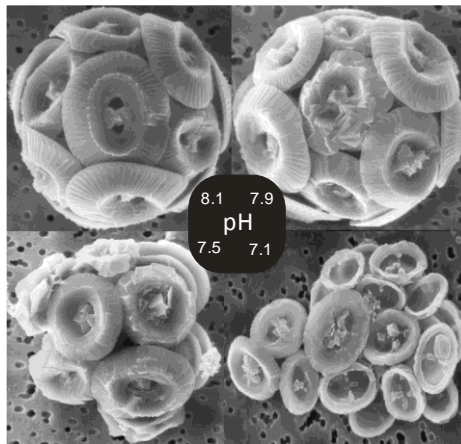


Coccolithophores in an Acidifying Ocean: from Single Strain to Multiple Species Approaches

Dissertation



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„Man schließt die Augen der Toten behutsam; nicht minder behutsam muss man die Augen der Lebenden öffnen.“

Jean Cocteau (1889-1963)

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SUMMARY

The human induced global climate change has severe consequences for the marine systems. Oceans have absorbed 50% of anthropogenic carbon dioxide emissions, consequently, attenuating global atmospheric warming. However, once entering the oceans, CO₂ loses its inert characteristics. By the reaction with water it forms carbonic acid resulting in the phenomenon latterly referred to as ocean acidification. In the last two centuries, with the beginning of the industrial revolution, the global mean surface pH was already reduced by 0.1 units. Within the next 90 years the acidity level is believed to drop by another 0.35 units.

Based on a simple causality a given atmospheric partial pressure of CO₂ can easily be transferred into surface ocean carbon chemistry. Nevertheless, implications for the marine biota caused by increasing ocean acidification are complex and difficult to assess. Although the effects of rising pCO₂ have been shown on single species of corals, pteropods, foraminifera, diverse phytoplankton species and larvae of echinoderms and fishes, research is far from understanding correlations between single species response and ecosystem functioning.

Based on the importance as the most prominent pelagic calcifier and their hypothesised functioning as carbon export ballast, coccolithophores are among the best evaluated species with respect to ocean acidification. Calcification and photosynthesis has turned out to be sensitive to future conditions, however, with highly variable responses among species and species strains. For most analysed species calcification declined with rising pCO₂, also the production of organic matter usually decreased, but turned out to rise for *Gephyrocapsa oceanica*. Surprisingly, the species *Coccolithus braarudii* appeared to be insensitive to an elevation of pCO₂ from 380 µatm to 750 µatm. Based on this intriguing picture of coccolithophore response to ocean acidification this dissertation was concerned with the following questions:

1. Does the insensitivity of *C. braarudii* to ocean acidification hold true for higher CO₂ concentrations and what is the reason for the different sensitivities of various species?
2. Does the total population carbon accumulation in the stationary phase reveal what could have been expected from the physiological ocean acidification response while undergoing exponential growth and are these results, gathered in the stationary phase dependent on the applied nutrient ratio?
3. Higher diversity is known to positively affect stability and resistance of ecosystems. Is it possible to extrapolate single species responses to ocean acidification to multi-species responses?

The study presented in chapter I affirms the insensitivity of *C. braarudii* for a pCO₂ range up to 800 µatm. Further increases to values of 2500 µatm, however, revealed a decrease in calcification. The biomass production by photosynthesis, showed an optimum at 1600 µatm

pCO₂. An optimum response has been observed earlier for the coccolithophore *Calcidiscus leptoporus*, however, for calcification. Based on these findings, an increase in dissolved inorganic carbon, i.e. increase in the substrate for calcification and photosynthesis was discussed as to be an advantage in the first place. At a certain CO₂ concentration the linked reduction in pH might negatively affect physiological processes and antagonise the positive effects of increasing substrate. For these reasons, the variable sensitivities of coccolithophores might not arise from different intracellular mechanisms but rather different optima for CO₂, HCO₃⁻ and H⁺.

Studies on coccolithophores in the exponential growth phase and their response to ocean acidification have proven to be useful to analyse underlying physiological mechanisms. Additional experiments concerning total population carbon accumulation in the stationary phase might allow for first estimates on ecosystem functioning of single species populations with respect to ocean acidification. Chapter II describes an experiment, allowing *Emiliana huxleyi* and *Gephyrocapsa oceanica* to deplete nutrients and stay for three days in the stationary phase. Under the stress of phosphate limitation and three different ocean acidification scenarios cells revealed an increase in cell size in the stationary phase. This increase was attenuated with rising pCO₂. Also the accumulations of calcite and organic carbon on a population level showed pronounced responses to increasing ocean acidification. These responses, however, were significantly dependent on the nutrient ratio the cells had to face. The biomass decrease for *Emiliana huxleyi* and increase for *Gephyrocapsa oceanica* as well as the decrease in calcite of both species were more pronounced for cells growing under a high N:P ratio compared to cells facing a Redfield ratio. This was contrary to the response on a population level. Due to changing nutrient uptake the more sensitive “High N:P” treatments were able to produce more cells with rising pCO₂, resulting in an attenuated calcite and biomass accumulation decrease. Based on these results, estimations of the future influence of coccolithophores on both atmospheric pCO₂ feedback and carbon export should take the affects of nutrient limitation on cell physiology stronger into consideration.

Despite their relevance for the physiological mechanisms of coccolithophores, hitherto published studies are far from assessing whole ecosystem functioning with respect to ocean acidification. Diversity is known to have positive effects on ecosystem stability and resistance, nevertheless, community interactions of coccolithophores and their collective response to rising pCO₂ were so far neglected. To gain first indications of the potential community interaction of coccolithophores, the experiment presented in chapter III allows three species, namely *G. oceanica*, *E. huxleyi* and *C. braarudii* to grow alone and within a community. Under the stress of nutrient limitation and three different pCO₂ single species cultures revealed a decrease in the population calcite and biomass accumulation. In contrary, the multiple species approach showed no significant variation in photosynthesis and calcification. This suggests a higher resilience caused by community interactions.

This dissertation underlines the importance of single species approaches to understand the underlying physiological processes with respect to ocean acidification. But it also shows our ignorance of marine ecosystem resilience and therefore, suggests that it might not be appropriate to extrapolate the changes in calcification rates of single species to ocean acidification onto a global scale. In near future it needs further experiments to evaluate the role of diversity and nutrient ratios as a positive factor for resilience and resistance of coccolithophore ecosystems.

ZUSAMMENFASSUNG

Der vom Menschen induzierte Klimawandel hat nicht nur eine Temperaturerhöhung der Atmosphäre und der Ozeane zur Folge. Wenn vielfach in den Medien bereits von dem „anderen CO₂-Problem“ gesprochen wird, dann ist die Rede von der Ozeanversauerung. Zwar ist Kohlendioxid in der Atmosphäre inert, reagiert allerdings mit Wasser zu Kohlensäure und führt somit in den Ozeanen zu einer Erniedrigung des pH-Wertes. Bis heute haben die Weltmeere 50% der vom Menschen verursachten Kohlendioxidemissionen aus fossilen Brennstoffen aufgenommen. Dies hat auf der einen Seite zu verringerten Anstieg der atmosphärischen CO₂-Konzentrationen geführt, andererseits wurde dadurch in den letzten 100 Jahren der globale Mittelwert des Oberflächen-pH-Wertes bereits um 0,1 Einheiten gesenkt. In diesem Jahrhundert könnte dieser Wert bei weiterschreitenden Emissionen um weitere 0,35 Einheiten sinken.

So einfach die Meereschemie bei bekannten atmosphärischen CO₂-Konzentrationen zu berechnen ist, so schwer ist es, die vielschichtigen möglichen biologischen Konsequenzen der Versauerung abzuschätzen und zu beurteilen. Vielfach konnten Auswirkungen auf die Biologie von Phytoplankton, Echinodermen- und Fischlarven, Pteropoden und Korallen gezeigt werden, dennoch ist die Wissenschaft noch weit davon entfernt, Zusammenhänge, die über die Reaktion von einzelnen Arten hinausgehen, zu verstehen und zu beurteilen. Ein besonderes Augenmerk lag in den vergangenen Jahren auf den Coccolithophoriden. Diese kalzifizierenden Einzeller produzieren neben den Flügelschnecken und Foraminiferen 50% des ozeanischen Kalks und spielen somit eine wichtige Rolle in der Ballast-Hypothese. In dieser Theorie wird der Export von organischer Materie maßgeblich von Mineralien getrieben. Zwar spielen Coccolithophoriden in Bezug auf die globale ozeanische Primärproduktion neben anderen Phytoplankton Arten, wie z. B. den Diatomeen, nur eine untergeordnete Rolle; sie sind dennoch die einzigen Primärproduzenten, die mit ihrer Kalzifizierung direkt die Pufferkapazität der Ozeane beeinflussen.

Bisherige Studien konnten zeigen, dass sowohl die Kalzifizierung als auch die Photosynthese von verschiedenen Coccolithophoriden durch die Ozeanversauerung beeinflusst werden. Allerdings zeigte sich zwischen den verschiedenen Arten, aber auch zwischen verschiedenen Stämmen derselben Art, Unterschiede in der Sensitivität. Insbesondere die Produktion von partikulärem organischem Kohlenstoff durch die Photosynthese zeigte sich bei einigen Arten durch Ozeanversauerung stimuliert, bei anderen wiederum gehemmt. Bei bis dato einer einzigen Art, *Coccolithus braarudii*, zeigte eine Erhöhung des pCO₂ von 380 auf 750 µatm keinerlei Auswirkungen auf die beiden Prozesse Photosynthese und Kalzifizierung.

Auf Grund dieser Ergebnisse beschäftigt sich die vorliegende Dissertation mit folgenden drei Fragen:

1. Bestätigt sich die gezeigte Insensibilität von *C. braarudii* auf Ozeanversauerung auch bei höheren $p\text{CO}_2$ -Werten und was ist der Grund für die unterschiedlichen Sensibilitäten verschiedener Arten?
2. Die bisherigen Ergebnisse zu den Effekten der Ozeanversauerung auf Coccolithophoriden wurden weitestgehend in der exponentiellen Wachstumsphase der Zellen erzielt. Zeigen Coccolithophoriden dieselben Reaktionen, wenn sie sich in der stationären Phase befinden und hat das Verhältnis der Nährstoffe vor der Limitation Auswirkungen auf die Ergebnisse?
3. Aus früheren Studien ist bekannt, dass erhöhte Diversität von Ökosystemen zu einer erhöhten Stabilität und Resistenz gegenüber Stress führt. Daraus ergibt sich die Frage, ob Mehr-Arten-Systeme die Ergebnisse liefern, die aus Einzelkulturen zu erwarten wären?

In Kapitel I dieser Dissertation konnte die Insensibilität von *C. braarudii* für CO_2 -Partialdrücke bis $800 \mu\text{atm}$ bestätigt werden. Wurden die Partialdrücke allerdings auf Werte bis $2500 \mu\text{atm}$ erhöht, konnte auch bei dieser Art eine Reduktion der Kalzifizierung beobachtet werden. Die Biomasse-Produktion offenbarte ein Optimum für $p\text{CO}_2$, dabei konnte bis etwa $1600 \mu\text{atm}$ ein Anstieg, für höhere CO_2 -Konzentrationen ein Abfall der Produktion verzeichnet werden. Schon bei *Calcidiscus leptoporus* wurde ein Optimum-Verlauf in Bezug auf Ozeanversauerung beobachtet, allerdings für die Kalzifizierung. Es wird daher diskutiert, dass der Anstieg im gelösten anorganischen Kohlenstoff und die damit verbundene Erhöhung des Substrats für Photosynthese und Kalzifizierung sich in erster Linie als Vorteil auswirken könnten. Ab einer gewissen CO_2 -Konzentration könnte sich die damit verknüpfte Reduktion des pH-Wertes als negativ herausstellen, wodurch Photosynthese und Kalzifizierung negativ beeinflusst werden könnten. Demzufolge wären die unterschiedlichen Reaktionen verschiedener Arten nicht auf unterschiedliche intrazelluläre Mechanismen zurück zu führen. Vielmehr könnten unterschiedliche Optima für CO_2 , HCO_3^- und H^+ der Grund für die verschiedenen Reaktionen auf Ozeanversauerung sein.

Ozeanversauerungsstudien an Coccolithophoriden in ihrer exponentiellen Wachstumsphase geben Einblicke in deren Physiologie und erlauben Vergleiche zwischen den Arten. Für eine Abschätzung der Folgen von Ozeanversauerung auf die gesamte Ökosystemleistung einer Population bringen Versauerungsstudien zu Coccolithophoriden in der stationären Phase zusätzliche Erkenntnisse. In Kapitel II wurden den Arten *Emiliania huxleyi* und *Gephyrocapsa oceanica* die Möglichkeit gegeben, ihre Nährstoffe aufzubreuchen und drei Tage in der stationären Phase zu verweilen. Dabei wurde die Größenzunahme der Zellen unter Phosphatlimitierung durch den CO_2 -Partialdruck gehemmt. Die Reaktionen von Kalzifizierung und Photosynthese auf zunehmende Ozeanversauerung waren allerdings abhängig von den Nährstoffverhältnissen, denen die Arten ausgesetzt wurden.

Zellen, die unter einem „Hoch N:P“-Nährstoffverhältnis gewachsen waren, zeigten auf die Versauerung deutlich ausgeprägtere Reaktionen in der Akkumulation von organischen und anorganischem Kohlenstoff, als solche Zellen, die einem Redfield-Verhältnis ausgesetzt wurden. Bezogen auf die Akkumulation von Kohlenstoff auf Populationsebenen, zeigte sich genau das entgegengesetzte Bild. Die empfindlicheren Zellen der „Hoch N:P“-Ansätze konnten auf Grund einer veränderten Nährstoffnutzung deutlich mehr Zellen bei erhöhtem CO₂ produzieren, wodurch sich die abfallenden Trends der Biomasse- und Kalk-Akkumulation auf Zellebene fast (Kalk) oder ganz (Biomasse) ausglich.

Für eine Beurteilung des zukünftigen Einflusses von Coccolithophoriden auf den atmosphärischen pCO₂ und ihre Funktion in den Kohlenstoffexport-Mechanismen sollten daher die Auswirkungen der Nährstofflimitierung auf die Zellphysiologie stärker berücksichtigt werden.

Die meisten bisherigen Studien sind trotz ihrer Bedeutung für die Erforschung der physiologischen Mechanismen von Coccolithophoriden noch weit von der Beurteilung ganzer Ökosysteme entfernt. Bis dato wurden Gemeinschaftsinteraktionen für die Beurteilung der Ozeanversauerung völlig außen vor gelassen. Dabei ist bekannt, dass sich eine erhöhte Diversität positiv auf die Ökosystemleistung und -stabilität auswirkt. Um einen ersten Eindruck zu erlangen, wurden daher in einem Experiment, beschrieben in Kapitel III, drei verschiedene Arten einzeln und als Gemeinschaft dem Stress der Ozeanversauerung ausgesetzt. Die Kalk- und Biomasse-Anteile, die jede Zelle in der stationären Phase aufbaute, verhielten sich unter Einfluss der Versauerung dabei in den Einzelkulturen so, wie man es aus früheren Studien vermuten würde. Alle drei Arten reduzierten ihren Kalkanteil, die Organik hingegen stieg für *G. oceanica* an und blieb unverändert für *E. huxleyi* und *C. braarudii*. Auf der Ebene der Population wurde sowohl die Organik als auch der Kalk pro Liter negativ durch eine Versauerung beeinflusst. Die Gesamtleistung der Gemeinschaft hingegen zeigte sich von der Versauerung unbeeinträchtigt. Weder in der Kalzifizierung, noch in der Photosynthese gab es wesentliche Unterschiede zwischen den verschiedenen pCO₂-Ansätzen. Damit deutet sich an, dass Mehr-Arten-Systeme widerstandsfähiger gegenüber dem Stress der Ozeanversauerung sein könnten.

Die Auswirkungen der Ozeanversauerung auf die Ökosysteme dieser Ozeane sind noch in keinsten Weise verstanden. Diese Dissertation unterstreicht die Bedeutung von Experimenten an einzelnen Arten, um deren physiologische Effekte besser zu verstehen. Sie zeigt aber auch auf, dass es wahrscheinlich nicht angebracht ist, die Effektstärke aus diesen Experimenten auf die globale Kalzifizierung zu extrapolieren. Es müssen in der nahen Zukunft weitere Experimente durchgeführt werden, um die Funktion der Diversität als Stresspuffer besser verstehen zu können.

INTRODUCTION

Global climate change

Only 20,000 years ago, our ancestors faced a very different Earth. It was the time of the last glacial maximum. Massive ice shields covered the north and the south of our planet and the sea level was believed to be 110 to 120 meters (Lambeck and Chappell 2001) and the global mean temperature around 3 – 5°K lower than present (Kohler et al. 2010). It took the ice crust 10,000 years to diminish to a similar state as today. The cause was a global warming, like it happened seven times before in the past 740,000 years (Augustin et al. 2004). But after the last big melting something was different. In those times, approximately one million humans lived on this planet and they changed their lifestyle. Within 5000 years most people stopped being hunters and gatherers and lived instead from agriculture (Mithen 2004). This was the beginning of the first, unintended and still ongoing human “geo-engineering project”.

Today, more than 7 billion people inhabit the continents and approximately 40% of the ice free land is used by intensive agriculture (Foley et al. 2005). Together with the never ending hunger for fossil fuels and cement mankind alters now significantly the face of Earth. In 2008, global carbon emissions by humans reached 8.7 Pg C yr⁻¹. This was an increase of 2% from the preceding year, an increase of 29% compared to the year 2000 and 41% above the emissions of 1990 (Le Quere et al. 2009). Due to the large increase in carbon emissions the atmospheric CO₂ concentration increased from approximately 280 to 395 µatm since the industrial revolution. The Earth’s atmosphere thus faces a higher CO₂ concentration than it has been for more than 800.000 years (Luthi et al. 2008). In a business-as-usual scenario, atmospheric CO₂ concentrations will reach 500 µatm by the middle of this century and exceed 800 µatm by 2100 (Friedlingstein et al. 2006).

The United Nations established in 1988 the Intergovernmental Panel on Climate Change (IPCC) in order to evaluate the risk of the increasing carbon emissions to the atmosphere. In their fourth assessment report the IPCC noted that "changes in atmospheric concentrations of greenhouse gases and aerosols, land cover and solar radiation alter the energy balance of the climate system", and concluded that "increases in anthropogenic greenhouse gas concentrations is very likely to have caused most of the increases in global average temperatures since the mid-20th century" (IPCC 2007). While the report extensively discusses the possible consequences of increasing temperatures on the atmosphere and the oceans (IPCC 2007 Chapter 4), it only briefly touches the fact of rising CO₂ concentrations in the oceans (IPCC 2007 Chapter 10.4.2).

The Ocean in a high CO₂ world

Even if humans majorly interact on land the effects of 7 billion people left its trace on the oceans as well. The number of ecosystems with unsustainable fishery is still increasing and the per capita catch in big ecosystems is more than twice the value to ensure a sustainable recovery (Coll et al. 2008). Large accumulations of plastic debris are found in the middle of the Atlantic (Law et al. 2010) and in the Pacific (Moore et al. 2001). Oil disasters like the recent loss of the BP oil rig “Deepwater Horizon” contaminate hundreds of kilometres of coast, billions of litres seawater and vast areas on the deep sea floor. But while these direct human impacts on the oceans are in most cases regional, the indirect consequences of the anthropogenic carbon emissions are global. Thereby, two different processes need to be distinguished. First, the rise in atmospheric temperature caused by the increase in atmospheric CO₂ concentrations leading to warmer oceans. Second, increasing CO₂ concentration in the oceans, resulting in an increase in carbonic acid, consequently acidifying the oceans.

Ocean warming

One decade ago, a global increase of the ocean heat content of the upper 300m was reported (Levitus et al. 2000) and attributed to the warming induced by anthropogenic carbon emissions (Levitus et al. 2001). In the fourth assessment report of the IPCC (2007) the mean warming rate was amounted to 0.5 W m⁻². However, in a recent study the rate increased to 0.64 W m⁻² (Lyman et al. 2010). On a physical basis this warming does not happen without consequences. The thermal expansion of warming waters led to a global sea level rise of 1.8 – 2.1 cm between 1961 and 2003 (Bindoff et al. 2007; Marcelja 2010). Climate Change models using the CO₂ emission scenarios of the IPCC, predict a further rise by 2.6 cm between 2000 and 2020 (Meehl et al. 2007; Marcelja 2010), which corresponds roughly to a doubling in the thermal expansion rate. Furthermore, a dramatic ice loss can be observed in the Arctic, where the warming is amplified (Screen and Simmonds 2010), leading to a freshening of the polar regions (Bethke et al. 2006). In the tropical and subtropical Atlantic higher evaporation rates caused by warmer atmosphere and waters lead to an increase in salinity (Hakkinen 2002; Boyer et al. 2005; Bethke et al. 2006). However, warmer waters do not only influence the physical structure of the oceans but have implications on biological aspects as well. Changes towards shallower and more stable stratification induced by ocean warming (Yeh et al. 2009) will reduce exchange with nutrient enriched deeper waters. Hence, nutrient limitation is intensified, resulting in severe effects for primary production. In general, temperature increases biological process rates of many organisms. Phytoplankton shows a factorial increase in their photosynthetic rates between 1 and 2 (Eppley 1972) and heterotrophic bacteria typically increase their activity by a factor of 2 to 3 in response to a 10°C temperature increase (Pomeroy and Wiebe 2001). Since

temperature affects organisms with different intensities, major shifts in the biogeochemical cycling can be expected. Indoor mesocosm experiments showed acceleration in the respiratory consumption compared to the CO₂ consuming primary production (Wohlers et al. 2009). This finding would have severe consequences for the ocean as a CO₂ sink. Enhanced respiration would increase surface CO₂ concentration, decrease the transport of biogenic material to the deeper ocean and hence would have a positive feedback on rising atmospheric CO₂.

Ocean acidification

Half of the anthropogenic CO₂ emissions, derived from the burning of fossil fuels, are absorbed by the oceans (Sabine et al. 2004). Thus, the role of the oceans as a CO₂ sink is crucial to buffer the ongoing global warming (Orr et al. 2001). However, with the dissolving in water CO₂ loses its inert characteristic and generates an equilibrium with bicarbonate and carbonate. In this process one proton for the formation of HCO₃⁻ and two protons for CO₃²⁻ arise; as shown in the following equation (1).



The increase in the proton concentration in the oceans is unavoidable. In the past 250 years, since the beginning of the industrial revolution, the global mean pH decreased by approximately 0.1 units, corresponding to an increase in seawater acidity of 30% (Caldeira and Wickett 2003). Since the effect of ocean acidification (OA) is a direct consequence of rising atmospheric CO₂ concentrations, predictions for future changes in seawater acidity are precise and independent from the uncertainties subjected to climate change (Feely et al. 2004). Because it is unlikely for humans to change their fossil fuel using behaviour on short notice seawater acidity could increase by 100% from the present state within the next 90 years. In this vein, we are going to reach a H⁺ concentration which Earth has not faced for at least the last 20 million years (Caldeira and Wickett 2003) and the rate of acidification is probably faster than in the last 60 million years (Pearson and Palmer 2000).

What does this mean for the living environment? Most biochemical pathways in cells are more or less pronounced sensitive to pH changes. Based on their general sensitivity to changing environmental conditions, it can be assumed that especially single cell organisms and juvenile stages of animals might be sensitive to OA. Since the saturation state of calcium carbonate (Ω) is decreasing with increasing acidification and might even reach undersaturation, the consequence for calcified structures would be dissolution. For this reason calcifying organisms would suffer the most from OA.

Research on the biological response to changes in the carbonate chemistry is still in its infancy and basically concentrates on the first of the three crucial questions:

1. What extent of OA is necessary to trigger a response in an organism and is this level reachable in the future ocean?
2. Are organisms able to acclimate or adapt to OA?
3. If organisms show a response to OA within a feasible range, what does this mean for the ecosystem and the biogeochemical cycling?

In laboratory experiments many organisms showed a distinct response to changes in the carbonate chemistry. It has been shown that increasing CO₂ concentrations and its consequences have adverse effects on the calcification rates of gastropods, echinoderms (Shirayama and Thornton 2005), tropical (Langdon and Atkinson 2005) and cold water corals (Maier et al. 2009). Likewise, the nitrogen fixation of a Baltic Sea cyanobacterium is negatively affected (Czerny et al. 2009). However, *Trichodesmium sp.*, the most important diazotrophic cyanobacterium of today's ocean doubles its growth rate when treated with a CO₂ concentration of 750 μatm compared to interglacial pCO₂ conditions (Ramos et al. 2007).

The biological response to OA is heterogeneous and every species shows its own sensitivity. Every class, genus or species is differently adapted to the carbonate speciation and changes within the carbonate system. Another reason for the enormous heterogeneity can be found within the complexity of OA. Not only is the proton concentration (i.e. the pH) changing, but the whole carbonate system speciation changes its equilibrium with the consequence of rising CO₂ and HCO₃⁻ and decreasing CO₃²⁻ concentrations. Especially, the increase in the substrate for photosynthesis (CO₂ and HCO₃⁻) can show positive effects on the primary production (e.g. Riebesell et al. 2000; Ramos et al. 2010). For reasons of different effects caused by OA and by rising dissolved inorganic carbon (DIC) concentrations, Ocean Carbonation (OC) should be considered as a different trigger of biological effects. However, since OA and OC are inextricably associated, the concept of OA usually covers both effects of rising pCO₂.

Especially the division of haptophytes, which includes coccolithophores, could be affected by the different effects of Ocean Acidification and Ocean Carbonation. In particular the formation of calcareous shell should be sensitive to shifts in pH, but the photosynthetic primary production on the other hand might benefit from an increase in the substrate.

Coccolithophores – Calcification under Ocean Acidification

In 1836, the German biologist and geologist Christian G. Ehrenberg climbed the chalk cliffs of Rügen and realised that this impressive formation was built by the debris of endless numbers of calcareous single celled organisms. This was both the hour of birth of the micropaleontology and the first hint to the most important pelagic calcifier. However, it took another 66 years before Lohmann introduced the name for the organisms producing the debris found by Ehrenberg: the coccolithophores (Lohmann 1902).

While the division of Haptophyta has its roots in the oldest branching groups of the eukaryotes (Baldauf 2003), the origin of the class Prymnesiophyceae and its subclass Calcihaptophycidae appeared first ~220Ma ago (Bown et al. 2004). During this time ~4000 morphologically different species of coccolithophores have diversified, whereof only ~280 still remain in the present oceans (DeVargas et al. 2007). Although, the number of coccolithophore species declined strongly during the last million years and their global calcification rate is only a fraction of what the ocean experienced during the Cretaceous (145 – 65 Ma ago), they are estimated to be responsible for more than 50% of today's oceanic precipitation of CaCO_3 (Milliman 1993; Broecker and Clark 2009).

In addition, coccolithophores are globally very successful primary producer. With the exception of the Polar Regions these calcifiers can be found in every ocean. In particular *Emiliania huxleyi* is a true cosmopolite. As a comparatively young coccolithophore, *E. huxleyi* evolved 268 kyr BP from the older genus *Gephyrocapsa oceanica* (Thierstein et al. 1977). Both species are known for their huge seasonal blooms extending over 100.000 km^2 . Much earlier *Coccolithus braarudii* evolved. It appeared in the early Paleocene (65Myr BP) and was first described by Wallich in 1877. It is one of the largest coccolithophore and commonly found in mid latitudes (Houdan et al. 2006).

Therewith the three coccolithophores used in this study, *E. huxleyi*, *G. oceanica* and *C. braarudii*, are among the most important pelagic calcifiers of the present ocean and considerably influence the marine carbon cycle.

Coccolithophores and the marine carbon cycle

The entire dissolved inorganic carbon (DIC) in the oceans can roughly be classified by two different major sources. On the one hand the exchange of CO_2 between oceans and atmosphere and on the other hand the eroded limestone (CaCO_3) that finds its way to the oceans by rivers or by remineralisation from the seabed. Especially the latter source of the oceans carbon budget is the reason, why the global oceans serve as a sink for atmospheric CO_2 . The dissolved CaCO_3 represents over 90% of the total alkalinity representing the degree of proton buffer capacity (further reading Zeebe and Wolf-Gladrow 2001). The more alkalinity the oceans contain, the better they are buffered against OA and against changes in the CO_2 concentration, i.e. oceans can absorb more of the atmospheric CO_2 , when the alkalinity is high (for further reading on the Revelle buffer factor: Revelle and Suess 1957; Broecker et al. 1979; Egleston et al. 2010). The uptake of CO_2 by the surface ocean is influenced by the biology. While the primary production reduces the DIC concentration, and therewith increases the uptake of atmospheric CO_2 , the consumption of the primary producers by heterotrophic organisms results in a respiratory increase in the CO_2 concentration.

In the context of the marine carbon cycle, the coccolithophores take a pride of place. They are the only important primary producers influencing the pelagic carbon flux in both directions. All primary producers use CO₂ (and / or HCO₃⁻ and / or CO₃²⁻) for photosynthesis, thereby increasing oceans function as a sink for CO₂ (2). The process of calcification, however, reduces the alkalinity and hence, the CO₂ buffer capacity (3) and thereby increases the CO₂ concentration.



The ratio of both processes determines if the feedback on the atmospheric CO₂ is positive or negative. With the support of heterotrophic, pelagic foraminifera and other minor relevant pelagic calcifiers like pteropods, the global pelagic production of CaCO₃ by coccolithophores is estimated to be between 0.7 Pg C a⁻¹ (Milliman and Droxler 1996) and 1.1 Pg C a⁻¹ (Lee 2001). Thus, calcification is only 2% of the 40 – 60 Pg C a⁻¹ produced by the global primary production (Carr et al. 2006). Accordingly, the global CO₂ production by calcification is negligible compared to the CO₂ drawdown by global photosynthesis. However, this is only true for the comparison of the production rates. Taking respiration and the effective carbon export into account the relevance of calcification rises. 75% of the organic matter is remineralised in the euphotic zone but only 12 Pg a⁻¹ organic carbon are exported below the thermocline (Laws et al. 2000; Falkowski et al. 2003). Consequently, the fraction of CaCO₃ increases to 6 – 8%. The importance of CaCO₃ for the carbon export to the deep ocean is, however, considerably larger as the small fraction gives reason to believe. Stokes's law for the sinking velocity of spheres (4) states that the sinking velocity (ω_{sink}) is proportional to both the square of the particle radius (r) and the difference between the density of the particle (ρ_1) and that of the surrounding media (ρ_2):

$$\omega_{\text{sink}} = \frac{2g \cdot r^2 \cdot (\rho_1 - \rho_2)}{9\eta} \quad (4)$$

where g is the gravitational acceleration and η the dynamic viscosity. Considering the difference between the density of particulate organic matter ($\rho_{\text{POM}} \approx 1060 \text{ kg m}^{-3}$ (Logan and Hunt 1987)) and the density of calcite ($\rho_{\text{calcite}} = 2710 \text{ kg m}^{-3}$) the estimated sinking velocity of a spherical 200 μm particle consisting only of POM in seawater at 15°C is 50 m d⁻¹. A 2% fraction of CaCO₃ doubles the velocity to 100 m d⁻¹ and an 8% one leads to a velocity of 250 m d⁻¹. Since a variation in the calcite fraction probably leads to a shift in the particle size distribution and Stoke's law is only a vague estimation for the sinking velocity under in situ conditions, an 8% calcite fraction will not lead to an increase in the export by a factor of five compared to an ocean with no CaCO₃. However, the study by Klaas and Archer (2002) showing that 80% of the organic matter fluxes below 2000 m are associated to CaCO₃ particles, suggests the importance of calcareous ballast for the export efficiency.

Although the direct feedback on the atmospheric CO₂ concentration by calcification and photosynthesis is probably negligible, coccolithophores might considerably influence carbon export mechanisms. Due to the effects of OA, however, this contribution could shrink in the waters of the future ocean (Biermann and Engel 2010).

Coccolithophores in an acidifying ocean

One decade ago, it was shown for the first time that the species *Emiliana huxleyi* and *Gephyrocapsa oceanica* are affected by OA. Based on these findings, on their importance as the most prominent pelagic calcifiers and on their relevance for the carbon export coccolithophores are next to corals the most examined organisms with respect to ocean acidification.

Riebesell et al. (2000) observed that the species *E. huxleyi* and *G. oceanica* reacted with increasing organic carbon production and reduced their calcification rates with decreasing pH. They reacted, however, with different sensitivity. Thereupon, the first attempts to extrapolate the global calcification rates using a batch culture experiment on a single species, suggested a negative feedback on the atmospheric CO₂ concentration (Zondervan et al. 2001). Further studies, however, showed the expected complexity of biological systems. Findings on the effects of OA on the coccolithophores *Coccolithus braarudii* and *Calcidiscus quadriperforatus* deviated from the linear responses, obtained with *E. huxleyi* and *G. oceanica*. The calcification of *C. quadriperforatus* revealed an optimum curve and *C. braarudii* showed no response within a range of pCO₂ of 180 µatm to 750 µatm (Langer et al. 2006). In addition, depending on the strain's origin of the same species, different sensitivities became obvious. Thus, all analysed strains of *E. huxleyi* with their origins in the Tasman Sea, North Pacific, South and North Atlantic showed differences in their response of growth rate, photosynthesis and calcification with respect to OA (Langer et al. 2009). Additionally, day lengths and irradiance strongly influenced the single strain response of photosynthesis and calcification to [CO₂] and/or pH with different strength (Zondervan et al. 2002). Furthermore, experiments with nitrate limitation (Sciandra et al. 2003) and temperature (Feng et al. 2008) on the physiological response of *E. huxleyi* to OA resulted in similar trends, but again with different characteristics.

First attempts of OA experiments on an ecological scale in large mesocosm enclosures provided, as well, challenging results for global calcification estimations. A mesocosm experiment in the Raune Fjord 2001 close to Bergen (Norway) where a bloom of diatoms followed by a bloom of *E. huxleyi* occurred; resulted in a decrease in calcification with rising pCO₂. However, no significant difference in the particulate organic carbon production was found (Delille et al. 2005). A similar experiment in the same fjord, again with a bloom of diatoms followed by a bloom of *E. huxleyi*, resulted in a increase in the organic carbon production with increasing OA, but, no variation in the calcification could be observed (Riebesell et al. 2007).

In view of results from both laboratory experiments and mesocosm studies it seems essential to understand the mechanistic principles behind the physiological response of coccolithophores to OA. Furthermore, laboratory approaches to close the gap between batch culture experiments and ecosystem setups, like mesocosm studies, are necessary to understand the potential of the diversity of coccolithophore ecosystems to adapt to the threat by Ocean Acidification. This PhD thesis analyses the aspects of both the physiological response of the coccolithophore *Coccolithus braarudii* and the first attempts to study the population and community response of coccolithophores.

Thesis Outline

This thesis is divided into three independent chapters addressing different aspects of coccolithophores and their response to ocean acidification. While the research presented in chapter I was partly funded by the EU-project EPOCA and the German program on Biological Impacts of Ocean Acidification (BIOACID), the studies described in chapter II and III were funded by the Leibniz Competitive Fund (SAW-Verfahren im Rahmen des Paktes für Forschung und Innovation 2010).

The overall aim of this thesis was to give new insights into single coccolithophore species physiology and to bring these into context with the total carrying capacities of both single species and of the whole coccolithophore communities.

Chapter I

Previous studies already suggested implications of ocean acidification for the marine biota. Especially the process of biogenic calcification was usually negatively affected by increasing pCO₂. As the most important pelagic calcifiers, the response of coccolithophores to increasing ocean acidification is subjected to special attention. The results, however, did not show a clear pattern. Responses to changing ocean carbonate chemistry appeared to differ among species and species strains. By applying a wider CO₂ range, this study looked closer into the previously reported insensitivity of calcification and photosynthesis of *Coccolithus braarudii* to seawater acidification. Since both photosynthesis and calcification are likely to have optimum concentrations for at least CO₂, HCO₃⁻ and H⁺ the observed differences in coccolithophore responses to OA were discussed as to be the result of different sensitivities to concentration changes of these ions.

Chapter II

Observations on coccolithophore sensitivity to ocean acidification were generally made during exponential growth. Unaffected by the depletion of nutrients and with a constant carbonate chemistry these experiments gave results on the physiological response of coccolithophore cells. These studies, however, delivered incomplete data if one wants to estimate the carrying capacity

of a whole coccolithophore population. The study presented in chapter II considers the previously shown variations in cell size, the particulate organic as well as inorganic carbon contents under phosphate limitation and the natural variation of the carbonate chemistry by photosynthesis and calcification. By applying these conditions the response of the coccolithophores *Emiliana huxleyi* and *Gephyrocapsa oceanica* to the stress of ocean acidification was observed and discussed.

Chapter III

In order to investigate the underlying physiological mechanisms in the response of coccolithophores to changing ocean carbonate chemistry, the focus on single species has been proven to be useful. Also variations in the carrying capacity of single coccolithophore populations gave new insights into the implications of ocean acidification. Both approaches, however, largely neglect potential indirect effects following changing community interactions.

Chapter III describes an experiment testing three species of coccolithophores, namely *Emiliana huxleyi*, *Gephyrocapsa oceanica* and *Coccolithus braarudii*, in monocultures and combined in a polyculture. With the aim to investigate possible interactions in the stationary phase, the cultures were allowed to deplete their nutrients and change the carbonate speciation by photosynthesis and calcification under the stress of three different pCO₂ levels. This study gives a first impression of the potential resistance of an artificial coccolithophore community.

CHAPTER I

Effects of changes in carbonate chemistry specifications on *Coccolithus braarudii*: a discussion on coccolithophore sensitivities

Sebastian A. Krug, Kai G. Schulz and Ulf Riebesell



Abstract

Ocean acidification and associated shifts in carbonate chemistry speciation induced by increasing levels of atmospheric carbon dioxide (CO₂) have the potential to impact marine biota in various ways. The process of biogenic calcification, for instance, is usually shown to be negatively affected. In coccolithophores, an important group of pelagic calcifiers, changes in cellular calcification rates in response to changing ocean carbonate chemistry appear to differ among species. By applying a wider CO₂ range we show that a species previously reported insensitive to seawater acidification, *Coccolithus braarudii*, responds both in terms of calcification and photosynthesis, although at higher thresholds. Thus, observed differences between species seem to be related to individual sensitivities while the underlying mechanisms could be the same. On this basis we develop a conceptual model of coccolithophorid calcification and photosynthesis in response to CO₂-induced changes in seawater carbonate chemistry speciation.

Introduction

Following the industrial revolution in the late 18th century, atmospheric carbon dioxide levels continuously increased from about 280 to presently 395 μatm , and are currently increasing further at a rate of 0.4% per year (IPCC 2007). The magnitude of this change would have been considerably larger if the surface oceans had not absorbed approximately 50% of the fossil fuel emissions to date (Sabine et al. 2004). The dissolution of anthropogenic CO_2 in water is followed by several chemical reactions leading to shifts in carbonate chemistry speciation resulting in decreasing pH, carbonate ion concentration, and saturation state (ocean acidification) and concomitant increases in the overall dissolved inorganic carbon inventory (ocean carbonation). This conceptual separation is of potential interest for understanding the basis of any physiological response to changes in seawater carbonate chemistry.

Coccolithophores are significant pelagic calcifiers, contributing about half to calcium carbonates preserved in open ocean sediments (Broecker and Clark 2009). They are among the best examined organisms with respect to their response to ocean acidification / carbonation (e.g. Riebesell et al. 2000; Zondervan et al. 2002; Sciandra et al. 2003; Langer et al. 2006; Feng et al. 2008; Langer et al. 2009; Shi et al. 2009; Ramos et al. 2010). Confusingly, the species analyzed so far have different response patterns for calcification and photosynthesis in response to changing carbonate chemistry speciation. While *Emiliana huxleyi* and *Gephyrocapsa oceanica* usually decreased calcification rates between a partial pressure of CO_2 (pCO_2) from about 180 to 800 μatm (Riebesell et al. 2000), *Calcidiscus quadriperforatus* showed an optimum curve response, with a maximum at current pCO_2 , and *Coccolithus braarudii* was not significantly changing calcification rates in this range (Langer et al. 2006). Similarly, there were also differences regarding organic carbon fixation rates by photosynthesis, questioning the operation of common cellular mechanisms for calcification and photosynthesis within the group of coccolithophores.

Here we investigate the response of *Coccolithus braarudii* to a broader pCO_2 range in two experiments, allowing to draw conclusions on the origin of observed differences between species.

Methods

The response of *Coccolithus braarudii* to changing seawater carbonate chemistry was analysed in two experiments. In the first experiment the pCO_2 was varied from 500 to 3500 μatm and in the second from 400 to 1700 μatm (compare Tab. 1.1).

Monospecific cultures of *Coccolithus braarudii* (strain RCC 1200, isolated in the South Atlantic off Namibia and kindly provided by the Roscoff Culture Collection <http://www.sb-roscoff.fr/Phyto/RCC/>) were incubated in triplicates as dilute batch cultures.

Cells were grown in sterile-filtered natural North Sea water in the first experiment, and for technical reasons in artificial seawater (Kester et al. 1967) in the second experiment, both at a temperature of 17°C and a salinity of 34. To ensure exponential growth without nutrient limitation 80 $\mu\text{mol kg}^{-1}$ of nitrate and 5 $\mu\text{mol kg}^{-1}$ of phosphate, trace metals, selenium and vitamins according to the f/2-medium (Guillard 1975) were added. The incident photon flux density (PFD) was 130 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at a 16/8 light/dark cycle.

Carbonate system manipulation

Manipulation of carbonate chemistry speciation to simulate ongoing ocean acidification can be achieved by either changing dissolved inorganic carbon (DIC) at constant total alkalinity (TA) or by varying TA at constant DIC (compare Schulz et al. 2009; Gattuso et al. 2010). The latter approach was chosen here for direct comparability with a previous study on *Coccolithus braarudii* (Langer et al. 2006). It is noted that there are differences in carbonate chemistry speciation between DIC and TA manipulation. In terms of magnitude and sign in concentration changes they are marginal for CO_2 ranges from 180 to 800 μatm , while towards higher levels there are increasing differences in terms of bicarbonate (HCO_3^-) concentration changes which start to decrease when manipulating TA instead of constantly increasing when manipulating DIC (Schulz et al. 2009). However, this was only the case at the highest pCO_2 level of about 3500 μatm (compare Tab.2). There are also increasing differences in CO_3^{2-} ion concentration changes towards such high pCO_2 levels, but CO_3^{2-} concentrations steadily decrease in both manipulations.

TA was manipulated by adding suitable amounts of 1M HCl or 1M NaOH to the 0.2 μm filtered seawater. Carbonate chemistry speciation was calculated from measured DIC and TA (see below) adopting the stoichiometric equilibrium constants for carbonic acid determined by Mehrbach (1973) and refitted by Dickson and Millero (1987) as a mean of initial and final values (compare Tab. 1.1).

Experimental setup and measurements

For acclimation, pre-cultures were grown exponentially for 7 days under the experimental conditions before transfer to experimental bottles. All bottles were incubated in triplicates without headspace. Depending on growth rate the cultures grew for 6-8 days. Only the five highest pCO_2 treatments of the first experiment were stopped after 17 days. To avoid pronounced variations in the carbonate system, maximum cell

numbers at the end of incubations did not exceed 2600 in the first and 4500 cells ml⁻¹ in the second experiment. This corresponds to a maximum DIC drawdown of less than 12%.

Table 1.1: Carbon chemistry speciation for experiment I and II, calculated as the mean of start and end values of measured DIC and TA. See materials and methods for details. It is noted that the higher variability of DIC concentrations in the second compared to the first experiment is due to inaccuracies in artificial seawater preparation and slightly higher variability in final cell densities.

Experiment I									
	DIC μmol kg ⁻¹	TA μmol kg ⁻¹	pCO ₂ μatm	[CO ₂] μmol kg ⁻¹	[HCO ₃ ⁻] μmol kg ⁻¹	[CO ₃ ²⁻] μmol kg ⁻¹	Ω _{calcite}	pH _{total}	[H ⁺] nmol kg ⁻¹
1	2048	2240	505	17.8	1887.9	142.3	3.4	7.96	10.96
2	2058	2249	512	18.0	1898.0	142.0	3.4	7.96	10.96
3	2062	2238	555	19.5	1909.9	132.6	3.2	7.92	12.02
4	2058	2135	1015	35.7	1947.0	75.3	1.8	7.67	21.38
5	2022	2077	1166	41.0	1917.4	63.6	1.5	7.60	25.12
6	2057	2103	1273	44.8	1951.8	60.4	1.4	7.57	26.92
7	2005	2021	1566	55.1	1903.2	46.7	1.1	7.47	33.88
8	2048	2052	1755	61.7	1942.9	43.4	1.0	7.43	37.15
9	2058	2041	2075	73.0	1948.1	36.9	0.9	7.36	43.65
10	2032	2008	2170	76.3	1921.3	34.3	0.8	7.33	46.77
11	2065	2030	2384	83.8	1949.0	32.2	0.8	7.30	50.12
12	2060	2025	2380	83.7	1944.2	32.0	0.8	7.30	50.12
13	2026	1943	3311	116.5	1887.8	21.7	0.5	7.14	72.44
14	2035	1940	3581	126.0	1888.9	20.1	0.5	7.11	77.62
15	2042	1945	3631	127.7	1894.4	19.9	0.5	7.10	79.43
Experiment II									
	DIC μmol kg ⁻¹	TA μmol kg ⁻¹	pCO ₂ μatm	[CO ₂] μmol kg ⁻¹	[HCO ₃ ⁻] μmol kg ⁻¹	[CO ₃ ²⁻] μmol kg ⁻¹	Ω _{calcite}	pH _{total}	[H ⁺] nmol kg ⁻¹
1	2132	2385	407	14.3	1932.7	185.0	4.4	8.06	8.66
2	2122	2370	413	14.5	1926.2	181.3	4.3	8.06	8.80
3	2131	2375	423	14.9	1937.2	178.9	4.3	8.05	8.97
4	2048	2230	532	18.7	1893.3	136.0	3.2	7.94	11.54
5	2053	2235	534	18.8	1898.1	136.1	3.2	7.94	11.55
6	2068	2243	560	19.7	1916.1	132.2	3.2	7.92	12.01
7	2061	2162	863	30.4	1942.5	88.2	2.1	7.74	18.25
8	2055	2149	901	31.7	1939.1	84.2	2.0	7.72	19.08
9	2059	2152	910	32.0	1943.2	83.8	2.0	7.72	19.22
10	1973	2028	1133	39.9	1870.8	62.3	1.5	7.60	24.87
11	1974	2018	1235	43.4	1873.2	57.4	1.4	7.57	27.06
12	1976	2017	1265	44.5	1875.4	56.1	1.3	7.56	27.70
13	2094	2108	1656	58.2	1987.6	48.1	1.1	7.47	34.21
14	2102	2107	1781	62.6	1994.3	45.1	1.0	7.44	36.67
15	2080	2083	1792	63.0	1973.1	43.8	1.0	7.43	37.30

Samples for DIC and TA were taken at the beginning and the end of both the acclimation and experimental phase. Suspended particulate inorganic carbon (PIC), otherwise interfering with the measurements, was removed by gentle pressure filtration (0.2 μm) from DIC and by vacuum filtration (GF/F, nominal pore size 0.7 μm) from TA samples. DIC was determined photometrically according to Stoll et al. (2001) (Stoll et al. 2001a) with an auto-analyzer (QUAATRO, Bran & L ubbe) at a precision of $\pm 20 \mu\text{mol kg}^{-1}$. TA was determined by potentiometric titration in duplicate samples at 20 C (Metrohm Basic Titrino 794) with 0.05 M HCl (Dickson 1981; Dickson et al. 2003a) at a precision of $\pm 3 \mu\text{mol kg}^{-1}$. Certified reference material (University of California, San Diego, Marine Physical Laboratory, Prof. A.G. Dickson) was measured alongside with the samples as an independent standard and used for corrections. Samples for cell abundance were determined with a Z2TM COULTER COUNTER[ ] cell and particle counter in the first and for technical reasons by light microscopy in the second experiment. With an accurate determination of the high cell abundance at the end of the acclimation phase and a precise transfer of the inoculum to the experiment allows for calculations of initial cell densities.

The cell abundances at the start (c_0) and at the end of incubations (c_1) were used to calculate daily growth rates (μ) during the experimental incubation period (Δt , incubation time in days) as:

$$\mu = \frac{\ln c_1 - \ln c_0}{\Delta t} \quad (1)$$

At the end of the experiment duplicate samples for total particulate carbon (TPC) and nitrogen (TPN) and particulate organic carbon (POC) and nitrogen (PON) were filtered onto pre-combusted (450 C for 8 hours) GF/F (Whatman) filters and frozen at -20 C until analysis. For POC determination, particulate inorganic carbon was removed by exposing the filters to fuming hydrochloric acid for 12 hours. Before measurement all filters were dried at 60 C. Afterwards they were folded and packed in tin cups and analysed in an elemental analyser with a heat conductivity detector (EuroVector EA) according to Sharp (1974).

Results

Manipulation of TA by about 300 $\mu\text{mol kg}^{-1}$ at constant DIC created a pCO_2 gradient from about 500 to 3500 μatm in the first experiment and from about 400 to 1700 μatm in the second experiment. Considering the combined pCO_2 range [CO_2], [CO_3^{2-}] and [H^+] changed relatively

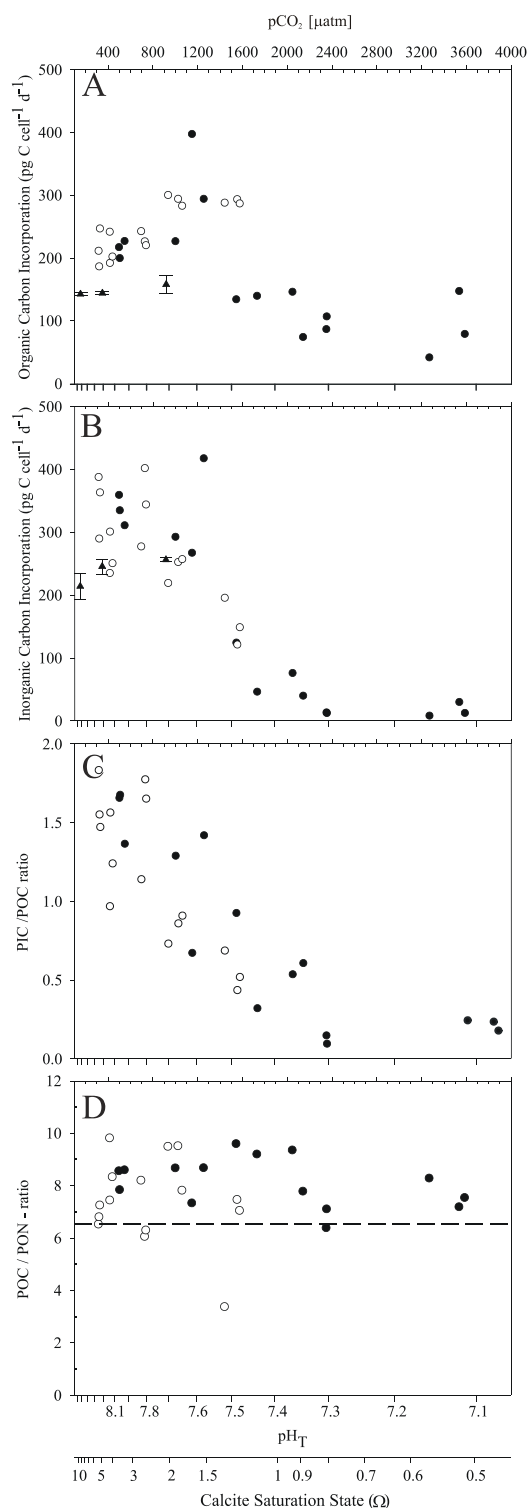


Figure 1.1: Particulate organic and inorganic carbon production rates (A and B, respectively), together with ratios of particulate inorganic to organic carbon (C) and particulate organic carbon to nitrogen (D) in response to changes in carbon chemistry speciation. Filled circles represent results of the first and open circles of the second experiment, while triangles those of Langer et al. (2006) for comparison.

proportional by a factor of about 9. While [CO₂] was rising from 14 to 124 μmol kg⁻¹ and [H⁺] from 6.9 to 63 nmol kg⁻¹, [CO₃²⁻] dropped from 181 to 21 μmol kg⁻¹. [HCO₃⁻] was much less affected within the same range and varied by a factor less than 1.06 between treatments (compare Tab. 1.1). Two out of ten triplicate treatments were clearly under-saturated with respect to calcite ($\Omega_{\text{calcite}} < 1$) while another two were only slightly above one.

POC production rates of *Coccolithus braarudii* were highest at intermediate pCO₂ (300 pg C cell⁻¹ d⁻¹) and declined towards lower and higher levels (200 and less than 100 pg C cell⁻¹ d⁻¹, respectively), although more pronounced in case of the latter (Fig. 1.1A). Calcification rates, although quite noisy, clearly decreased towards higher pCO₂ levels from about 300-400 pg C cell⁻¹ d⁻¹ at the lower end to less than 25 pg C cell⁻¹ d⁻¹ at the higher end of the pCO₂ range (Fig. 1.1B). The considerably stronger decrease in PIC compared to POC production led to a pronounced drop in PIC/POC (Fig. 1.1C). The variations in POC production, however, were not reflected in POC/PON, which generally fluctuated slightly above the Redfield ratio of 6.6 in all treatments (Fig. 1.1D).

Growth rates ranged between 0.6 and 0.9 d⁻¹ at lower pCO₂ and decreased to less than 0.2 d⁻¹ towards the higher levels (Fig. 1.2A), while cell diameter decreased almost by a factor of two (Fig. 1.2B).

Discussion

The response of coccolithophorid calcification and photosynthetic carbon fixation rates to changing carbonate chemistry speciation, more specifically to a $p\text{CO}_2$ range from about 180 to 800 μatm , has been shown to differ between species, challenging the concept of common cellular mechanisms for these two processes in coccolithophores. While calcification rates were found to decrease with increasing $p\text{CO}_2$ in *Emiliana huxleyi* and *Gephyrocapsa oceanica* (Riebesell et al. 2000; Zondervan et al. 2002; Sciandra et al. 2003; Ramos et al. 2010), *Calcidiscus quadriperforatus* showed an optimum curve response, and *Coccolithus braarudii* no sign of change (Langer et al. 2006). Similarly, photosynthetic carbon fixation rates were found to increase in *Emiliana huxleyi* and *Gephyrocapsa oceanica*, while not being significantly affected in *Calcidiscus quadriperforatus* and *Coccolithus braarudii*. Extending the $p\text{CO}_2$ range towards higher values, however,

revealed that the processes of calcification and photosynthesis in *Coccolithus braarudii* are indeed prone to changes in carbonate chemistry speciation (Fig. 1.1A,B). Calcification rates clearly decreased with increasing $p\text{CO}_2$ like in *Emiliana huxleyi* and *Gephyrocapsa oceanica*, facing lowest production rates at the onset of CaCO_3 undersaturation (Fig. 1.1, $\Omega < 1$). The assumed incipient calcite dissolution could not be determined, it is therefore not possible to say, if the calcite production rate was low or if calcite dissolved. For this reason the shown calcite production rates do only reflect the net production for $\Omega < 1$.

Calcification rates, however, do only decrease at higher levels, while photosynthetic carbon fixation rate was highest at intermediate $p\text{CO}_2$, suggesting an optimum curve response like seen for calcification in *Calcidiscus quadriperforatus*. When extending the $p\text{CO}_2$ range

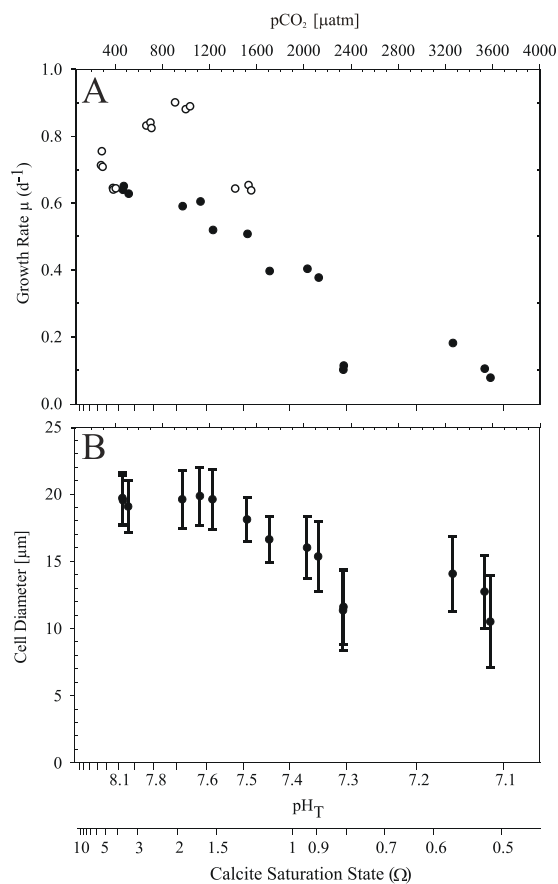


Figure 1.2: Growth rates (μ) of *Coccolithus braarudii* in the first (filled circles) and second experiment (open circles) and together with cell diameter as measured in the first experiment in response to changing carbon chemistry speciation (A and B, respectively).

towards lower levels calcification and photosynthetic carbon fixation rates have been shown to decrease with decreasing $p\text{CO}_2$ in *Emiliana huxleyi*, although seemingly stronger in case of the latter (Buitenhuis et al. 1999).

A conceptual model of carbonate chemistry dependant photosynthesis and calcification in coccolithophores

Putting all pieces of information together we propose that changes in calcification and photosynthetic carbon fixation rates in response to changing carbonate chemistry are likely to have the same underlying mechanisms in all coccolithophores, with both processes characterized by an optimum curve response (compare Fig. 1.3). The observed species specific differences would reflect species specific carbonate chemistry sensitivities rather than different cellular mechanisms. This concept could also explain strain-specific differences observed for *Emiliana huxleyi* (Langer et al. 2009).

Identifying the carbonate chemistry parameters responsible for calcification and photosynthetic carbon fixation rate changes towards both ends of the optima of the proposed model is difficult. While towards the lower end concentrations of CO_2 , HCO_3^- and H^+ are decreasing and pH , CO_3^{2-} concentration and calcite saturation state (Ω_{calc}) are increasing, these trends are reversed towards the higher end (compare Fig. 1.3). This poses the question for the optimum conditions in terms of carbonate chemistry speciation for calcification and photosynthesis.

Maximizing any biological rate requires the availability of saturating substrate concentrations and optimal ambient conditions. During photosynthetic carbon fixation CO_2 is fixed by the enzyme Ribulose-1,5-Bisphosphat-Carboxylase/Oxygenase (RuBisCO) in the chloroplast, while calcification is sustained by inorganic precipitation of Ca^{2+} and CO_3^{2-} ions forming calcite in the coccolith producing vesicle. *Emiliana huxleyi*, like probably most phytoplankton species, is known to fuel photosynthesis by active uptake of both CO_2 and HCO_3^- from the surrounding seawater medium (Rost et al. 2003; Schulz et al. 2007a), however, simultaneous CO_3^{2-} ion uptake by HCO_3^- transporters can also not be ruled out (Mackinder et al. 2010). Although there is no convincing proof for the actual substrate taken up for calcification it is difficult to conceive

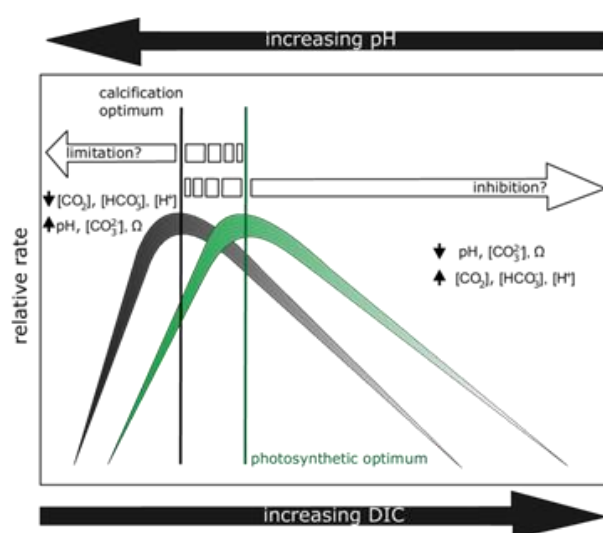


Figure 1.3: Conceptual model of coccolithophorid photosynthesis and calcification in response to changes in carbonate chemistry speciation due to increasing DIC at decreasing pH.

how cells would be able to operate an internal carbon pool for calcification separated from that used in photosynthesis. Therefore it seems reasonable to state that calcification is sustained by the uptake of some form of dissolved inorganic carbon, probably a mixture like for photosynthesis. This means that there should be thresholds for the concentrations of at least two of the dissolved inorganic carbon species in seawater (CO_2 , HCO_3^-) below which uptake and ultimately supply to photosynthesis and calcification becomes sub-saturating. Furthermore, the concentration of CO_2 in seawater influences the operation of carbon concentrating mechanisms (CCMs) in marine phytoplankton additionally. Decreasing concentrations can be thought to increase the gradient between seawater and cytosol, resulting in enhanced net diffusive loss of CO_2 out of the cytosol, and, if not compensated for, decreasing internal CO_2 concentrations (Schulz et al. 2007b). This implies that if cytosolic pH is not simultaneously increasing there would be less dissolved inorganic carbon ultimately available as substrate, eventually resulting in reduced rates of photosynthesis and calcification. The enhanced leakiness at low seawater CO_2 concentrations can also be thought to increase metabolic costs of carbon supply to photosynthesis and calcification.

Concerning optimal conditions for these two processes cellular pH, or H^+ concentration, is certainly a factor. Photosynthetic carbon fixation is mediated by a pH dependant enzymatic reaction (Portis et al. 1986) and calcium carbonate precipitation rate is depending on calcium carbonate saturation state (Morse et al. 2007), highly influenced by pH. Although the chemical process of precipitation is not equivalent to the biological calcification, a pH sensitivity is likely for the latter process as well. Decreasing seawater pH has been shown to result in lower intracellular pH levels in *Emiliana huxleyi* (Suffrian et al. 2010 in press). Changes in the intracellular pH reported by Suffrian (2010) can be perceived as a mixed signal of all compartments. Therefore, a pH change for the calcification vesicle can neither be affirmed nor be disproved. But increasing intracellular H^+ concentrations affects the proton gradient between calcification vesicle and cytosol. A negative effect on the calcification in the vesicle is therefore possible. Variations in seawater pH therefore have the potential to directly impact rates of photosynthesis and calcification.

In the proposed model, changes in carbonate chemistry speciation such as increasing DIC at decreasing pH could be thought to enhance calcification and photosynthesis by supplying increasing amounts of substrate until a certain threshold beyond which pH levels become unfavourable (compare Fig. 1.3). Ocean carbonation (increasing DIC, but most importantly CO_2 and HCO_3^-) and acidification (decreasing pH) could therefore act differently on these two processes. However, at this point contributions of pH to the shape of the optimum curve on the left side of the optimum and of CO_2 on the right side cannot be ruled out either. In any case, both

photosynthesis and calcification are likely to have optimum concentrations for at least CO_2 , HCO_3^- and H^+ . Observed differences in coccolithophorid photosynthesis and calcification to changes in carbonate chemistry speciation could then be the result of different optimum concentrations for these ions. In other words, different sensitivities rather than different intracellular mechanisms for photosynthesis and calcification in species and maybe even strains can explain the differences described for *Emiliana huxleyi* / *Gephyrocapsa oceanica*, *Calcidiscus leptoporus* and *Coccolithus braarudii*.

Acknowledgements

We thank our working group for inspiring discussions, Michael Meyerhöfer for DIC measurements, and Ian Probert for kindly supplying the *Coccolithus braarudii* strain. We would also like to thank the EPOCA project, funded by the European Commission and the German program on Biological Impacts of Ocean Acidification (BIOACID) for the financial support.

CHAPTER II

High nitrate to phosphorus ratio attenuates negative effects of rising $p\text{CO}_2$ on net population carbon accumulation

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Abstract

The ongoing rise in atmospheric $p\text{CO}_2$ and the consequent increase in ocean acidification have direct effects on marine calcifying phytoplankton which potentially translates to altered carbon export. To date it remains unclear how nutrient ratio interacts with $p\text{CO}_2$. In this study two species, namely *Emiliania huxleyi* and *Gephyrocapsa oceanica*, were full-factorially exposed to two different N:P ratios (Redfield and high N:P) and three different $p\text{CO}_2$ levels. Effects on net inorganic population carbon accumulation were measured after species reached stationary phase by taking cell sizes and total cell abundance into account. Moreover, this study also allows for natural variation of carbon chemistry by changing DIC and alkalinity. For both species results show a significant negative cellular PIC response to increasing $p\text{CO}_2$ which is strongest under high N:P ratio. In contrast, net PIC population accumulation is significantly attenuated under high N:P ratio. We suggest that less cellular nutrient accumulation allows for higher cell abundances which compensates for the strong cellular responses of PIC to $p\text{CO}_2$. We are also able to show that at high initial $p\text{CO}_2$ the natural variation of $p\text{CO}_2$ during the experimental runtime is regulated by biomass. In contrast, at low initial $p\text{CO}_2$ the PIC/POC ratio is responsible for variations in $p\text{CO}_2$.

Our results point to the fact that the physiological (i.e. solely cellular) PIC response to ocean acidification cannot necessarily be assumed to be true for the total population response. It is therefore recommended to consider the effects of nutrient limitation on cell physiology and translate these to net population carbon accumulation when predicting the influence of coccolithophores on both, the atmospheric $p\text{CO}_2$ feedback and their function in carbon export mechanisms.

Introduction

At present earth faces an atmospheric CO₂ partial pressure of 398 μatm which already is approximately 100 μatm higher than at preindustrial times. This fraction, however, would be considerably larger if the surface oceans had not absorbed approximately 50% of previous fossil fuel CO₂ emissions (Sabine et al. 2004). This leads to an attenuation of global warming on the one hand but causes the effect known as ocean acidification on the other hand (Caldeira and Wickett 2003). The ongoing increase in atmospheric pCO₂ results in decreasing surface ocean pH and [CO₃²⁻] and increasing HCO₃⁻ and CO₂-concentrations. These variations in ocean carbonate chemistry have direct implications on physiological processes, like photosynthesis and calcification of many organisms (Turley et al. 2010). Especially calcifiers at the surface ocean such as coccolithophores, foraminifera and pteropods are threatened of malformation and / or dissolution (Fabry 2008). Since about half of the pelagic calcification is accomplished by coccolithophores (Broecker and Clark 2009) and the sinking of their calcareous coccoliths might play a crucial role in carbon export mechanisms (Klaas and Archer 2002) the physiological response of coccolithophores to ocean acidification is of special interest. Therefore, coccolithophores are among the best examined organisms with respect to their response to ocean acidification. These responses in calcification and photosynthesis of various coccolithophore species and species strains, however, were mostly measured per unit cell in the exponential growth phase and under constant carbonate speciation during the experimental runtime (e.g. Riebesell et al. 2000; Zondervan et al. 2001; 2002; Langer et al. 2006; Langer et al. 2009; Shi et al. 2009; Krug et al. 2011). At this, the effect strength on individual calcification strongly differed in response among species and species strains analysed so far. Different optimum concentrations at least for CO₂, HCO₃⁻ and H⁺ than different intra-cellular mechanisms were hypothesized to be the reason for varying responses in photosynthesis and calcification rates among species and strains (Krug et al. 2011).

Research progress on the physiology of pelagic calcifiers caused by ocean acidification cannot be imagined without the previously mentioned studies. However, they only partly consider three irrevocable points for drawing conclusions on the consequences of ocean acidification on net population carrying capacities of particulate carbon. Therefore, current extrapolations on the global carbon cycle are presumably overlooking important information. These points being: (1) The natural changes in the carbonate system by photosynthesis and calcification (Taylor et al. 1992; Robertson et al. 1993; Purdie and Finch 1994). Thereby, photosynthesis decreases pCO₂ by consuming dissolved inorganic carbon (DIC) and calcification increases pCO₂ by reducing total alkalinity (TA). (2) Variations in the cellular carbon content caused by nutrient limitations (Paasche 1998; Riegman et al. 2000). And (3) the number of cells in a population. Only the consideration of change in cell size under nutrient limitation, i.e. the increase in cell size under

phosphate limitation (Riegman et al. 2000; Müller et al. 2008) and the decrease in cell size under nitrate limitation (Riegman et al. 2000; Sciandra et al. 2003; Müller et al. 2008), together with the number of cells in a population allows to draw conclusions on the implications of ocean acidification on total carrying capacity of particulate carbon. But rather than nitrogen limitation, which is in general referred as to be the limiting resource of phytoplankton (Falkowski 1997), the effects of phosphate limitation have to be considered as the prevailing limitation factor for at least *Emiliana huxleyi* (Egge and Heimdal 1994; Tyrrell and Taylor 1996).

In this study we experimentally set out to test whether the effects of different initial CO₂ concentrations on net population carbon accumulation of two coccolithophore species, namely *E. huxleyi* and *Gephyrocapsa oceanica* is dependent on the phosphate limitation level. This hypothesis was tested by following growth to the depletion of phosphate and monitoring the change in carbonate speciation by the consumption of DIC and the reduction of TA. To the best of our knowledge this study allows for the first time to draw conclusions about direct effects of ocean acidification on net population carbon accumulation of single coccolithophore species.

Methods

Experimental design

A batch culture experiment was designed in order to analyse the influence of nutrient limitation and pCO₂ on the net calcification population response of *Emiliana huxleyi* and *Gephyrocapsa oceanica*. Both species were exposed to three different pCO₂-levels and two different NO₃⁻ to PO₄³⁻ ratios which resulted in a fully crossed two-factorial design. Each treatment was replicated four times, resulting in 48 experimental units.

The experiment took place in 2000 ml polycarbonate bottles randomly distributed across four climate cabinets. Before the onset of the experiment, 200 cells ml⁻¹ were transferred into each experimental unit. Cells were acclimated to the respective experimental pCO₂ levels (see below), temperature (16°C) and light (130 μmol photons m⁻² sec⁻¹ and 14h / 10h light/dark cycle)

Table 2.1 Carbonate specifications at the beginning of the experiment. pCO₂ is given in μatm, all other parameters (with exception of pH and Ω) are given in μmol kg⁻¹.

pCO ₂	TA	DIC	pH	[CO ₂]	[HCO ₃ ⁻]	[CO ₃ ²⁻]	Ω
459 ± 7	2700 ± 2	2447 ± 3	8.15 ± 0.01	17 ± 0.3	2233 ± 5	197 ± 2	4.80
1046 ± 7	2700 ± 2	2595 ± 4	7.84 ± 0.00	39 ± 0.	2452 ± 1	104 ± 1	2.54
1283 ± 18	2700 ± 2	2627 ± 2	7.75 ± 0.01	47 ± 0.7	2491 ± 3	88 ± 1	2.14

Table 2.2 Start and end concentrations of nitrate, ammonium, their sum, phosphate and the ratio of total N to P for both species at three different pCO₂. At the end the concentrations of ammonia and phosphate were always below detection limit (b.d.). Concentrations are given in μmol kg⁻¹.

	Ratio	pCO ₂	Start concentration					End conc.	
			NO ₃ ⁻	NH ₄ ⁺	Σ N	PO ₄ ³⁻	N/P	NO ₃ ⁻	PO ₄ ³⁻
<i>E. huxleyi</i>	„Redfield“	460	3.88 ± 0.08	4.8 ± 0.2	8.68	0.5 ± 0.03	17	b.d.	b.d.
		1046	3.88 ± 0.08	4.8 ± 0.2	8.68	0.5 ± 0.03	17	b.d.	b.d.
		1280	3.86 ± 0.08	4.8 ± 0.2	8.66	0.5 ± 0.03	17	b.d.	b.d.
	„High N:P“	460	12.41 ± 0.2	4.8 ± 0.2	17.21	0.2 ± 0.01	75	7.98	b.d.
		1046	12.49 ± 0.23	4.8 ± 0.2	17.29	0.2 ± 0.03	75	8.23	b.d.
		1280	12.49 ± 0.23	4.8 ± 0.2	17.29	0.2 ± 0.01	75	10.29	b.d.
<i>G. oceanica</i>	„Redfield“	460	3.88 ± 0.08	4.8 ± 0.2	8.68	0.5 ± 0.03	17	b.d.	b.d.
		1046	3.86 ± 0.08	4.8 ± 0.2	8.66	0.5 ± 0.04	17	b.d.	b.d.
		1280	3.88 ± 0.08	4.8 ± 0.2	8.68	0.5 ± 0.02	17	b.d.	b.d.
	„High N:P“	460	12.49 ± 0.23	4.8 ± 0.2	17.29	0.2 ± 0.02	75	9.75	b.d.
		1046	12.49 ± 0.23	4.8 ± 0.2	17.29	0.2 ± 0.03	75	9.41	b.d.
		1280	12.49 ± 0.23	4.8 ± 0.2	17.29	0.2 ± 0.01	75	11.07	b.d.

conditions for six to eight generations. In order to limit sedimentation during the acclimation process and the subsequent experiment, bottles were carefully rotated three times a day each time with 15 rotations. The duration of the experiment was determined by species capability to use up nutrients and reach the stationary phase. After being three days in the stationary phase the cultures were sampled and prepared for analysis.

Treatments and medium preparation

Both coccolithophore species were freshly isolated from waters originated from the island Terceira (Azores, North Atlantic, 38°39'22" N 27°14'08" W) and have been in culture for not longer than five months.

Trace metals and vitamins according to a tenth of a common f/2-medium (Guillard 1975) were added to 100 l of 1.4 μm pre-filtrated North Sea water with a salinity of 32. Macronutrients in a concentration of 3.9 μmol L⁻¹ nitrate, 4.8 μmol L⁻¹ ammonium and 0.23 μmol L⁻¹ phosphate were added. By the addition of Na₂CO₃, total alkalinity was elevated to 2700 μmol kg⁻¹ to abate expected variations in the carbonate specification. Subsequently, the medium was 0.2 μm sterile filtrated and equally dispersed into six sterile 20 l polycarbonate bottles. Three different CO₂ levels were established by aeration with enriched air, according to pCO₂ of 460, 1046 and 1280 μatm, respectively (Table 2.1).

In order to manipulate two different nutrient ratios, additional nitrate was added in one 20 l bottle of each pCO₂ level to reach a concentration of 12.5 μmol L⁻¹. The residual bottles were enriched with additional phosphate to achieve concentrations of 0.54 μmol L⁻¹. Final ratios of total nitrogen to phosphate of 75:1 (17.3 μmol N kg⁻¹, 0.23 μmol P kg⁻¹) and 17:1 (8.7 μmol N kg⁻¹ : 0.54 μmol P kg⁻¹) were established (Table 2.2).

Sampling and response variables

At the end of the experiment samples were taken (Whatman GF/F filters 25 mm Ø) in order to determine the content of particulate organic nitrogen (PON), total particulate carbon (TPC) and particulate organic carbon (POC). For the latter, the particulate inorganic carbon (PIC) was removed by exposing filters containing TPC to fuming hydrochloric acid for 2 hours. All filters were dried at 60°C and analysed in an elemental analyser with a heat conductivity detector (Thermo Flash 2000) according to Sharp (1974). The PIC content was calculated by the subtraction of POC from TPC. In order to determine the particulate carbon content per cell, the concentration was divided by the cell abundance. The divisions of PIC by POC and POC by PON resulted in the PIC/POC- and C/N - ratio, respectively.

Cell abundance was measured every day with a Z2™ COULTER COUNTER® cell and particle counter. The decision to terminate a culture was based on the statistical significant fit to the growth model

$$n_t = a / (1 + ((a-b)/b) \cdot e^{-\mu \cdot t})$$

with n_t indicating the cell number after t days, a the maximum cell abundance, b the start cell number and μ the growth rate. The first day, the growth curve of a culture significantly fitted to the model, i.e. reached the stationary phase, was defined as the first of three days in the stationary phase, after which the cultures were terminated.

The growth rate was calculated by

$$\mu = \frac{\ln(n_2) - \ln(n_1)}{t_2 - t_1}$$

with n indicating the cell abundance and t the time from one day to the other. The presented values correspond to the mean of μ during the exponential phase. Additionally, cell size was measured with the Z2™ COULTER COUNTER®. The resulting diameter was used to calculate the biovolume following Hillebrand et al. (1999b). Cell size increase of *G. oceanica* at 1046 μatm and 1280 μatm pCO₂ was referred to the cell size at the exponential phase of 460 μatm.

Because of the strong decline in growth rate at the two highest pCO₂ treatments resulting in a larger number of undivided cells, the cell size in the exponential phase was higher.

The total biovolume (carrying capacity) was achieved by the multiplication of cell abundance by cell volume.

Additional samples for PO_4^{3-} , NO_3^{2-} and NH_4^+ were taken and filtered through GF/F filters. The filtrates were frozen in polyethylene bottles. Duplicate samples from each bottle were analysed colorimetrically with an accuracy of $\pm 0.1 \mu\text{mol}$ (Hansen and Koroleff 1999).

Samples for DIC and total alkalinity were taken at the beginning and at the end of the experiment. The DIC measurements were carried out photometrically in a Bran & L ubbe QUAATRO analyzer equipped with a XY-2 sampling unit (Stoll et al. 2001b).

For TA determination by potentiometric titration, duplicate samples (25ml) were filtered (Whatman GF/F filters $0.2 \mu\text{m}$) and titrated at 20°C in an automated titration device (Metrohm Basic Titrino 794) with 0.05 M HCl -solution (Dickson 1981; Dickson et al. 2003b) and a precision of $\pm 3 \mu\text{mol kg}^{-1}$. Certified reference material (University of California (San Diego), Marine Physical Laboratory, Prof. A.G. Dickson) was used as a standard and measured every day before and after measuring the samples. The corresponding partial pressure of CO_2 and the residual parameters of the carbonate system were calculated with the equilibrium constants for carbonic acid by Mehrbach et al. (1973) refitted by Dickson and Millero (1987).

Statistics

Prior to statistical analyses data were tested for normality and homogeneity of variances. If data were not normally distributed and / or variances were not homogenous data were log- or square root transformed. Addressing our hypotheses, effects of pCO_2 , nutrient ratio and their interactions on POC and PIC content per cell as well as per volume of culture medium, on cell size and on total biovolume were tested by calculating a General Linear Model (GLM) for both species separately. Nutrient ratio was used as a categorical and factors. The differences between ΔpCO_2 , TPC and PIC/POC ratio of all treatments pCO_2 as a continuous factor. Due to significant interactions between the factors nutrient ratio and pCO_2 a further GLM was calculated after separation for the interacting were compared by Tukey HSD.

Please note, that due to problems during the sampling procedure we omitted two replicates of *E. huxleyi*, (460 μatm at "high N:P" and 1280 μatm at Redfield Ratio) and one replicate of *G. oceanica* (1050 μatm at Redfield ratio). The two outliers in growth rate of the *G. oceanica* 1280 μatm treatment were ignored for the calculation of growth curve and population carrying capacity but not for all other parameters.

All statistical tables can be found in the appendix of this chapter.

Results

Particulate matter

For both species particulate inorganic and organic carbon (PIC and POC) and organic nitrogen (PON) on the cellular as well as on the population level were significantly affected by the manipulated factors pCO₂ and nutrient ratio (Table 2.3). PIC content of both species decreased on both the cellular and the population level in response to increasing pCO₂ (Table 2.4). All together POC content of *E. huxleyi* significantly decreased with pCO₂. POC content of *G. oceanica* also decreased on the population but increased on the cellular level (Table 2.3, Table 2.4, Figure 2.1, Figure 2.2). Cellular PON content generally increased with pCO₂ for *E. huxleyi* and *G. oceanica* (Table 2.3) but revealed a decrease for the “High N:P” treatments of *E. huxleyi* (Table 2.4, Figure 2.1).

Also nutrient ratio significantly affected POC, PIC and PON content on the cellular level (Table 2.3). In all three cases the cellular content was significantly higher for the “High N:P” treatments (Figure 2.1). On a population level only the PIC and POC but not PON was significantly affected by the nutrient ratio. Vice versa to the cellular content on the population level the “Redfield” treatments were higher. In general the response of cellular PIC content to increasing pCO₂ of both species significantly depended on the nutrient ratio. That is, cellular PIC content for

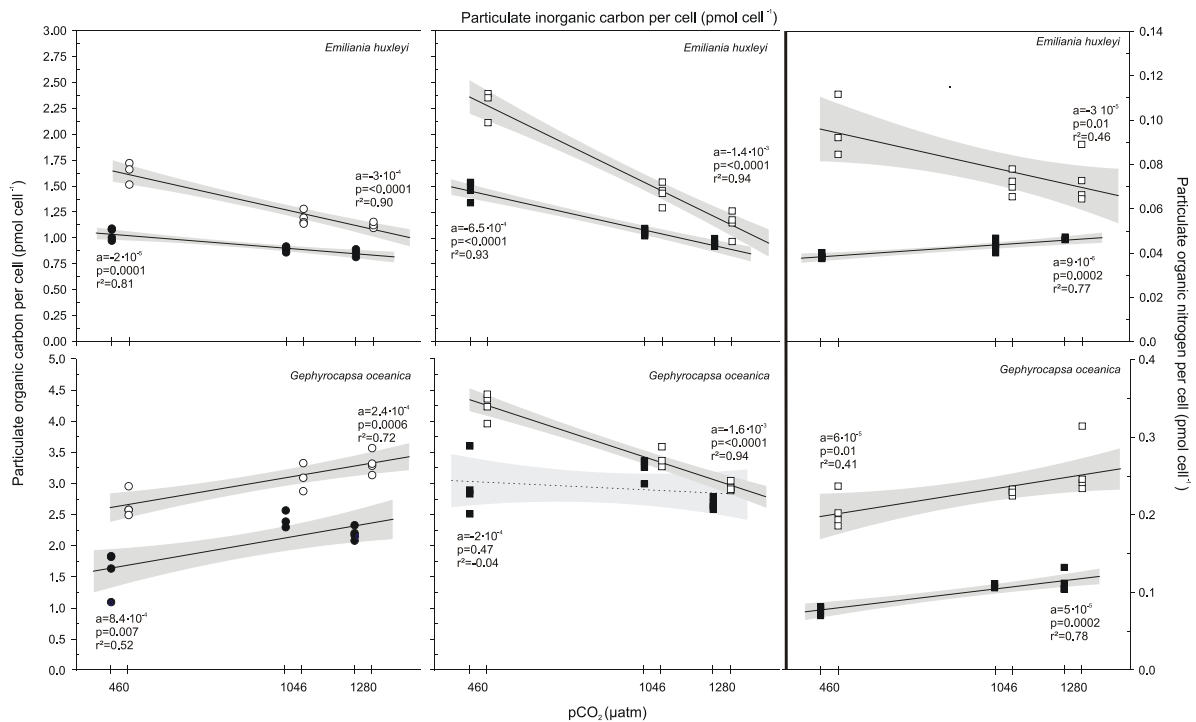


Figure 2.1: POC (left), PIC (middle) and PON (right) content per cell for *E. huxleyi* (upper row) and *G. oceanica* (lower row). Closed and open symbols indicate the “Redfield” treatments and the “Higher N:P” treatments, respectively. Lines reflect the regression with *a* being the slope and *r*² the adjusted coefficient of determination. The gray shading reflects the 95% confidence interval with *p* being the significance.

G. oceanica significantly decreased only under “High N:P” conditions but not in the Redfield medium. For *E. huxleyi*, correspondingly, the regression slope in the “High N:P” medium was steeper than under Redfield conditions (Table 2.4, Fig. 2.1). On the population level the regression slopes for both species were steeper for the Redfield treatments (Table 2.4, Figure 2.2).

The dependence of the pCO₂ response on nutrient ratio occurred also for the POC fraction of *E. huxleyi*, but not of *G. oceanica* (Table 2.3, Table 2.4, Figure 2.1). As for PIC content the regression slope for the cellular POC content was steeper in the “High N:P” treatment. Vice versa on the population level only the Redfield cultures significantly decreased in response to pCO₂ (Table 2.4, Figure 2.2).

Although, there was no significant interaction between pCO₂ and nutrient ratio (Table 2.3), the PON responses of *E. huxleyi* revealed opposite trends for the two nutrient treatments. Thereby, cells growing under “High N:P” conditions significantly decreased their PON content in response to pCO₂, whereas cell growing under Redfield conditions increased in PON content (Table 2.4, Figure 2.1).

The C/N ratio significantly decreased with pCO₂ for both species. Nutrient ratios had an effect only on the C/N ratio of *G. oceanica*, that is C/N-ration was significantly higher in the Redfield treatments (Table 2.5, Figure 2.3).

For the PIC/POC ratio of *E. huxleyi* the general linear model did not show any significant effects

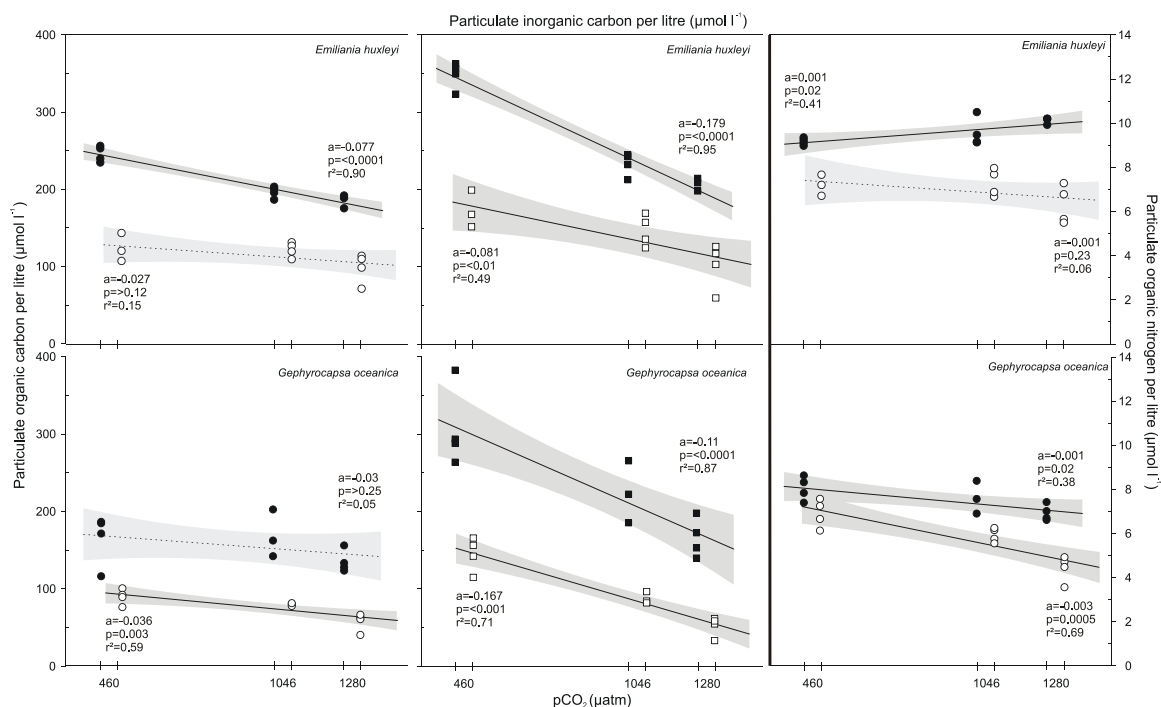


Figure 2.2: POC (left), PIC(middle) and PON (right) per litre for *E. huxleyi* (upper row) and *G. oceanica* (lower row). Closed and open symbols indicate the “Redfield” treatments and the “Higher N:P” treatments, respectively. Lines reflect the regression with a being the slope and r² the adjusted coefficient of determination. The gray shading reflects the 95% confidence interval with p being the significance.

by the manipulated factors and their interaction (Table 2.5). However, regression analyses separated for Redfield and high N:P treatments with pCO₂ as a predictor revealed a decrease in the ratio for both nutrient ratios (Table 2.6, Figure 2.3). PIC/POC ratio of *G. oceanica* was significantly affected by both manipulated factors, but not by their interaction (Table 2.5). As for *E. huxleyi*, all nutrient treatments decreased their PIC/POC ratio with increasing pCO₂ (Table 2.6, Figure 2.3).

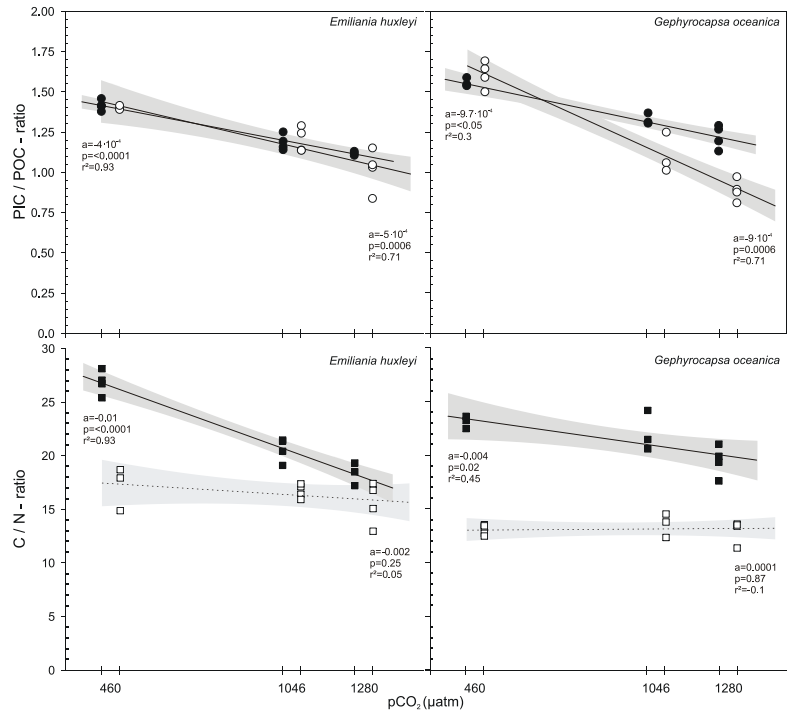


Figure 2.3: Ratio of calcification to photosynthesis (upper graphs) and the ratio of carbon to nitrogen content in the cells for *E. huxleyi* (left side) and *G. oceanica* (right side). Closed and open symbols indicate the “Redfield” treatments and the “Higher N:P” treatments, respectively. Lines reflect the regression with *a* being the slope and *r*² the adjusted coefficient of determination. The grey shading reflects the 95% confidence interval with *p* being the significance. Dotted lines reflect non significant regression lines.

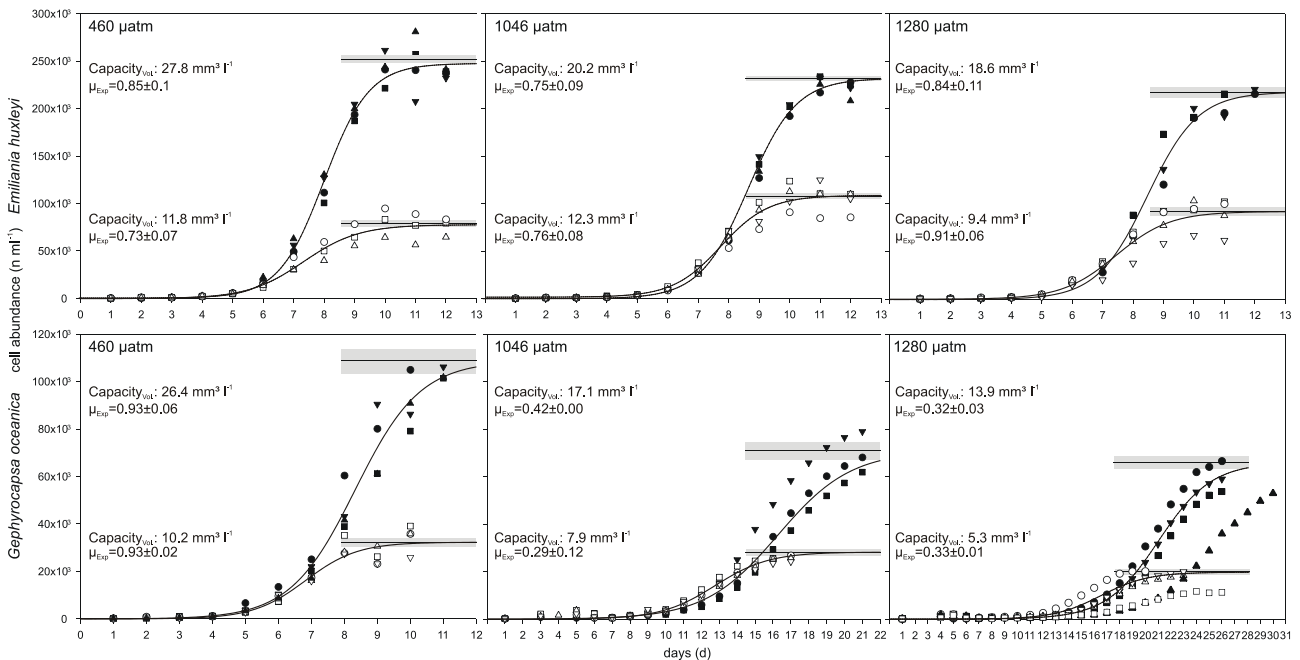


Figure 2.4: Cell abundance of *E. huxleyi* (upper graphics) and *G. oceanica* (lower graphics) at three different pCO₂ levels. Closed symbols indicate the four replicates of the Redfield ratio, open symbols that of the high N:P ratio. The total carrying capacity is given as the biovolume in mm³ per liter. The growth rate (μ) is calculated only for the exponential phase.

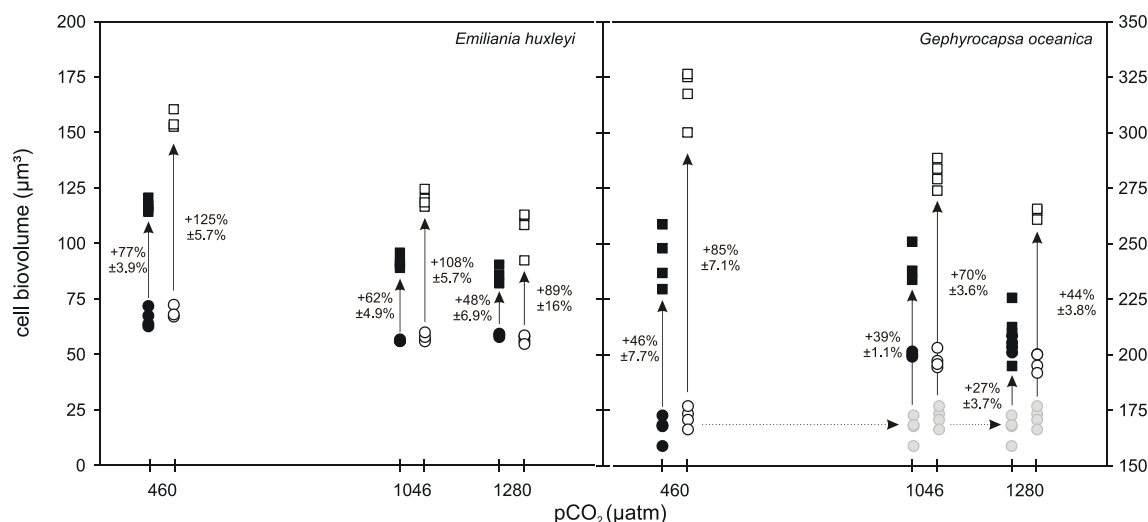


Figure 2.5: Single cell biovolume of *E. huxleyi* (left graph) and *G. oceanica* (right graph). Circles indicate the cell volume in the exponential phase, squares the volume in the stationary phase. Closed and open symbols indicate the “Redfield” treatments and the “Higher N:P” treatments, respectively. Numbers express the percental increase plus/minus the standard deviation. Due to a cell size artefact (see methods) the cell size increase of the two highest pCO₂ treatments of *G. oceanica* were calculated with respect to the exponential phase volume at 460 µatm pCO₂ (grey circles).

Cell growth, size and cell volume carrying capacity

Growth rate of *E. huxleyi* increased with pCO₂ only in the “High N:P” scenario (Table 2.6, Fig. 2.4). Growth rate of *G. oceanica* was significantly influenced by pCO₂ (Table 2.5) and decreased in both nutrient regimes (Table 2.6, Fig. 2.4).

All cell sizes increased in the stationary phase from what was measured in the exponential phase (Figure 2.5). For both species and both nutrient treatments this increase, however, was less pronounced with increasing pCO₂ (Table 2.5, Table 2.6, Figure 2.5). Nutrient ratio influenced cell volume significantly in all cases and led to larger cells at the “High N:P” treatments (Table 2.5, Table 2.6, Figure 2.5).

Also carrying capacity was significantly influenced by the manipulated factors and by their interaction (Table 2.5). Increasing pCO₂ significantly reduced carrying capacity of both species (Table 2.5, Table 2.6, Fig. 2.4). This was, however, not significant for the “High N:P” treatment of *E. huxleyi* (Table 2.6, Fig. 2.4).

pCO₂ variations

The prepared pCO₂ of the medium was varied by the growth of the coccolithophores to a different extent (Figure 2.6). While both nutrient treatments of *E. huxleyi* with a start pCO₂ of 460 µatm faced no change (“Redfield”: t=-0.27; p=0.8 “High N:P”: t=-1.57; p=0.26), treatments of *G. oceanica* increased pCO₂ towards the end of the experiment (“Redfield”: t=-9.8; p=0.002; “High N:P”: t=-4.4; p=0.02). All higher pCO₂ treatments of both species and both nutrient ratios reduced pCO₂ during the experiment. With the exception of the *G. oceanica* “Redfield” 1280 µatm

and “High N:P” 1060 μatm treatments (“Redfield”: $t=2.02$; $p=0.18$; “High N:P”: $t=1.51$; $p=0.23$), all differences between start and end $p\text{CO}_2$ were significant ($t\geq 3.84$; $p\leq 0.03$).

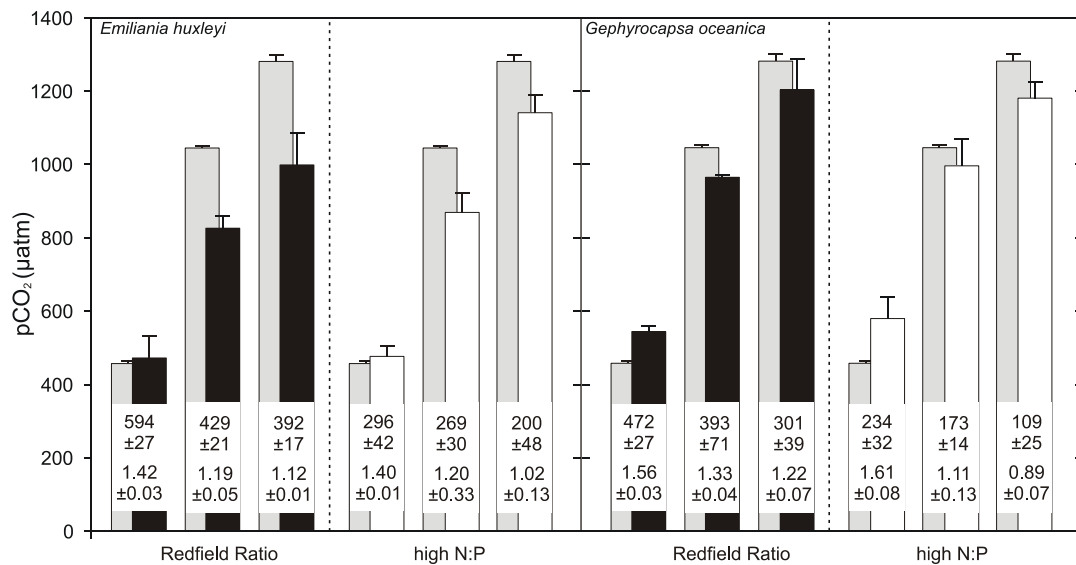


Figure 2.6: Start $p\text{CO}_2$ values of the experiment (grey bars) for *E. huxleyi* (left side) and *G. oceanica* (right side) and the associated end values for the Redfield ratio (black) treatments and for those treatments facing a high N:P ratio (white). Written values reflect the TPC in μmol per litre and the PIC/POC ratio, both with its standard deviation.

Discussion

Although, nitrogen is in general referred to be the limiting resource for phytoplankton growth (Falkowski 1997), *Emiliana huxleyi* preferably grow and bloom in parts of the ocean with relative high N:P ratios (Egge and Heimdal 1994; Tyrrell and Taylor 1996). Especially, under the consideration of the future extent of P-limited areas (Ammerman et al. 2003), simulating two different phosphate limitation scenarios provide complementary results to earlier ocean acidification studies on coccolithophores (Riebesell et al. 2000; Zondervan et al. 2001; Zondervan et al. 2002; Sciandra et al. 2003; Langer et al. 2006; Langer et al. 2009; Shi et al. 2009; Krug et al. 2011). To the best of our knowledge, this study shows for the first time different sensitivities to ocean acidification caused by different nutrient ratios for net population carbon accumulation of the species *E. huxleyi* and *G. oceanica*. That is, the decline of net population calcification in response to $p\text{CO}_2$ is significantly attenuated at high N:P ratio. However, based on complications caused by potential precipitation of inorganic phosphorus (see discussion below), the direct effects of rising $p\text{CO}_2$ and its interaction with different nutrient ratios can only be evaluated for *E. huxleyi* for which all cultures had similar experimental runtimes and thus did not confound with potential inorganic phosphorus precipitation.

Interaction between pCO₂ and N:P ratio for *E. huxleyi*

For *E. huxleyi* our results suggest that a more efficient nutrient use (i.e. less PON per cell) at a high N:P ratio attenuated the negative effect of ocean acidification on total population POC, PIC and PON accumulation. Despite a more pronounced response in cellular POC, PIC and PON accumulation to increasing pCO₂, cells growing under a higher N:P ratio revealed no response in the net population POC and PON accumulation and a less distinct decrease in total PIC accumulation compared to “Redfield” treatments. Vice versa to the increasing cellular PON content of the “Redfield” cells, cellular PON is decreasing for “high N:P” treatments. Since only phosphate was depleted and nitrogen was still available, the results suggest that not increasing cellular PON but rather particulate organic phosphorus (POP) is likely to cause variations in cell abundance. We suggest that similar to low values of PON per cell the available inorganic phosphorus was also transferred into less cellular POP which in turn allowed for higher cell abundance. This would explain not only the increasing cell abundance but also the insignificant differences in total biovolume (i.e. cell abundance compensated for decreasing cell size) with respect to increasing pCO₂. This in turn translated into an insignificant response of population POC and only a moderate decline in PIC. In contrast, while the cellular PIC and POC content of cells facing a Redfield ratio is significantly less affected with rising pCO₂ compared to cells exposed to a higher N:P ratio, their cellular PON content increases. Since here both nutrients nitrogen and phosphate are depleted, the increase in cellular PON might explain the drop in the maximum cell abundance but does not exclude additional regulation by cellular POP. Thus in the Redfield treatments together with a decreasing cell volume the drop in cell abundance results in an amplified reduction in total biovolume, resulting in a strong decline in total POC and PIC accumulation.

In general the response of population net photosynthesis (i.e. POC) and net calcification (i.e. PIC) of both species to rising pCO₂ in this study confirmed the results of earlier experiments (Langer et al. 2009). Likewise, the cell responses to phosphate limitation coincide with previous studies. Caused by an oversupply of nitrogen and the production of biomass without the possibility to divide, cells exposed to phosphate limitation grew considerably larger in volume compared to their size in the exponential phase (Riegman et al. 2000; Müller et al. 2008). Cells growing under the Redfield ratio depleted their nitrogen sources, therefore cells facing a higher N:P ratio, got significantly larger in volume. As a consequences of decreasing cellular PIC and POC the increase in cell size caused by nutrient limitation is attenuated by rising pCO₂. Thereby, it is not clear if pCO₂ decreases the maximum cell size or just the cellular growth rate. It cannot fully be excluded that cells of all pCO₂ treatments would reach the same size after being in the stationary phase for longer time. The growth rate (μ) of *E. huxleyi* is not changing or even increasing with pCO₂. This makes a constant cellular growth rate and a changing maximum cell size more likely.

We are aware that nutrient concentration, i.e. higher phosphate concentrations in the Redfield treatment potentially confounds with nutrient ratio. However, for conclusion on the cellular level the absolute concentrations were insignificant, the nutrient ratio alone caused the observed variations in response to pCO₂. The absolute concentrations accounted for the significant variations in the particulate matter between the two nutrient ratio treatments on a population level. However, for our conclusion only the slopes and not absolute carbon accumulation in response to pCO₂ were compared.

Natural variations in pCO₂

The results of both species can, also, be consulted to interpret the natural variations in pCO₂, caused by the consumption of DIC and the reduction of TA. The loss of DIC by the primary production of phytoplankton blooms leads to a decrease in pCO₂ which cannot immediately be balanced by atmospheric CO₂ (Taylor et al. 1992; Robertson et al. 1993; Purdie and Finch 1994). Reduction of TA by calcification of coccolithophores in turn leads to an increase in pCO₂ and therewith counteracts the reduction of pCO₂ by photosynthesis (Purdie and Finch 1994).

Thus, the ratio of calcification and photosynthesis, approximately given by the PIC/POC ratio, determines whether pCO₂ in a bloom of coccolithophores is changing. Comparison of the different treatments at 460 and 1046 µatm pCO₂ (Table 2.7, Figure 2.6) shows that an insignificant difference in ΔpCO₂ is attended by an insignificant change in PIC/POC ratio and a significant change in TPC. At 1280 µatm pCO₂, however, a significant change in ΔpCO₂ is attended by a significant change in TPC ratio and an insignificant change in PIC/POC. This visualisation indicates that the ratio of PIC and POC balances variations in the pCO₂ at lower start pCO₂ values and thus the amount of total biomass is insignificant. At higher pCO₂ starting conditions the decrease in calcification is not able to compensate for changes caused by POC accumulation. Therewith, total biomass is the leading factor for pCO₂ variations at higher pCO₂ conditions. This effect is accelerated by a decreasing buffer capacity, i.e. increasing Revelle factor in the future oceans (for further reading: Revelle and Suess 1957; Broecker et al. 1979; Egleston et al. 2010).. Thereby, primary production will cause larger fluctuations in the surface pCO₂ (Riebesell et al. 2007). With rising atmospheric pCO₂ the reduction of DIC by photosynthesis leads to a stronger decline in the aqueous CO₂ concentration as the same level of calcification would lead to an increase.

Potential precipitation of inorganic phosphorus

In case of *G. oceanica*, however, an change in the N:P ratio for the different pCO₂ treatments by inorganic precipitation of phosphorus cannot be fully excluded. Just recently found indications led to the assumption that within a F/2 medium by Guillard (1975) with 4 µmol kg⁻¹ phosphate over a time of ten days approximately 10% of the initial phosphate concentration precipitate

inorganically (Febiri et al. pers com.). This finding would have severe implications for all phosphate limitation experiments in the past and in the future. Although, it is not completely solved what kind of precipitation occurs, the strong decline in growth rate with rising pCO₂ of *G. oceanica* and the therewith longer experimental duration would lead to a pronounced precipitation at higher pCO₂. The thereby artificially caused variation in the nutrient ratios makes an evaluation of the interaction of N:P ratio and pCO₂ not possible. At the same time, the precipitation of phosphate accounts for the strong decline in the carrying capacity between the low and the two highest pCO₂ treatments.

All treatments of *E. huxleyi*, had the same experimental duration of eleven to twelve days. Since the effect seems not to be dependent on changes in pH within the here applied range of ocean acidification (Febiri et al. pers. com.), an equal precipitation of phosphate leads to an increase in the phosphate limitation, but does not affect the interaction of nutrient ratio and pCO₂.

Conclusion

The attenuated effects of ocean acidification on the accumulation of total particulate matter by a high N:P nutrient ratio points out that variations in the cellular POC and PIC cannot easily be extrapolated to the population level. Considering these results, estimations on the future carbon cycle should not only account for pCO₂ and the response of coccolithophores to ocean acidification in exponential growth but also for the effects of future conditions on the carrying capacity of a species. Although the function of calcite as ballast for global ocean carbon export is not completely clear, for future predictions on carbon export in an acidified ocean our study reveals the necessity to consider nutrient ratios and their capability to attenuate negative responses of net population calcite accumulation.

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Appendix

Statistical Tables

Table 2.3 Results of a two factorial general linear model for the response variables POC, PIC and PON per cell and per litre for both species, respectively.

	Factor	df	<i>Emiliana huxleyi</i>					<i>Gephyrocapsa oceanica</i>					
			R ²	Slope	MS	F	p	df	R ²	Slope	MS	F	p
sqrt POC cell ⁻¹	Whole model	3,18	0.95		0.001	123	<0.001	3,19	0.85		0.06	40.1	<0.001
	Nutrients	1,18			0.066	95.0	<0.001	1,19			0.73	12.2	0.003
	pCO ₂	1,18		-2•10 ⁻⁴	0.095	136	<0.001	1,19		0.001	1.93	32.2	<0.001
	Inter.	1,18			0.02	21.6	<0.001	1,19			4•10 ⁻⁴	0.001	0.94
PIC cell ⁻¹	Whole model	3,18	0.95		0.01	137	<0.001	3,19	0.77		0.08	24.8	<0.001
	Nutrients	1,18			0.86	95	<0.001	1,19			32.7	32.7	<0.001
	pCO ₂	1,18		-0.001	2.62	291	<0.001	1,19		-9•10 ⁻⁴	29.8	29.8	<0.001
	Inter.	1,18			0.36	40	<0.001	1,19			16.6	16.6	<0.001
PON cell ⁻¹	Whole model	3,18	0.95		2.04	139	<0.001	3,19			2.1	158	<0.001
	Nutrients	1,18			65.2	32	<0.001	1,19			79.1	38.4	<0.001
	pCO ₂	1,18		0.01	183	90	<0.001	1,19		0.009	213	103.5	<0.001
	Inter.	1,18			0.02	0.01	0.93	1,19			0.8	0.39	0.54
POC litre ⁻¹	Whole model	3,18	0.94		190	107	<0.001	3,19	0.79		460	27.7	<0.001
	Nutrients	1,18			12468	65.6	<0.001	1,19			3972	8.63	<0.01
	pCO ₂	1,18		-0.05	6572	34.6	<0.001	1,19		-0.03	2955	6.43	0.02
	Inter.	1,18			1585	8.3	<0.01	1,19			31.2	0.07	0.80
PIC litre ⁻¹	Whole model	3,18	0.93		480	99	<0.001	3,19	0.89		898	60	<0.001
	Nutrients	1,18			28153	58.7	<0.001	1,19			26187	29.2	<0.001
	pCO ₂	1,18		-0.13	41764	87.1	<0.001	1,19		-0.14	54682	60.9	<0.001
	Inter.	1,18			5943	12.4	<0.001	1,19			2254	2.5	<0.001
sqrt PON litre ⁻¹	Whole model	3,18	0.01		161511	1.17	0.33	3,19	-0.01		98937	0.95	0.44
	Nutrients	1,18			11443	0.07	0.79	1,19			60801	0.61	0.44
	pCO ₂	1,18		-0.12	77452	0.48	0.49	1,19		0.09	23811	0.24	0.63
	Inter.	1,18			119043	0.74	0.40	1,19			150437	1.52	0.23

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Table 2.4 Results of regression analyses with pCO₂ as predictor on the PIC, POC and PON quota per cell as well as per litre of *E. huxleyi* and *G. oceanica* at both nutrient ratios.

		<i>Emiliana huxleyi</i>					<i>Gephyrocapsa oceanica</i>				
	Factor	R ²	F _{1,10}	p	Slope	SE	R ²	F _{1,10}	p	Slope	SE
POC cell ⁻¹	<i>Redfield</i>	0.81	42.3	<0.001	-10 ⁻⁴	2·10 ⁻⁵	0.51	11	0.008	3·10 ⁻⁴	10 ⁻⁴
	<i>High N:P</i>	0.9	92	<0.001	-3·10 ⁻⁴	3·10 ⁻⁵	0.72	26	<0.001	2·10 ⁻⁴	5·10 ⁻⁵
PIC cell ⁻¹	<i>Redfield</i>	0.97	137	<0.001	-6·10 ⁻⁴	6·10 ⁻⁵	-0.05	0.6	0.5	-2·10 ⁻⁴	3·10 ⁻⁴
	<i>High N:P</i>	0.94	168	<0.001	-0.001	10 ⁻¹	0.93	144	<0.001	-0.002	10 ⁻⁴
PON cell ⁻¹	<i>Redfield</i>	0.77	34.2	<0.001	9·10 ⁻⁶	2·10 ⁻⁶	0.78	37.5	<0.001	5·10 ⁻⁵	7·10 ⁻⁶
	<i>High N:P</i>	0.46	9.5	0.01	-3·10 ⁻⁵	10 ⁻⁵	0.4	8.7	0.01	6·10 ⁻⁵	2·10 ⁻⁵
POC litre ⁻¹	<i>Redfield</i>	0.9	95	<0.001	-0.08	0.008	0.05	1.5	0.25	-0.03	0.02
	<i>High N:P</i>	0.15	2.8	0.13	-0.03	0.02	0.59	16	0.003	-0.04	0.009
PIC litre ⁻¹	<i>Redfield</i>	0.95	174	<0.001	-0.18	0.014	0.71	25.5	<0.001	-0.17	0.03
	<i>High N:P</i>	0.49	10.7	<0.01	-0.08	0.02	0.87	68.8	<0.001	-0.11	0.01
PON litre ⁻¹	<i>Redfield</i>	0.41	7.82	0.02	0.001	4·10 ⁻⁴	0.38	7.2	0.02	-0.001	5·10 ⁻⁴
	<i>High N:P</i>	0.06	1.64	>0.2	-9·10 ⁻⁴	7·10 ⁻⁴	0.69	25.8	<0.001	-0.003	5·10 ⁻⁴

Table 2.5 Results of a two factorial general linear model for the response variables C/N, PIC/POC, growth rate (μ), the increase in cell size and the volume carrying capacity for both species, respectively.

	Factor	df	<i>Emiliana huxleyi</i>					<i>Gephyrocapsa oceanica</i>					
			R ²	Slope	MS	F	p	df	R ²	Slope	MS	F	p
C/N ratio	Whole model	3,18	0.2		115	4.4	<0.01	3,19	0.94		1.89	95	<0.001
	Nutrients	1,18			105	0.92	0.34	1,19			42	22.2	<0.001
	pCO ₂	1,18		-0.01	517	4.51	0.04	1,19		-0.01	111	58.6	<0.001
	Inter.	1,18			0.65	0.006	0.94	1,19			0.09	0.05	0.83
Sqrt PIC/POC ratio	Whole model	3,18	-0.01		122413	0.85	0.47	3,19	0.95		1.52	120	<0.001
	Nutrients	1,18			202092	1.65	0.21	1,19			33.8	22.2	<0.001
	pCO ₂	1,18		-0.086	3565	0.29	0.59	1,19		-0.01	117.7	77.3	<0.001
	Inter.	1,18			103255	0.84	0.36	1,19			3.2	2.1	0.17
Growth rate	Whole model	3,18	0.10		0.009	1.77	0.12	3,19	0.9		0.009	68	<0.001
	Nutrients	1,18			0.04	4.1	0.06	1,19			3•10 ⁻⁵	0.003	0.96
	pCO ₂	1,18		7•10 ⁻⁵	0.01	1.3	0.26	1,19		-8•10 ⁻⁴	1.8	201	<0.001
	Inter.	1,18			0.04	4.1	0.06	1,19			0.004	0.39	0.54
Δ Size	Whole model	3,18	0.93		24.4	94	<0.001	3,19	0.92		175	81	<0.001
	Nutrients	1,18			1159	47.5	<0.001	1,19			4224	24	<0.001
	pCO ₂	1,18		-0.04	3129	128	<0.001	1,19		-0.09	25073	143	<0.001
	Inter.	1,18			151	6.2	0.02	1,19			351	2	0.17
Volume capacity	Whole model	3,18	0.93		3•10 ⁻⁶	95	<0.001	3,19	0.96		2•10 ⁻⁶	175	<0.001
	Nutrients	1,18			2•10 ⁻⁴	77	<0.001	1,19			2•10 ⁻⁴	108	<0.001
	pCO ₂	1,18		-7•10 ⁻⁶	10 ⁻⁴	38	<0.001	1,19		-4•10 ⁻⁶	4•10 ⁻⁴	185	<0.001
	Inter.	1,18			5•10 ⁻⁵	17.5	<0.001	1,19			4•10 ⁻⁵	19	<0.001

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Table 2.6 Results of regression analyses with pCO₂ as predictor on the C/N ratio, the PIC/POC ratio, the growth rate (μ), the increase in cell size and the volume carrying capacity of *E. huxleyi* and *G. oceanica* at both nutrient ratios.

		<i>Emiliana huxleyi</i>					<i>Gephyrocapsa oceanica</i>				
	Factor	R ²	F _{1,10}	p	Slope	SE	R ²	F _{1,10}	p	Slope	SE
C/N	<i>Redfield</i>	0.93	130	<0.001	-0.01	9·10 ⁻⁴	0.45	8.5	0.02	-0.004	0.001
	<i>High N:P</i>	0.05	1.5	>0.2	-0.002	0.001	-0.1	0.03	0.9	10 ⁻⁴	8·10 ⁻⁴
PIC/POC	<i>Redfield</i>	0.93	143	<0.001	-4·10 ⁻⁴	3·10 ⁻⁵	0.3	5.2	<0.05	-0.001	4·10 ⁻⁴
	<i>High N:P</i>	0.71	26	<0.001	-5·10 ⁻⁴	9·10 ⁻⁵	0.94	148	<0.001	-9·10 ⁻⁴	7·10 ⁻⁵
μ	<i>Redfield</i>	-0.07	0.32	0.58	-5·10 ⁻⁵	5·10 ⁻⁹	0.97	295	<0.001	-8·10 ⁻⁴	5·10 ⁻⁵
	<i>High N:P</i>	0.35	6.4	0.03	2·10 ⁻⁴	8·10 ⁻⁵	0.86	68	<0.001	-0.001	10 ⁻⁴
Δ Size	<i>Redfield</i>	0.89	82	<0.001	-0.03	0.003	0.81	44	<0.001	-0.08	0.01
	<i>High N:P</i>	0.86	61	<0.001	-0.04	0.006	0.91	117	<0.001	-0.1	0.01
Capacity	<i>Redfield</i>	0.93	135	<0.001	-10 ⁻⁵	10 ⁻⁶	0.94	150	<0.001	-2·10 ⁻⁵	10 ⁻⁶
	<i>High N:P</i>	0.02	6·10 ⁻⁶	0.31	-2·10 ⁻⁶	6·10 ⁻⁶	0.80	45	<0.001	-8·10 ⁻⁶	10 ⁻⁶

Table 2.7 p-values of the Post-Hoc Test (Tukey's test) on the variation in the pCO₂ during the experiment, the PIC/POC ratio and the TPC per cell content at the three different start pCO₂ for the species *Emiliana huxleyi* (E.hux) and *Gephyrocapsa oceanica* (G.oc) species at both nutrient ratios. The encircled Δ pCO₂ values support the hypothesis of a PIC/POC ratio driven variation in pCO₂.

		Species	Nutrients	E. hux Redfield	E. hux High N:P	G. oc Redfield
		460 μ atm	Δ pCO ₂	E. hux	High N:P	0.662
G. oc	Redfield			0.061	0.024	
G. oc	High N:P			0.028	0.039	0.154
PIC/POC	E. hux		High N:P	0.508		
	G. oc		Redfield	0.233	0.303	
	G. oc		High N:P	0.005	0.009	0.411
TPC	E. hux		High N:P	0.000		
	G. oc		Redfield	0.001	0.001	
	G. oc		High N:P	0.000	0.076	0.000
1046 μ atm	Δ pCO ₂	E. hux	High N:P	0.386		
		G. oc	Redfield	0.001	0.018	
		G. oc	High N:P	0.009	0.032	0.699
	PIC/POC	E. hux	High N:P	0.737		
		G. oc	Redfield	0.008	0.049	
		G. oc	High N:P	0.292	0.266	0.042
	TPC	E. hux	High N:P	0.000		
		G. oc	Redfield	0.364	0.023	
		G. oc	High N:P	0.000	0.001	0.002
1280 μ atm	Δ pCO ₂	E. hux	High N:P	0.017		
		G. oc	Redfield	0.044	0.624	
		G. oc	High N:P	0.008	0.280	0.803
	PIC/POC	E. hux	High N:P	0.248		
		G. oc	Redfield	0.064	0.034	
		G. oc	High N:P	0.002	0.130	0.001
	TPC	E. hux	High N:P	0.002		
		G. oc	Redfield	0.014	0.018	
		G. oc	High N:P	0.000	0.016	0.000

CHAPTER III

Polyculture positively affects ocean acidification resistance compared to average monoculture

Sebastian A. Krug and Birte Matthiessen



Abstract

It is often described that cells in monoculture respond to external influences in a different way compared to multi-type culture systems. Therefore, we investigated whether due to changing species interactions a coccolithophore community shows differing reactions to ocean acidification on organic and inorganic carbon per cell and total community carbon production compared to the respective species growing in monoculture. Seawater carbonate chemistry was manipulated to full-factorially simulate three levels of CO₂-induced seawater acidification in monocultures of *Emiliana huxleyi*, *Gephyrocapsa oceanica* and *Coccolithus braarudii* and in polycultures containing all three species. The possibility of cells to deplete nutrients and stay a certain time in stationary phase as well as the possibility to interact with each other led to new insights on the particulate carbon assimilation with respect to future ocean acidification. Both factors (pCO₂ and culture identity) significantly influenced per capita and total population/community production when measured at mid-term and at the end of the experiment. While the per capita POC of *C. braarudii* and *E. huxleyi* monocultures and the polyculture showed no change and *G. oceanica* exhibited a higher content with increasing ocean acidification, the per capita PIC decreased in all treatments. On a population level all monocultures revealed a decrease in POC and PIC. The community production of the polyculture was unaffected by ocean acidification and hence, significantly different from what could have been expected from the population level in the monocultures. Moreover, the production on the community/population level showed pronounced differences to the responses on a per capita base. A significant reduction in cell abundance will be discussed as the cause for a strong decline in the population/community productivity.

Based on these results we emphasize the need for an ecosystem approach when investigating the potential effects of global anthropogenic changes on marine ecosystems.

Introduction

A large fraction of the carbon dioxide released into the atmosphere by human activities is absorbed by the surface oceans (Sabine et al. 2004). This initiates the process known as ocean acidification and will continue to change seawater carbonate chemistry as long as CO₂ emissions continue. In the case of unabated CO₂ emissions ocean acidification will be reflected by decrease in seawater pH during this century which is probably unprecedented for the past 20 million years (Pearson and Palmer 2000). While the changes in ocean chemistry can be predicted with reasonable certainty, their impacts on the marine biota are still largely unknown (Raven et al. 2005).

Calcifying marine organisms are particularly vulnerable to ocean acidification (Fabry 2008). Among these, coccolithophores are one of the best studied groups in terms of their sensitivity to ocean acidification (Riebesell et al. 2000; Zondervan et al. 2002; Sciandra et al. 2003; Langer et al. 2006; Zondervan 2007; Feng et al. 2008; Langer et al. 2009; Ramos et al. 2010). To date, however, most of the laboratory experiments on CO₂ effects, both in coccolithophores and other marine organisms, concentrated on direct effects on single species and species strains. Although the focus on single species has proven to be useful in identifying underlying physiological mechanisms linking changes in atmospheric pCO₂ levels and species specific responses, it largely neglects potential indirect effects following changing community interactions (Hillebrand and Matthiessen 2009) and the view on total community response. Hence, approaches on single species allow for limited predictability of consequences following changes in atmospheric pCO₂ levels, and overlook the potential of communities to compensate and maintain primary production and calcification by shifting dominance. For this reason and in contrast to the majority of previous phytoplankton experiments focusing on per capita CO₂ effects in the exponential growth phase, a certain retention time in the stationary phase along with nutrient depletion is necessary to allow for inter- and intraspecies community interactions.

Resilience is the capacity of a community to absorb disturbance and re-organize while undergoing change, and still keep essentially the same structure and ecosystem function (but see Folke et al. 2004; Walker et al. 2009). Theoretically and experimentally it has been shown that diversity as a source of functional response to stress plays a crucial role in maintaining resilience. The concept of response diversity (Elmqvist et al. 2003) shows that community functioning (such as primary production) often is maintained by few dominant species, however, diversity of non abundant species can provide insurance to maintain community functioning when dominant species are reduced or lost due to environmental stress (Norberg et al. 2001).

Currently, coccolithophores contribute about half to the marine biogenic CaCO₃ precipitation (Broecker and Clark 2009) and are important primary producers especially in the oligotrophic

oceans (Thierstein and Young 2004). Previous studies on single species strains in batch culture experiments showed that within the range from 380 to 750 μ atm pCO₂ particulate organic carbon (POC) per cell of *Emiliana huxleyi* and *Gephyrocapsa oceanica* increased while calcification rates (i.e. particulate inorganic carbon (PIC) production) decreased. However, the effect sizes varied, both between species and strains (reviewed by Ridgwell et al. 2009).

Consolidating results from research on diversity effects and its role for ecosystem resilience showing varying effects of increasing pCO₂ on single coccolithophore species led us to experimentally investigate which role species interactions may play for total community functional response upon the effects of increasing pCO₂. We conducted a microcosm experiment manipulating pCO₂ in mono- and polycultures of the three well studied and in nature co-occurring coccolithophore species *Emiliana huxleyi*, *Gephyrocapsa oceanica* and *Coccolithus braarudii* and measured particulate organic as well as inorganic carbon production on both the individual and the community level. Identifying possible shifts in species composition requires longer experimental duration. Therefore, semi continuous cultures instead of batch cultures were used and analysed during the phase of stationary cell growth instead of measuring during the exponential growth phase where normally no species interactions take place (Weis et al. 2007). Using this approach, we tolerated the natural alteration in carbonate chemistry caused by the consumption of dissolved inorganic carbon (DIC) by photosynthesis and calcification and by the decrease in total alkalinity (TA) by calcification. Hereby, it can be expected that, due to a significant reduction in the buffer capacity in high CO₂ treatments, alterations will be more pronounced compared to those treatments representing the present day carbonate chemistry (Delille et al. 2005; Riebesell et al. 2007).

The functional responses of the monocultures are discussed with respect to nutrient uptake ratios and their capability to alter the surrounding seawater chemistry as well as the potential to build up total community biomass. The results from the monocultures are compared to what is observed in the polyculture and discussed in the framework of community resilience theory.

Methods

Experiment

Three naturally coexisting coccolithophore species, i.e. *E. huxleyi*, *G. oceanica* and *C. braarudii* were used in the experiment. Cultures were provided by the Roscoff Culture Collection and originate from strains RCC1228 (*E. huxleyi*), RCC1303 (*G. oceanica*) and RCC1203 (*C. braarudii*). The factors culture identity and rising pCO₂ were full-factorially manipulated. Culture identity was manipulated in four levels comprising each species in monoculture and all three species in a polyculture. Seawater carbonate chemistry was manipulated to obtain three start pCO₂ levels

(420, 730 and 1220 μatm). Experimental manipulations were fourfold replicated resulting in 48 experimental units.

Prior to the experiment algae were acclimated to experimental conditions for seven generations. The three species, however, clearly differed in average cell biovolume. The measured diameter resulted in a volume of 100 μm^3 for *E. huxleyi*, 150 μm^3 for *G. oceanica* and 2030 μm^3 for *C. braarudii*, calculated following Hillebrand et al. (1999a). To avoid unequal starting conditions these differences in volume were balanced by the initial cell concentration. To reach a volume of 40,600 μm^3 of biovolume ml^{-1} each, 20 cells ml^{-1} of *C. braarudii*, 270 cells ml^{-1} of *G. Oceanica*, 406 cells ml^{-1} of *E. huxleyi* and a third of the start cell abundance of each species in the polyculture treatment were added.

The experimental duration was more or less two times one week corresponding to approximately five to eight generations per week. The exact duration of each culture was determined by the capability to use up the nutrients and reach the stationary phase. That is, after being in the stationary phase for three days, 100 ml (7.3%) of the media was transferred to a new cell flask with fresh medium and allowed to grow for another week. After being in the stationary phase for another three days the experiment was terminated.

Media

At the beginning of the experiment 1.3 L cell culture flasks were filled with sterile filtered (0.2 μm pore size) North Sea water with a salinity of 35. Nutrients in concentrations of 6.8 $\mu\text{mol L}^{-1}$ nitrate and 0.4 $\mu\text{mol L}^{-1}$ phosphate, corresponding to a molar ratio of dissolved N to dissolved P of 17 : 1 were added. Vitamins and trace metal concentration arose from a tenth of a common f/2-medium (Guillard 1975). Light followed a sixteen to eight hours light/dark cycle and an intensity of 130 $\mu\text{mol photons m}^{-2} \text{sec}^{-1}$.

The desired carbonate chemistry was achieved by adding Na_2CO_3 and 0.4 N HCl to a constant start alkalinity value of $2423 \pm 9 \mu\text{mol kg}^{-1}$ and dissolved inorganic carbon concentration values of 2156 ± 2 , 2271 ± 6 and $2352 \pm 1 \mu\text{mol kg}^{-1}$. Thus, at the beginning of the experiment, the corresponding partial pressure of CO_2 , calculated with the equilibrium constants for carbonic acid by Mehrbach et al. (1973) refitted by Dickson and Millero (1987), was 422 ± 8 , 732 ± 21 and $1226 \pm 9 \mu\text{atm}$.

Samples for DIC and total alkalinity were taken at the beginning and at the end of both the acclimation phase and each semi-continuous culture. The DIC measurements were carried out photometrically in a Bran & L ubbe QUAATRO analyzer equipped with a XY-2 sampling unit (Stoll et al. 2001c). For TA determination by potentiometric titration, duplicate samples (100ml) were filtered (Whatman GF/F filters 0.2 μm) and titrated at 20°C in an automated titration device (Metrohm Basic Titrino 794) with 0.05 N HCl-solution (Dickson 1981; Dickson et al. 2003) and a precision of $\pm 3 \mu\text{mol kg}^{-1}$. Certified reference material (University of California (San Diego),

Marine Physical Laboratory, Prof. A.G. Dickson) was used as a standard and measured every day before and after measuring the samples.

Response variables

As response variables for the experiment the per capita content and the carrying capacity of particulate organic carbon (POC) and particulate inorganic carbon (PIC) as well as the total cell abundance and the nutrient uptake ratio were determined. At mid-term and at the end of the experiments samples for total particulate carbon (TPC) and particulate organic carbon were taken (Whatman GF/F filters 25 mm Ø). For the latter, the particulate inorganic carbon was removed by exposing the filters to fuming hydrochloric acid for 2 hours. Before measurement all filters were dried at 60°C. Afterwards they were folded and packed in tin cups and analysed in an elemental analyser with a heat conductivity detector (Thermo Flash 2000) according to Sharp (1974). The particulate inorganic carbon content was calculated by the subtraction of POC from TPC. To obtain the per capita particulate carbon content, the community / population production per litre was divided by the cell abundance.

Cell abundance was measured every day with a Z2™ COULTER COUNTER® cell and particle counter. The decision to terminate a culture was based on the statistically significant fit to the growth model. According to

$$n_t = a / (1 + ((a - b) / b) \cdot e^{-\mu t})$$

with n_t being the cell number after t days, a being the maximum cell abundance (carrying capacity (CC)), b the start cell number and μ the growth rate, the CC was calculated. The first day the growth curve of a culture could be significantly fit to the model, i.e. reached the stationary, phase was defined as the first of three days in the stationary phase.

Samples for PO_4^{3-} and NO_3^{2-} were taken from each bottle and filtered through GF/F filters. The filtrate was frozen in polyethylene bottles. Duplicate samples from each bottle were analysed colometrically with an accuracy of $\pm 0.1 \mu\text{mol}$ (Hansen and Koroleff 1999). The nutrient consumption was calculated as the subtraction of the remaining nutrient concentrations from the start concentration.

Likewise, the alteration of the pCO_2 was calculated by the subtraction of start and end value.

The analysis of the community composition in the polycultures was conducted by scanning electron microscopy (SEM) at the end of the experiment. For each treatment 830 ± 140 cells were counted on average.

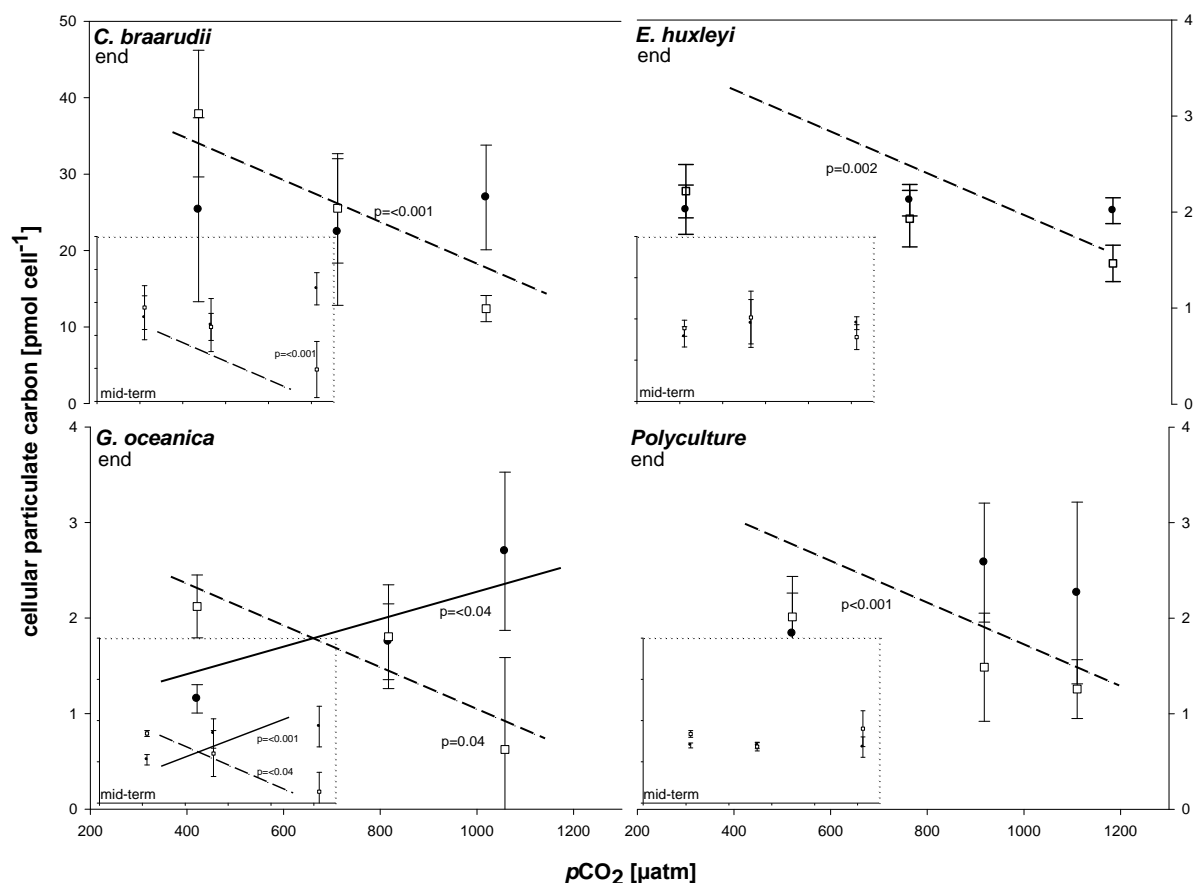


Figure 3.1 Particulate organic (closed signs) as well as inorganic carbon (open signs) per cell of the three monocultures and the polyculture with respect to pCO₂. The big figures present the end of the experiment, the small figures show responses at mid-term. The scaling of the small figures is equal to that of the corresponding bigger figure. Lines indicate the sign and that POC (full line) or PIC (broken line) significantly increased or decreased with pCO₂.

Statistical analyses

Prior to the statistical analyses data were tested for homogeneity of variances. If variances were not homogeneous data were log- or square root transformed. Effects of pCO₂ and culture identity and their interaction on the organic and inorganic carbon content (POC, PIC) per cell as well as per litre, on the cell abundance and the nutrient consumption ratio were tested with a General Linear Model (GLM) after terminating the cultures after the first phase and at the end of the experiment. Culture identity was used as a categorical and pCO₂ as a continuous factor. According to significant interaction terms between the factors culture identity and pCO₂, effects of pCO₂ in each level of culture identity were tested separately. Differences in the variation of pCO₂ and PIC and POC per cell and per L among cultures were tested within each level of pCO₂ by using a one-factorial ANOVA and Post-Hoc Test (Tukey's HSD-test) with culture identity as factor.

Please note, that due to problems during the sampling procedure we omitted one replicate of *C. braarudii* 420 µatm and 1220 µatm, one of *G. oceanica* 420 µatm and one of the polyculture 1200 µatm pCO₂. All statistical tables can be found in the appendix of this chapter.

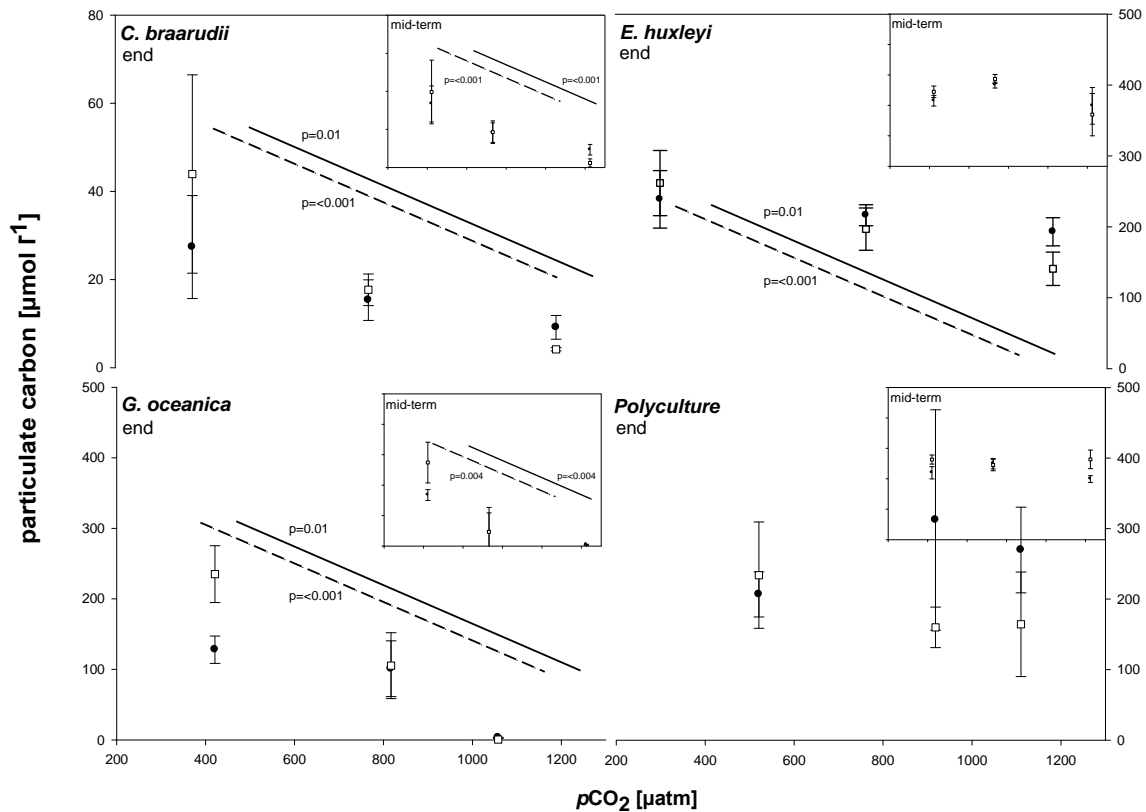


Figure 3.2 Particulate organic (closed signs) as well as inorganic carbon (open signs) per litre of the three monocultures and the polyculture with respect to rising pCO_2 . The big figures present the end of the experiment; the small figures show responses at mid-term. The scaling of the small figures is equal to that of the corresponding bigger figure. Lines indicate the sign and that POC (full line) or PIC (broken line) significantly increased or decreased with pCO_2 .

Results

Carbon assimilation

Both factors pCO_2 and culture identity significantly affected particulate inorganic and organic carbon per capita and per litre. For all carbon measures, at least 86% of the variances could be explained by the manipulated factors (Table 3.4). Per capita PIC generally decreased (Table 3.4). After the first phase this was significant only for *C. braarudii* and *G. oceanica* (Figure 3.1, Table 3.2). At the end of the experiment PIC per cell significantly decreased in all three monocultures as well as in the polycultures (Figure 3.1, Table 3.5). The pCO_2 also positively affected per capita POC across all cultures (Table 3.4). However, this effect was driven by the strong positive effect on POC per cell of *G. oceanica* (Figure 3.1, Table 3.5). Per capita results are indicating that pCO_2 showed different effects in the different cultures for PIC as well as POC after mid-term and at the end of the experiment. In contrast to the per cell level, the overall production of both organic and inorganic particulate carbon per litre dropped for *C. braarudii* and *G. oceanica* at mid-term and for all monocultures at the end of the second phase with rising CO_2 concentrations (Figure 3.2, Table 3.5). The particulate carbon in the polyculture, however, was not affected by ocean acidification at any phase (Figure 3.2, Table 3.5).

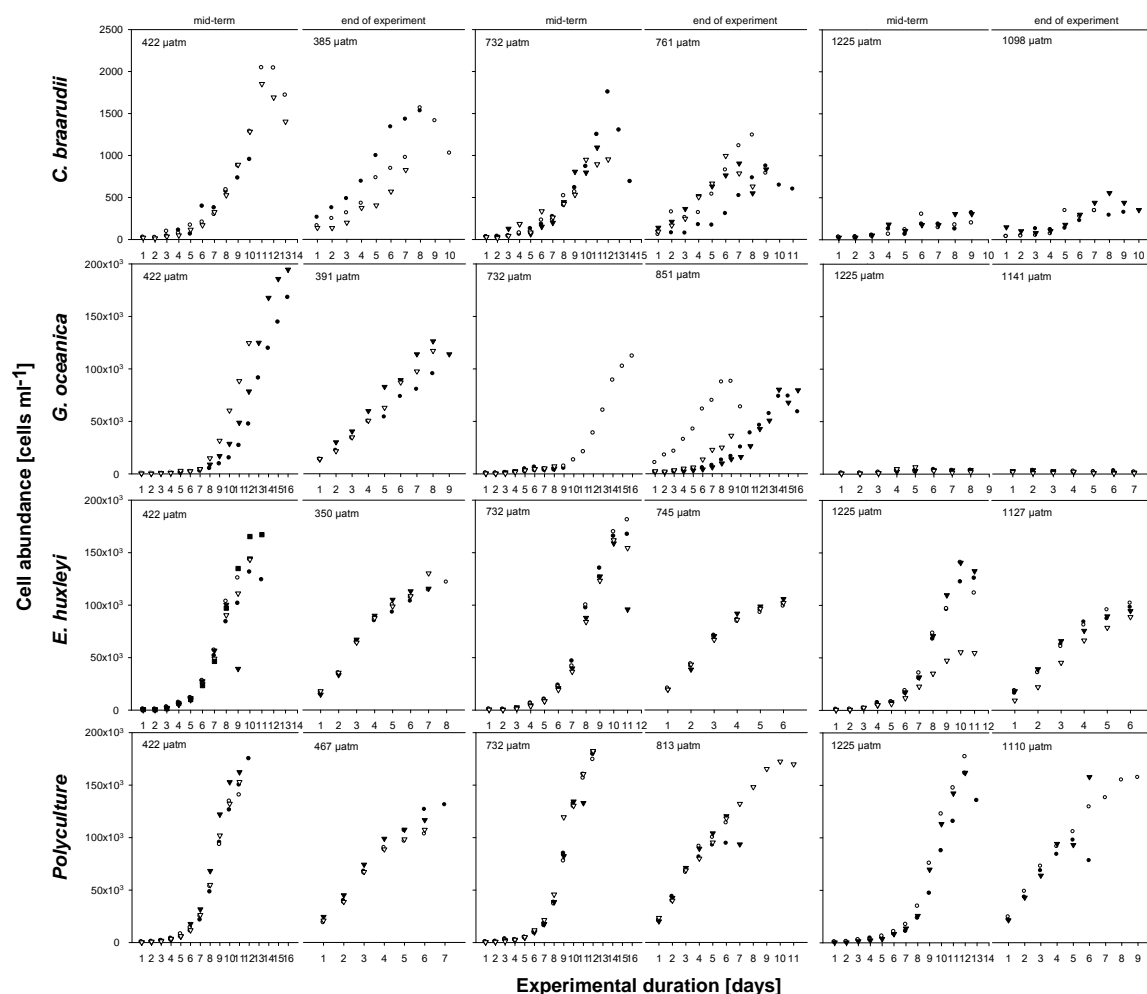


Figure 3.3 Cell abundances of the three monocultures and the polyculture over the time each presented by four replicates. To the left are the lowest pCO₂ values, to the right the highest values. The figures for the two different phases alternate.

Cell abundance

The maximum cell abundance significantly differed among culture identities and overall decreased with increasing pCO₂ level (Table 3.4). While the comparatively large cells of *C. braarudii* reached only 1700 cells ml⁻¹ at the end of the first phase, the much smaller cells of *E. huxleyi*, *G. oceanica* and the polyculture reached 140,000 cells ml⁻¹ on average (Figure 3.3). At both phases of the experiment cell abundance in monocultures of *C. braarudii* and *G. oceanica* significantly decreased with respect to increasing pCO₂ (Figure 3.3). *E. huxleyi*, however, showed no significant drop in cell abundance at mid-term but at the end of the second phase (Figure 3.3, Table 3.5). As for community level measures of PIC and POC there was no significant effect of pCO₂ on the cell abundances in the polycultures (Figure 3.3, Table 3.5). For all cultures a more or less pronounced reduction in cell abundance was found from mid-term to the end of the experiment (Figure 3.3). This drop can partly be explained by a small reduction in the nutrient concentration at the second phase of the experiment. Due to the transfer of 100 ml phosphate depleted media with cells, the media in the new cell flasks faced a phosphate reduction by 7%.

Table 3.1 Mean species composition of the polyculture (%) at the end of the experiment.

	pCO ₂ [µatm]	<i>E. huxleyi</i> % ±StDv	<i>C. braarudii</i> % ±StDv	<i>G. oceanica</i> % ±StDv
Poly.	521±140	98.8±1.5	0.0±0.0	1.2±1.5
	918±161	98.7±2.2	0.1±0.2	1.2±2.0
	1286±172	99.5±0.6	0.0±0.1	0.5±0.6

Table 3.2 Mean Nitrate and Phosphate consumptions. The uptake ratio is given as the dividend of N:P. Start concentration was 6.8 µmol l⁻¹ for Nitrate and 0.4 µmol l⁻¹ for Phosphate. All values are given in µmol l⁻¹ ± standard deviation.

	Mid-term				End of Experiment			
	Begin pCO ₂	NO ₃ ²⁻ uptake	PO ₄ ³⁻ uptake	Uptake ratio	Begin pCO ₂	NO ₃ ²⁻ uptake	PO ₄ ³⁻ uptake	Uptake ratio
<i>C. bra.</i>	421±11	3.2±1.5	0.4	7.7±3.6	426±101	3.49±1.0	0.4	9.1±2.6
	732±24	2.5±1.0	0.4	6.2±2.4	714±137	3.10±0.4	0.4	8.0±1.1
	1226±12	1.9±1.2	0.4	4.7±2.9	1021±46	2.07±0.1	0.4	5.4±0.4
<i>E. hux.</i>	422±9	6.7±0.1	0.4	16.3±0.1	482±103	6.12±0.1	0.4	16.2±0.1
	732±24	6.6±0.0	0.4	16.2±0.1	859±148	6.18±0.1	0.4	16.3±0.1
	1225±10	6.5±0.2	0.4	15.8±0.6	1201±88	6.16±0.0	0.4	16.2±0.0
<i>G. oc.</i>	424±9	6.8±0.0	0.4	16.5±0.1	421±64	6.19±0.0	0.4	16.4±0.1
	732±24	2.9±2.6	0.4	6.9±6.4	817±45	6.23±0.5	0.4	15.7±1.3
	1225±10	0.4±0.3	0.4	1.1±0.7	1058±33	0.70±0.3	0.4	1.8±0.8
Poly.	422±9	6.6±0.0	0.4	16.2±0.1	521±140	6.18±0.0	0.4	16.3±0.1
	732±24	6.7±0.1	0.4	16.3±0.1	918±161	6.17±0.1	0.4	16.3±0.2
	1225±10	6.7±0.1	0.4	16.3±0.2	1110±79	6.20±0.1	0.4	16.3±0.1

Unfortunately, no satisfying explanation can be given for the cell abundance differences of the four replicates of *G. oceanica*, 730 µatm pCO₂ at mid-term. At this treatment three of four replicates were only able to grow around 5700 cells ml⁻¹ with the given nutrients. One replicate, however, was growing up to 110000 cells ml⁻¹ (Figure 3.3).

The species composition of the polycultures at the end did not differ between pCO₂ level. All replicates of each pCO₂ level consisted of at least 95% *E. huxleyi*. The two other species played only a marginal role in the community structure at the end of the experiment (Table 3.1).

Nutrient consumption

The accuracy of both phosphate and nitrate measurement was ±0.1µmol l⁻¹. With a start concentration of 0.4 µmol l⁻¹ phosphate and a consumption of 0.37 µmol l⁻¹ during both phases it can be assumed that all treatments were exhausted in inorganic phosphate by the end of each phase (Table 3.2). Hence, in the stationary phase all cultures were phosphate limited. The nitrate consumption, however, varied strongly between species and with increasing ocean acidification (Table 3.2, Table 3.4 and Table 3.5).

Table 3.3 Calculated pCO₂ values at beginning and end of each phase of the experiment and their differences. All values are given in $\mu\text{atm} \pm$ standard deviation.

	Start pCO ₂	Mid-term	Δ pCO ₂	End of Experiment		
		End pCO ₂		Start pCO ₂	End pCO ₂	Δ pCO ₂
<i>C. bra.</i>	421±11	408±13	-13±17	426±101	442±55	15±47
	732±24	716±43	-16±24	714±137	729±126	14±74
	1226±12	1171±31	-56±28	1021±46	951±20	-70±29
<i>E. hux.</i>	422±9	321±32	-101±33	482±103	314±59	-168±47
	732±24	538±48	-194±40	859±148	465±36	-395±157
	1225±10	798±48	-429±57	1201±88	577±28	-636±71
<i>G. oc.</i>	424±9	525±145	100±138	421±64	461±64	40±61
	732±24	701±30	-31±16	817±45	737±62	-80±32
	1225±10	1213±69	-12±72	1058±33	979±63	-14±75
Poly.	422±9	335±29	-87±23	521±140	450±198	-146±301
	732±24	493±77	-239±89	918±161	407±172	-552±168
	1225±10	--	--	1110±79	426±131	-663±256

E. huxleyi and the polycultures showed an N:P uptake ratio of 16:1 in every phase and at every level of ocean acidification. *G. oceanica* followed the Redfield ratio of 16:1 only at the lowest pCO₂ level. With rising acidification the ratio significantly drops rapidly to almost one (Table 3.2). At high CO₂ concentrations *G. oceanica* needed almost as much phosphate as it needed nitrogen. As the only species of the here tested, *C. braarudii* did not follow a Redfield uptake ratio at the lowest pCO₂ values. With a mean ratio of approximately 8 the consumption of nitrate was reduced compared to that of other tested species and the polyculture (Table 3.2, Table 3.5). Additionally, ocean acidification induced a further reduction of the N:P uptake ratio. Due to a high standard deviation in the first phase, however, the results for *C. braarudii* were only significant at the end of the experiment (Table 3.2, Table 3.5).

Carbonate Chemistry specification

With low standard deviations the desired pCO₂ gradient was applied to all cultures at the beginning of the first phase (Table 3.3). Since the manipulation occurred in bigger bottles and the water was carefully pumped in the culture flask all treatments had the same variability (Table 3.3). In the second phase, however, the standard deviations were much more pronounced. This is again partly due to the 100ml water from the first phase which was transferred into the new flask, but also because of the fact that the media for the second phase was not manipulated at once. This might have lead to the observed higher standard deviations. Therefore, the cultures of the second phase faced a slightly different pCO₂ gradient.

Although all treatments started with the same nutrient concentration and the carbonate chemistry was similar, the alteration of the carbonate chemistry within one manipulated level

significantly differed among cultures (Table 3.3, Table 3.6). The treatments at 420 μ atm pCO₂ at the end of the experiment were the only exception.

While the cultures of *C. braarudii* and *G. oceanica* were only able for a rather small population production (Figure 3.2) their alteration in carbon chemistry speciation are small compared to the high productive cultures of *E. huxleyi* and the polycultures (Figure 3.2, Table 3.3). Particularly the polycultures but also *E. huxleyi* showed an increase in the amplitude of carbon chemistry alteration with respect to the increasing start pCO₂ (Figure 3.2, Table 3.3).

Discussion

The overall assimilation of particulate carbon per litre of a population and/or community depends on both the carbon content per cell (i.e. the physiological scale) and the actual cell abundance (i.e. the population or community scale). To the best of our knowledge, this study is among the first culture experiments which consider both scales while testing for effects of ocean acidification. Moreover, focused responses were not limited on single species strains but also on a total community response in a polyculture in which community interactions potentially alter the expected responses due to indirect effects of changing species interactions. By applying these new perspectives this study has three major outcomes. First, measures of per capita PIC and POC with respect to increasing pCO₂ differ from responses on the population scale. Second, the total community response in PIC and POC of the polyculture differs from what could be expected from the monocultures. And finally third, the capability to manipulate their own environment by changing carbonate chemistry differs between species and polyculture.

First, whereas on the population scale in the monocultures both PIC and POC content were negatively affected by increasing pCO₂, the response of per capita POC content was insignificant or even changed sign (*G. oceanica*) towards a positive response. Concerning the per capita result of this experiment, it confirms results of previous studies analysing the effects of ocean acidification on the carbon per cell content. These studies showed strong decreases in PIC and increases in POC of *G. oceanica* (Riebesell et al. 2000), less but significant effects on the production of *E. huxleyi* (Riebesell et al. 2000; Zondervan et al. 2001) and only little implications for the biggest coccolithophore, *C. braarudii* (Krug et al. 2011). Since crucial differences in between the experimental set-ups exist, one must be careful to compare this study with previous experiments. Differences are on the one hand the possibility to alter the equilibrium of the carbon chemistry specifications by photosynthesis and calcification. On the other hand the experiment was not stopped in the exponential phase but after three days in the stationary phase. According to their limitation it can be expected that cells develop differently. Müller et al. (2007) presented results on *E. huxleyi* cells facing both nitrogen and phosphate limitations. As a

result cells got significant smaller under nitrogen limitation and bigger with increased PIC production rates under phosphate limitation which affects PIC and POC content and ratio.

Tying together population/community- and per capita-level the differences become evident. Reflecting the effects of ocean acidification on *G. oceanica* in the stationary phase it shows, that even an increase in the organic carbon per cell cannot compensate the strong decline in cell abundance and therewith the decrease in the community production of particulate carbons. Only the PIC content showed for the monocultures the same tendency on a per capita and on the population base. In all cases the calcification decreased with ocean acidification. The strong decline in the population productivity of all monocultures caused by a decrease in cell abundance might be linked to a change in the nutrient utilisation with ocean acidification (chapter II). At higher pCO₂ levels and with the same amount of nutrients especially *C. braarudii* and *G. oceanica*, in the second phase also *E. huxleyi* were not able to reach cell abundances of lower pCO₂ values. The reason for this is not yet clear and highly speculative. An enhanced growth of the associated bacteria community might have the ability to reduce the N:P ratio a bit (Vrede et al. 2002) but is most certainly not able to reduce the ratio as it was observed in this experiment. One possibility might be an increase of the surface-absorbed phosphate. For several classes of phytoplankton it is known that the fraction of surface-absorbed phosphate can be bigger than 50% of the total as particulate organic measured phosphorus (Sanudo-Wilhelmy et al. 2004). The observed phosphate loss might be partly due to a rise in the fraction of surface-absorbed P with rising CO₂ concentrations and needs therefore further investigations.

Just recently found indications led to the assumption that within a F/2 medium by Guillard (1975) with 4 µmol kg⁻¹ phosphate over a time of ten days approximately 10% of the initial phosphate concentration precipitate inorganically (Febiri et al. in prep.). This finding would have severe implications for all phosphate limitation experiments in the past and in the future. Since the variance of the experimental durations of the different treatments was less than ten days, a marginal phosphate precipitation would not cause the observed differences in the total population carbon accumulation.

The small but significant reduction in particulate carbon per L of *E. huxleyi* at the end of the experiment is not reflected in the nutrient measurements. This might be due to the insufficient sensitivity of the analytic method. Hence, it can only be speculated if a decrease in the nutrient uptake ratio might be the cause for this effect on *E. huxleyi* monocultures, as well.

Only the polyculture does not show any decrease in the community production of particulate carbons, neither the organic nor the inorganic. The decreasing calcification on a per capita base at the end of the experiment was even compensated by the cell abundance, i.e. on the community base. Even so, the polyculture was represented by more than 95% of *E. huxleyi*, the response to ocean acidification differed to what could have been expected from the monocultures.

Remaining a certain time in the stationary phase enables the cells additionally to interact both on an intraspecific level in the monocultures and on an interspecific level in the polycultures. The reason why the community production of the polyculture does not react with respect to ocean acidification is highly speculative. Particularly, the insignificant abundance of *G. oceanica* and *C. braarudii* in the polycultures at the end of the experiment makes the difference to the *E. huxleyi* monocultures difficult to interpret.

By accumulate the maximum possible biomass the coccolithophores exert influence on the carbon chemistry. The natural alteration in the carbon chemistry speciation is attended by the consumption of DIC by photosynthesis and calcification and the reduction of alkalinity only by calcification. The gas exchange with the atmosphere, however, cannot compensate for these variations in DIC during a bloom and of course it cannot for TA at all (Riebesell et al. 2007). With those changes in the carbon chemistry an alteration in pCO₂ during a phytoplankton bloom is unavoidable. Although both TA and DIC decrease during a bloom, the pCO₂ can increase or decrease, according to the ratio of photosynthesis and calcification. The fact that the cultures in this study were able to change their carbon chemistry environment brings this experiment closer to in situ conditions as it would be with a constant carbon chemistry system.

The species, however, used their possibility to build up biomass with a defined amount of nutrients with varying efficiencies. Due to a significantly lower N:P uptake ratio the biggest species, *C. braarudii*, was only able to build up approximately 20% of the *E. huxleyi* biomass at present pCO₂ conditions. Hence, a rather small community production of *C. braarudii* as well as *G. oceanica* under high pCO₂ conditions led to marginal alterations in the carbon chemistry speciation compared to the highly productive cultures of *E. huxleyi* and the polyculture.

Carbon chemistry variations increase with respect to rising pCO₂ start conditions for polycultures and *E. huxleyi* monocultures. The reason for this trend is twofold. On the one hand the drop in pCO₂ with increasing ocean acidification is related to a decrease in the buffer capacity of sea water under these conditions (Zeebe and Wolf-Gladrow 2001). Alterations in DIC and Alkalinity, as they occur during coccolithophore blooms would therefore cause bigger fluctuations in pCO₂ in a high CO₂ world as they would at a present CO₂ concentration. On the other hand the rather equal community production of PIC and POC of the polyculture and *E. huxleyi* (only at mid-term) with respect to increasing start pCO₂ values did not counteract the strong pCO₂ variations. On first sight the constant community production of *E. huxleyi* and the polyculture seems to be obvious. 50% of the biomass was built with the last division of the cells under conditions which did not face any or at least only marginal difference in the carbon chemistry. Also the start pCO₂ was very different; the fast acclimation to the new adjusted carbon chemistry (Ramos et al. 2010) would therefore cause similar production rates towards the end of the experiment.

But to reach a state of comparable environments in the end no strong reactions to the starting conditions should occur.

In the case of *C. braarudii* and *G. oceanica* the effect of stronger pCO₂ variations with increasing acidification were compensated by a strong decrease in the PIC and POC population production. Hence, the reduced DIC consumption and the small variations in total alkalinity caused by a strong decrease in calcification were not able to lower high pCO₂ values to moderate levels.

Even so the observed variations in the carbonate system during this experiment are partly more distinct as they would occur in natural environments, this study gives new insights of three coccolithophore species dealing with limiting nutrients and ocean acidification. Only growth to the stationary phase made the difference of the carbon content on a per capita base compared to the overall population production per litre visible. Additionally the, for reason as yet unexplained, unaffected polyculture emphasizes the need for an ecosystem approach when investigating the potential effects of global anthropogenic changes on marine ecosystems.

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Appendix

Statistical Tables

Table 3.4 Outcome of General linear models (GLM) testing the effects of pCO₂ as continuous and culture identity as categorical factors and their interaction (CultID x pCO₂) on the cell abundance, the nutrient uptake ratio, the particulate inorganic and organic carbon content per cell as well as per litre after the first phase (mid-term) and at the end of the experiment.

	Factor	df	Mid-term					End of experiment				
			R ²	Slope	MS	F	p	R ²	Slope	MS	F	p
POC cell ⁻¹	Whole model	7.36	0.97			201	<0.001	0.93			80.4	<0.001
	CultID	3.36			0.50	59.7	<0.001			0.45	27.3	<0.001
	pCO ₂	1.36		0.0001	0.07	9.0	<0.005		0.0001	0.07	4.2	<0.05
	CultIDx pCO ₂	3.36			0.01	1.7	>0.1			0.02	1.5	>0.2
PIC cell ⁻¹	Whole model	7.36	0.92			65.8	<0.001	0.94			94.8	<0.001
	CultID	3.36			0.50	23.2	<0.001			0.61	38.1	<0.001
	pCO ₂	1.36		-0.0003	0.37	15.8	<0.001		-0.001	0.63	39.4	<0.001
	CultIDx pCO ₂	3.36			0.13	5.7	<0.004			0.03	2.0	>0.1
POC l ⁻¹	Whole model	7.36	0.89			48.7	<0.001	0.88			45.3	<0.001
	CultID	3.36			29.4	8.3	<0.001			34.4	8.7	<0.001
	pCO ₂	1.36		-0.004	80.0	22.6	<0.001		-0.004	50.6	12.9	<0.001
	CultIDx pCO ₂	3.36			31.8	9.0	<0.001			43.5	11.1	<0.001
PIC l ⁻¹	Whole model	7.36	0.86			36.6	<0.001	0.88			47.5	<0.001
	CultID	3.36			41.8	6.7	0.001			55.9	16.0	0.001
	pCO ₂	1.36		-0.007	211.3	33.9	<0.001		-0.009	298.7	85.7	<0.001
	CultIDx pCO ₂	3.36			65.1	10.5	<0.001			54.2	15.5	<0.001
Cell abundance	Whole model	7.36	0.83			31.5	<0.001	0.95			127.8	<0.001
	CultID	3.36			10 ¹⁰	11.9	<0.001			1.9	41.2	<0.001
	pCO ₂	1.36		-55	10 ¹⁰	14.5	<0.001		-39.4	2.8	59.8	<0.001
	CultIDx pCO ₂	3.36			8·10 ⁹	8.0	<0.001			1.5	32.6	<0.001
NO ₃ ⁻ /PO ₄ ³⁻ uptake	Whole model	7.36	0.84			33.7	<0.001	0.88			47.2	<0.001
	CultID	3.36			43.6	7.6	<0.001			61.3	19.5	<0.001
	pCO ₂	1.36		-0.006	139.2	24.4	<0.001		-0.006	159.4	50.8	<0.001
	CultIDx pCO ₂	3.36			86.5	15.1	<0.001			97.8	31.1	<0.001

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Table 3.5 Results of regression analyses with pCO₂ as predictor on the cell abundance, the NO₃⁻/PO₄³⁻ uptake ratio and the particulate inorganic and organic carbon content per cell as well as per litre of species in mono- and of the polycultures as response variables.

	Factor	Mid-term					End of Experiment				
		R ²	F _{1,10}	p	Slope	SE	R ²	F _{1,10}	p	Slope	SE
POC cell ⁻¹	<i>C. bra.</i>	0.18	2.91	>0.1	0.01	7·10 ⁻³	-0.1	0.03	>0.8	-2·10 ⁻³	0.01
	<i>E. hux.</i>	0.03	1.36	>0.2	4·10 ⁻⁴	3·10 ⁻⁴	-0.1	0.001	>0.9	10 ⁻⁶	4·10 ⁻⁵
	<i>G. oc.</i>	0.33	6.01	<0.04	10⁻³	4·10⁻⁴	0.29	5.58	<0.04	0.002	7·10⁻⁴
	Poly.	-0.1	0.19	>0.6	-5·10 ⁻⁵	10 ⁻⁴	-0.01	0.96	>0.3	7·10 ⁻⁴	7·10 ⁻⁴
PIC cell ⁻¹	<i>C. bra.</i>	0.62	15.45	<0.001	-0.02	6·10⁻³	0.76	25.85	<0.001	-0.03	6·10⁻³
	<i>E. hux.</i>	-0.02	0.76	>0.4	-4·10 ⁻⁴	4·10 ⁻⁴	0.62	18.58	0.002	-2·10⁻⁴	-6·10⁻⁵
	<i>G. oc.</i>	0.66	20.41	<0.001	-2·10⁻³	4·10⁻⁴	0.36	6.1	0.04	-4·10⁻⁴	2·10⁻⁴
	Poly.	-0.11	0.08	>0.7	2·10 ⁻⁵	8·10 ⁻⁵	0.31	5.47	<0.05	-10⁻³	4·10⁻⁴
POC l ⁻¹	<i>C. bra.</i>	0.70	56.5	<0.001	-0.03	7·10⁻³	0.53	11.12	0.01	-0.02	7·10⁻³
	<i>E. hux.</i>	-0.03	0.63	>0.4	-0.03	0.04	0.32	6.17	0.03	-0.06	0.02
	<i>G. oc.</i>	0.59	15.24	<0.004	-0.18	0.05	0.76	36.66	<0.001	-0.17	0.03
	Poly.	-0.05	0.57	>0.4	-0.02	0.03	-0.01	0.90	>0.8	0.1	0.1
PIC l ⁻¹	<i>C. bra.</i>	0.87	45.8	<0.001	-10⁻³	2·10⁻⁴	0.88	66.53	<0.001	-10⁻³	-2·10⁻⁴
	<i>E. hux.</i>	0.23	4.23	>0.06	-3·10 ⁻⁴	10 ⁻⁴	0.71	27.54	<0.001	-0.16	0.03
	<i>G. oc.</i>	0.58	14.69	0.004	-0.30	0.08	0.93	137.65	<0.001	-0.33	0.03
	Poly.	-0.12	0.004	>0.9	-0.001	0.02	0.14	2.64	>0.1	-0.1	0.06
Cell abundance	<i>C. bra.</i>	0.85	50.8	<0.001	-9·10⁻⁴	10⁻⁴	0.72	24.7	0.001	-1.1	0.21
	<i>E. hux.</i>	0.14	2.74	>0.1	-45	27	0.61	18.3	<0.002	-30	7
	<i>G. oc.</i>	0.57	14.5	0.004	-176	46.1	0.95	206	<0.001	-148	10
	Poly.	-0.1	3·10 ⁻⁴	>0.9	-0.3	16.6	-0.1	0.44	>0.5	21	32
NO ₃ ⁻ /PO ₄ ³⁻ uptake	<i>C. bra.</i>	0.07	1.7	0.23	-0.003	0.002	0.49	9.7	0.01	-3·10⁻⁴	9·10⁻⁵
	<i>E. hux.</i>	0.27	3.4	0.1	-6·10 ⁻⁴	3·10 ⁻⁴	-0.1	0.03	>0.8	2·10 ⁻⁴	9·10 ⁻⁵
	<i>G. oc.</i>	0.68	22.0	0.001	-0.02	0.004	0.71	25.2	<0.001	-0.02	0.004
	Poly.	0.02	1.2	>0.3	2·10 ⁻⁴	10 ⁻⁴	-0.01	0.95	0.3	1·10 ⁻⁴	1·10 ⁻⁴

Table 3.6 Results of a one-factorial ANOVA for the differences in pCO₂ and PIC and POC per cell and per L among cultures within each level of pCO₂

Factor	Start pCO ₂	Mid-term				End of experiment			
		R ²	MS	F _{1,10}	p	R ²	MS	F _{1,10}	p
Δ pCO ₂	420	0.73	9873.8	11.8	0.002	0.06	31411	1.2	0.35
	730	0.79	51160	19.7	<0.001	0.78	280928	18.9	<0.001
	1220	0.88	241071	27.8	<0.001	0.88	295416	25.0	<0.001
POC cell ⁻¹	420	0.98	1.28	265.3	<0.001	0.95	1.06	85.3	<0.001
	730	0.96	1.3	122.3	<0.001	0.93	1.05	63.3	<0.001
	1220	0.98	1.29	196.7	<0.001	0.87	0.79	28.9	<0.001
PIC cell ⁻¹	420	0.99	1.16	368.1	<0.001	0.98	1.21	241.8	<0.001
	730	0.94	1.46	81.7	<0.001	0.94	1.35	81.7	<0.001
	1220	0.78	0.9	11.8	0.006	0.90	0.96	44.2	<0.001
POC l ⁻¹	420	0.97	59.2	125.2	<0.001	0.92	69.9	52.5	<0.001
	730	0.87	169.7	34.3	<0.001	0.86	136.4	30.8	<0.001
	1220	0.95	140.3	73.7	<0.001	0.94	186.3	76.9	<0.001
PIC l ⁻¹	420	0.93	77.3	54.7	<0.001	0.83	66.9	21.9	<0.001
	730	0.83	181.4	25.1	<0.001	0.93	78.0	70.3	<0.001
	1220	0.93	188.6	50.4	<0.001	0.87	133.2	32.4	<0.001

FUTURE PERSPECTIVES

In summary, the results presented in this thesis imply a more complex and intriguing picture of coccolithophores and their response to ocean acidification. While the study in chapter I offers a hypothesis explaining the different sensitivities of coccolithophores, the studies presented in chapters II and III allow for new angles of view on coccolithophore-acidification-research. By extending the experiments to the stationary phase with variable carbonate chemistry and the interaction of several species, the latter two studies point out that research on physiological response on OA is important to understand underlying mechanisms but are alone not sufficient for the extrapolation to population and community levels. Nevertheless, the simulation of population or even community carbon accumulation in batch culture experiments is only the beginning of a synergistic research effort linking biogeochemistry and experimental ecology. Given the predicted future expansion of phosphate limitation (Ammerman et al. 2003) and reduced nutrient availability by increasing stratification (Yeh et al. 2009), it would be useful to examine the combined influence of nutrient ratios and ocean acidification (chapter II) on more complex communities. The performance of a two factorial mesocosm study, with pCO₂ and nutrient ratio as factors would give new insights on ecosystem functioning.

Furthermore, three ideas might be useful for future research.

An “Eppley Curve” for Calcification?

Chapter I complements the current knowledge on single species response to rising pCO₂. The earlier presented insensitivity of the coccolithophore *C. braarudii* could have been ruled out when applying wider ranges of pCO₂. All species analysed so far responded to OA either by in- or decreasing PIC and / or POC production, however the effect size was variable. The partly oppositional response in PIC and / or POC production of different coccolithophore species was discussed as to be the effect of different optimum concentrations of CO₂, HCO₃⁻ and H⁺.

Considering optimum responses for all production rates of coccolithophores, Ridgwell et al (2009) suggested that the “Eppley Curve” concept could be adapted to OA. This concept by Eppley (1972; re-evaluated by Bissinger et al. 2008) delineated the different growth-temperature-optima of a community with a sufficient diversity in one simple function of temperature and growth. Many global carbon cycle and phytoplankton models include the “Eppley Curve” to predict growth rates (Moisan et al. 2002; Schmittner et al. 2008). Such a function for the optimum responses of coccolithophore calcification rates to OA could help to predict global effects on the CO₂ – calcification feedback. For three reasons the development of such an equation, however, might be difficult.

1. The dependency of calcification on temperature (Feng et al. 2008; 2009) requires a combined OA - temperature - calcification function.
2. Evaluating the calcification in the stationary phase suggests an additional dependency between effects of OA and the applied nutrient ratio (chapter II).
3. The right choice of underlying data sets indicating calcification sensitivity to OA should be applied to the adapted “Eppley curve”. Most studies, including the experiment described in chapter I, deliver data of variations in cell physiology with respect to OA, collected in the exponential growth phase. Current models of the calcification feedback on atmospheric pCO₂ rely on these results (Heinze 2004; Gehlen et al. 2007; Ridgwell et al. 2007). Undergoing cell alterations in the stationary phase (like increasing POC and PIC), the significant interaction with the supplied nutrient ratio and the associated variations in the response to OA, compared to the exponential phase (chapter II) have the potential to question the relevance of hitherto selected data for carbon cycle models.

Implications for organic carbon export

Aside from the direct feedback on atmospheric pCO₂ by calcification and photosynthesis, calcified cells might play an important function as mineral ballast in the transport of organic matter below the mixed layer (Klaas and Archer 2002). A recent study presented reduced sinking velocities due to decreasing PIC / POC ratios under future conditions (Biermann and Engel 2010). But again these variations were observed under exponential cell growth with replete nutrients. Coccolithophores prefer phosphate limited conditions (Egge and Heimdal 1994; Tyrrell and Taylor 1996), resulting in an increase in cell volume, and PIC and POC accumulation in the stationary phase (Riegman et al. 2000; Müller et al. 2008). The resultant increase in PIC/POC ratio, i.e. the increasing particle density and the increasing particle diameter of single coccolithophores, both lead to an accelerated sinking velocity.

Observations presented in chapter II indicate attenuated cell size growth with increasing OA and, similar to results observed in the exponential phase, decreasing PIC/POC ratio. Thus, the sinking velocity of single cells is negatively affected by OA.

It is likely, however, that the size of single coccolithophore cells does not significantly affect the sinking velocity of bigger particles and export mechanisms. The production of large fecal pellets by herbivore organisms and the conglutination of detritus and living cells produce significantly larger particles with higher velocities than single cells (Silver et al. 1978; Honjo et al. 1982). Size, density and abundance of these particles mainly define the carbon export (Clegg and Whitfield 1990). Thus, the calcareous fractions of coccolithophores, not the cell size would affect the sinking velocity by changing particle density of larger particles (Klaas and Archer 2002; Francois

et al. 2002; Armstrong et al. 2002). If the PIC/POC ratio of single cells changes, but phosphate limitation (chapter II) and / or the interaction with other species (chapter III) leads to an increase in total cell abundance, i.e. total biomass accumulation, an OA induced decrease in total calcite production might be attenuated. Consequently, the sinking velocity of larger particles should be less affected by changing calcification rates of coccolithophores. For this reason it might be necessary to re-evaluate the observed decline in the sinking velocity of particles produced by *E. huxleyi* in the exponential phase under replete nutrient condition (Biermann and Engel 2010).

Winter and summer stress

Although phytoplankton exists throughout the year, OA research has so far only accounted for spring bloom situations. The effects of rising pCO₂ on single species under optimum conditions of light, temperature and usually nutrients, allow only for limited conclusions on the ecosystem functioning. An important issue is the hibernation of phytoplankton under winter stress, meaning low light, higher stratification and lower temperature. Only those species with sufficient surviving cell abundance will be able to bloom upon the arrival of spring. Variations in temperature, stratification and the linked variability in nutrient availability are known to influence survivability of species, resulting in different spring communities (Goffart et al. 2002). Additional stress by future ocean acidification might affect winter diversity with severe consequences for the development of spring blooms. Furthermore, species may respond differently. If coccolithophores are not able to withstand winter acidification to the same extent as diatoms, bloom successions might develop differently. An experimental design to test the implications of winter and OA stress situations on the development of a bloom would lead to further elucidation of the wide field of OA.

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EIGENANTEIL

Die Kapitel dieser Dissertation sind bereits in wissenschaftlichen Fachzeitschriften erschienen (Kapitel I) oder in Vorbereitung eingereicht zu werden (Kapitel II u. III).

Da die Publikationen von mehreren Autoren veröffentlicht werden, soll hier der Eigenanteil des Kandidaten Krug an seiner Dissertation verdeutlicht werden:

Kapitel I:

Effects of changes in carbonate chemistry specifications on *Coccolithus braarudii*: a discussion on coccolithophore sensitivities

Autoren: Sebastian A. Krug (SK), Kai G. Schulz (KS) und Ulf Riebesell (UR)

Veröffentlich: *Biogeosciences*, 8, 771–777, 2011; doi:10.5194/bg-8-771-2011

Anteile: SK und KS entwickelten die Idee für die Studie, SK führte das Experiment durch, SK analysierte die Daten; SK, KS und UR diskutierten die Ergebnisse, SK und KS verfassten das Manuskript.

Kapitel II:

High nitrate to phosphorus ratio attenuates negative effects of rising pCO₂ on net population carbon accumulation

Autoren: Sebastian A. Krug (SK), Sarah Lena Eggers (LE) und Birte Matthiessen (BM)

In Vorbereitung eingereicht zu werden

Anteile: SK und BM entwickelten die Idee für die Studie, SK und LE führten das Experiment durch, SK analysierte die Daten; SK, LE und BM diskutierten die Ergebnisse, SK verfasste das Manuskript.

Kapitel III:

Polyculture positively affects ocean acidification resistance compared to average monoculture

Autoren: Sebastian A. Krug (SK) und Birte Matthiessen (BM)

In Vorbereitung eingereicht zu werden

Anteile: SK und BM entwickelten die Idee für die Studie, SK und BM führten das Experiment durch, SK analysierte die Daten; SK und BM diskutierten die Ergebnisse, SK verfasste das Manuskript.

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Work experience:

July 2006 – April 2011	Leibniz Institute of Marine Sciences Graduation at “marine Biogeochemistry”
July 2009 – July 2010	Leibniz Institute of Marine Sciences Planning and conducting the logistics of an arctic expedition (40 participants from 9 nations)
December 2008 – June 2009	Leibniz Institute of Marine Sciences Planning and Conduction the logistics of a mesocosm field experiment in the Bay of Kiel (40 participants from 3 German research institutes)
October 2000 – April 2006	Leibniz Institute of Marine Sciences, various employments as student assistant in physical Oceanography, fishery Biology, marine Ecology, marine Biogeochemistry

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October 2004 – March 2005	University of Aberdeen (Scotland) Chemistry Department (Prof. Jörg Feldmann) Project thesis: Arsenic compounds in the blood of the Galapagos Iguanas
October 2000 – April 2006	Christian Albrechts University to Kiel, Diploma thesis: Impact of ocean acidification on the coccolithophore <i>Coccolithus pelagicus</i> Degree: Diploma in Biology
August 1999 – April 2000	Military Service (Medical Corps Soldier – Hauptgefreiter)
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April 2006	ZTS Student award for outstanding performance and public engagement

ERKLÄRUNG (STATEMENT)

Hiermit erkläre ich an Eides statt, dass die vorliegende Dissertation, abgesehen von der Beratung durch meinen akademischen Lehrer, nach Inhalt und Form meine eigene Arbeit ist und ich keine anderen als die angegebenen Quellen und Hilfsmittel verwendet habe. Des Weiteren versichere ich, dass die vorliegende Dissertation weder im Ganzen noch zum Teil einer anderen Stelle im Rahmen eines Prüfungsverfahrens vorgelegen hat und unter Einhaltung der Regeln guter wissenschaftlicher Praxis entstanden ist.

Kiel, den 30. März 2011

Sebastian Krug

