The demise of lifesaving organisms? Measuring the effects of ocean acidification on algae growth in situ

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Amsterdam, 3 januari 2020

Beste lezer,

Exact twee jaar geleden begonnen we met de grondbeginselen van wat later een heel groot project bleek te worden. Onze plannen zijn na de eerste opzet nog 200 keer veranderd, maar uiteindelijk is dit wetenschappelijk artikel ons resultaat.
We begonnen met de inzameling van het geld, we wilden immers op een reis van twee maanden. We hebben de meest bizarre manieren bedacht om geld in te zamelen; we zijn op de radio geweest, we hebben in de stromende regen op de markt gestaan, we hebben een gigantisch diner georganiseerd en we hebben elk energiebedrijf in Nederland gemaild met de hoop op sponsoring. Deze reis van twee maanden op een zeilschip over de Atlantische Oceaan maakten wij om onze metingen voor ons onderzoek op te halen. Dit was een buitengewone ervaring, waar we niet alleen veel hebben geleerd maar ook veel lol hebben gehad.
Ook hebben we de wetenschappelijke wereld ontdekt op de Universiteit van Amsterdam met de hulp van onze geweldige mentor, Katja Peijnenburg. Zij heeft ons in contact gebracht met ongeveer het gehele *Institute for Biodiversity and Ecosystem Dynamics* en ze heeft ons geholpen met de opzet en uitvoering van het onderzoek. Maar ook op school kregen we hulp van een zeer inspirerende begeleider, Jens Dirkse, die ons heeft uitgedaagd om een hoger niveau van onderzoek te bereiken. We willen hierbij Katja en Jens heel graag bedanken voor hun hulp. Echter zijn zij niet de enigen die we moeten bedanken; alle mensen en bedrijven die hebben gesponsord hebben ook heel erg veel bijgedragen aan ons project. Verder willen we alle mensen bedanken die de tijd hebben genomen voor al onze vragen.
Al met al zijn we heel trots met wat we bereikt hebben de afgelopen jaren. Dus we kunnen nu met veel voldoening ons eerste *research paper* inleveren!

Veel leesplezier!

Groetjes,

Eline en Nikki

The demise of lifesaving organisms? Measuring the effects of ocean acidification on algae growth in situ

Eline Haak & Nikki Cappendijk

In this study we examined in what ways ocean acidification affects algae growth. According to prior laboratory studies, algae, a group of mostly calcifying organisms will suffer from decalcification if ocean acidity increases. However, our measurements in the ocean environment (in situ) and subsequent analyses indicate that ocean acidification has a positive effect on algae growth. Furthermore, ocean nutrients do not show a significant response on ocean acidification. We have been able to find the same correlation between acidity and algae growth, even after correcting for other factors that could influence algae growth, like seawater temperature.
We have analysed the overall algae growth and nutrient impact, and we have not accounted for differences between different algae species. Daily measurements were taken over a six week period on the Atlantic Ocean between 15° and 28° latitude.

1 Introduction

1.1 Background: more CO2 leads to more acidification of the ocean

An influx of anthropogenic carbon dioxide (emissions of carbon dioxide associated with human activities) causes a decrease of the average seawater pH, which affects the growth of calcifying algae.

By the end of this century, the average surface ocean pH could be lower than it has been for more than 50 million years (Rhein *et al.* 2013). Due to an influx of anthropogenic carbon dioxide, the carbonate system of the world oceans is changing rapidly (Feely *et al.* 2004) and the average seawater pH is decreasing, which leads to decalcification of marine life. The rate of current and projected increases in atmospheric CO_2 is approximately 100x faster than has occurred in at least 650,000 years (Siegenthaler *et al.* 2005). The oceans have absorbed ca. 30% of the anthropogenic carbon dioxide.

The major controls on seawater pH are atmospheric CO2 exchange, the production and respiration of dissolved and particulate organic matter in the water column, and the formation and dissolution of calcium carbonate minerals (Rhein *et al*. 2013).

1.2 Relevance: acidification affects marine life

The relevance of this paper has everything to do with future environmental issues. An increase of pH in seawater has a major impact on marine life. Not only fish are affected by ocean acidification, but also different species of algae.

A decrease in pH causes the calcium skeletons of some species of algae to dissolve, which leads to shortage of algae. Algae are food for many marine organisms and when a shortage of algae occurs, it can negatively affect the growth of these marine organisms. For example, the pink salmon.

Algae are widely defined, but one definition is that algae have chlorophyll as their primary photosynthetic pigment (Lee, 2008). Algae can be separated in two different groups: calcifying and non-calcifying algae. The main difference is their physical structure, only calcifying algae are affected by ocean acidification, due to their spine of calcium.

However, not only calcifying algae are affected by ocean acidification. This can impact marine organisms both via decreased calcium carbonate ($CaCO_3$) saturation, which affects calcification rates, and via disturbance to acid-base (metabolic) physiology (Sabine *et al.* 2004). For example, due to a decrease of calcifying algae, in this case Pteropods, the growth of juvenile salmon is obstructed. Pteropods are a group of holoplanktonic heterobranch gastropod mollusks, which are CO2 and also pH sensitive.

Juvenile pink salmon, sockeye salmon, pollock, and other commercially important fishes feed mainly on pteropods. A study of Armstrong *et al*. shows that a variability in the diet of juvenile pink salmon, with a single species of pteropod comprising 15 to 63% by weight of pink salmon diets during a 3-year study. Because Pacific pink salmon have a short, 2-year life cycle, prey quality and abundance during the salmon's juvenile stage may strongly influence the pink salmon's adult population size and biomass according to Aydin *et al.* (Kroeker *et al. 2013*).

Decreased calcification would presumably compromise the fitness of calcifying organisms and could shift the competitive advantage towards non-calcifiers. It is important to have a firm understanding of the degree to which ocean acidification influences critical physiological processes such as nutrient dynamics, as these processes are important drivers of calcification, ecosystem structure, biodiversity, and ultimately ecosystem health (Guinotte *et al.* 2008).

Many studies have already looked into the relation of algae and ocean acidification, but those studies were all under laboratory circumstances. Studies on acidification reveal consistent reductions in calcification, growth and development of a range of calcified marine organisms, despite the variability in their biology (Kroeker *et al.* 2013). It is yet unclear what the effects of acidification are in nature (in situ). So the scientific relevance of this research is to examine if those outcomes are also true in nature, because the great advantage of in situ observational studies is their increased realism, both in terms of long duration and inclusion of all elements of the ecosystem (Riebesell *et al.* 2010).

2 Theory

2.1 Central thesis and problem statement

The main question to be answered is: 'In what ways has ocean acidification influenced the growth of algae?' The foregoing issue has been broken apart in a number of sub-questions. These questions will be consecutively answered and read: 'Does ocean acidification affect the amount of nutrition needed for algae?', 'Do differences in nutrition correlate with amounts of algae?', 'Does seawater temperature effect algae growth?'

2.2 Scope

Given the limited scope of this research, a big part of the effects of ocean acidification is not accounted for.

Algae are treated as one species; differences between algae species are not considered. Furthermore, measurements were taken in the Atlantic Ocean in the tropical and subtropical zone, leading to a limited temperature range of 21℃ to 28℃. Also, any fluctuations of pH during different hours of the day are not taken into account.

Finally, ocean depth may influence algae growth but as this study has taken samples only at constant depth of 1 meter, this is not taken into account.

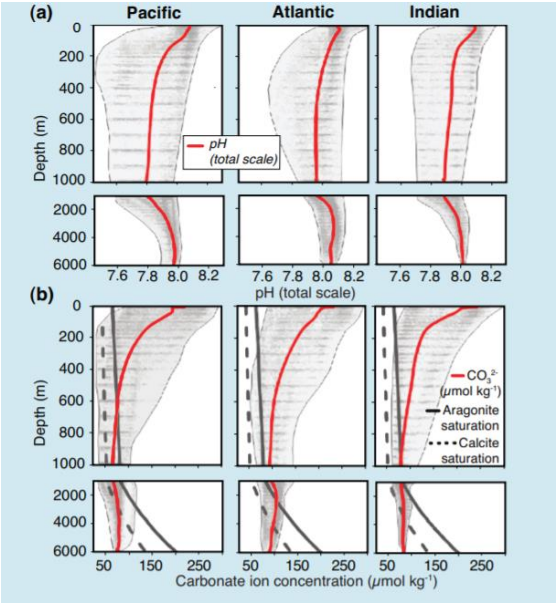


Figure 2: Distribution of (a) pH and (b) carbonate (CO3 2–) ion concentration in the Pacific, Atlantic and Indian Oceans. (Rhein *et al.* 2013)

According to a study of Rhein *et al.*, the lines of figure 2 show the mean pH (red solid line, top panel), mean CO_3^{2-} (red solid line, bottom panel), and aragonite and calcite (black solid and dashed lines, bottom panel) saturation values for each of these basins. The shaded areas show the range of values within the ocean basins. Dissolution of aragonite and calcite shells and skeletons occurs when CO_3^{2-} concentrations drop below the saturation level, reducing the ability of calcifying organisms to produce their shells and skeletons. Figure 2 also shows that around the surface of the Pacific, Atlantic and Indian oceans the pH rises in comparison with deeper ocean water.

2.3 Relevant chemical reactions

Dissolved inorganic carbon is mostly present in three inorganic forms in seawater: free aqueous carbon dioxide ($CO_{2(aq)}$), bicarbonate (HCO_3^-), and carbonate ions (CO_3^{2-}) (Gattuso, Hansson *et al.* 2012). Thus when carbon dioxide dissolves in seawater it can be considered to react with the water in accordance with the following series of chemical equilibria (figure 1):

$$CO_2\,(g) = CO_2\,(aq) \quad (1.1)$$

$$CO_2\,(aq) + H_2O\,(l) = H^+(aq) + HCO_3^-(aq) \quad (1.2)$$

$$HCO_3^-(aq) = H^+(aq) + CO_3^{2-}(aq) \quad (1.3)$$

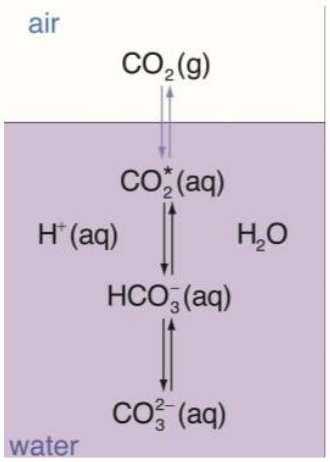


Figure 1: The chemical equilibria of the carbon dioxide system in seawater (Riebesell *et al.* 2010)

Figure 1 displays the carbon dioxide system in seawater. Which indicates that when the amount of carbon dioxide in the air increases, the amount of bicarbonate will increase ass well, and thus the average seawater pH will decrease.

2.4 Other potential factors impacting algae growth

The main hypothesis has already been outlined above: will lower pH lead to less algae growth in situ? There are other factors that influence algae growth that need to be taken into account.

2.4.1 Lower pH leads to less available nutrients

Calcifying algae can be affected through variations in amounts of carbon dioxide in the air, but also when other environmental parameters are changed, such as nutrient availability and temperature. A nutrient is a substance used by an organism to survive, grow and reproduce. Therefore it is important to look at nutrient levels as well (oligotrophic vs. eutrophic) (Langer *et al. 2012*). The effect of elevated pCO2 levels on nutrients has not been

studied often, though research has shown that ocean acidification can affect nutrient uptake of algae (Celis-Pla *et al.* 2015; Langer *et al.* 2012). Because of the lack of research and statements it cannot be concluded that ocean acidification has a direct impact on the amount of nutrients in the ocean. Fluctuations in nutrients cannot be linked directly to lower pH, but algae which are affected by ocean acidification do have an impact on nutrients so there is expected to be a link between ocean acidification, algae and nutrients.

2.4.2 Less available nutrients lead to a decrease of algae growth

A study of Rouco *et al.* showed that the volume of coccolithophorid *Emiliana Huxley* had decreased under limited nutrient conditions of nitrate and phosphate. This was measured under various pCO2 conditions. It showed that in both limited nutrient cultures and limited phosphate cultures volume decreased more in elevated pCO2 conditions. This corresponds to a study of Langer *et al.* which showed a 33% decrease in growth rate of the *Calcidiscus Leptoporus* under both nitrate and phosphate limitation.
This would indicate that cell division rate of calcifying algae decreases in environments with fewer nutrients.

2.4.3 Lower temperature leads to a decrease of algae growth

It is virtually certain that the upper ocean (0 to 700 m) warmed from 1971 to 2010. [...] High agreement among analyses provides medium confidence that oxygen concentrations have decreased in the open ocean thermocline in many ocean regions since the 1960s. The general decline is consistent with the expectation that warming-induced stratification leads to a decrease in the supply of oxygen to the thermocline from near surface waters, that warmer waters can hold less oxygen, and that changes in wind-driven circulation affect oxygen concentrations (Rhein *et al.* 2013). So when temperature in upper ocean increases, the water can hold less oxygen. According to a study of Dromgoole on the effects of decreased oxygen pressure on several species of algae in a pool, the oxygen responses indicate that spatial or temporal changes in oxygen tension of the environment could have a significant influence on the production of macroalgae. Warmer waters can hold less oxygen, which influences the production of micro-algae. So it is expected that algae growth will be slightly but not significantly obstructed by ocean water temperature.

3 Method

3.1 Data and samples collection

A total of 78 samples were collected on the Atlantic ocean. The locations of samples are shown in figure 2. PH was measured with a pH/mV/ORP/ISE/temp.-meter with BNC, CAL Check and Memory. Chlorophyll samples were taken with a Whatman filter ME 25/21. Nutrients samples were collected in a CELLSTAR® Tube.

Figure 3: Locations of samples

The pH values were measured at 8.00 AM each morning. First of all, a bucket of ca. 5 liters surface water was collected. Furthermore, all our seawater samples of the same station are taken from the same bucket. Secondly, the pH-meter electrode was put into the water of the collected seawater and the first dataset (pH nr. 1) was measured when the pH-value was around 7,900. This was because of the highly fluctuating results displayed on the pH-meter. So it was decided to start measurements around

a pH of 7,900, to avoid significant measurement errors. The second dataset (pH nr. 2) was measured ten minutes afterwards. (Riebesell *et al.* 2010)

The chlorophyll samples were taken with a filtration system, see figure 4. Two Nalgene™ Reusable Filter Holders were used in order to filter the seawater. To create a vacuum atmosphere inside the filter holders, a LABOPORT® mini series vacuum pump was utilized. Two datasets were collected (Filter A and Filter B) with the Whatman filter ME 25/21. The first filter was filtered with two liters seawater, the second filter was filtered with one liter seawater. (Chittaro *et al.* 2006; Suzuki *et al.* 1998)

Figure 4: Filtration system setup

The final sample was taken afterwards. Around 12 ml of the filtered seawater was collected in a CELLSTAR® Tube. The filters as well as the tube were put in a freezer in an environment around -18°C.

On top of that, metadata, such as weather, outside temperature, velocity, was also collected at each station to take the abiotic milieu into account. The boat was sailing, so the samples did not come into contact with possible engine pollution.

3.2 Analysis of samples

The mass of Chlorophyll was determined through a spectrophotometric determination. Filtrer A was put in a Microcentrifuge Tube, Skirted w/ Screw Thread together with 1500 µl organic solution (75% methanol / 25% dimethylsulfoxide) and miniature glass beads. The tube was beaten (BeadBeater) a couple of times until the beads had completely shattered the filter. Thereafter, the tube was centrifuged (Thermo Fisher centrifuge) two times for 5 minutes in order to settle down the remainders of the filter and the glass balls. A polystyrene Sarstedt cuvette was filled with the supernatant. Next, the cuvette was placed in a NOVASPEC® III+ spectrophotometer at two different wavelengths (665 nm and 645 nm) to measure the absorption. (Garty *et al.* 1997; Espinoza *et al.* 2006). It was not possible to collect the dataset at a wavelength of 645 nm.
To calculate the mass of Chlorophyll, a standard formula is used:

$$[ChlA] = (13{,}9 * [A665] - 2{,}16 * [A645]) * Ve/Vm$$

With [ChlA] in mg/l, [A665] as the result of the spectrophotometer at a wavelength of 665 nm and [A645] as the result of the spectrophotometer at a wavelength of 645 nm. Ve is the extraction volume in liter. Vm is the sample volume in liter.

At a wavelength of 665 nm the amount of chlorophyll A as well as the amount of chlorophyll B is measured and at a wavelength of 645 nm only the amount of chlorophyll B is measured, this eventually leads to the total amount of chlorophyll A. Because of the limited scope of this paper it was decided to neglect the amount of chlorophyll B (A645), so in this paper this formula was used:

$$[ChlA] = (13{,}9 * A665) * Ve/Vm$$

The Skalar San++ Automated Wet Chemistry Analyzer was used to determine the amount of $NO_2 + NO_3$, NO_2, NH_4 and PO_4 in our samples. The sample is transferred into a test-tube and placed in the machine.

				Biomass/algae	Nutrients			
	pH (no. 1)	*pH (no. 2)*	*Temperature*	*Chlorophyll*	*$NO_2 + NO_3$*	*NO_2*	*NH_4*	*PO_4*
pH (no. 1)	1,00							
pH (no. 2)	0,61	1,00						
Temperature	0,23	0,46	1,00					
Chlorophyll	-0,29	-0,56	-0,28	1,00				
$NO_2 + NO_3$	-0,31	-0,62	-0,71	0,26	1,00			
NO_2	-0,23	-0,24	-0,23	0,24	0,56	1,00		
NH_4	-0,12	-0,05	-0,27	0,04	0,46	0,88	1,00	
PO_4	-0,31	-0,42	-0,49	0,15	0,43	0,36	0,35	1,00

Figure 5: Data correlated ($\hat{\rho}$)

Inside the machine by means of chemical reactions with the sample a color is formed. The brighter the color, the higher the concentration of $NO_2 + NO_3$, NO_2, NH_4 and PO_4. The San++ FlowAccess™ V3 data acquisition Windows® software package controls the complete analyzer and has eventually calculated the datasets of $NO_2 + NO_3$, NO_2, NH_4 and PO_4. (Sumner *et al.* 2007; Kwaansa-Ansah *et al.* 2017)

4 Result

The data of this paper can be divided into two sets of data. The first set is known as the metadata, these are the measurements and observations which surround the samples. The second data set has the actual samples relevant to the content of this paper. All data and metadata can be found at Mendeley Data at http://dx.doi.org/10.17632/yjwz7s6shw.1.

We measured the following sample data: pH, temperature, biomass (chlorophyll A), $NO_2 + NO_3$, NO_2, NH_4 and PO_4. We wanted to understand which environment variable impacts biomass the most. Therefore we correlated pH, temperature, biomass, $NO_2 + NO_3$, NO_2, NH_4 and PO_4 with biomass (figure 5).

The correlation was calculated with this formula:

$$\hat{\rho} = \frac{\overline{xy} - \bar{x}\bar{y}}{\sigma_x \sigma_Y}$$

The correlation coefficient within a sample can be taken as an estimate of the correlation of two variables. (Barlow, 1989)

The highest correlation appears between pH no. 1 and no. 2 (figure 5). So it is *likely* that the quality of pH-measurements is high. The different nutrients correlate highly. The figure also shows a strong correlation between temperature and $NO_2 + NO_3$. And pH (no. 2) has a strong correlation with temperature, chlorophyll and $NO_2 + NO_3$. There is little to no correlation between NH_4 and pH (no. 2) as well as chlorophyll. So NH_4 is not a determining factor in further analysis. The following data will be used for subsequent analysis: pH (no.2), temperature, chlorophyll and $NO_2 + NO_3$.

First of all, the correlation between biomass and pH is summarised in figure 6. As is seen in the chart, the correlation between the amount of chlorophyll A and pH is negative. What can be observed is that more algae grow in more acidic water. In this chart the correlation, R^2 of 0,41, can be found.

R-squared was calculated with this formula:

$$R^2 = \frac{\sum(y - \bar{y})^2 - \sum(y - \hat{y})^2}{\sum(y - \bar{y})^2}$$

This measures the proportion of the total variation in Y that is explained by the simultaneous predictive power of all the explanatory variables, through the multiple regression model. (Agresti, Finlay, 1997)

Secondly, the correlation between pH and nutriënts is summarised in figure 7. The

correlation is negative. The amount of correlation is relatively high (R^2 of 0,38), so there is a *plausible* relation between pH and nutrients.

Thirdly, figure 8 shows the correlation between chlorophyll and pH when chlorophyll is normalised for temperature. This was calculated with this formula (Agresti, Finlay, 1997):

$$E(Y) = \alpha + \beta_1 X_1 + \beta_2 X_2 + \ldots + \beta_k X_k$$

The correlation remains negative. When chlorophyll is normalised for temperature, it reduces R^2 in comparison with figure 7. So it is very *likely* there are no positive effects of temperature on the correlation between pH and chlorophyll.

Finally, the relation of temperature and nutrients is summarised in figure 9. The correlation appears to be negative. The relation is relatively strong, according to the R^2 of 0,50. Thus it is possible that temperature has a negative impact on available nutrients.

As is seen in figure 5, there appears to be little correlation between nutrients and biomass, which indicates lack of effect of nutrients on the correlation between biomass and pH. It can be stated that there is little relation between nutrition and algae growth.

So, it can be observed that it is very *likely* that ocean acidification effects the amount of nutrients. However, it is not certain if the relation between nutrients and pH is influenced by the amounts of chlorophyll.

Last, it is stongly suggested ocean temperature has no visual effects on algae growth (within the temperature limits of the measurements).

5 Conclusion

Altogether, it is very *likely* ocean acidification positively affects algae growth. As is stated before differences in pH have a positive effect on the amount of biomass in seawater. Ocean acidification also positively affects nutrients, which can thereby be related to biomass. In conclusion, there appears to be a positive relation between ocean acidification and algae growth which is directly opposite to the hypothesis.

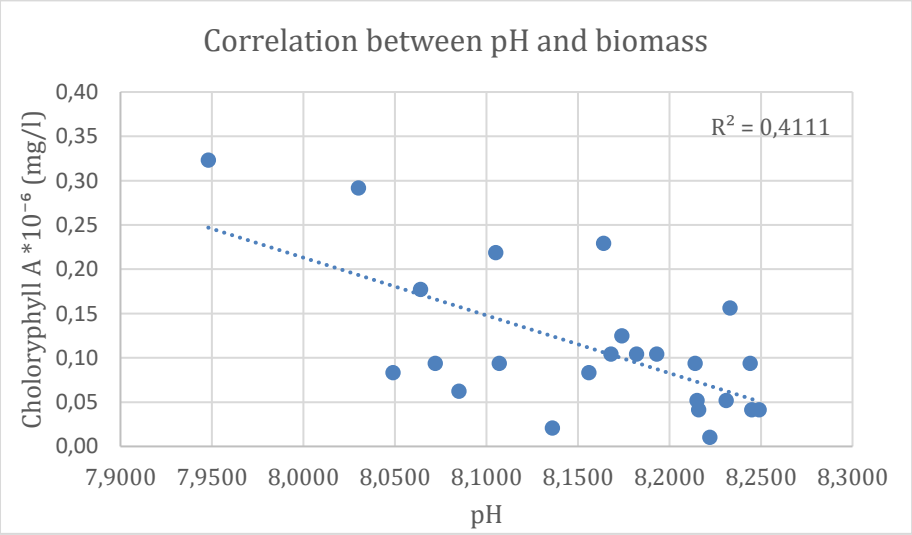
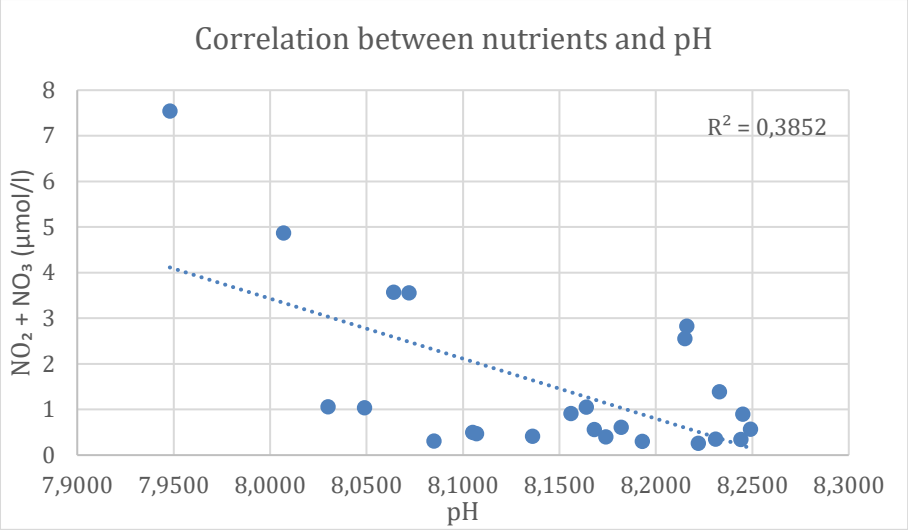


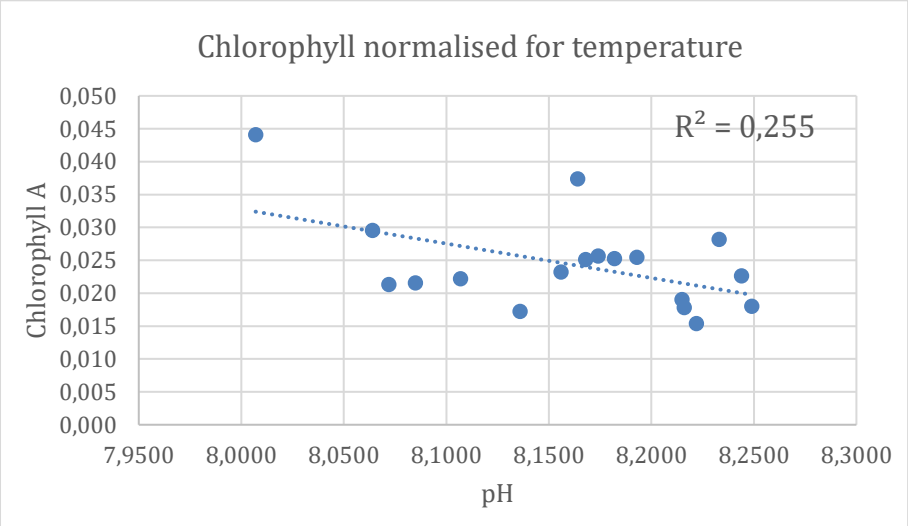
Figure 6: Correlation between pH and biomass

Decrease of pH leads to increase of biomass.



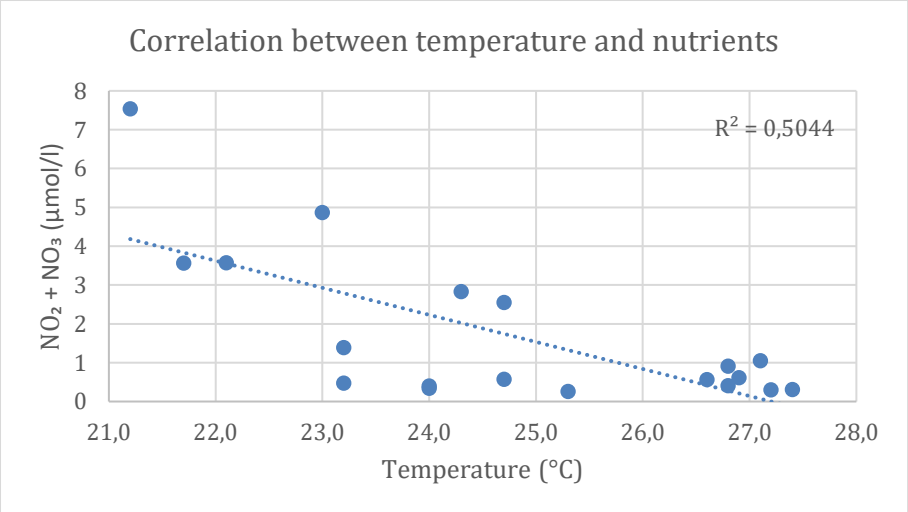
Decrease of pH leads to increase of nutrients.

Figure 7: Correlation between pH and nutrient



Decrease of pH (and temperature) leads to increase of biomass.

Figure 8: Chlorophyll normalised for temperature



Increase of temperature leads to decrease of nutrients.

Figure 9: Correlation between temperature and nutrients

6 Discussion

In the discussion these topics will be mentioned: differences between algae species and morphology, geographical location, weakness of *in situ* observational studies, quality of measurements and fluctuations in pH.

There appears to be a negative relation between the pH and the chlorophyll in our results. This is not only in contrast to our expectations, but also in contrast to different lab studies which have shown that ocean acidification has adversely affected calcifying algae (Kroeker, Guinotte, Fabry, Baginni *et al.*). We do not know which types of algae were present and therefore also not how much of these algae were calcifying algae. Some species of algae have shown to be positively affected by lower pH-values, such as *Padina* and *cystoseira* (Johnson *et al.*; Baggini *et al.*). This could indicate that certain species of non-calcifying algae benefit from ocean acidification, which can exceed the amount of calcifying algae that suffer from it. This could cause an increase in chlorophyll. Important to remember is that this is merely a possible cause. More research to different species of algae should be done to prove this.
This negative relation can also be due to other factors which we haven't discussed yet, such as salinity, which we have not measured.

An important parameter which was not analysed in this study, is the morphology of calcifying algae. Only the volume of algae was measured. However, it appears that not only the amount of algae is affected by acidification, but also the morphology of calcifying algae is impacted (Langer *et al.* 2012; Rouco *et al.* 2013). Coccolith length is an important parameter, as it shows that the calcification rate might be hampered.

As the data sets used for this analysis do not account for the difference between various algae species, the question remains as to how different species response to ocean acidification in the natural environment. This can be clarified in subsequent research.

Another cause for the negative relation between pH and chlorophyll can be explained through the geographical location where the sample data was collected.

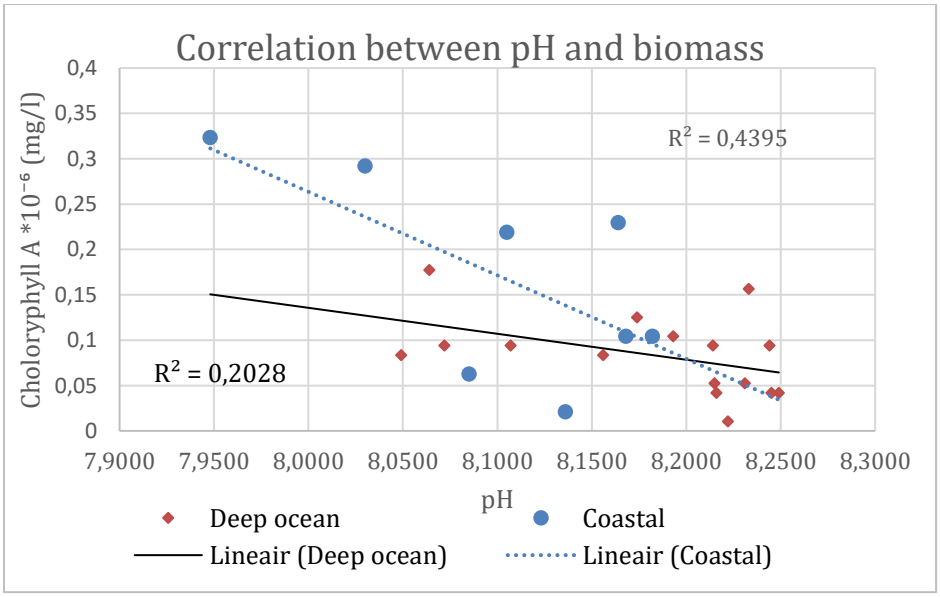


Figure 10: Coastal measurements compared to deep ocean measurements

What stood out was the significant difference between ocean samples and coastal samples (taken in harbours). In the ocean the pH measurements as well as the chlorophyll results were significantly lower than in coastal regions (figure 10). This could have resulted in biased correlations between pH and chlorophyll.

The main weakness of *in situ* observational studies is the potential for significant, but hard to detect effects of confounding factors that vary among locations with (or independent of) seawater carbonate chemistry. Waters emanating from deep-sea vent sites, for example, are rich in carbon dioxide, but often have high concentrations of methane, sulfide, heat and other parameters (Riebesell *et al.* 2010).

At the time of acquisition of the samples, all measurements went through the same procedure. The second pH-measurement was taken at least 10 minutes after the sea water was collected. This could've resulted in the temperature of the sea water dropping, or rising, whereas pH results have to be corrected back to the *in situ* temperature of the ocean. Because these differences in temperature were considered too insignificant, this has not been done.

During analysis of the samples, we found it not possible to collect the dataset at a wavelength of 645 nm. This is most likely due to a chemical reaction between the cuvette and the filter membrane. The used membranes were different from the membranes that are usually used, Fisherbrand™ Grade 122 Cellulose General Purpose Filter Paper. Since the wavelength of 665 nm was measured instantly after the tubes had been beaten, it can be assumed that this chemical reaction had not, or only slightly taken place. Although it should be taken into account that this has effected the measurements of the chlorophyll A. As mentioned earlier, chlorophyll B has not been measured.

In this research any fluctuations of pH during different hours of the day is not taken into account, which leads to possible natural fluctuations of pH not accounted for.

7 Acknowledgements

University of Amsterdam, Institute for Biodiversity and Ecosystem Dynamics: Katja Peijnenburg. Naturalis Biodiversity Center. Het 4e Gymnasium: Jens Dirkse. Prins Bernhard Cultuurfonds. Hanna Instruments. HenQ. Even Naturals. Wolfsbergen & Osnabrug.

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