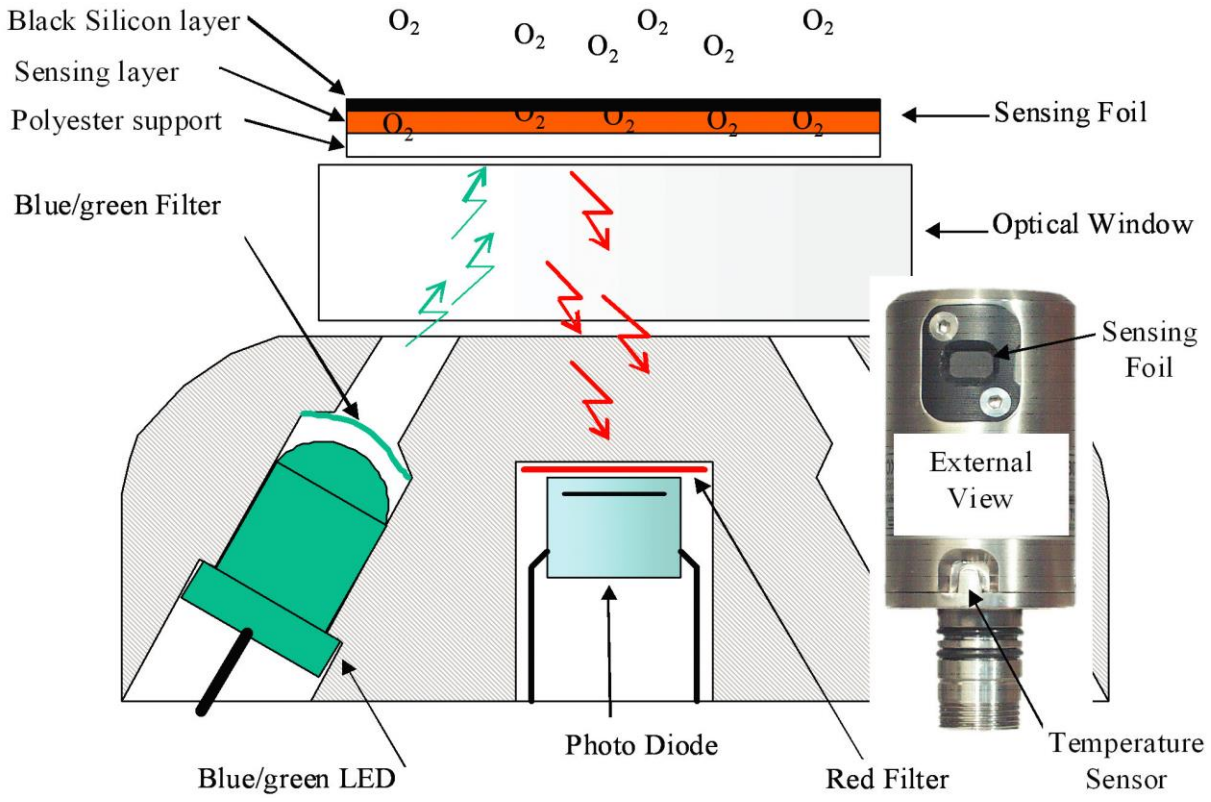

Figure 11 - Optical design and outside view of an optode-based oxygen sensor (Tengberg et al. 2006).

From each experimental condition, cells were harvested by gentle filtration of 400 – 500 ml culture volume over a 2 µm membrane filter (Isopore, Millipore) to obtain a volume of about 4 – 6 ml (equal to a Chla concentration of 2 – 3 µg mL^{-1}). Subsequently, the concentrated cell suspension was transferred into a special custom-made cuvette where an implantable oxygen microsensor was placed (PreSens) and maintained at 2 °C under continuous gentle stirring. For the measurement of the oxygen-based P-E curves, *Chaetoceros* sp. cells were dark-adapted for 10 min and then exposed each for 5 min to five increasing light intensities (45, 90,185, 302, 455 µmol photons m^{-2} s^{-1}), alternating with subsequent dark phase of the same duration. The light intensities were provided by a light projector equipped with neutral density filters. Each irradiance level was checked with a light sensor as described above (paragraph 2.2). After the measurements, samples for determination of Chla concentration of the cell's concentrate were taken, filtered onto GF/F filters and stored at -80 °C. Chla was subsequently extracted in 1,6 mL acetone (overnight in darkness, 4 °C) and determined with a Turner Designs fluorometer (Model 10-000 R, Mt. View, Canada).

For the calculation of photosynthetic rates the oxygen solubility in dependence of the medium salinity and the measuring temperature (Benson and Krause 1984) was used. Gross oxygen production, GP, and net oxygen production, NP are here reported in µmol O_2 (mg Chla h)$^{-1}$. GP was derived by correcting net oxygen evolution rates for the corresponding dark respiration rate (R; µmol O_2 (mg Chla h)$^{-1}$). GP_{max} and NP_{max} in the following sections referred to GP and NP values obtained at the maximal light intensity investigated (i.e. 455 µmol photons m^{-2} s^{-1}). The P-E curves based on gross oxygen production were fitted according to Eilers and Peeters (1988).

2.2.4. Growth and cell size determination

Cell samples were fixed with Lugol's solution and stored at 2 °C in the dark until counting. Cell numbers were determined using Utermöhl chambers (Hydrobios, Altenholz, Germany) on an inverted microscope (Zeiss Axiovert 200). After a settling time of at least 24 hours, *Chaetoceros* sp. cells were counted in stripes in an Utermöhl chamber until at least 400 cells had been counted. A magnification of 400x in combination with a 1.6x optovar was used for counting. The cell numbers were plotted on a logarithmic scale and the slope of the linear regression was used to determine growth rates (Fanesi et al. 2016). Cellular biovolume was calculated according to Hillebrand et al. (1999), measuring at least 50 cells for treatment.

2.2.5. Particulate organic carbon and nitrogen

For the quantification of particulate organic carbon and particulate organic nitrogen (POC and PON, respectively), at the end of the experiment 750 mL of each *Chaetoceros* sp. culture flask were filtered onto pre-combusted (500 °C, 15 h) 25-mm GF/F filters (Whatman). One filter blank was taken for each bottle. Samples were stored in combusted glass petri dishes at -20 °C until sample preparation. Prior the analysis, filters were dried at 60 °C overnight before they were acidified with 200 µL of 0.2 M HCl to remove the inorganic carbon. After being dried again at 60 °C overnight, filters were coated in tin foil and compressed into small pellets. Samples were analysed with an automated carbon nitrogen elemental analyser (Euro EA - CN Elemental Analyzer, HEKAtech GmbH, Wegberg, Germany). POC and PON contents per cell were corrected for blank measurements and normalized to cell density and the filtered volume to yield cellular quotas. Daily POC production rates were calculated by multiplication of the cellular quota with the specific growth rate of the respective treatment. The molar ratio of cellular carbon to nitrogen (C : N) was calculated by dividing the content of POC per cell (mol) by the content of PON per cell in (mol).

2.2.6. Pigments

For the analysis of pigments, 750 mL of the *Chaetoceros* sp. cultures were filtered onto 25 mm glass fiber filters (GF/F, Whatman). The filters were frozen immediately in liquid nitrogen (N_2) and stored at -80 °C. Before analysis, pigments were extracted from the GF/F filter for 24 h at 4 °C in the dark using 90% acetone (v/v). After centrifugation (5 min, 4 °C, 13.000 *g*) and filtration through a 0.45 µm pore size nylon syringe filter (Nalgene®, Nalge Nunc 241 International, Rochester, NY, USA), total pigment concentrations were determined via reverse HPLC (LaChromElite system, VWR, Darmstadt, Germany). A Spherisorb ODS-2 column (5 µm particle size; Waters, Milford, MA, USA) was used for the separation of the pigments, applying a gradient following Wright et al. (1991). Peaks were detected at 440 nm and identified as well as quantified by co-chromatography with standards (DHI Lab Products, Hørsholm, Denmark) using the software EZChrom Elite ver. 3.1.3. (Agilent Technologies, Santa Clara, CA, USA).

More specifically, concentrations of the light harvesting pigments (LHP): chlorophyll *a* (Chla) and c2 (Chl c2), fucoxanthin (Fuco), and the light protective pigments (LPP): diatoxanthin (Dt), diadinoxanthin (Dd) and ß-carotene were determined and normalized to filtered volume and cell numbers to yield cellular quotas.

2.3. Statistical analysis

2.3.1. Temperature and salinity

The physiological data were tested statistically for significance using two-way analysis of variance (ANOVA) followed by Bonferroni post-tests (p-value < 0.05). The different salinity and temperature conditions were used as treatment factors. The data set was checked for normality by Shapiro-Wilk test (SigmaPlot 12.5), and all random samples passed the test.

In addition, to test for significance between two particular data sets (e.g. all values of rP/R in *Chaetoceros* sp. versus *P. antarctica*) standard t-test was used.

2.3.2. Iron limitation

The effect of iron availability (Control vs. +Fe) on all experimental parameters was statistically analyzed using one-way analyses of variance (ANOVA) with Bonferroni's multiple comparison post-tests. All statistical analyses were performed using the program GraphPad Prism (Version 5.00 for Windows, Graph Pad Software, San Diego California, USA) and the significance testing was done at the $p < 0.05$ level.

3. Results

3.1. Temperature and salinity

3.1.1. Physiological key parameters

For the physiological characterisation of phytoplankton cells, the photosynthetic capacity and the potential of light-protective mechanisms were measured. **Figure 9** depicts an example of the Photosynthesis-irradiance (P-E) curves (shown as net oxygen evolution rate) based on the measurement of oxygen evolution (**Figure 9a**) and based on PAM-fluorescence (**Figure 9b**) in comparison of *Chaetoceros* sp. and *Phaeocystis antarctica*. The oxygen evolution rates were light saturated at a relatively low irradiance with a light saturation index (E_k value) of 50 µmol photons m^{-2} s^{-1} (**Figure 9a**). Moreover, the maximum values of net photosynthesis were higher in *Chaetoceros* sp. than in *P. antarctica*. This difference is partly due to the different respiratory activities of the phytoplankton species. In contrast to oxygen evolution, the fluorescence-based photosynthesis rates were saturated at a higher irradiance (E_k = 115 µmol photons m^{-2} s^{-1}) in both species. Another prominent difference in the comparison of *Chaetoceros* sp. and *P. antarctica* was observed in NPQ values (**Figure 9c**). Here, much higher maximum NPQ values were observed in *Chaetoceros* sp. than in *P. antarctica*.

For further analysis, the data from P-E curves were used to compare the maximum gross photosynthetic rates (P_{max}), respiration rates (R), ratio rP/R, NPQ_{max}, and ratio of maximum fluorescence-based/maximum oxygen-based photosynthesis rates (P_F/P_O) for all experimental conditions and for the three algal strains used in this study (see below). It has to be mentioned that at a growth temperature of -1 °C the two strains of *P. antarctica* did not grow sufficiently well at 70 PSU to obtain sufficient biomass for physiological measurements. Therefore, under this temperature/salinity combination physiological measurements were performed for *Chaetoceros* sp. only. In addition to the determination of physiological parameters, data of P-E curves were also used to apply a curve fit according to Eilers and Peeters (1988) and to finally estimate the effects of changes in rP/R on NPP for different environmental scenarios (see below).

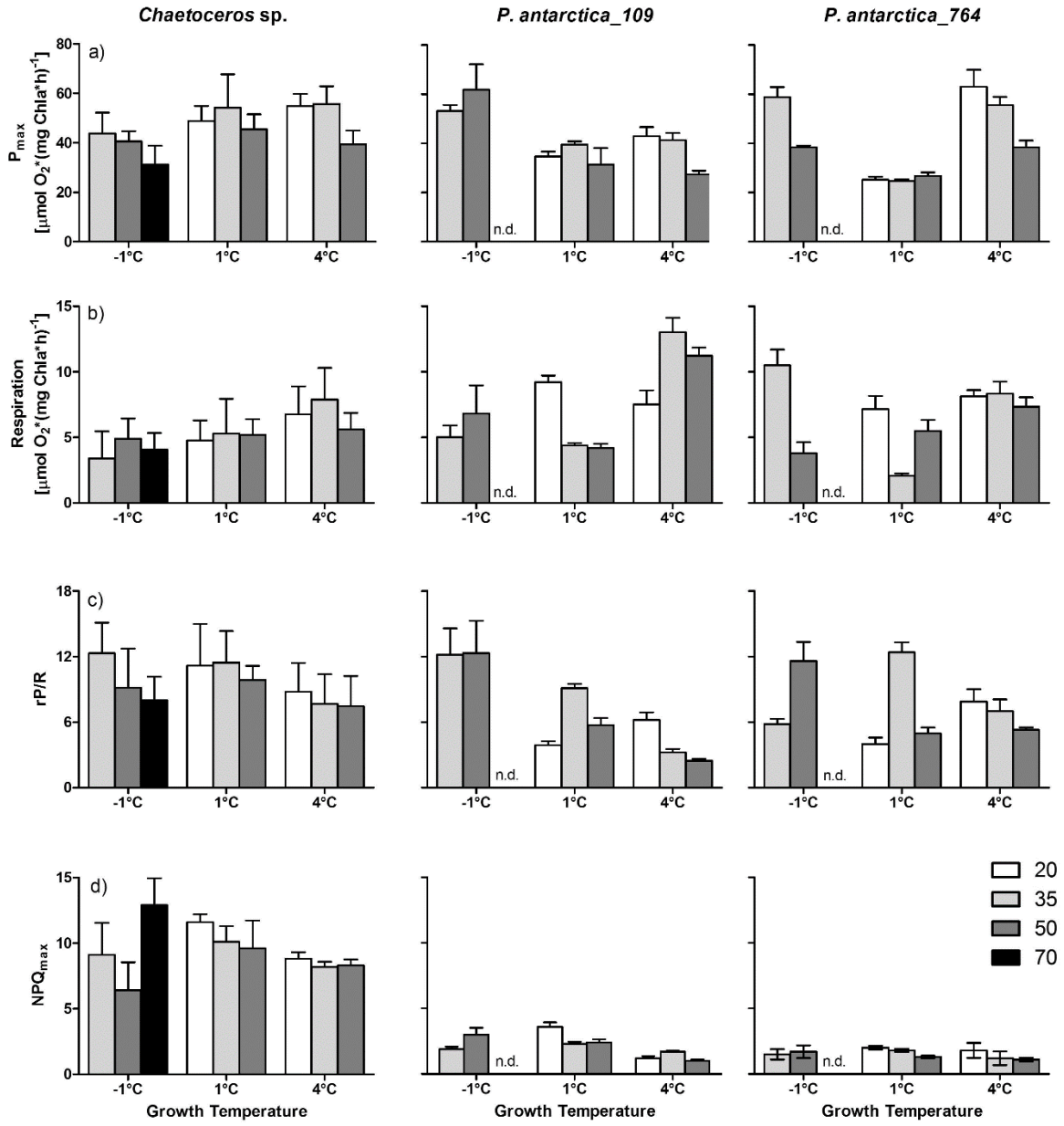

Figure 12 - Physiological key parameters (P_{max}, R, rP/R, NPQ_{max}) of *Chaetoceros* sp. and *Phaeocystis antarctica.* Mean values (± standard deviation) of physiological parameters measured in *Chaetoceros* sp. and *Phaeocystis antarctica* (strains 109 and 764) grown under different temperatures (-1, 1, 4 °C) and salinity of growth medium (20, 35, 50, 70 PSU; white, light grey, dark grey, black bars, respectively): a) Maximum gross oxygen-based photosynthesis (P_{max}, [µmol O_2 (mg Chla)$^{-1}$ h^{-1}], n = 4 – 11), b) Respiration rate (R, [µmol O_2 (mg Chla)$^{-1}$ h^{-1}], n = 4 – 11), c) Ratio gross photosynthesis/respiration (rP/R, n = 4 – 11), d) Maximum value of non-photochemical quenching (NPQ_{max}, n = 4 – 11), 'n' depicts the number of biological replicates. For *P. antarctica* no data were obtained at the condition -1 °C/70 PSU (marked with 'n.d.').

Figure 12a shows the mean values of P_{max} (Gross oxygen-based photosynthesis) at three different growth temperatures and in combination with different salinities. For *Chaetoceros* sp. no significant effect of temperature on P_{max} was observed, which is in contrast to *P. antarctica*. *P. antarctica* strain 109 showed significantly higher P_{max} at -1 °C than at 1 and 4 °C, all in combination with 50 PSU ($p < 0.001$). This temperature effect on P_{max} was also observed for the comparison of -1 and 1 °C at 35 PSU ($p < 0.05$), whereas no such temperature effect was detected for the comparison of P_{max} values measured at 1 and 4 °C. A different influence of temperature on P_{max} was observed in *P. antarctica* strain 764. Here, significantly higher P_{max} values were measured at 4 °C than at 1 °C, at 20 and 35 PSU ($p < 0.001$), although P_{max} was higher at -1 °C than at 1 °C only at 35 PSU ($p < 0.001$).

For all tested species an influence of salinity on P_{max} was observed at 4 °C, with significantly lower P_{max} values at 50 PSU than at 20 PSU ($p < 0.01$) and 35 PSU ($p < 0.05$). This salinity effect on P_{max} was additionally observed at -1 °C, in *P. antarctica* strain 764 comparing 50 to 35 PSU ($p < 0.001$), and in *Chaetoceros* sp. comparing 70 and 35 PSU ($p < 0.001$).

It should be highlighted that P_{max} values of *Chaetoceros* sp. at 1 °C were significantly higher than in both strains of *P. antarctica* ($p < 0.01$). At 4 °C, P_{max} in *Chaetoceros* sp. was significantly higher than in *P. antarctica* strain 109 ($p < 0.01$). Significant differences in P_{max} in comparison of strain 109 and 764 of *P. antarctica* were observed only at 4 °C ($p < 0.05$).

In **Figure 12b** depicts the respiration rates under the applied experimental conditions. In *Chaetoceros* sp., there was a trend of increasing respiration rates with temperature at a salinity of 35 PSU, with significant differences between -1 and 4 °C ($p < 0.001$). In *P. antarctica* strain 109 a comparable effect was observed at 35 and 50 PSU with a significant increase of respiration rates at 4 °C compared to 1 °C ($p < 0.01$) and at 4 °C compared to -1 °C ($p < 0.01$). In *P. antarctica* strain 764 a significant increase of respiration rates with temperature was observed only at a salinity of 35 PSU in the comparison of 4 to 1 °C ($p < 0.001$). In the comparison of the different species the most prominent result is the significantly higher respiration rate at 4 °C / 35 and 50 PSU in P. antarctica strain 109 compared to strain 764 ($p < 0.01$) and to *Chaetoceros* sp. ($p < 0.001$).

Figure 12c represents the ratio of gross P_{max} over respiration (rP/R). For all investigated species a decreasing ratio P/R at 35 PSU was observed in the comparison of 1 to 4 °C ($p < 0.05$). This trend of decreasing rP/R with increasing temperature was measured in *Chaetoceros* sp. and *P. antarctica* strain 109 also in the comparison of -1 to 4 °C (at 35 PSU; $p < 0.01$).

Salinity was of minor importance on changes in rP/R. In both strains of *P. antarctica*, only at a growth temperature of 1 °C a significantly higher rP/R was observed at 35 PSU compared to both 20 and 50 PSU ($p < 0.01$).

As a consequence, significantly higher rP/R values were detected in *Chaetoceros* sp. at 1 °C / 20 and 50 PSU than in both strains of *P. antarctica*. In addition, rP/R was significantly higher in *Chaetoceros* sp. than in *P. antarctica* strain 109 at 4 °C / 35 and 50 PSU ($p < 0.01$). It should be highlighted that the differences in rP/R were mainly due to changes in respiration rates but not to changes in gross P_{max} (**Figure 13**).

The comparison of NPQ_{max} values revealed the largest interspecies differences between *Chaetoceros* sp. and *P. antarctica* (**Figure 12d**). At all growth conditions, NPQ_{max} values in *Chaetoceros* sp. were significantly higher (1 and 4 °C with $p < 0.001$; -1 °C with $p < 0.05$) than in *P. antarctica*. In contrast, there was no significant influence of temperature or salinity on NPQ_{max} in neither *Chaetoceros* sp. nor in both *Phaeocystis* strains. The species-specific differences in NPQ_{max} were further supported by the ratio of the half-saturation irradiance of NPQ_{max} (E_{50}) over the photo-acclimation parameter E_k (derived from fluorescence-based photosynthesis rates P_F). Thus, the ratio E_{50}/E_k describes the light-dependent NPQ induction status in relation to the saturation level of the electron transport chain. It is evident that the mean value E_{50}/E_k for all experimental conditions was significantly higher in *Chaetoceros* sp. (mean $E_{50}/E_k = 4.0$) compared to both strains of *Phaeocystis* (mean $E_{50}/E_k = 2.0$; **Figure 14**).

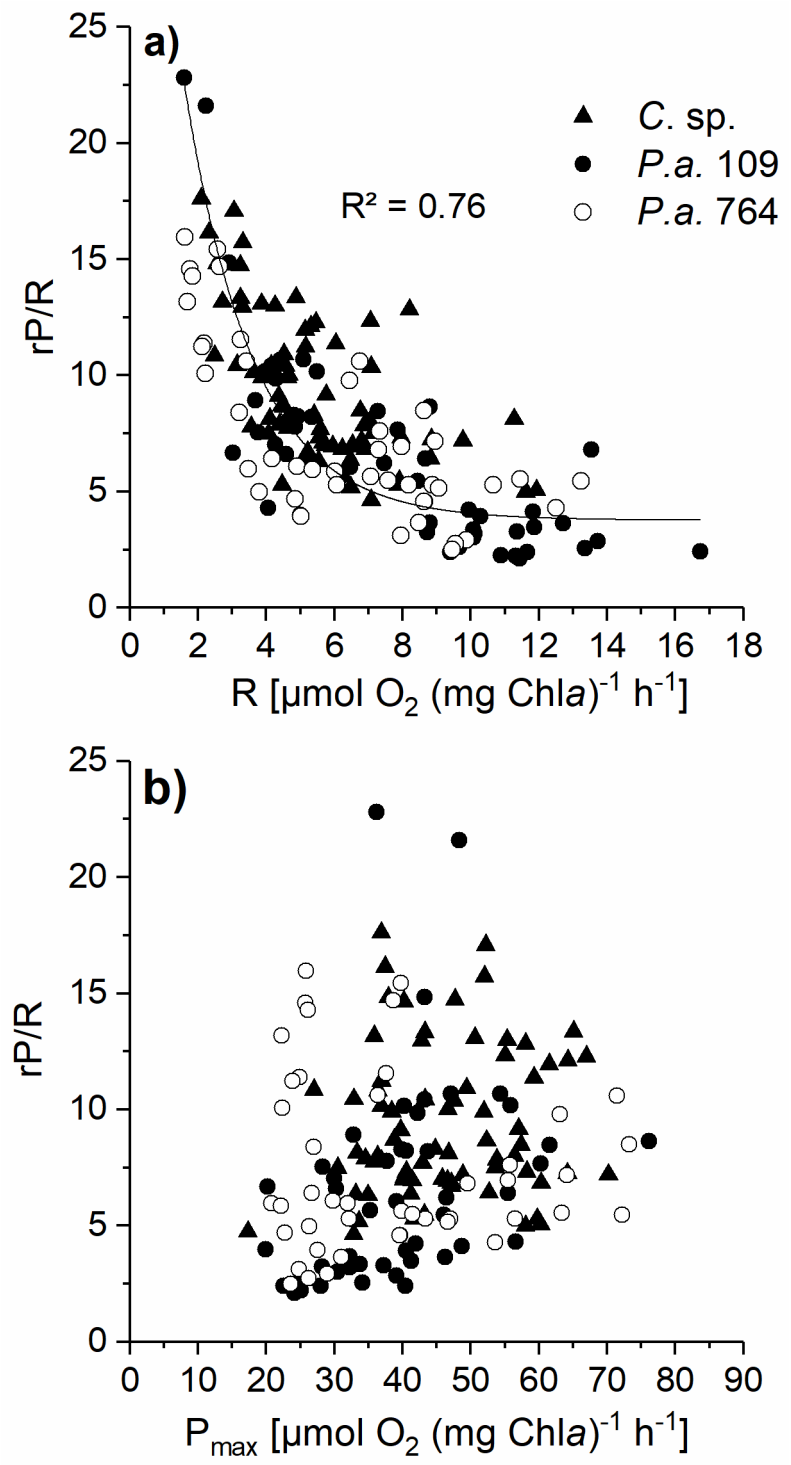

Figure 13 - Correlation between a) ratio of gross photosynthesis to respiration (rP/R) and respiration and b) rP/R and maximum gross photosynthetic rates (P_{max}) in *Chaetoceros* sp. and *Phaeocystis antarctica* (strains 109 and 764). Cultures of *Chaetoceros* sp. (filled triangles), *P. antarctica* strain 109 (filled circles), and strain 764 (open circles) were grown under different combinations of temperature (-1, 1, 4 °C) and salinity of the growth medium (20, 35, 50 PSU). Data in a) were fitted with an exponential regression.

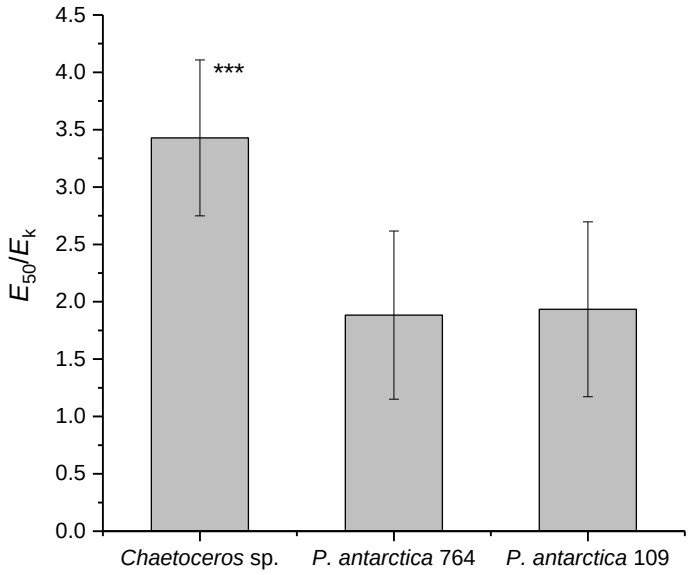

Figure 14 - Ratio of half-saturation irradiance of maximum NPQ (E_{50}) over characteristic irradiance E_k derived from fluorescence-based photosynthesis-irradiance curves in *Chaetoceros* sp., *Phaeocystis antarctica* strain 764 and *P. antarctica* strain 109. Level of significance is indicated by *** ($p < 0.001$).

The ratio of maximum fluorescence-based to maximum oxygen-based gross photosynthetic rate describes the activity of alternative electron pathways. From the data shown in **Figure 15a,** it is evident that P_F/P_O was very constant at a value of approximately 3.5 for almost all experimental conditions in *Chaetoceros* sp. (except data at -1 °C, 50 PSU). Similar to the observed species-dependence of NPQ_{max} and a^*_{phy}, the P_F/P_O values were significantly higher in *Chaetoceros* sp. than in both *Phaeocystis* strains at 1 and 4 °C ($p < 0.01$; except for condition 1 °C / 20 PSU in strain 764). Moreover, there was no significant influence of salinity on P_F/P_O at 1 and 4 °C.

The mean value of the Chl-specific *in vivo*-absorption (a*phy) describes the absorption efficiency of algal cells. Under the experimental conditions, *Chaetoceros* sp. showed the lowest variation of a*phy values with no significant influence of neither temperature nor salinity (**Figure 15b**). Similarly, there was no significant influence of salinity on the absorption efficiency of both strains of *P. antarctica*. On the other hand, in both strains of *P. antarctica* a large variation of a*phy values was observed. Accordingly, at a growth temperature of 1 and 4 °C, the a^*_{phy} values were significantly lower in both strains of *P. antarctica* at all salinities than in *Chaetoceros* sp. ($p < 0.001$). In addition, *P. antarctica* strain 764 showed significantly higher a^*_{phy} values than strain 109 at a growth temperature of 4 °C. In contrast, at -1 °C growth temperature a^*_{phy} values were in a comparable range for all three algal species.

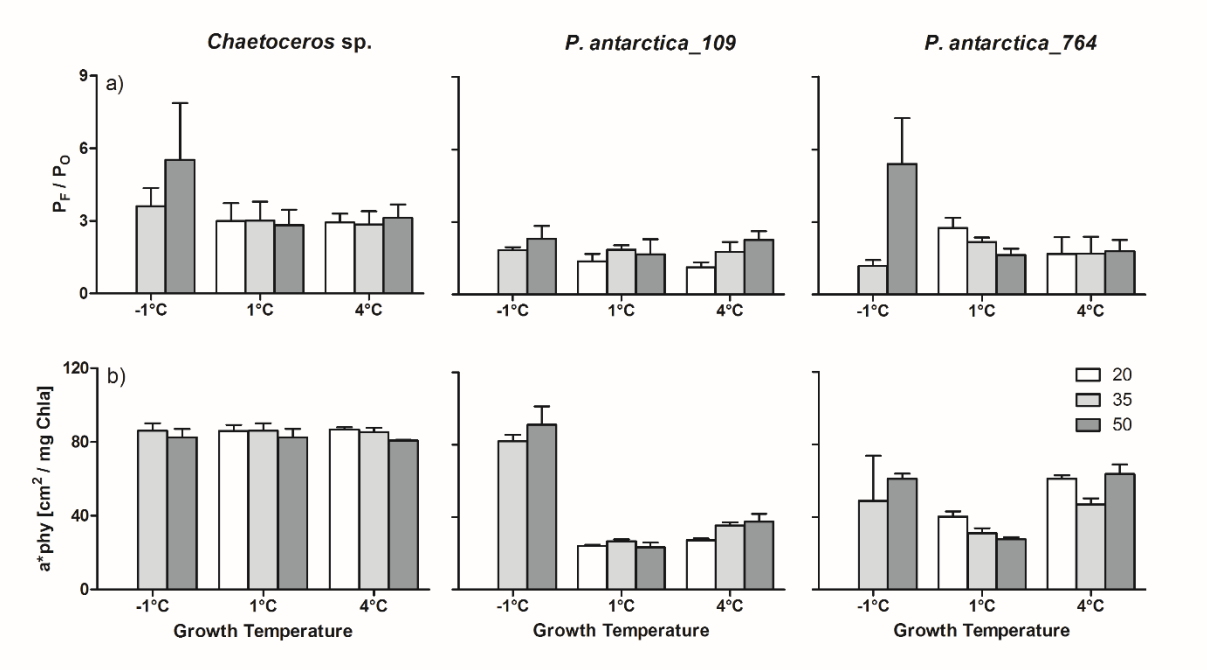

Figure 15 - Physiological key parameters (P_F/P_O, a^*_{phy}) of *Chaetoceros* sp. and *Phaeocystis antarctica.* Mean values (± standard deviation) of physiological parameters measured in *Chaetoceros* sp. and *Phaeocystis antarctica* (strains 109 and 764) grown under different temperatures (-1, 1, 4 °C) and salinity of growth medium (20, 35, 50, PSU; white, light grey, dark grey, respectively): a) Ratio maximum fluorescence-/maximum oxygen-based photosynthesis rate (P_F/P_O, n = 4 – 11), b) Chlorophyll-specific absorption coefficient (a^*_{phy}, [cm^2 (mg Chla)$^{-1}$], n = 3). 'n' depicts the number of biological replicates. For *P. antarctica* no data were obtained at the condition -1 °C / 70 PSU (marked with 'n.d.'). The same column colours with respect to medium salinity were applied for all subfigures.

In summary, for all investigated species an effect of temperature on at least some physiological parameters is evident. In contrast, salinity did influence physiological parameters, albeit only at specific conditions. Moreover, for a number of parameters an interspecific difference between *Chaetoceros* sp. and *P. antarctica* was observed.

3.1.2. Effect of rP/R on NPP

In field samples, it is often not possible to measure respiratory losses in phytoplankton cells (see above). However, for the estimation of daily-integrated NPP, consideration of respiratory losses is necessary. Since no existing data are available, respiratory losses are assumed to be in the range of 10 – 30% (see above). In the present study, it was intended to assess the potential deviation of integrated NPP under the assumption of different respiratory losses and to compare it to a respective NPP estimation with measured rP/R. Therefore, from the applied experimental conditions the data from specific experimental combinations (**Table 3**) were chosen to estimate NPP rates for different seasonal conditions. In addition, NPP was estimated on the basis of *in situ* light conditions (adopted from Petrou & Ralph, 2011) that are typical for different seasons in the SO. The light conditions differ in maximum irradiance, shape of illumination, and daylength (**Figure 10**). From the fitted oxygen-based P-E curves the daily integrated NPP (µmol O_2 [mg Chla d]$^{-1}$) was estimated from GP minus measured respiratory losses and compared to NPP calculated from GP minus assumed respiratory losses of 10, 20, and 30% (equals to rP/R 10, 5, 3.3), respectively (**Figure 16**).

As expected from *in situ* light conditions, the highest NPP based on measured respiratory losses was estimated for spring and summer conditions, whereby NPP rates were always higher in *Chaetoceros* sp. than in *P. antarctica* (**Figure 16a-b**). Otherwise, lowest NPP (based on measured respiration) was estimated for winter condition (**Figure 16d**) with still positive values for *Chaetoceros* sp. and *Phaeocystis* strain 109, whereas for *Phaeocystis* strain 764 slightly negative NPP values were estimated. Regarding the effect of different rP/R on NPP, it was observed that under spring and summer conditions the assumption of 10 to 20% respiratory losses over-/underestimated NPP only slightly, respectively. This is in contrast to autumn and winter conditions (**Figure 16c-d**), where the assumption of respiratory losses >10% yielded a severe underestimation of NPP in comparison to NPP estimates for all investigated species.

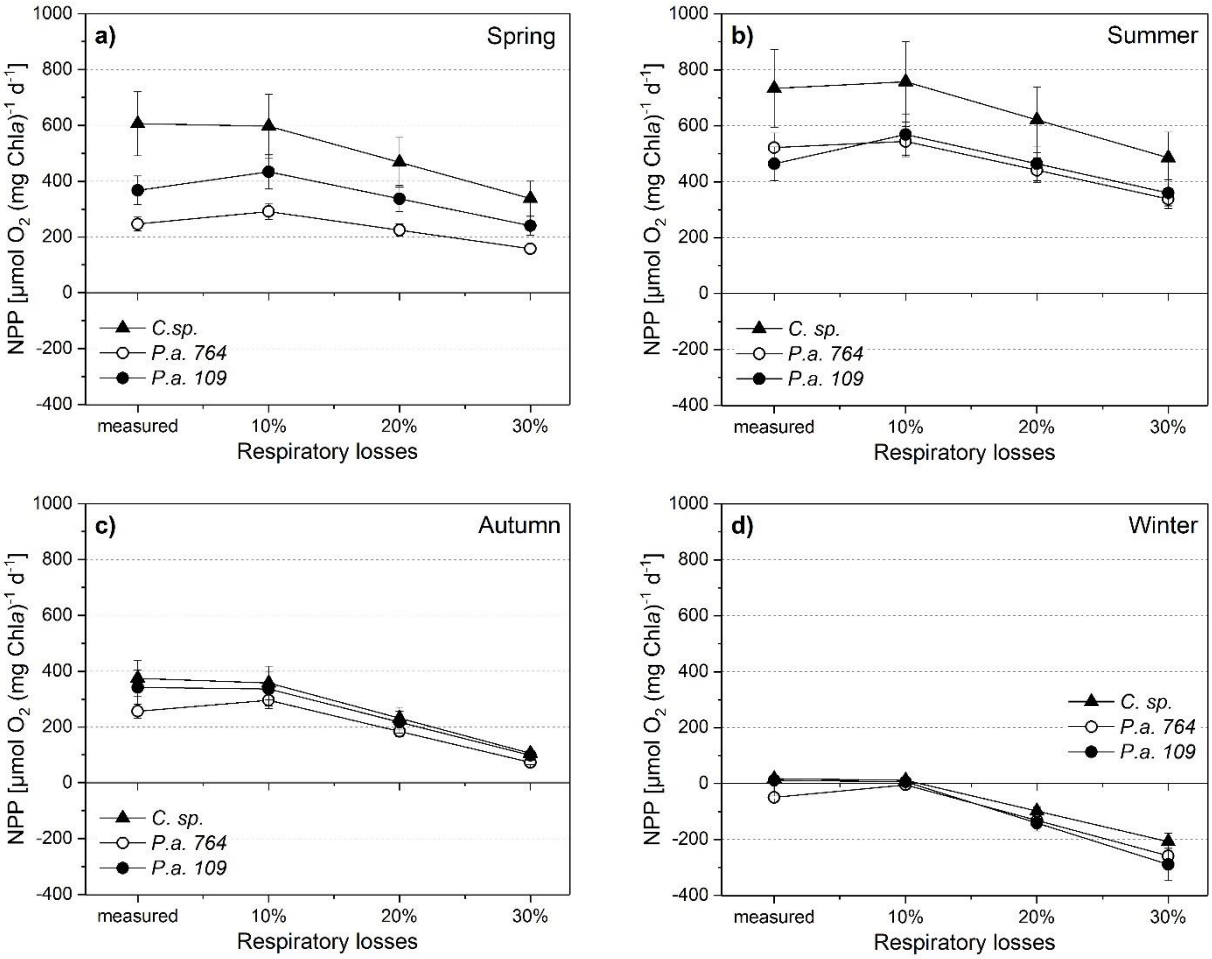

Figure 16 - Daily integrated Net Primary Production (NPP, [µmol O_2 (mg Chla)$^{-1}$ h^{-1}]) estimated from Gross oxygen Production (GP) minus measured respiratory losses and compared to NPP calculated from GP minus assumed respiratory losses of 10, 20, and 30% in *Fragilariopsis cylindrus* (*F.c.*), *Phaeocystis antarctica* (*P.a.*, strains 764 and 109). Estimation of NPP is based on mean values (± sd) of fitted Photosynthesis-Irradiance curves for different experimental conditions that represent specific seasonal *in situ*-conditions: a) Spring, b) Summer, c) Autumn, d) Winter (see text for details).

In addition to daily NPP estimates from oxygen-based photosynthesis (NPP_O), daily NPP was also estimated from fluorescence-based photosynthesis rates (NPP_F). The ratio NPP_F/NPP_O depicts the potential overestimation of daily NPP measured by fluorescence in relation to oxygen-based NPP estimates (**Figure 17a**). As with the ratio P_F/P_O, the ratio NPP_F/NPP_O depends on the activity of alternative electron sinks that can be addressed by fluorescence-based measurement of photosynthesis rates but not by oxygen evolution rates. Additionally, NPP_F/NPP_O can be influenced by the proportion of respiratory losses whereby high respiratory losses tend to increase NPP_F/NPP_O. Whereas the NPP_F/NPP_O was relatively stable between values of 2.5 to 2.9 in *Chaetoceros* sp., a trend of declining values of NPP_F/NPP_O from 'Summer' to 'Winter' conditions was observed in *P. antarctica*.

Thus, under 'Autumn' and 'Winter' conditions, NPP_F/NPP_O was significantly lower in both strains of *P. antarctica* than in *Chaetoceros* sp. ($p < 0.01$).

The estimation of P_F (and subsequently also NPP_F) takes into account the absorptivity of phytoplankton cells (measured as a^*_{phy}). **Figure 17b** depicts the a^*_{phy} values of *Chaetoceros* sp. and *P. antarctica* that correspond to the specific experimental condition chosen for NPP estimation. It is evident that a^*_{phy} values in *Chaetoceros* sp. were very constant for all conditions, which is in line with the stable ratio NPP_F/NPP_O. In contrast, a^*_{phy} values in both strains of *Phaeocystis* strongly increased from 'Summer' to 'Autumn' and 'Winter' conditions. This was a rather unexpected result since the increase of a^*_{phy} usually coincides with a higher absorptivity of the cells and should increase the ratio NPP_F/NPP_O, respectively. The latter was, however, not the case in cells of *P. antarctica*. Thus, it could be assumed that respiratory losses exert larger influence on the ratio NPP_F/NPP_O under these conditions than a^*_{phy}.

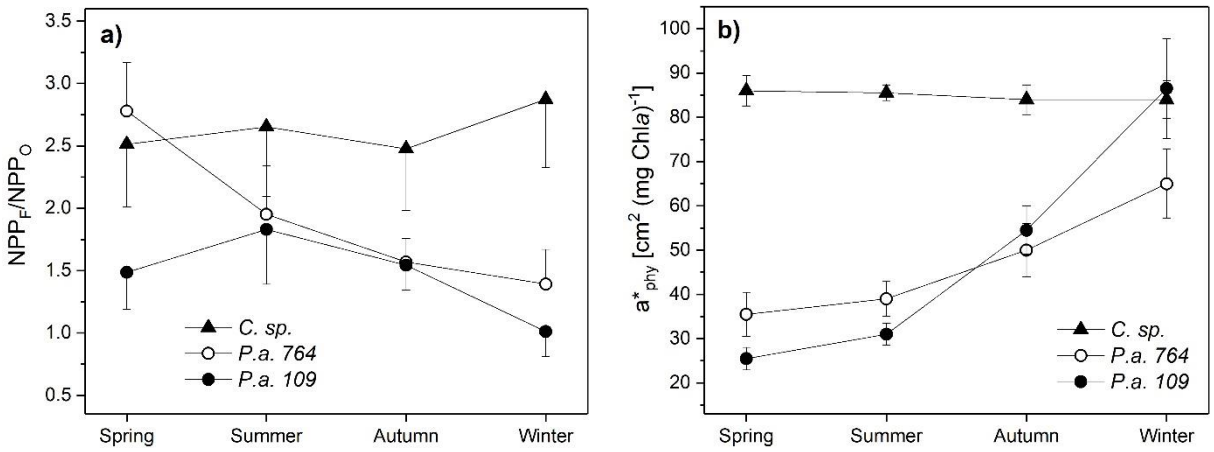

Figure 17 - Fluorescence-based/oxygen-based estimation of net primary production and specific absorption. a) The ratio fluorescence-based/oxygen-based estimation of Net Primary Production (NPP_F/NPP_O) under specific experimental conditions that represent specific seasonal *in situ*-conditions: Spring, Summer, Autumn, Winter (see text for details) in *Chaetoceros* sp. (*C.* sp.), *Phaeocystis antarctica* (*P.a.*, strains 764 and 109). b) Chlorophyll *a*-specific absorption coefficient (a^*_{phy}, [cm^2 (mg Chla)$^{-1}$]).

3.2. Iron limitation

3.2.1. Cell parameters

The particular organic carbon (POC) and particular organic nitrogen (PON) per cell varied significantly between the two treatments. Both POC and PON were higher by 18% ($p < 0.01$) and 28% ($p < 0.001$) in +Fe than Control conditions, respectively. The molar carbon to nitrogen ratios of cells grown under Control had a slightly higher ($p < 0.01$) C:N ratio, as reported in **Table 5**. Furthermore, with decreasing Fe availability daily carbon production rates declined (**Table 5**). If the POC values are normalized over volume (Stefels and van Leeuwe 1998), instead of over cells, differences between the two treatments are not visible anymore (**Table 5).**

The growth rates of *Chaetoceros* sp. increased by 9% from 0.53 to 0.58 d^{-1} under Control and +Fe conditions, respectively (**Table 5**). Correspondingly, the cell length/volume increased in iron enriched cells compared to iron deplete cells.

	Control	**+Fe**
POC (pg C $cell^{-1}$)	7.63 ± 0.42	9.26 ± 0.71*
PON (pg N $cell^{-1}$)	1.37 ± 0.06	1.76 ± 0.12*
C:N (mol:mol)	6.48 ± 0.10	6.12 ± 0.16*
Daily POC production rate (pg C $cell^{-1}$ d^{-1})	4.04 ± 0.20	5.39 ± 0.86*
μ (d^{-1})	0.53 ± 0.02	0.58 ± 0.02*
Cell length (µm)	$5.63 \pm 0{,}28$	6.44 ± 0.11*
Cell volume (μm^3)	46.37 ± 5.75	58.39 ± 1.80*
POC_{vol} (pg C μm^{-3})	0.17 ± 0.03	0.16 ± 0.01

Table 5 - Elemental composition, growth and cell size of Chaetoceros sp. for Control and +Fe conditions. Values represent mean ± standard deviation (n=3). Significant changes ($p<0.05$, ANOVA) relative to the Control condition are denoted by *.

3.2.2. Pigments

Generally, the pigment content of *Chaetoceros* sp. did not differed significantly in the two iron treatments, except for Chla, 20% lower in Control than under +Fe treatment ($p < 0.01$), and ß-carotene, decreased by 43% in Control vs. +Fe treatment ($p < 0.001$), as reported in **Table 6**.

	Control	**+Fe**
Chla (fg cell^{-1})	126.5 ± 8.8	158.7 ± 16.5*
$Chlc_2$ (fg cell^{-1})	17.9 ± 1.7	19.9 ± 3.6
Fuco (fg cell^{-1})	75.1 ± 6.1	80.5 ± 12.0
ß-car (fg cell^{-1})	2.8 ± 0.3	4.9 ± 0.6*
Dd (fg cell^{-1})	22.8 ± 1.7	25.5 ± 8.2
Dt (fg cell^{-1})	0.4 ± 0.1	0.5 ± 0.3
LHP/LPP	9.5 ± 0.4	10.5 ± 2.7

Table 6 - Cellular concentrations (fg cell^{-1}) of the light harvesting pigments (LHP): chlorophyll *a* (Chla), chlorophyll c_2 (Chl c_2), fucoxanthin (Fuco), as well as of the light protective pigments (LPP): ß-carotene (ß-car), diadinoxanthin (Dd), diatoxanthin (Dt) and the ratio of LHP/LPP for *Chaetoceros* sp. grown under Control and +Fe conditions. Values represent mean ± standard deviation (n=3). Significant changes ($p<0.01$, ANOVA) in pigment concentrations relative to the controls denoted by *.

3.2.3. Chla based fluorescence parameters

Iron availability influenced strongly the photophysiology of *Chaetoceros* sp., as shown in **Table 7**. The maximum PSII efficiency of dark-adapted cells (Fv/Fm) was significantly higher ($p < 0.001$) in the +Fe treatment compared with the Control treatment, being 0.50 ± 0.01 and 0.38 ± 0.01, respectively. The same trend was observed also for the connectivity between adjacent photosystems P, with a significantly 27% increase in the +Fe compared to Control treatment (0.44 ± 0.02 and 0.32 ± 0.03, $p < 0.001$). Similarly, the concentrations of functional reaction centers of PSII per cell $RCII_{cell}$ decreased significantly by 26% in the Control ($p < 0.001$). In contrast, the functional absorption cross section of PSII, σ_{PSII}, was 10% higher under Control than under +Fe.

	Control	**+Fe**
Fv/Fm (rel. unit)	0.38 ± 0.01	0.50 ± 0.01*
P (rel. unit)	0.32 ± 0.03	0.44 ± 0.02*
$RCII_{cell}$ (amol $cell^{-1}$)	0.60 ± 0.02	0.81 ± 0.05*
σ_{PSII} (nm^2)	5.35 ± 0.11	4.77 ± 0.06*

Table 7 - Chla fluorescence-based photophysiological parameters in Chaetoceros sp. for Control and +Fe conditions. Values represent mean ± standard deviation (n=3). Significant changes ($p < 0.05$, ANOVA) relative to the Control condition are denoted by *.

The same positive effect is visible also in electron transport rates (ETR): absolute ETR (**Figure 18**) showed a clear difference between Control and +Fe treatment. Particularly, the maximum absolute electron transport rates ($aETR_{max}$) almost doubled from Control to +Fe, rising from 156.5 ± 22.4 to 304.2 ± 24.3 e^- $PSII^{-1}$ s^{-1} (**Table 8**).

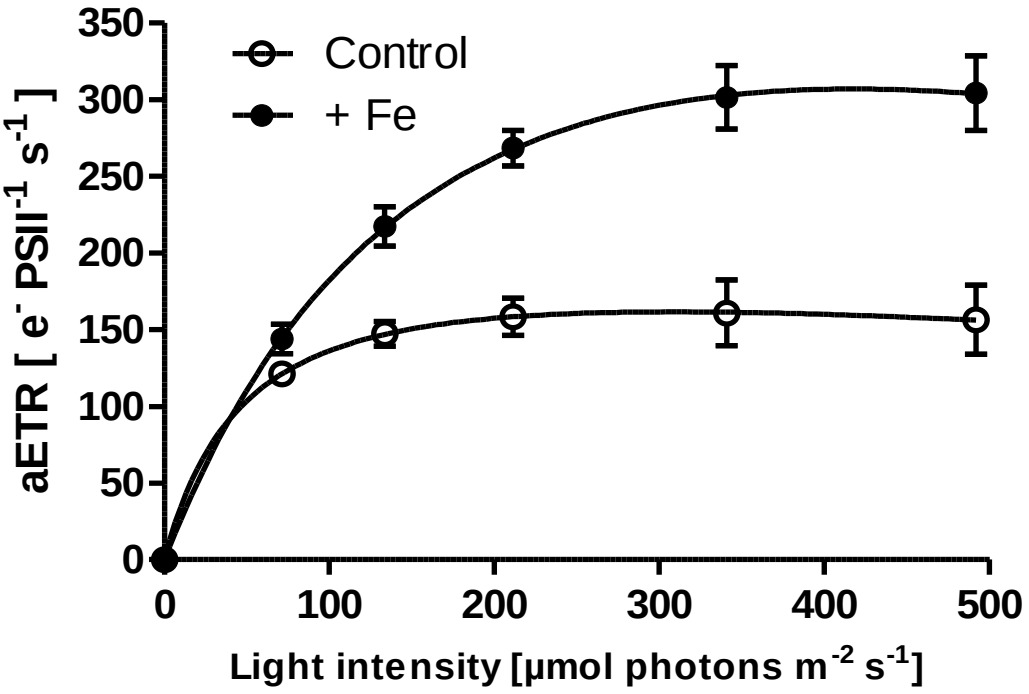

Figure 18 - Absolute electron transport rates (aETR) in *Chaetoceros* sp. grown under Control (open circle) and +Fe (filled circle) conditions. Values represent mean ± standard deviation (n=3).

The photo-acclimation parameter E_k (derived from fluorescence-based photosynthesis rates P_F) revealed a very different light acclimation status of *Chaetoceros* cells, depending on the iron condition. Accordingly, the E_k value increased more than 3-fold from Control to +Fe conditions (**Table 8**). However, this did not affect the sensitivity to photoinhibition as deduced from the values of Fv/Fm recovery (**Table 8**).

	Control	**+Fe**
$aETR_{max}$ (e^- $PSII^{-1}$ s^{-1})	156.5 ± 22.4	304.2 ± 24.3*
E_k value	33.8 ± 8.2	124.2 ± 30.0*
Fv/Fm recovery (%)	46.9 ± 1.5	51.0 ± 1.8

Table 8 - $aETR_{max}$, photoacclimation parameter E_k and Fv/Fm recovery (%) in *Chaetoceros* sp. for Control and +Fe conditions. Values represent mean ± standard deviation (n=3). Significant changes ($p < 0.01$, ANOVA) relative to the Control condition are denoted by *.

Neither the light-dependent induction kinetics nor the NPQ at the highest light intensity in *Chaetoceros* was influenced by the different iron conditions (**Figure 19**).

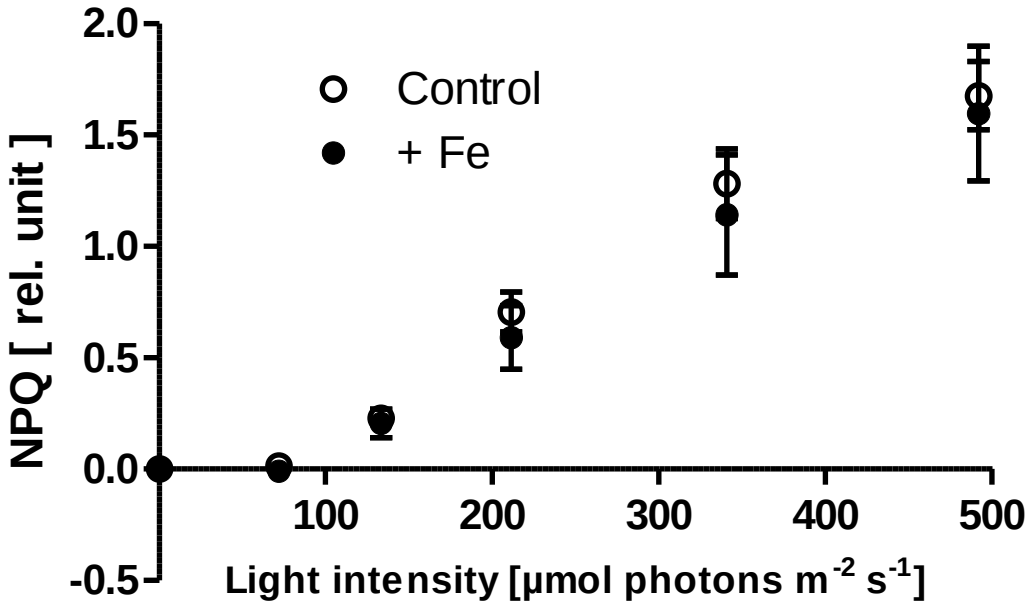

Figure 19 - Non photochemical quenching (NPQ) in *Chaetoceros* sp. for Control (open circle) and +Fe (filled circle) conditions. Values represent mean ± standard deviation (n=3).

3.2.4. Oxygen-based photosynthesis and respiration rates

The different iron treatments induced similar effects on gross oxygen production rates (**Figure 20a**) as observed for the fluorescence-based aETR (**Table 8** and **Figure 18**). As depicted in **Figure 20a**, GP_{max} in iron deplete condition was only 24% of the GP_{max} in the iron replete condition. The +Fe treatment reached gross photosynthesis (GP) rates of 397 µmol O_2 mg $Chla^{-1}$ h^{-1}, while the Control conditions produced at the highest 96 µmol O_2 mg $Chla^{-1}$ h^{-1}. An even greater difference between +Fe and Control conditions was measured in net photosynthesis (NP) rates (**Figure 20b**), with NP_{max} values equal to 287 and 55 µmol O_2 mg $Chla^{-1}$ h^{-1}, respectively. Unfortunately, the relatively low Chla concentration used in these measurements caused a low signal/noise ratio and resulted in a large standard deviation. Nevertheless, the measured differences between both iron conditions follow the same trend as aETR measured by fluorescence, i.e. the Control treatment caused a significant decrease in GP and NP compared to +Fe treatment, like aETR (see above).

Similarly, respiration (R) was significantly lower under Control conditions ($p < 0.05$, **Table 9**). The ratio of GP to R (GP/R, **Table 9**) was also calculated, interestingly showing no variations between the two analyzed conditions. Both the values reported in **Table 9** are referring to growth light intensity (i.e. 100 μmol photons m^{-2} s^{-1}).

	Control	**+Fe**
Respiration (R) [μmol O_2 (mg Chla h)$^{-1}$]	-39.8 ± 8.9	-112.3 ± 56.3*
GP/R	2.9 ± 1.7	2.7 ± 1.6

Table 9 - Respiration (R) and GP/R in *Chaetoceros* sp. for Control and +Fe conditions. Values represent mean ± standard deviation (n=3). Significant changes ($p<0.05$, ANOVA) relative to the Control condition are denoted by *.

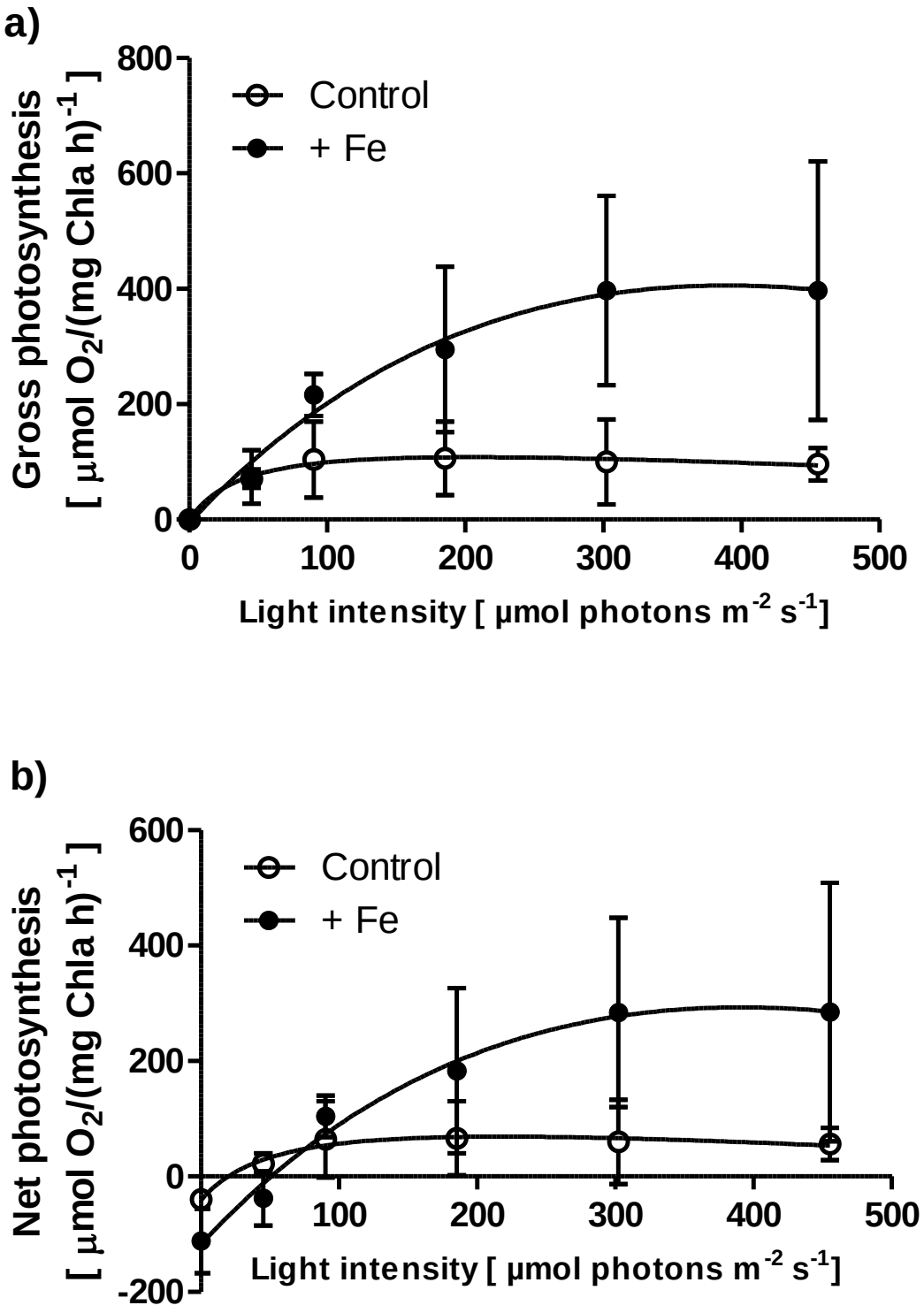

Figure 20 - Gross (a) and net (b) photosynthesis in *Chaetoceros* sp. for Control (open circle) and +Fe (filled circle) conditions. Values represent mean ± standard deviation (n=3).

4. Discussion

4.1. Temperature and salinity

4.1.1. Effects on photosynthetic rates

Although numerous studies have highlighted the importance of the phytoplankton contribution on microbial respiration and gross carbon production in the SO (Arístegui et al. 1996), the quantitative extent of respiratory losses by phytoplankton is not well understood. Additionally, to our knowledge there is no study dealing with the influence of multiple stressors on both, photosynthesis and respiration rates, in Antarctic phytoplankton. Thus, the aim of this study was the investigation of variations in the ratio photosynthesis to respiration, in dependence on combined temperature and salinity changes in two typical phytoplankton strains of the SO.

The analysis of photosynthetic rates revealed no clear correlation between temperature and P_{max} values in *Chaetoceros* sp. and *Phaeocystis antarctica* over all temperatures tested. This observation is rather unexpected, even though in accordance with previous studies (Tilzer and Dubinsky 1987; Thomas et al. 1992), because P_{max} is mainly defined by the activity of the enzyme RubisCO, whose activity should be directly correlated with temperature. A possible explanation for this contradictory observation might be that CO_2 solubility increases more than O_2 solubility when temperature diminishes (Kranz et al. 2014). Moreover, the temperature effect can be compensated by a higher cellular RubisCO content at lower temperature (Young et al. 2015).

For salinity conditions, only at a growth temperature of 4 °C was the trend of decreasing P_{max} at higher salinity observed in all investigated species. It should be noted, however, that the natural variability of water salinity at 4 °C is rather small in the SO. Thus, natural phytoplankton will most likely not be confronted with a combination of higher temperature and salinity values > 40 PSU. On the other hand, the fact that only *Chaetoceros* sp. but not *P. antarctica* was able to grow at a combination of -1 °C and a salinity of 70 PSU shows that the ability to acclimate to such conditions is restricted to phytoplankton species usually found in sea ice.

The comparison of the results of different studies with respect to the physiological acclimation of phytoplankton to different salinity conditions reveals a huge variability and partly

contradictory results (Palmisano et al. 1987; Arrigo and Sullivan 1992; Petrou and Ralph 2011). This could be due to the species-specific acclimation potential, but also to the different experimental conditions in the mentioned studies. Species-specific photosynthetic capacity in our study highlights different sensitivities to temperature and salinity changes, constituting an important mechanism in ecological niche adaptation. Precisely, *Chaetoceros* sp. was better adapted to sea-ice-like environment (low temperature, high salinity), while *P. antarctica* showed a lack of plasticity, dealing with the same conditions. The ability to acclimatise to changing environmental conditions might be crucial for the survival or extinction of Antarctic species. A new equilibrium in the phytoplankton community could occured, as consequence of climate change, with the overcoming of some species among others.

The mean values of P_{max} in *P. antarctica* (43 µmol O_2 (mg Chla h)$^{-1}$) and in *Chaetoceros* sp. (50 µmol O_2 (mg Chla h)$^{-1}$) are close to photosynthesis rates of approximately 60 O_2 (mg Chla h)$^{-1}$ measured in phytoplankton from SO under low-light conditions given in (Tilzer and Dubinsky 1987; Thomas et al. 1992; Gleitz and Thomas 1992; Thomas and Dieckmann 2002). Thus, the relatively low P_{max} values of the present study compared to some other studies (Petrou et al. 2011) are most probably due to the low growth light intensity used in the present study. This hypothesis is supported also by a recent study with different Antarctic species, where the increase in P_{max} with high light was confirmed (Kulk et al. 2019).

It was rather surprising that no photo-inhibitory effects (e.g. decrease of photosynthesis rates) were observed in the measurements of P-E curves at high irradiances with up to 700 µmol photons m^{-2} s^{-1}. In addition, NPQ_{max} was reached always at the highest applied irradiance within P-E curves (713 µmol photons m^{-2} s^{-1}), while photosynthetic rates were saturated at least at about 400 µmol photons m^{-2} s^{-1}. This could be interpreted in the way that the full potential of light protection in the investigated species was required at very high irradiance only, which is an indication of a very high overall potential of light protection. Thus, in our opinion, the higher NPQ_{max} values in *Chaetoceros* sp. are not an indication of photo-inhibitory stress but of a high photoprotective potential. These results are in contrast to the study of (Petrou and Ralph 2011), where strong photo-inhibitory effects were observed in *Chaetoceros* sp. in measurements of oxygen evolution. It could be speculated that these differences were induced by the different lengths of acclimation period to experimental conditions, with three days in (Petrou et al. 2011) and at least one week in the present study. Longer acclimation periods to changing temperature and salinity could have induced photoprotective mechanisms (Lyon and Mock 2014) that prevented photo-inhibitory effects at high irradiance.

4.1.2. Effects on respiratory losses and rP/R

No linear correlation between respiration rates and the salinity of the growth medium was revealed, like with P_{max}. In contrast to photosynthetic rates, a general trend of increasing respiration rates with the increase of growth temperature from 1 to 4 °C at a salinity of 35 PSU was observed in both strains, *Chaetoceros* sp. and *P. antarctica*. In *P. antarctica,* this trend was also found at a salinity of 50 PSU. These changes in respiration rates also influenced the temperature-dependent changes of the ratio of gross photosynthesis to respiration (rP/R). At a salinity of 35 PSU, *Chaetoceros* sp. and both strains of *P. antarctica* showed decreasing rP/R with increasing growth temperature from 1 to 4 °C. From these results two major conclusions could be drawn: first, the changes in rP/R were primarily due to variations in respiration but not in photosynthetic rates, and second, rP/R is primarily temperature-dependent, whereas the impact of the salinity is of minor importance for rP/R.

These results strongly support the few previous observations of a temperature dependence of respiration rates in phytoplankton of the SO (Tilzer and Dubinsky 1987; Thomas et al. 1992; Regaudie-de-Gioux and Duarte 2012). The novel finding of the present study is that salinity influenced this temperature dependence of respiration only to a very small degree in *Chaetoceros* sp., whereas in *P. antarctica* an effect of salinity was observed specifically in the combination with low salinity (20 PSU).

Moreover, this study provides values for taxon-specific respiratory losses in SO phytoplankton. Indeed, it should be noted that the degree of temperature dependence of respiration was different in the comparison of *Chaetoceros* sp. and the two strains of *P. antarctica*. While *Chaetoceros* sp. did show the highest mean values of rP/R with the least variability in dependence on experimental conditions, a distinctly larger variability of rP/R values was observed in *P. antarctica* in the comparison of -1 and 4 °C growth temperature.

Accordingly, for all investigated experimental conditions, the respiratory losses in relation to GP were in the range of 8 – 14% in *Chaetoceros* sp., 8 – 25% in *P. antarctica* strain 764, and 8 – 33% in P. antarctica strain 109, with the lowest and highest losses at -1 and 4 °C, respectively. This is similar to the range of respiratory losses in natural phytoplankton of the SO during austral summer given in (Arístegui et al. 1996).

The species-specific differences of rP/R were also evident under different seasonal conditions where *P. antarctica* showed significantly higher rP/R values in autumn/winter compared to spring/summer whereas, the season-specific rP/R values varied not significantly in *Chaetoceros* sp..

In phytoplankton from temperate habitats, diatoms show the higher maximum respiratory losses (up to 50%) than Haptophyte (up to 14%; Geider and Osborne 1989). Our study shows that this observation cannot be generalized. To our knowledge there are no other taxon-specific investigations of respiratory losses in SO phytoplankton.

4.1.3. Respiratory losses and net primary production

An important aim of the present study was the evaluation of the impact of different rP/R on NPP estimates in representative phytoplankton species from the SO. Therefore, the present data set was used to obtain general information on the effect of different rP/R on NPP estimates for specific irradiance, temperature, and salinity combinations that represent different seasonal conditions. The comparison of species-specific NPP for the different seasons showed a comparable pattern for all investigated species. The highest NPP was calculated for the 'Summer' condition with high irradiance, short dark period, and high water temperatures. Consequently, there was no negative effect of assumed respiratory losses up to a value of 20% on NPP estimates in comparison to NPP estimates with measured respiratory losses. Only with the assumption of 30% respiratory losses was there a significant underestimation of NPP for *Chaetoceros* sp. and *P. antarctica* strain 764 compared to NPP estimates with measured respiratory losses, respectively. Due to the saturation of photosynthesis at relatively low irradiance in the investigated algal strains, the NPP estimates did not depend on the differences in the maximum irradiance at the different seasonal conditions, but were mainly influenced by the length of the daily illumination period and the thus correlated respiratory losses. Therefore, the 'Spring' condition (16/8h, light (L) /dark (D) period) yielded slightly lower NPP estimates compared to the 'Summer' condition (19/5h, L/D), whereas NPP estimates under the 'Autumn' condition (11/13h, L/D) were significantly reduced. In the latter condition, it is noteworthy that the assumption of respiratory losses > 10% yielded significant underestimates of NPP compared to the estimation with measured respiratory losses. These seasonal differences in NPP estimates of the present study are comparable to the NPP estimation in Petrou and Ralph (2011).

The strongest impact of respiratory losses was observed in NPP estimates for the 'Winter' condition (6/18h, L/D). Here, a positive NPP (estimated on the basis of measured respiratory losses) was observed for *Chaetoceros* sp. and *P. antarctica* strain 109, only. Due to the very short light period, the total photosynthesis rate and carbon assimilation were strongly reduced. This means that these algal strains were able to keep respiratory losses at a minimum and would survive unfavourable 'Winter' conditions. This is in line with results of (Thomas et al. 1992) where strongly reduced but still positive carbon uptake rates were measured under a combination of low irradiance (5 µmol m^{-2} s^{-1}) and low temperature (-1.5 °C) in the diatom *Chaetoceros*. The importance of minimized respiratory losses under winter conditions is also deducible from the assumption that respiratory losses >10% would yield negative NPP estimates and would strongly impede the ability of phytoplankton cells to survive such conditions. The same holds true for the *P. antarctica* strain 764 for which a negative NPP was estimated on the basis of measured respiratory losses. It has to be mentioned that increased salinity is also an important factor under these conditions. Whereas *Chaetoceros* sp. grew even at a salinity of 70 PSU, there was no growth observed in *P. antarctica* at this salinity, with 50 PSU being the highest salinity to promote growth in *P. antarctica*. Obviously, there were species-dependent and even strain-dependent differences in the acclimation to the combination of low temperature, low irradiance, and increased salinity. On the other hand, these observations are in line with the ecological niche in which these algae are found: *Chaetoceros* sp. is a typical ice alga of the SO, whereas *P. antarctica* prefers open ocean areas as outlined above.

To the best of our knowledge, the independence of NPP from variations of rP/R due to different environmental conditions was not shown before.

4.1.4. Species specific differences in acclimation to variations in temperature and salinity

One of the major results from this study is the observation of species- and even strain-specific differences in several parameters measured. The first to mention is the maximum NPP estimate particularly under 'Spring' and 'Summer' conditions. In general, the highest NPP estimates were observed for *Chaetoceros* sp., whereas NPP for *P. antarctica* strains was significantly lower. Thus, the patchiness observed in field measurements of NPP in the SO (Arístegui et al. 1996) could be caused by different species composition in combination with hydrogeographical differences at the investigated field stations.

The strongest interspecies differences were observed in the maximum NPQ values. High mean NPQ_{max} values between 6.4 and 12.9 were observed in *Chaetoceros* sp., whereas NPQ_{max} in *P. antarctica* was in the range of 1 to 2.4 (with the exception of NPQ_{max} = 3.6 for strain 764 at 1 °C / 20 PSU). Particularly, the NPQ_{max} values for *Chaetoceros* sp. were distinctly higher in the present study compared to previous publications (Park and Lee 2010; Petrou et al. 2011; Petrou et al. 2014). This could be caused by methodological differences in the measurement of NPQ, e.g. Rapid light curves applied in the studies mentioned above vs. P-E curves with steady state-conditions as used in the present study. On the other hand, the species dependence of NPQ_{max} values in the comparison of different Antarctic phytoplankton species and, in particular, the higher NPQ_{max} in diatoms than NPQ_{max} in *P. antarctica* was also shown in previous publications (Kropuenske et al. 2009; Alderkamp et al. 2012; Petrou et al. 2011). The non-photochemical quenching is designated as a very important mechanism to adapt to dynamic light conditions as experienced by the phytoplankton in their natural habitats (Lavaud et al. 2007). Thus, the differences in NPQ_{max} in Antarctic phytoplankton were explained by the acclimation of the species to different environmental conditions, e.g. to sea ice or highly stratified water conditions in the case of *Chaetoceros* sp., in contrast to deeply mixed waters in the pelagic zone in the case of *P. antarctica* (Petrou et al. 2011).

With respect to an acclimation to dynamic environmental conditions, the extent of alternative electron transport is of importance. Alternative electrons are not used for the reduction of $NADP^+$. Instead, they contribute to e.g. cyclic electron transport at PSII and PSI, to the water-water cycle, to photorespiration, to the reduction of nitrate and sulphate (Halsey and Jones 2015;

Wagner et al. 2017), and, thus, to the generation of the trans-thylakoid pH gradient. Therefore, it is assumed that the activity of alternative electron transport changes the photosynthetic NADPH/ATP ratio in favour of ATP (STREB et al. 2005). It is, however, not known whether this additional ATP production could compensate for ATP production by e.g. lowering respiration rates. The number of alternative electrons is estimated by the difference between fluorescence-based and oxygen-based photosynthesis rate (P_F/P_O). Particularly in diatoms, the activity of alternative electron pathways contributes to photoprotection under variable environmental conditions (Wagner et al. 2016). In the present study, it is noteworthy that the diatom *Chaetoceros* sp. showed a relatively constant but higher ratio P_F/P_O than the strains of *P. antarctica*. This is in line with the significantly higher Chl-specific absorptivity (a^*_{phy}) of the cells of *Chaetoceros* sp. at 1 and 4 °C growth temperature compared to *P. antarctica*. The a^*_{phy} value describes the wavelength-dependent and Chla-normalized absorptivity (spectrally integrated optical absorption cross section) of phytoplankton cells. It depends not only on Chl concentration but also on the content of accessory pigments and the package effect which is usually adjusted by phytoplankton cells in response to light or nutrient acclimation (Kirk 2010). In this way, the α-slope of P-E curves is directly correlated with a^*_{phy}. In the present study, a^*_{phy} was used to convert rETR into fluorescence-based photosynthesis rates (P_F). Thus, the higher a^*_{phy} values in *Chaetoceros* sp. resulted in higher P_F/P_O, and it could be assumed that *Chaetoceros* sp. was forced to dissipate a larger fraction of absorbed light by alternative electron pathways than was necessary in *P. antarctica*. These differences were even more pronounced when the season-specific ratio of fluorescence-based to oxygen-based NPP estimated were compared. For the 'Spring' condition, there were significant differences in the NPP_F/NPP_O ratio between *Chaetoceros* sp. and *P. antarctica*. Since in *P. antarctica* these differences were in contrast to the season-specific changes in a^*_{phy} it has to be concluded that the relative extent of respiratory losses under the shorter daylight periods in 'Autumn' and 'Winter' conditions is of major importance for the ratio NPP_F/NPP_O.

Thus, the investigated algal strains showed very different strategies to cope with changing environmental conditions. Accordingly, *Chaetoceros* sp. appears to be less flexible in the regulation of the extent of photoprotective mechanisms (NPQ and alternative electrons), but the photoprotective level is generally higher in *Chaetoceros* sp. than in *P. antarctica*.

4.2. Iron limitation

4.2.1. Iron limitation effects on the physiology of *Chaetoceros* sp.

Iron is essential for redox-based reactions and is required for photosynthesis, respiration, and the nitrate and sulfur utilization in phytoplankton (Behrenfeld and Milligan 2013; Raven 2013). Since iron is important for the proteins of the electron transport chain (ETC), iron deficiency directly influences the photosynthetic performance of the cells (Behrenfeld and Milligan 2013). In agreement with this, the strongest responses of the here tested Antarctic diatom *Chaetoceros* sp. to iron depletion were found in PSII. Hence, the quantum efficiency of photosynthesis declined from 0.50 to 0.38 (**Table 7**). Similarly, the number of functional reaction centers per cell, $RCII_{cell}$, was reduced under Control, as well as the connectivity between photosystems II, P, a response commonly observed under iron limitation. Conversely, the functional absorption cross section of PSII, σ_{PSII}, increased in the cells under the latter condition. Such a response is attributed to an increase in the ratio of antenna complexes relative to the reaction center core complexes (Greene et al. 1991). It was suggested that Southern Ocean phytoplankton species in particular counteract the diminished number of iron-rich reactions centers with a larger σ_{PSII} (Strzepek et al. 2012), as frequently observed for other Antarctic *Chaetoceros* species (Timmermans et al. 2001; van Oijen et al. 2004; Petrou et al. 2014; Trimborn et al. 2019). This statement is supported by the comparison of the initial slope of the P-E curve, with α values being higher in Control compared to +Fe treatment (**Figure 18**).

In the photosynthetic apparatus, both PSI and cytochrome b_6f have high iron requirements. The limiting components of the electron flow in the thylakoid and mitochondrial membranes are the cytochrome b_6f and the bc complex, respectively. It has been shown (Wilhelm and Wild 1984) that the amount of Rubisco and cytochrome f (cyt f) are regulated in a coordinated manner and therefore, the concentration of cyt f correlates with GP_{max}. Both cytochrome complexes, i.e. b_6f and bc, contain 3 cytochrome molecules, each with an iron atom. Under iron limitation, iron cannot be replaced and the cytochrome complex cannot be substituted by alternative electron carriers. Thus, iron limitation strongly limits the capacity of aETR (**Figure 18**); precisely, the $aETR_{max}$ in the Control was almost the half compared to +Fe treatment (**Table 8**).

The decrease in photosynthetic rates under iron deficiency was even more impressive considering GP_{max} and $NP_{max.}$ Specifically, the maximum photosynthetic values in the Control compared to +Fe treatment were only 20% and 25% for NP and GP (**Figure 20**), respectively. The differences in light-saturated GP could be accounted for by a lowered Rubisco content in Control condition. Indeed, it was already shown that the relative abundance of the carboxylating enzyme (Rubisco) decreased in response to iron starvation (Geider et al. 1993). Anyhow, the significantly lower values of GP_{max}, $aETR_{max}$ and E_k (discussed later) in Control compared to +Fe conditions are in line with other data reported in literature for *Chaetoceros* (Petrou et al. 2014).

In addition, iron deficiency reduced the light saturation index E_k (**Table 8**), showing the inability of these cells to use higher irradiance for increased carbon assimilation, as shown by the daily POC production (**Table 5**). This aspect is in line with the reduction in growth rates (μ; **Table 5**) under Control conditions, clearly indicating an energy problem.

The lower daily carbon production rates, POC and PON content per cells (**Table 5**) could be caused by a less efficient energy transfer from photochemistry to biomass production under Control conditions, as already suggested for *Chaetoceros debilis* (Trimborn et al. 2019). Neverthless, if POC data are normalized to cell volume (POC_{vol}, **Table 5**) rather than to number of cells, the reduction in POC production is not visible anymore. Therefore, we can suggest that the cell size reduction compensated and ensured similar high POC production in the two treatments. The relevant decrease in cell volume, Chla quotas, rates of POC production under iron limitation, have been measured not only in Antarctic diatoms, but also in other Antarctic taxa such as prymnesiophytes e.g. *P. antarctica* (Koch et al. 2019).

As a consequence of the above mentioned lowered amount of cyt b_6f, it was further suggested that Fe limitation in diatoms lowers the photoprotection by NPQ (Strzepek and Harrison 2004). This is due to the function of cyt b_6f complexes as proton translocators across the thylakoid membrane. A diminished amount of cyt b_6f should also lower the proton translocation rate and thus, the extent of the proton gradient across the thylakoid membrane. Nevertheless, the $\triangle$pH is an indispensable prerequisite for the formation and the extent of NPQ (Tian et al. 2019).

Indeed, no significant differences in non-photochemical quenching, NPQ, were observed between the two treatments (**Figure 19**). This is in contrast with what was reported by Petrou et al. 2014 where a strong difference was reported. However, variations in the experimental set up should be considered. Firstly, *Chaetoceros* cells were grown at different light intensity, namely 30 µmol photons m^{-2} s^{-1} in Petrou et al., while the light intensity in the present study was significantly higher (i.e. 100 µmol photons m^{-2} s^{-1}). Secondly, the NPQ_{max} values in Petrou et al. were obtained with a light intensity 5 times higher than in this study (2260 µmol photons m^{-2} s^{-1} and 492 µmol photons m^{-2} s^{-1}, respectively). Having a closer look at the data in former study, the NPQ values in iron limited cells are even lower than in the present study until 500 µmol photons m^{-2} s^{-1}, while only at higher light intensities, NPQ strongly increased during the PI curve. Recently, also Trimborn et al. (2019) observed an enhanced NPQ for Control relative to +Fe cells in *Chaetoceros debilis* cells, suggesting this was due to the operation of PTOX.

The large decrease of the electron transport capacity (**Figure 18** and **Figure 20**) should have forced the Control cells to re-balance the light absorption capacity with the lower capacity of photosynthetic energy usage in cell's metabolism. Indeed, with a severe decrease of cellular Chla content (**Table 6**) Fe-deplete cells aimed to re-balance the reduced light use capacity and the light supply by the adjustment of absorption capacity, rather than dissipate the excess of light through NPQ. This re-balance of light supply and light use was obviously quite efficient, since the cells were neither forced to increase their LPP, nor their NPQ capacity (**Table 6** and **Figure 19**). A similar acclimation strategy can be observed under other nutrient deprivation, e.g. nitrate limitation (Young and Beardall 2003). Neither diadinoxanthin, nor diatoxanthin were significantly different in the two treatments (**Table 6**), so we can assume that NPQ was based on qE and not on other NPQ components, as e.g. qI (photoinhibition). Moreover, the P-E curve (**Figure 20**) and the Fv/Fm recovery rate (**Table 8**) did not reveal any indications for an increased sensitivity to high irradiances in cells under Control conditions.

Noteworthy is the strong decrease of the ß-carotene content under Control conditions (**Table 6**), possibly due to the decrease in $RCII_{cell}$ (**Table 7**). Since ß-carotene is attached to the reaction centers of PSII in the photosynthetic apparatus, a diminution of the latter could be correlated positively with the former. To the best of our knowledge, among the different studies analyzing pigments in Antarctic diatom, no data about β-carotene content were reported.

4.2.2. Respiratory losses and rP/R

Under natural conditions, the estimation of NPP is hindered by difficulties in the measurement of respiratory loss rates of phytoplankton in dark periods. Therefore, calculations of NPP have to employ algorithms with fixed respiration rates, or models with very noisy respiration data (Moisan and Mitchell 2018).

This constraint is mainly due to methodological limitations (reviewed in Marra 2009). Net community respiration can be measured from the consumption of O_2 in samples incubated in the dark. However, respiration by phytoplankton alone cannot be separated by heterotrophs' respiration. Nowadays, respiration is still measured either from laboratory culture or, indirectly, from other kinds of measurements.

Therefore, a novel aspect of this study was the measurement of the respiration rates in the Antarctic diatom *Chaetoceros* sp. (**Table 9**). The iron-limited conditions induced a drastic decrease of R by 65% compared to +Fe conditions at the growth light intensity. This had a strong influence on NP rates (**Figure 20b**), for example in the light compensation point, which in Control conditions was 70% lower than in Fe replete conditions. Surprisingly, under low irradiance (below 80 µmol photons m^{-2} s^{-1}) the NP rates in Control were considerable higher than in +Fe condition. Contrarily, at growth light intensity (100 µmol photons m^{-2} s^{-1}), the NP rate of cells under Control conditions was only slightly lower than in cells under +Fe conditions. However, GP rates show that the general photosynthetic capacity was strongly reduced in Control cells, with GP_{max} being 76% lower than in Fe replete cells (**Figure 20b**). Therefore, Fe deplete cells showed a very efficient acclimation to iron limitation by decreasing their respiratory losses. This compensates for the inevitable limitations of photosynthesis under Control conditions. This is also reflected by the fact that the ratio of photosynthesis to respiration (GP/R, **Table 9**) did not change in response to iron-deplete conditions. Thus, up to an irradiance of 100 µmol photons m^{-2} s^{-1} the iron limitation is not a major issue for the growth rate of *Chaetoceros* sp. due to very efficient acclimation.

5. Conclusions and Future Perspectives

The increase of atmospheric CO_2 has been predicted to impact the seasonal cycle of inorganic carbon in the global ocean (Le Quéré et al. 2018) and, indeed, the amplitude of this cycle has increased already over the last decades (2018). Especially at high latitudes, like in the SO, biological CO_2 draw-down represents a key factor and leads to an oceanic pCO_2 (partial pressure of CO_2) minimum in summer, when biological productivity is high (Hauck 2018). However, biological production is highly seasonal and physical changes in the growing season determine its response to climate change (Hauck et al. 2015). Nevertheless, oceanographers lack an understanding of fundamental properties of plankton dynamics, leaving a large gap in our knowledge of how biological processes contribute to the ocean's C cycle (Marra 2009). One of these crucial properties is respiration, which, until now, has always been inferred from laboratory culture or, indirectly, from other kinds of measurements. Furthermore, current methods usually estimate community respiration, without distinction between autotrophic and heterotrophic respiration.

In light of the importance of the SO for the atmospheric CO_2 level (Landschützer et al. 2015; Gruber et al. 2019), it is essential to understand the influence of combined changes of environmental factors on respiratory losses in relation to the photosynthetic activity of the phytoplankton. Knowing autotrophic respiration, we can more accurately predict phytoplankton growth rates and their role in the microbial food web.

Therefore, the present work aims to fulfill this gap in our knowledge. Experimental data on the variability of photosynthesis to respiration ratio (rP/R) in ecological Antarctic phytoplankton species were successfully collected. In this respect, two key species from the Southern Ocean were investigated: the diatom *Chaetoceros* sp. and the prymnesiophyte *Phaeocystis antarctica*. Three hypotheses were tested, analysing this ratio under different environmental conditions and three parameters were also investigated, testing this basic hypothesis: the P/R ratio varies as a function of temperature and nutrient availability in an ecotype-dependent manner.

Laboratory-based methods were employed to measure photosynthetic and respiration rates, fluorescence and cell parameters. More specifically, the effect of temperature and salt concentration were examined, analysing the following hypotheses:

1. P/R ratios are temperature dependent in a way that the daily carbon assimilation rate is significantly influenced.
2. Temperature dependency of rP/R persists under different salinity conditions.

It was proved that the P/R ratio is temperature dependent. Particularly, the changes in this ratio were principally due to variations in respiration, rather than in photosynthesis. A few studies have reported a temperature dependence of respiration rates in SO phytoplankton (Tilzer and Dubinsky 1987; Thomas et al. 1992; Regaudie-de-Gioux and Duarte 2012), but no information is available about salinity effects. This parameter was the subject of study of our second statement, where the interpretation of the results was complicated. More specifically, salinity has only a secondary importance on the rP/R variations, although with species-specific differences. Different salinities, indeed, influence the temperature dependence of respiration only to a minor degree in *Chaetoceros* sp., while totally new was the observed effect of low salinity (20 PSU) on the same parameter in *P. antarctica*. Furthermore, *Chaetoceros* sp. showed the highest rP/R mean values, with the least variability in dependence on experimental conditions, whereas a distinctly larger variability of rP/R values was observed in *P. antarctica* in the comparison of -1 and 4 °C growth temperatures. The species- and even strain-specific differences in rP/R were also evident in other physiological parameters measured.

Thus, the investigated algal strains showed very different strategies to cope with changing environmental conditions. Accordingly, *Chaetoceros* sp. appears to be less flexible in the regulation of the extent of photoprotective mechanisms (non-photochemical quenching and alternative electrons), but the photoprotective level is generally higher in *Chaetoceros* sp. than in *P. antarctica*. The characteristic photoacclimation strategies are perfectly in line with the ecological niche occupancy of the two taxonomic groups. Precisely, *Chaetoceros* sp. is better adapted to sea-ice-like environment (low temperature, high salinity), while *P. antarctica* showed a lack of plasticity in dealing with the same conditions.

However, the rP/R variability had only a small influence on estimated NPP rates for specific seasonal conditions. This was due to the finding that phytoplankton cells were able to keep respiratory losses relatively low. According to the collected data it is deduced, that respiratory losses in the range of 10 – 15% should represent realistic values to convert measured GPP into NPP under field conditions. With respect to the low level of respiratory losses, the accurate determination of photosynthesis rates becomes even more important.

In this respect, the observation of a very different extent of alternative electron pathways in the comparison of *Chaetoceros* sp. and *P. antarctica* is a remarkable result. Thus, the estimation of NPP by the measurement of, for example, variable Chl fluorescence in populations with different species composition and at different seasonal conditions could be significantly influenced by the activity of these alternative electron pathways. Nevertheless, it should be emphasized that changes of other environmental factors (e.g. nutrient availability, grazing pressure) may induce stronger variation of rP/R. In this case, the impact on NPP needs to be re-evaluated.

The investigation of the third parameter, i.e. iron limitation, was very challenging. Working in an iron free environment is a tricky task, not only because iron is everywhere, but also because algae with limited iron supply are very difficult to cultivate and grow much more slowly than those in rich media. For this reason, only rP/R in *Chaetoceros* sp. was measured, with highly interesting preliminary results. *Chaetoceros* sp. cells showed a very efficient acclimation to iron limitation by decreasing their respiratory losses. This compensates for the inevitable limitations of photosynthesis and the ratio of photosynthesis to respiration did not change in response to iron-deplete conditions.

In this thesis, it was attempted to measure the same parameters with *P. antarctica*, but either problems with iron contamination in iron limited cells, or too low signal-to-noise ratio, prevented successful results with this species. Differences in *Chaetoceros* and *P. antarctica* species under iron limitation were observed in a very recent study (Trimborn et al. 2019), but important information about respiration have not been reported yet. Therefore, further experiments with other important SO species, like *P. antarctica*, are necessary to understand the species-specificity of these effects.

Nonentheless, at least with *Chaetoceros* sp., we proved our third hypothesis, namely:

3. P/R ratio remains constant under iron-limiting conditions

For the first time, a combination of chlorophyll *a* fluorescence, O_2 evolution and particulate organic carbon production measurements were used to understand the effect of low iron availability on the usage of photosynthetic electrons in cell metabolism and finally in carbon production in *Chaetoceros* sp. More studies are needed to infer the influence of P/R ratio on NPP in dependence on iron availability.

6. References

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Education

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Supervisors: Prof. Dr. Christian Wilhelm and Dr. Torsten Jakob (Germany)
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The experimental work for this thesis was performed mainly in Germany in the Plant physiology laboratory of the University of Leipzig.
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The Effect of Climate Change on the Carbon Balance in Microalgae

- 17th Scientific Conference of the Section Phycology (DBG), 13.03.2018, Berchtesgaden, Germany

Deborah Bozzato, Torsten Jakob, Christian Wilhelm (2017):
Photosynthesis/respiration ratio in Antarctic algae under global climate change conditions

- 15. Mitteldeutsche Pflanzenphysiologie-Tagung, 10.02.2017, Lutherstadt Wittenberg, Germany

Deborah Bozzato, Torsten Jakob, Christian Wilhelm (2016):
Glycolate excretion in *Chlamydomonas* in response to different environmental conditions

- 14. Mitteldeutsche Pflanzenphysiologie-Tagung, 02.04.2013, Jena, Germany

Poster

Deborah Bozzato, Torsten Jakob, Christian Wilhelm (2018):
The Effect of Climate Change on the Carbon Balance in Microalgae

- 27th International Polar Conference, 25-29.03.2018, Rostock, Germany
- SCAR/IASC Open Science Conference at POLAR 2018, 19-23.06.2018, Davos, Switzerland
- Coordination-Workshop SPP 1158, 12-14.09.2018, Gießen, Germany

Deborah Bozzato, Torsten Jakob, Christian Wilhelm (2017):
The effect of climate change on the carbon balance between photosynthesis and respiration in planktonic and benthic microalgae

- XIIth SCAR Biology Symposium, 10-14 July 2017, Löwen, Belgium
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The effect of climate change on the carbon balance between photosynthesis and respiration in planktonic and benthic microalgae

- Coordination-Workshop SPP 1158, 14-16.09.2016, Rostock, Germany

Declaration of independent work

I, Deborah Bozzato, herewith declare that:

I have written the present thesis without improper aid and without the use of other resources than stated. I have marked any direct and indirect citations according to the guidelines of good scientific practice.

No other person provided support and contributed to this thesis. No PhD consultants were used, and no third party received direct or indirect financial benefits in goods and services for work related to the present thesis.

Furthermore, I certify that this research thesis or any part of it has not been previously submitted for a degree or any other qualification at the University of Leipzig or any other institution in Germany or abroad.

Deborah Bozzato

Leipzig, 01.10.2019

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