Global CO_2 Rise: A blessing or a curse for marine microalgae using carbon concentrating mechanisms during blooms

Bachelor Thesis
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Abstract

Global anthropogenic CO_2 rise is currently creating new environments and niches for microalgae living in the coastal and open oceans. These microalgae harness carbon concentrating mechanims (CCM) as a means of increasing their dissolved inorganic carbon uptake to overcome inherent problems that the enzyme RubisCO possesses. With algal blooms apparently becoming more frequent in the oceans, the possible harmful effects that they can cause are becoming a bigger problem for the local environment and populace. In this thesis brief summaries of the following three subjects are given: global CO_2 rise and its most direct implications for algae (CO_2 concentration and pH decrease); the reasons as to why algae use carbon concentrating mechanisms and its probable energetic costs, and finally algal bloom characteristics, specifically bloom onset, pH effects and ecological impacts. While these three themes are inherently more complex it is predicted that CO_2 rise will impact the bloom species at several levels. Algae like *Emiliana huxleyi* will suffer from the pH decrease, however at present times it still is a dominating blooming species due to other characteristics, like a highly regulated CCM-system, high growth rate and recycling of HCO_3^-.

All else being equal in time it will probably be the algae with a strongly regulated or possibly no CCM, who posses a better RubisCO enzyme than other algae, who draw the longest straw due to decreased energetic costs. Research on these algae has only just started.

However, CO_2 rise will not be solely responsible for dramatic species shifts in algal blooms due to aforementioned complexity of these described subjects. To gain insight in the interactions within and between the three subjects, studies should combine both focus on large scale experiments and on the molecular level.

Introduction

Ever since the start of the Industrial Age around the end of the 18th century CO_2 levels have been rising significantly. This global increase in atmospheric CO_2 levels is most likely a result of human industrial efforts (IPCC 2007).

The increase in CO_2 levels will resonate in the worlds' oceans. Based on the predictions made by the Intergovernmental Panel on Climate Change (IPCC) the levels of CO_2 will rise above 700 parts per million (ppm) within the next 50 to 100 years. When looking at the oceans this means a concentration increase of 30 μmol per kg^{-1} dissolved CO_2 in the ocean ecosystems (Feng et al. 2009). Most of this CO_2 is estimated to stay in the upper layers of the ocean for a long time. In fact, around 50% of the CO_2 that has been taken up by the oceans since the start of the Industrial Age is still in the seawater up to 400 metres deep (Sabine et al. 2004).

Since photosynthetic organisms living in the oceans are limited to the euphotic zone, it is likely that the rise of CO_2 has an impact on the organisms who live in these waters. Algae are the major contributors to the oceans primary production. In order to survive, a photosynthetic alga needs to have light and a steady supply of nutrients, and as such it heavily relies on dissolved inorganic carbon (DIC) as a carbon source . The distribution of DIC is usually in an equilibrium; 90% consists of HCO_3^-, 9% CO_3^{2-} and only 1% is CO_2. This naturally low amount of CO_2 due to the equilibrium poses a problem for a lot of algae. CO_2 diffuses very slowly into the oceans but is easily obtained by algae via their plasmalemma. However, the molecular structure of HCO_3^-, a source of carbon more readily available, is too large for natural diffusion. Another problem lies in the enzyme ribulose-1,5-biphosphate carboxylase/oxygenase (RubisCO). This enzyme needs CO_2 as a substrate, but only has a very low affinity for it.

Many algae had to develop ways to circumvent these problems and as such Carbon Concentrating Mechanims (CCM's) have evolved. These mechanisms operate by increasing the active uptake of HCO_3^- and CO_2^- into the cells. CCM's can be used both internally and externally. In this way algae can increase the concentrations of CO_2 near the active site of RubisCO in order to overcome the low affinity of the enzyme for CO_2. The specific ways in which all the different CCM's operate are still unclear. Several theories have been proposed, ranging from active HCO_3^- uptake to Crassulacean acid metabolism (CAM) like pathways also found in terrestrial plants (Giordiano et al. 2005). Despite a lot of these differences, one enzyme in particular has been found in a lot of CCM's; Carbonic Anhydrase (CA). CA works as a catalyst for the conversion of HCO_3^- to CO_2 and vice versa. By releasing CA externally an alga can increase the concentration of CO_2 nearby to increase the natural diffusion of CO_2 into the cell. Once inside the cell, internal CA's can convert this CO_2 back into HCO_3^{2-} in order to decrease the natural efflux of CO_2 back into the seawater. The question whether CCM's can prove to be an advantage or disadvantage to algae in times of CO_2 rise will be looked at in this paper.

Algal growth in seas and oceans is limited by a lot of factors, including the above mentioned DIC problems. However, at several points in time the overall high availability of nutrients facilitate rapid growth of a benefitting alga. Environmental conditions such as a change in water temperature or the physical concentration of a species in a certain area due to local patterns in water circulation can also influence the growth rate of an alga. At a certain point these benefitting algae start dominating the plankton community. The

precise working of the underlying mechanisms are still poorly understood (Vargo, 2009). After a while the algal bloom outgrows itself and can't sustain itself and collapses again. These blooms occur in both coastal areas as well as in the open oceans. Freshwater algae are known to bloom as well (Anderson et al 2002).

The effects of an algal bloom on the regional ecosystem vary. Especially toxic algal species can cause serious ecological, and socioeconomical problems, like the toxic microalga *Karina brevis* causing the famous "Red Tides". During this red tide *Karina brevis* emits so much poison in the surrounding waters that many fish die. (Vargo, 2009). Ecological problems can also arise without the production of poison. The coccolitophoric alga *Emiliana huxleyi* is one of the most notorious bloomers. Though his environmental impact is not directly related to active factors like poison release, the sheer impact of the biomass generated does leave its mark on the ecosystem. Toby Tyrrell and Agostino Merico discuss several of the studies conducted by others of these effects, like increased calciumcarbonate release in the environment, increased turbidity due to light scattering properties of the coccoliths and increased light and heat trapping due to biomass increase (Tyrrell & Merico, 2004).

By increasing the worlds' atmospheric CO_2 mankind seems to be creating a new problem for itself, alongside all the other scenarios predicted by the IPCC like rising water levels and increases in droughts.

With all the adaptations that microalgae have evolved over the years to optimize their growth, including the mentioned carbon concentrating mechanism, the effects of the rise of CO_2 on algal growth are still undergoing a lot of study. By altering the conditions in the oceans we are now possibly creating new niches for algal growth. The ways in which microalgae apply their carbon collecting mechanisms may be one of the factors in this. When new microalgae can now dominate the ecosystem due to rising CO_2 levels by becoming a bloom species, new environmental and socioeconomical problems can arise. In combination with other global warming effects this may prove disastrous for the local communities. Will there be any new effects?

In order to bring clarity into this matter, this paper will focus on the underlying mechanisms of CCM's, Algal Blooms and CO_2 rise. First the three mentioned topics will be described more thoroughly after which this paper will try to combine these topics to give insight into the interactions between these topics and how this will affect our oceans, coastal waters and the socioeconomical problems that can arise.

Anthropogenic CO_2 rise in relation to Oceanic CO_2 and pH changes

In past years the Intergovernmental Panel on Climate Change (IPCC) has been constituted to assess the issues regarding anthropogenic CO_2 and climate change. This panel is comprised of hundreds of scientists from different fields all around the world. In 2007 they published their most recent report. This report has been recognized by governments all over the world as a leading voice in global warming.

The 2007 IPCC concludes that they are "highly confident" that the observed shifts in regime and abundance concerning algae, plankton and fish are due to anthropogenic reasons (IPPC 2007). A regime shift occurs when an ecological system reaches a certain treshold (in example increased amounts of a species of alga) and as a consequence changes from its former stable state in a new one, thereby dramatically altering the former ecosystem.

CO_2 rise is mentioned as the most important greenhouse gas in the greenhouse effect. The concentrations of CO_2, next to methane (CH_4) and nitrous oxide (N_2O) have been rising significantly since 1750. The rise in CO_2 is mostly caused by the burning of fossil fuels for industrial (factories, transport etc) and personal (cars, housing energy etc.) uses (IPPC 2007).

Global pH decrease of the oceans and coastal waters due to atmopsheric CO_2 diffusion into these waters is also considered a consequence of global warming. They conclude an average decrease of pH units of 0.1. Projections leads them to expect an average decrease of global surface ocean pH between 0.14 and 0.35 units over the 21st century (IPCC 2007). The IPCC does not include real conclusions on this matter as to what this decrease in pH will facilitate in the oceans. They do however mention coral corrossion and trophic cascades due to the decline of calcifying organisms.

However, research has actually been rather intense on this topic. In a recent study changes in adult survival and recruitment in the barnacle *Semibalanus balanoides* were observed due to higher CO_2 levels (Findlay et al 2009). Apparently the rise in CO_2 does not only affect algae or corals, but also organisms in higher trophic levels.

In the case of algae there has been a lot of investigation on CO_2 rise, mostly focusing on the coccolithophoric *Emiliana huxleyi*. The detrimental effects of CO_2 rise on the mineral structure of their calcified "shells" protecting the algae are considerable. By manipulating pCO_2 and pH values in an experimental setting these studies showed a decrease in coccolithophores on the algae to a maximum of 31% at the highest pCO_2 levels. Next to this the impact of UV-light was considered; results showed an increase of harmful UVR transmission of 26% (Gao et al, 2009).
This result shows how all of the mechanisms considering growth and reproduction in marine organisms seem to be interconnected.
In a large survey combining three experiments this connection was studied as well. In two Ecostat experimental settings four scenarios were described; Ambient (12 degrees, 390 ppm CO_2), High CO_2 (12 degrees, 690 ppm), high temperature (16 degrees, 390 ppm) and greenhouse (16 degrees, 690 ppm). In all of the treatments different community

structures were found, with diatoms thriving best in the high CO_2 environment, and coccolithophores in the greenhouse treatment. (Feng et al, 2009).

It is therefore also highly likely that a global increase in pCO_2 as predicted by the IPCC will affect the organims living in the euphotic zone. Consequences for the organisms living in the euphotic zone will be observed at the communital, environmental and cellular level.

This paper will therefore now focus on these levels; Cellular, by looking at carbon concentrating mechanisms in relation to CO_2 and the environmental/community level by looking at algal bloom specifics in relation to CO_2.

Carbon Concentrating Mechanisms – eCA expression and energetic costs

Carbon concentrating mechanisms

Algae and land plants evolved ways of carbon assimilation. By directly fixing dissolved inorganic carbon, the enzyme RubisCO plays a major role in the carbon assimilation process in many species. However, RubisCO is both a carboxylase and an oxygenase; which poses a problem. The affinity for CO_2 is low in RubisCO, therefore the rate of assimilation of carbon is mostly dependant on the pCO_2 concentration around the enzyme in order to prevent RubisCO making use of its oxygenase properties. (Giordiano, Beardall and Raven, 2005). Many organisms, including algae, have evolved Carbon Concentrating Mechanisms in order to overcome these assimilation problems.

CCM's differ in several ways amongst the organisms (Giordiano, Beardall and Raven, 2005, Roberts et al., 2007). For the purpose of narrowing down the subject, this paper will focus mostly on the enzyme Carbonic Anhydrase. This enzyme plays a large role in many CCM's. The occurrence and use of CA within algae is versatile. By enhancing the rate of conversion of HCO_3^- into CO_2 and vice versa, CA is found in many layers of eukaryotic species. For instance, CA can be found in the external environment, periplasm, cytoplasm, the endoplasmatic reticulum, pyrenoids and thylakoids (Fig.1: Roberts et al, 2007). In most cases CA facilitates carbon assimilation by converting the carbon source in order to let it diffuse through either active pumps or via natural diffusion. Since the current rise of pCO_2 in seawater will affect cells on the extracellular level first, this paper focuses mainly on extracellular carbonic anhydrase (eCA).

Fig 1. Schematic illustration showing possible CCM's in diatoms. (K Roberts et al. 2007)

eCA

Extracellular carbonic anhydrase has been studied extensively. By dehydrating HCO_3^-, eCA enhances the potential for CO_2 uptake (Beardall et al, 2009). Most algae are able to furthermore acquire their need for C_i (inorganic Carbon) by natural diffusion and/or active uptake of CO_2. HCO_3^- active uptake via pumps exists as well.
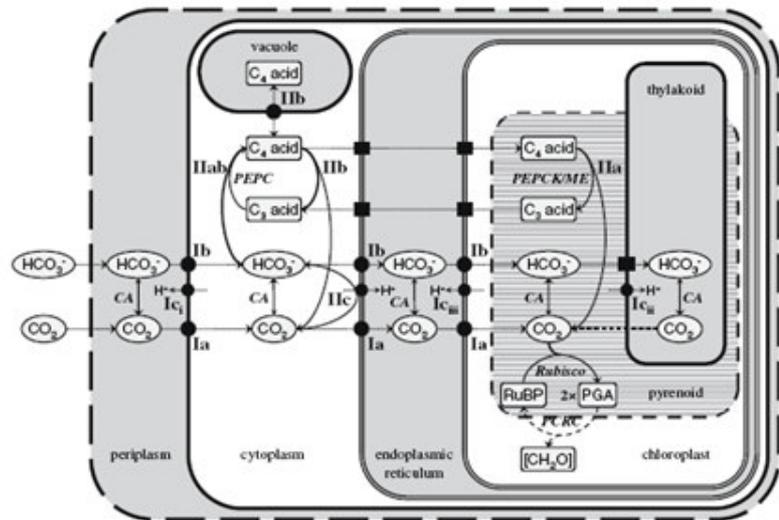
Studies show that eCA activity is limited by both light and pCO_2 conditions. For example, the alga *Skeletonema costatum* showed an increase in eCA activity under limiting light and CO_2 conditions. In limiting pCO_2 conditions but high light conditions in combination with higher eCA activities *S. costatum* was still capable of photosynthesis (Xiongwen & Kunshan, 2003). Repeated experiments showed the same for several species. In an experiment concerning the bloom forming species *Eucampia zoodiacus, Skeletonema costatum*, *Thalassionema nitzschioides* and the non-bloom-forming *Thalassiosira pseudonana*, exposure to different pCO_2 levels showed no differences in their maximal photosynthetic rates. Individual properties like eCA activity however differed amongst the species. The highest eCA activity shifts were found in the bloom forming species under low pCO_2 conditions, while the non-bloom forming *T. pseudonana's* eCA activity shifts remained comparatively low, indicating a lessened ability to regulate its eCA expression. (Fig.2: Trimborn et al, 2009).
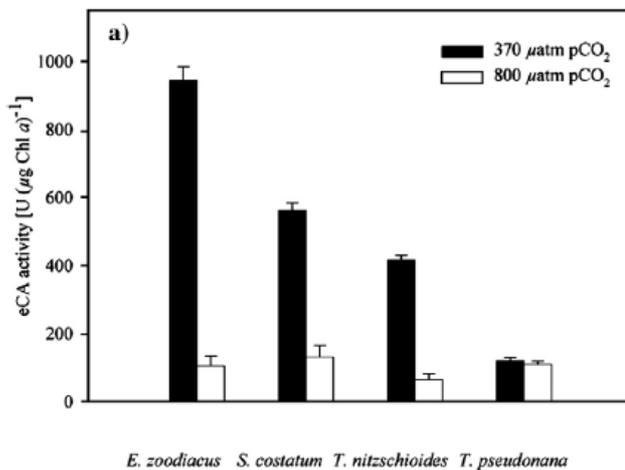

Fig 2: Activities of eCA under different pCO_2 regimes. (Trimborn et al, 2009)

However, in other species of algae and certain cyanobacteria the down-regulation of eCA expression is controlled by the concentration of HCO_3^- instead of CO_2, (Beardall & Giordano, 2002) making the overall utilization of eCA as complex as the differences between CCM's itself. Next to these problems, the expression of eCA isn't entirely dependant on CO_2/HCO_3^- concentrations. Acidification or alkalisation caused by pH shifts, temperature and the availability of nutrients all have effects on the expression of CCM's, with eCA in particular being a highly regulated enzyme (Beardall & Giordano, 2002).
pH is a considerable factor in eCA expression, as shown in a study on the alga *Mougeotia* (Chlorophyta, Charophyceae). Here pH was manipulated in an experimental setting, showing an increase of CA expression at higher pH levels. (Arancibia-Avila et al, 2000)

Table 1: Comparison of CA activity under different pH regimes (P Arancibia-Avila et al, 2000)

Experiment	Sample number	Relative CA activity/[chl a] (mg/mL)
Total CA, pH 8	3	4.45 ± 0.86
Total CA, pH 5	3	1.00 ± 0.14*
External CA, pH 8	10	2.37 ± 0.76
External CA, pH 5	5	0.99 ± 0.74*
Internal CA, pH 8[†]	10	4.09 ± 1.46
Internal CA, pH 5[†]	3	3.52 ± 2.74

Algae living in coastal waters show a higher amount of CCM activity than oceanic algae. This activity increase might make them more resillient to the greater differences in CO_2 influx in coastal waters than in open seas (Trimborn et al, 2009).

Energetic Costs

Little is known about the energetic costs involved in CCM induction and maintenance. In general it is assumed that the production of eCA and other components in CCM do have costs (Riebesell, 2004). A study similar to the one conducted by Trimborn et al (2009) with the alga *Chlamydomonas acidophila* showed that under higher pCO_2 conditions the algae switched off or highly suppressed its CCM, while increasing its maximum yield (Spijkerman, 2008). This study shows a correlation between higher maximum yield and lower CCM expressions, indicating at least some sort of trade-off between CCM and growth rates. That this difference is due to energetic costs has not been thoroughly studied, but it seems likely.

Active carbon acquisition with the use of pumps is a process which requires either the use of ATP or via a H^+ electron transport system. Apparently the energetic costs of maintaining these pumps outbenefit the use of only relying on natural diffusion of CO_2 across the plasmalemma. Nonetheless, there is reasonable evidence some algae possess no way of actively taking up HCO_3^-, relying only on natural diffusion and no external CA, thereby reducing their energetic costs involved in making extra CA. Examples of these algae that appear to have neither eCA or a CCM are certain freshwater Rhodophytes, crysophytes and synurophytes. In the marine ecosystem there are some marine red algae which live at low light levels where fewer CO_2 is needed. At present times there are not yet any marine microalgae known to fall into these categories. (Giordano et al, 2005). A reason for the survival of these species might be due to the chemical properties of RubisCO in these algae. The CO_2/O_2 affinity of RubisCO in these species might be higher than that in algae using eCA, corresponding to a system found in higher land plants as well (Raven et al, 2005). Few studies have been executed in order to find out if these algae still make active use of HCO_3^- since net photosynthetic rates found in these algae can't be explained only by CO_2 acquisition. One theory suggests leaking of

intracellular CA from inside the cells to outside the plasmalemma thus acting as eCA (Raven et al, 2005).
 Another problem is the difficulty of differentiating between regular HCO_3^- and HCO_3^- catalyzed by CA (Sültemeyer et al, 2001).

eCA and active uptake in relation to blooms

Colman et al (2002) give a short summary of known information on the use of CO_2 and HCO_3^- active uptake in relation to eCA (Tables 2 and 3). Note however a discrepancy between table 2 and figure 2. The algae *Skeletonoma costatum* and *Thalassiosira pseudonana* are used in the experiments conducted by Trimborn et al (Fig 2., 2009) and there its eCA activity is measured. In table 2 however both algae are reported as not having eCA. This might be due to new insights regarding this alga following the year 2002. Other reported values are assumed to be correct in this paper.

Table 2: Algae with multiple Ci uptake systems

CA, carbonic anhydrase (expression in air-grown cells); +, present; -, absent

Species	Transport system		External CA	Reference
	HCO_3^-	CO_2		
Chlorophyta				
Chlamydomonas reinhardtii	+	+	+	Sültemeyer *et al*. 1989
Dunaliella terteolecta	+	+	+	Amoroso *et al*. 1998
Scenedesmus obliquus	+	+	+	Palmqvist *et al*. 1994
Chlorella saccharophila	+	+	+	Rotatore and Colman 1991*b, c*
Chlorella ellipsoidea	+	+	-	Rotatore and Colman 1991*b, c*
Chlorella kesslerii	+	+	-	Bozzo *et al*. 2000
Bacillariophyta				
Navicula pelliculosa	+	+	-	Colman and Rotatore 1988; Rotatore and Colman 1992
Phaeodactylum tricornutum	+	+	+/-	Colman and Rotatore 1995
Cyclotella sp.	+	+	+	Rotatore *et al*. 1995
Ditylum brightwellii	+	+	-	Korb *et al*. 1997
Skeletonema costatum	+	+	-	Korb *et al*. 1997
Chaetoceros calcitrans	+	+	-	Korb *et al*. 1997
Thalassiosira punctigera	?	+	-	Elzenga *et al*. 2000
Thalassiosira pseudonanna	+	?	-	Elzenga *et al*. 2000
Rhodophyta				
Porphyridium cruentum	+	+	+/-	Colman and Gehl 1983; Huertas *et al*. 2000*a*
Haptophyta				
Emiliania huxleyi	?	+	+/-	Elzenga *et al*. 2000
Dicrateria inornata	+	+	+	This paper
Isochrysis galbana	+	+	+	This paper
Phaeocystis globosa	?	+	+	Elzenga *et al*. 2000
Eustigmatophyta				
Eustigmatos vischeri	+	+	-	I. E. Huertas, unpublished data
Vischeria stellata	+	+	-	Huertas *et al*. 2002*b*

Concerning algae with a multiple uptake system, two of the more notorious bloomers are members of the Haptophyta; *Emiliana huxleyi* and *Phaeocystis globosa*. Both exhibit a

use of eCA as well. This multitude in characteristics might make them such powerfull bloomers. However, many members of the Chlorophyta have a similar triple working but do not exhibit blooms to the extent of that of the Haptophyta. Most of these Chlorophyte blooms (and species) appear to be located in the arctic regions (Moro et al, 2002). This while blooms caused by *E. Huxleyi* for example can be found in several parts of the oceans.

Bacillariophyta also exhibit a low amount of blooming. They however don't use eCA very often as seen in table 2. Even for those algae with the discrepancies between studies described above are no real bloomers. *T. Pseudonana*, even though reported as having eCA is still a poor eCA user.

Interestingly, algae making use of only 1 system of active uptake show no use of eCA (Table 3). The Dinophyceae group harbours several harmful algal bloomers. A study conducted by Ratti et al (2007) also shows that the alga *Protoceratium reticulatum* (belonging to the Dinophyceae group) does not make use of eCA. Its iCA however is of great importance to maintain a steady photosynthetic rate at ambient CO_2. At a higher CO_2 rate the growth of the alga collapsed (Ratti et al 2007).

Table 3: Algal species with a single Ci uptake system

Note that none of these species express an external carbonic anhydrase. +, present; -, absent

| Species | Transport system | | Reference |
	HCO$_3^-$	CO$_2$	
Chlorophyta			
Eremosphaera viridis	-	+	Rotatore *et al.* 1992
Nannochloris atomus	-	+	Huertas and Lubian 1998; Huertas *et al.* 2000*a*
Nannochloris maculata	-	+	Huertas and Lubian 1998; Huertas *et al.* 2000*a*
Dinophyceae			
Amphidinium carterae	-	+	This paper
Heterocapsa oceanica	-	+	This paper
Eustigmatophyceae			
Nannochloropsis gaditana	+	-	Huertas and Lubian 1998; Huertas *et al.* 2000*b*
Nannochloropsis oculata	+	-	Huertas and Lubian 1998; Huertas *et al.* 2000*b*
Monodus subterraneus	-	+	Huertas *et al.* 2002*b*

Since both eCA users as non-eCA users appear to be able to form an algal bloom, a link between eCA useage and blooming seems to be rudimentary at best.

However, with so many factors influencing the expression of CCM's and eCA in particular, the effects of the rising CO_2 levels in the atmosphere are probably going to have an effect on algae using CCMs. The properties of algae lacking a CCM, like having a RubisCO system equivalent to higher land plants, might give them an edge in competing with other algae due to decreased energetic costs.

Thus, the regulating properties of algae on their CCMs show a high dependence on the environmental condition of the medium surrounding the algae. In an algal-bloom, conditions vary significantly over time, posing new questions on having a CCM during a bloom.

Algal Blooms

Onset

The exact reasons as to why algal blooms occur are still poorly understood. Many different models have been suggested over the years. At the hypothetical level an algal bloom is the result of a set of ideal circumstances for an alga that can as such dominate the local ecosystem. In the case of phytoplankton these circumstances concern mostly nutrient availability, temperature and increase in light (Aberle et al, 2007).
Nutrient availability in itself can be influenced by either natural or human effort. Natural reasons concern the seasonal cycles of nutrients driven by forces like turbulence and upwelling, whilst human impacts can be as diverse as sewage and animal waste, groundwater pollution and fertilizer runoff (Anderson et al, 2002).
Other biological factors like temperature, light and CO_2 levels are also of direct influence on algal blooms. *Emiliani huxleyi*, a highly investigated bloomer, for instance shows a high resistance to photo-inhibition caused by high light conditions and conversely a high dependence on silicates (Tyrell & Merico, 2004). It is probable that the reasons why a bloom occurs are the interactions between such advantages and disadvantages.

Although these kinds of theories in itself are explanatory on a larger scale (and quite accurate in predicting algal blooms), they don't focus on more specific dynamics concerning algal blooms. In a recent study it was proposed to start looking at the cellular level of algae to give more insight. The study concerns the earlier mentioned alga *Karenia brevis*. Circadian rhythms appear to have a great impact on when *K. brevis* enters its S-phase, essential to vegetative growth. The algae in the bloom also seem to display a high genetic variability, allowing for a more flexible response to environmental changes. Alongside this gene-expression level the study also emphasizes behavior as a trigger for blooming, especially the alga's high amount of vertical migration (Fig.3: Van Dola, 2008).
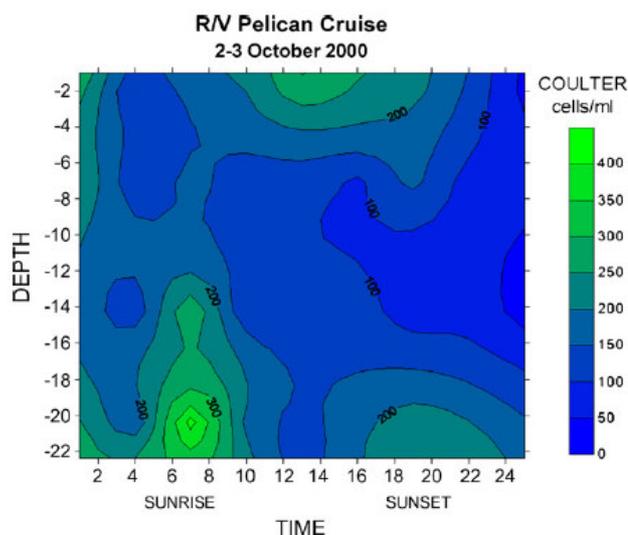

Fig 3. *K. brevis* cell counts over time, showing a strong migration pattern (Van Dola, 2008)

Due to this strong migrational pattern, *K. brevis* can readily adapt to different light conditions. This may be a factor in reacting to bloom encouraging conditions.

Another, more specific cause is the maximum growth rate of the blooming species. It is reported that *E. huxleyi* for instance can, under favouring conditions, achieve a growth rate of up to 2.8 doublings per day (Brand & Guillard, 1981). The calcified structures that cover *E. huxleyi* may also be a reason for its status as a dominant blooming species. By taking up HCO_3^- for calcification, *E. huxleyi* is possibly able to thrive better at low CO_2 conditions than other algae by recycling the used HCO_3^- for carbon assimilation (Tyrell & Taylor, 1995)

Studies like those conducted by Tyrell and Taylor show how CO_2 is also one of the factors influencing algal bloom development. Even though there are numerous reasons as to why a bloom occurs, during the bloom itself these conditions may change over time. Since research concerning this topic is still in its infancy, this part is omitted in this paper, but should be incorporated in the equation once more studies have been conducted. For this reasons, this paper will further solely discuss the pH effects on algae, since global CO_2 rise is also considered to have an impact on the overall pH of the oceans. Afterwards, some of the reported impacts on human economy and ecology are mentioned.

pH effects on Algae

The effects of pH on algal growth have been studied quite thoroughly. In a stable environment, the pH value can partly influence the ecological community. One study compares these differences in community structure as a result of pH values interactions found by other researchers as follows: At pH 7 and above, more diatom species are present, whilst at a pH level of 4 and lower, fewer species are present (Oberholster et al, 2009)

In a field experiment in a freshwater pond in Varanasi, India pH also influenced the kinds of species present. Fluctuations in pH affect the way carbon is able to be utilized by eukaryotes and cyanobacteria. Acidification of the water leads to an increase in eukaryotic species, whilst a higher pH is a more suitable environment for cyanobacteria (Sharma et al, 2009).

Even though the importance of pH on algal growth has only recently been recognized, lab experiments that solely concern pH as a factor have already been conducted in previous years. One of these shows an increasing inhibition of photosynthetic capabilities and growth in *Thallasiosira pseudonana* and *Thallasiosira oceanica* when pH values were heigthened above a value of 7 (Fig 4: Chen & Durbin, 1994).
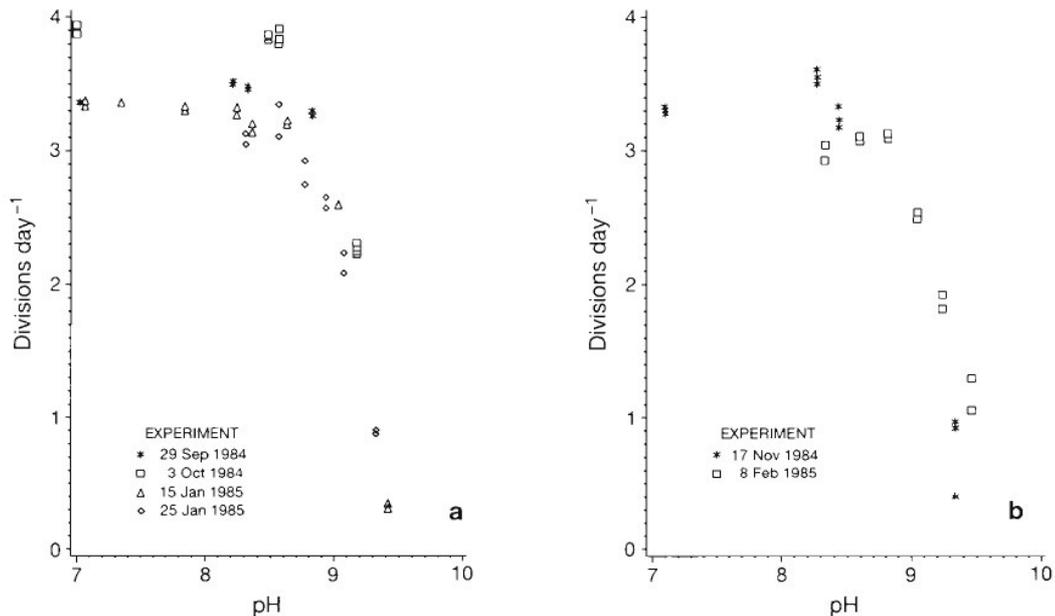
Fig 4. Growth rate (calculated by measurements of fluorescence over 40 hours) of *T. pseudonana* under different pH Regimes. (Chen & Durbin, 1994)

The relationship between CO_2, HCO_3^- and pH might be a factor in algal blooms. Even though CO_2 might rise globally, in the local environment of a bloom CO_2 gets assimilated rather quickly. This decrease in CO_2 might in turn elevate pH levels locally; causing possible problems to the algae, constituting a collapse of the bloom. Evidence on this is scarce however.

Impacts

The impacts of an algal bloom, and for humans the so-called harmful algal blooms in particular, are not to be underestimated. Red tides caused by *Karina brevis* are found in a wide array of countries all over the world. Red tides can cause Paralytic Shellfish Poisoning (PSP) or Diarrhetic Shellfish Poisoning (DSP). The range of countries where these blooms originate and wreak havoc amongst the local populace, both human and ecological, is diverse; New England, the Faroe Islands near Argentina, Thailand and Newfoundland Canada. In all of these occurrences it were probably dinoflagellates to blaim.
Reports of so-called Brown Tides (tides caused by brown algae) are also made. These in itself can also be poisonous for shellfish and marine vegetation and were found in Tasmania, Taiwan, Guatamala, Korea, Hong Kong and Venezuela (Anderson, 1989). Even though this list is already quite diverse, other parts of the world are probably frequently engulfed in these tides as well.

One reason for the great dispersal of these algae causing problems is human introduction. In a shellfish poisoning which killed 26 people in Champerico, Guatamala an alga named *Pyrodunium bahamense* was to blame. This organism however was never before found

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in those regions (Anderson, 1989). Anderson also describes a case of brown tide by the alga *Aureococcus anophagefferens* which devastated the local multi-million dollar shellfish industry of the North Eastern United States.

Not only humans are the ones suffering from these blooms. Deaths by algal blooms in the human populace are often indirect effects; eating poisoned fish or shellfish are the main causes. Harmful algal blooms at first influence their local surroundings. Brown tides caused by the alga *Aureococcus anophagefferens* also severely impact the benthos at the mid-west Atlantic coast of North America (Bricelj & Lonsdale 1997). Mostly eelgrass (*Zostera marina*), but also bay scallops (*Argopecten ivrudicms*) and blue mussels (*Mytilus erlulis*) are affected.

Near the coast of Florida *K. brevis* is once again responsible for destroying the local ecosystem. It is estimated that over 96% of the occurring fish deaths between 2003 and 2007 are caused by Red Tides, an astounding amount that affected the overall community structure fundamentally (Gannon et al, 2009).

The examples mentioned above are just the tip of the iceberg. Many more detrimental, sublethal or even deadly effects have been reported over the years and are too numerous to summarize here. Trends show an increase of harmful algal blooms, along with a higher amount of dispersion (Anderson et al, 2002). Considering the implications on the socioeconomical systems in countries affected by these blooms, the increase in HAB's is a dark foresight. Many third world countries count largely on fisheries for their income. Is global warming one of the reasons for these increases in blooms? In combination with the other problems arising in third world countries due to global warming, these countries can plummit into an economical disaster state.

Will the global increase in anthropogenic CO_2 and its coupled decrease in pH affect the current compositions and occurrence of blooms? How are the mechanisms responsible for carbon acquisition in algae going to help or impair them in these times of CO_2 rise? Is it possible that some previously "non-blooming" species might become a dominant factor?

Discussion

The complexity of all these mechanisms pose a problem in creating a coherent theory on how the three subjects will interact in the upcoming years. Providing insight in this matter is relevant, considering the examined major impacts algal blooms can have on both environmental and human wellbeing.

With all the research done on algae and their growth rates under elevated pCO_2 levels it is likely that the global rise will have an impact on the oceanic and coastal ecosystems. The ways in which algae will react to this rise in DIC is dependent on their ability to down- or upregulate their CCM's, in particular eCA. When looking at the organismal level, the rise in CO_2 seems to be favouring algae which are predominantly fixing CO_2 for their carbon resource, while coccolithophoric algae will be suffering from a repression of their biogenic calcification due to increased acidification (U Riebesell, 2004). *E. huxleyi* is the model organism used most to assert the effects of CO_2 rise on calcification. The decrease of stability of the coccolithophoric shells make them susceptible to all sort of side effects, like the mentioned increased damage taken from ultraviolet light. Still *E. huxleyi* remains one of the most dominant bloomers.

Reasons for this might be the strong regulatory systems occurring in blooming algae considering their CCMs. *E. huxleyi* is still able to bloom because of its ability to decrease the energetic costs of maintaining a CCM, its high maximum growth rate and the ability to reuse carbon created in the calcification process. At present CO_2 levels, these abilities seem to outweigh the negatives of decalcification due to pH decrease and a constant energy cost of eCA expression, even at its lowest level. In the study by P Arancibia-Avila (2000) a link has been established between eCA activity and pH shifts. When pH levels drop due to the increase in CO_2 eCA expression will both be affected by the pH drop as the CO_2 increase, enforcing each others' effects.

Notably, even though the global CO_2 levels are rising, levels within a bloom seem to drop rather fast, constituting better results for algae harnessing CCM's over species with less or absent regulatory systems.

Since most algae rely on a CCM for their carbon assimilation one way or another it appears that it are these kinds of extra positives in the case of *E. huxleyi* that make these algae a dominant bloomer.

Algae like *Thallasiosira pseudonana* exhibit lower levels of eCA regulation, making it less possible for them to adapt to quickly changing environments. This in combination of the absence of several advantages such as HCO_3^- re-using makes them highly susceptible to being outcompeted in the near future, even though their respective photosynthetic rates in different CO_2 conditions appear to remain around the same values.

However, when *T. pseudonana* is exposed to heightened pH values, their photosynthetic rates collapse as mentioned earlier. In the competitive environment of an algal bloom this might pose another problem, although more research is needed on local conditions in a bloom to get more constitutive results on how the environment affects the algae present in the timespan of a bloom.

Chlorophyta express fewer CCM activities then the Haptophyta (to which *E. Huxleyi* belongs). They also express less algal blooms. Whether or not this is due to their weakened CCM's remains unclear. In the following decades they might get a little boost due to the CO_2 rise in their growth rates since they also expand less energy for maintaining a CCM.

Are it then the algae without an extensive CCM that might come out on top? Some researchers suggest their RubisCO form is alike those of higher land plants, showing a higher affinity for CO_2 than the RubisCO forms used in most of the other algae. Most of these algae however are freshwater algae or macroalgae/
Noteably, the evidence for these algae (both freshwater as the marine macroalgae) having no CCM at all is still in its infancy (J A Raven et al, 2005).
In the hypothetical situation that there are microalgae that don't have CCM"s, it might make them highly placed candidates for becoming new dominant bloom species. Without the use of a CCM, these algae can spend more of their energy in growth instead of enzyme expression. Also, algae suspected in this category are mostly non-calcifying organisms. When competing with coccolitophores, even despite the apparent current advantages coccolitophores possess in favour of other algae, they might constitute the winning hand. This because even though they lack several of those advantages, in the end they don't suffer the drawbacks of acidification, at least not on the calcification process level. It should be noted that these kinds of "upper-hand situations" are not to be seen as ways in which the algae totally outcompete each other, but rather a shift in who's the biggest bloomer at a certain place. These interpretations only take in CO_2 and the CCM systems in response to CO_2, but it aren't just these systems which are the sole responsible forces in a bloom. *E. huxleyi* for instance is probably also better at living at phosphate limiting conditions then most other algae.

So why is it then that algal blooms belonging to the Dynophycaea are starting to increase their numbers? Algae like *K. Brevis* and *Protoceratium reticulatum* are allready known to bloom more frequently. Even though they do posses a CCM (Internal CA in the case of *P. reticulatum*) that they rely on; an important factor seems to be which kind of active uptake pump they are using. It are probably the Dynophycaea that rely on active CO_2 pumps solely that are now reaping their benefits from only using iCA and no eCA. The iCA will keep their carbon source located inside their cell bodies, whilst they don't have to invest energy in eCA. By increasing athmospheric CO_2 it makes their lifes easier, in comparison to the other algae.

It is rather clear that CO_2 rise in itself will not be solely responsible for the regime shifts expected to occur. In the case of algal blooms this predictions become even harder. Even though algae in lab experiments react highly to different CO_2 conditions, these conditions do not necessarily reflect the natural nutrient availabilities in an algal bloom or the ways in which species during a bloom interact. Mesocosm experiments should be conducted to give more insight in these interactions. The proposal by F M Van Dola (2008) to start describing these interactions at more cellular levels, like a better understanding of the energetic costs involved in utilizing a CCM seems to be a correct approach in both CO_2 effects as bloom-dynamics. By identifying all factors involved in an algal bloom, more accurate predictions can be made on the future situation. As an example of these smaller

interactions: When *E. huxleyi* and *K. brevis* are hypothetically both present in a pre-bloom situation, who will outcompete the other and how? Little research has been conducted on CCMs in *K. brevis,* however the other ways in which they form a bloom are of importance as well. When *E. huxleyi* for example might have the head-start due to its maximum growth rate the algae can then impair *K. brevis* growing potential by creating a "canopy" of *E. huxleyi*. This canopy might disturb *K. brevis´* circadian rhythm by blocking sunlight. This example though is a very hypothetical situation, but constitutes a way of showing the numerous possibilities as to why and when a species dominates a system on a small scale, next to the larger factors.

Because of the slow diffusion of atmospheric CO_2 into the oceans, CO_2 levels in the oceans will continue to rise in the coming years even though CO_2 emissions are trying to be cut back. When looking at the effects a bloom of *K. brevis* might have on the local populace and ecosystem, it is of high importance to start investigation and policies to tone these blooms down. Since reports have been made that *K. brevis* is occuring more frequently in the past few years, research in its CCM's mechanisms might prove to be of high value. A differentation should be made in increasing blooms and the ways in which they actively aquire their DIC (Multiple pumps, sole pumps and eCA), as well as an explanation for the relative low abundance of "Chlorophyta" blooms.

Since CO_2 rise right now looks to be one of the more explanatory factors and is one of the more "easily" controlled factors in terms of human intervention, for that we can influence CO_2 is already established over the past hundreds of year.

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