We thank anonymous Referee #1 for his/her constructive criticism and valuable comments. In the following we address the points brought up, with referee comments in boldface and author responses in normal typeface.

The main comment I have is that whereas some limitations of lab experiments are acknowledged (age of cultures, lack of evolution), other major ones are not, and need to be. In particular, the experience with OA impacts on N2-fixation suggests that meta-analysis is not always the best way of elucidating the right answer. The large majority of experiments conducted have found that OA stimulates an increase in N2-fixation. However, they were all carried out at elevated iron concentrations compared to the open ocean. The study of Shi et al (2012), at more realistic iron, found the opposite effect. This should ring alarm bells for meta-analysis studies because even if an infinite number of lab studies were to be carried out then the overriding majority conclusion could still be completely wrong if they were mostly carried out under unrealistic environmental conditions, and if that difference alters the response obtained. This doesn't mean that the results of this study are not valuable (no single approach to studying OA is perfect), but it is helpful in the interpretation to consider the possibility of this sort of error. Another potential source of error is that lab experiments are carried out on monocultures isolated from the ecosystem with which they normally closely interact. However, this does not hold for the mesocosm experiments.

We agree with the referee. We will extent the paragraph about limitations of the single lab-experiments used in our meta-analysis and highlight the points mentioned above.
One should note, however, that the field of carbonate chemistry research has evolved over more than a decade and that many of the experiments used in our meta-analysis were conducted after standardized procedures, following the "Guide to Best Practices for Ocean Acidification Research and Data Reporting" (Riebesell et al. 2010).

14858/23: the other limitations should also be acknowledged.

The sentence will be changed to: "As the data sets used in this meta-analysis do not account for adaptive responses, ecological fitness and ecosystem interactions, the questions remains how these physiological responses play out in the natural environment."
In order to keep the abstract short and straightforward, we will address limitations of the single carbonate chemistry experiments in the discussion section.

14859/13: the impacts are not likely to be large on a centennial scale, see for instance the work of Christoph Heinze. The word "Thus" is any case not justified, because showing that there is an effect is very different from showing that the effect is significant.

The sentence will be rephrased.

14859-14860: it would be helpful if at this point it could also be explained how this paper differs from previous studies by Findlay et al and Ridgwell et al.

We will also refer to differences between meta-analyses by Findlay et al. and Ridgwell et al.

14860/15: how many studies?

The number of studies included in our meta-analysis is specified in the result section.

14862/15: how many experiments were excluded on this basis?

The number of experiments excluded on this basis will be added.

14863-14864: the precise equation/method for allocating weights should be provided, and the weights listed as an extra column in table 1.

We agree and will rewrite the paragraph as follows: “Using the variance v_i and the mean of the response ratio L_i for each experiment *i*, Cochran‘s Q (Cochran, 1954) was computed. With the help of Q an estimate of the between experiment variance (σ^2_λ) was obtained (Hedges et al., 1999). The weighted mean of the log response ratio $\overline{L^*}$ is given by:

$$\overline{L^*} = \frac{\sum_{i=1}^{k} w_i^* L_i}{\sum_{i=1}^{k} w_i^*} \qquad (2)$$

where *k* is the number of studies and $w_i = 1/(v_i + \sigma^2_\lambda)$.

Subsequently, the standard error of the weighted mean was estimated (see Eq. 7 in Hedges et al., 1999) and the confidence intervals were calculated.

Including weights in table 1 would seriously impair the clarity of the chart, as all the studies needed to be separated by experiment and CO_2 manipulation level. As the table is supposed to focus on the overall responses of coccolithophores within the studies, we would like to keep it as lucid as possible.

table 1: the criterion for distinguishing "some response" from "no response" should be described in the caption or the main text.

To clarify this, we will change the caption of the table to: “Summary of the available carbonate chemistry manipulation experiments and the responses of *Emiliania huxleyi* as reported by the authors of those studies. Symbols indicate: — no significant response, ╱ increased response, ⌒ non-linear response and ╲ decreased response.”

14870/2: "Another proposed explanation for the high difference in variance between..."

This will be changed as suggested.

14871/3: "2009), overall there is nevertheless a generally negative..." (these results do not say anything about how large the strain-specific variations are)

This sentence will be rephrased: “Although some strains of *E. huxleyi* appear to be less sensitive to ocean acidification (Langer et al., 2009), the species shows a negative response towards reduced pCO_2 levels in our meta-analysis, suggesting that strain-specific variations are small compared to the generally negative effect of ocean acidification on this species.”

14871/11: "lead to a reduction in" rather than "minimize"

Will be changed to “decrease the confidence interval”

14871/21: although it should be noted that this effect is not observed in E. huxleyi, which has been most intensively studied.

We agree with the referee. For this reason, we already draw a distinction by saying: “[…] – at least for *Gephyrocapsa oceanica* – [...]”

14872/5: an increase in calcification rate at high CO2 does not necessarily mean that the species is benefitting from the high CO2 (resources can be reallocated, e.g. at the expense of reproduction rate).

This will be clarified.

14872/21-25: I think there are insufficient data (N=3) to conclude that the most prevalent species are the ones most affected.

We agree with this comment and will accommodate this in the revised version.

14874/17-19: but this would imply that they calcify to no purpose, which is hardly likely.
We will revise this section.

table 1: add extra colum for weightings. spelling mistakes in specifics column. "- no response" in caption.
The mistakes will be corrected. Concerning the weightings please see the reply above.

figs 1-3: remind readers in the captions that these are responses relative to 280 ppmv.
This will be changed.

References:
Riebesell U., Fabry V. J., Hansson L. & Gattuso J.-P. (Eds.), 2010. Guide to best practices for ocean acidification research and data reporting, 260 p. Luxembourg: Publications Office of the European Union.

We thank anonymous Referee #2 for his/her constructive criticism and valuable comments. In the following we address the points brought up, with referee comments in boldface and author responses in normal typeface.

A main critical question that the authors should clarify is about the need and innovative aspects of this work since there are other meta-analyses ex- ercises performed and published on this topic; for examples see Findlay et al. (2011). The authors mentioned the accordance with the Findlay et al., 2011 meta-analyses results. The general negative effects of OA on Ehux calcification and PIC/POC has already been shown. What can we learn more on the coccolithophore response to OA mainly from the meta-analyses of monoclonal culture experimental results that haven't already been published? In addition, the large majority of data available and presented here are from Ehux and for all other tested species there are not enough data to do a meaningful meta-analysis.

As mentioned in our manuscript, other meta-analyses did not specifically focus on coccolithophores, except for the work by Findlay et al. (2011). However, these authors only analyzed how ocean acidification influences the ratio of PIC to POC in *E. huxleyi* and only included 15 single experiments in their meta-analysis. Besides PIC/POC, we also included calcification and photosynthesis responses in our analysis and used 48 single experiments to do so. The meta-analyses by Hendriks et al. (2010) and Kroeker et al. (2010, 2013) also analyzed calcification and photosynthesis responses of coccolithophores but included only 2-19 single experiments in their analyses (see manuscript for a detailed listing of experiments used in their studies). We are confident that the much larger dataset used in our meta-analysis justifies the publication of our work.

Although the majority of data dealing with coccolithophore responses to ocean acidification examined *E. huxleyi*, we found it important to make a distinction between the single species, as their responses to OA is quite diverse. This difference can clearly be identified with the help of our analysis, although the available datasets for *C. braarudii* and *G. oceanica* are rather limited.

Since a main justification for having this article published is to use a larger set of experiments to allow a more robust prediction of the impacts of OA on coccolithophores, it is key that the authors clearly make a comparison with the number of data previously used and the benefit of having this new meta-analysis.

This has been done in the last paragraph of the introduction, where the number of data used in the meta-analyses by Hendriks et al. (2010) and Kroeker et al. (2010, 2013) are listed. The number of data used in the meta-analysis by Findlay et al. (2011) and differences between their and our analysis will also be added.

I found the title -Responses of coccolithophores to ocean acidification: a meta-analysis- misleading, since the number of living coccolithophore species is >200 and having the responses of 4 tested heterococcolithophore species mainly from culture experiments doesn't resolve the response of coccolithophores to OA.

We believe that using the plural „coccolithophores“ does not imply that we are resolving responses of all living coccolithophore species to OA. It rather refers to coccolithophores that have been the subject of ocean acidification research, which also becomes apparent in the abstract. If possible we would prefer not to change the title.

It is mentioned that "The perturbation method appears to affect photosynthesis, as responses varied significantly between total alkalinity (TA) and dissolved inorganic carbon (DIC) manipulations". This needs to be clarified since it is hard to conclude this on the basis of a meta-analysis. This hypothesis should be probably tested in a controlled experiment for example as, shown in Hoppe et al. (2011).

We agree that it is prematurely to firmly conclude that the perturbation method affects photosynthesis. That is why we discuss the topic with great care and conclude that the subject needs to be further clarified. However, we will revise the paragraph and highlight the points mentioned by the referee.

The results shown in Table 2 should be checked carefully since it is suggested that the C. braarudii results in Krug et al. (2011) and Langer et al. (2006) are completely different when instead they are very similar.
It is not clear to us why the reviewer comes to the conclusion that responses of *C. braarudii* are very similar in the studies mentioned. Responses depicted in table 2 are directly taken from the respective papers.
Langer et al. (2006) state: "In the *Coccolithus pelagicus* [n.b. presently referred to as *Coccolithus braarudii*] cultures neither PIC nor POC content per cell changes significantly over the CO_2 range tested […], yielding a stable PIC/POC ratio."

Krug et a. (2011) state: "POC production rates of *Coccolithus braarudii* were highest at intermediate *p*CO2 [...] and declined towards lower and higher levels [...], although more pronounced in case of the latter [...]. Calcification rates, although quite noisy, clearly decreased towards higher *p*CO2 levels [...]. The considerably stronger decrease in PIC compared to POC production led to a pronounced drop in PIC/POC […]."

It would be important to add the Conclusions section to summarize the main findings and the differences with previous similar meta-analysis exercises.
We feel that this is dealt with in the discussion section and that it is redundant to add a conclusion.

Findlay et al., 2011 is not listed in the references.
The reference his will be added.

'PIC/POC ratio' should be changed to 'PIC/POC'; 'ratio' is redundant.
This will be changed.

In the introduction when mentioning the ballasting properties of coccolithophores the paper by Ziveri et al., 2007 could be mentioned.
Good remark, this will be added.

Responses of coccolithophores to ocean acidification: a meta-analysis

J. Meyer* and U. Riebesell

(GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany)

*Judith Meyer, e-mail: jumeyer@geomar.de

Abstract

Concerning their sensitivity to ocean acidification, coccolithophores, a group of calcifying single-celled phytoplankton, are one of the best-studied groups of marine organisms. However, in spite of the large number of studies investigating coccolithophore physiological responses to ocean acidification, uncertainties still remain due to variable and partly contradictory results. In the present study we have used all existing data in a meta-analysis to estimate the effect size of future pCO_2 changes on the rates of calcification and photosynthesis and the ratio of particulate inorganic to organic carbon (PIC/POC) in different coccolithophore species. Our results indicate that ocean acidification has a negative effect on calcification and the cellular PIC/POC ratio in the most abundant coccolithophore species *Emiliania huxleyi* and *Gephyrocapsa oceanica*. In contrast the more heavily calcified species *Coccolithus braarudii* did not show a distinct response when exposed to elevated pCO_2/reduced pH. Photosynthesis in *Gephyrocapsa oceanica* was positively affected by high CO_2, while no effect was observed for the other coccolithophore species. There was no indication that the method of carbonate chemistry manipulation was responsible for the inconsistent results regarding observed responses in calcification and the PIC/POC ratio. The perturbation method, however, appears to affect photosynthesis, as responses varied significantly between total alkalinity (TA) and dissolved inorganic carbon (DIC) manipulations. These results emphasize that coccolithophore species respond differently to ocean acidification, both in terms of calcification and photosynthesis. Where negative effects occur, they become evident at CO_2 levels in the range projected for this century in case of unabated CO_2 emissions. As the data sets used in this meta-analysis do not account for adaptive responses, ~~and~~ ecological fitness and ecosystem interactions, the questions remains how these physiological responses play out in the natural environment.

1. Introduction

Coccolithophores, a globally distributed group of marine haptophytes, are major primary producers in the ocean and the most prolific calcifying organisms on our planet (Brownlee & Taylor, 2004; Shutler et al., 2010). By performing photosynthesis and calcification, they contribute to both biological carbon pumps, the soft tissue pump and the carbonate counter pump. While the former supports carbon sequestration in the ocean through production and sinking of organic matter to depth, the latter decreases the ocean's capacity to take up CO_2 due to the reduction of surface layer alkalinity. Moreover, by providing ballast material, which accelerates sinking velocities of organic particles to depth, coccolithophore-derived calcite contributes to enhancing carbon sequestration to depth (Klaas & Archer, 2002; Armstrong et al., 2002; Ziveri et al., 2007). Thus, changes in the contribution of coccolithophores to ocean primary production could~~an~~ ~~significantly~~ potentially impact global carbon cycling (Riebesell et al., 2009).

In the face of global change phytoplankton are subjected to rapid alterations in their environmental conditions. Due to the sensitivity of calcification to ocean acidification, coccolithophores are considered to be among those, which may be adversely affected in a high CO_2 future ocean. While impacts of ocean acidification on coccolithophores have been studied extensively (for review see e.g. Riebesell & Tortell, 2011), variable and partly conflicting responses were observed in different perturbation studies (for a summary see Tables 1 and 2). Differences in experimental conditions, such as in light intensity, temperature, salinity, nutrient concentration and $p\mathrm{CO}_2$ levels have been attributed as possible causes for those variations. But even studies with comparable experimental conditions provided deviating responses of coccolithophores. Some of this divergence was shown to be related to species- and strain-specific differences (Langer et al., 2006, 2009). But also the method of carbonate chemistry manipulation, whether through changes in total alkalinity (TA) or dissolved inorganic carbon (DIC), was discussed as possible cause for some of the observed discrepancies (Iglesias-Rodriguez et al., 2008; Shi et al., 2009).

Building on the extensive literature on coccolithophore responses to ocean acidification, the present study aims to provide statistically and methodologically robust estimates for those responses. In particular, we intend to answer the question whether increasing seawater acidity alters calcification, photosynthesis and the PIC to POC ratio in acclimated cultures of coccolithophores. We further assess whether the observed responses are affected by the carbonate chemistry manipulation method and if they differ between coccolithophore species, thus trying to address some of the inconsistencies in the existing studies. Recent meta-analyses conducted by Kroeker et al. (2010, 2013) and Hendriks et al. (2010) did not specifically focus on coccolithophores but analyzed responses of many different taxa to ocean acidification. Although coccolithophores were included in those meta-analyses, only a few experiments (Kroeker et al., 2010: 13 experiments, Hendriks et al., 2010: 2 experiments for calcification responses, 12 experiments for photosynthetic responses, Kroeker et al., 2013: 19 experiments) were considered and no distinction was made between different coccolithophore species. The meta-analysis by Findlay et al. (2011) focused on *Emiliania*, *huxleyi*, but only investigated the species' PIC/POC response to ocean acidification (15 experiments were included in the analysis). ~~Hence, i~~In our approach a larger set of experiments and response variables was~~as~~ analyzed, allowing for a more robust prediction of the impact of ocean acidification and~~ the~~ related changes in seawater chemistry on coccolithophore physiological performance.

2. Materials and Methods

2.1 Literature search

A literature search was conducted to assemble all published data sets on CO_2/pH sensitivities of coccolithophore calcification and photosynthesis. As a first step the ISI database Web of Science (www.webofknowledge.com) was scanned for appropriate studies. Additional literature was obtained from the EPOCA (European Project on OCean Acidification) database (www.epoca-project.eu) and from the associated blog (www.oceanacidification.wordpress.com). Subsequently, the reference lists of all studies identified by this approach were scanned for other relevant literature.

Experimental data were extracted directly from the published papers or, if not reported therein, from the PANGEA® archive (www.pangaea.de). If the information could not be retrieved from either source, the first author of the study was contacted directly.

2.2 Data selection

All studies in which the carbonate system was altered and the effect on coccolithophores reported, comprising both laboratory and field experiments, were selected for this meta-analysis. Studies that varied other environmental factors in addition to seawater carbonate chemistry, such as light intensity, day length, temperature or nutrient availability, were also incorporated. Data of particulate inorganic (PIC) and organic carbon (POC) production rates, *p*H values, carbonate system parameters and experimental conditions (light level, day length, temperature, nutrients) were obtained for the control (ambient or pre-industrial pCO_2 level) and the experimental treatments (elevated pCO_2 level). If PIC and POC were provided as quota values on a per-cell basis, production rates were calculated by multiplying the growth rates (μ) with the cell quota of organic or inorganic carbon.

The following pCO_2 levels were chosen to compare the responses of *Emiliania huxleyi* to pre-industrial carbon dioxide concentrations of ~ 280 parts per million (ppm):

(1) ~ 380 ppm – reflecting the present day pCO_2 level,

(2) ~ 780 ppm – the pCO_2 level projected for the end of this century under the SRES A1B scenario, IPCC Report 2000 (Nakicenovic et al., 2000), and

(3) ~ 1000 ppm – the pCO_2 level projected for the end of the century under the 'worst case' emission scenario A1FI, IPCC Report 2000 (Nakicenovic et al., 2000).

Since there was not a sufficient number of studies investigating the responses of *Coccolithus braarudii* and *Gephyrocapsa oceanica* at pCO_2 levels around 780 ppm, only concentrations of ~ 380 ppm and ~ 1000 ppm were used to compare the responses of these species. All experiments where the pCO_2 levels deviated no more than ± 50 ppm from the targeted 380 ppm and no more than ±100 ppm from the targeted 780 ppm and 1000 ppm were included in the analysis. Since the studies by Lefebvre et al. (2012) and Jones et al. (2013) did not meet these specifications, they were excluded from the meta-analysis.

Manipulation of the seawater carbonate chemistry can be achieved in various ways. First, the carbonate system can be adjusted by bubbling with CO_2. This approach increases [CO_2], [HCO_3^-] and DIC, decreases pH and [CO_3^{2-}] and does not change the alkalinity. Second, acid can be added, which increases [CO_2] and [HCO_3^-], decreases the alkalinity and [CO_3^{2-}] and does not change DIC. In both manipulations the saturation state (Ω) decreases as well. Although there are other ways to adjust the carbonate system, the above-mentioned methods are the ones most commonly used. It was noted which manipulation method was applied to decrease the pH in each study. Subsequently, a separate meta-analysis was conducted in order to analyse whether responses of coccolithophores varied between the methods. Here, only responses to a pCO_2 elevation from pre-industrial levels to 780 ppm and 1000 ppm were included in the analysis. On this basis 22 experiments were excluded.

When studies reported results from multiple carbonate system perturbation experiments, all individual experiments were included in the analysis. The same applied when there were different experiments with various species or strains.

If not only the carbonate system, but also other factors such as light intensity or day length were changed in a study, the approach of Kroeker et al. (2010) was adopted and the ambient level of the factor, defined by the authors of the primary study, was used to ensure the comparability between the experiments. If the observed responses of a study did not differ significantly for the ambient and non-ambient levels of a given environmental factor (always regarding the same pCO_2 value), both experiments were included.

The data on PIC and POC production obtained by Iglesias-Rodriguez et al. (2008) were normalized to POC biomass, following the approach suggested by Riebesell et al. (2008). Data shown in Table 1 represent the original measurements reported by Iglesias-Rodriguez et al. (2008) prior to normalization. Müller et al. (2010) did not report PIC and POC production rates in their study, since the sampling time for those data varied and created a bias in the data. By averaging the PIC and POC production rates over time, the bias was minimized and the data were suitable to be included in this meta-analysis.

2.3 Data analysis

Determining differences between the control and treatment groups in response to changes in carbonate chemistry was the first step in our analysis. For this purpose the logarithmically transformed response ratio (L) was calculated for each experiment and response variable (PIC, POC and PIC/POC) as:

$$L = \ln(RR) = \ln(\bar{X}_E) - \ln(\bar{X}_C) \quad (1)$$

where $\bar{X}$ is the mean of a treatment (E) and a control (C) group. The response ratio is logarithmically transformed and unit-less, thus allowing a comparison of data between experiments, which report responses in different units. The effect size is an easy measure of relative change between the control and the treatment group. When $L < 0$, the effect of acidification in the treatment group is negative and when $L > 0$, the effect is positive. A response ratio of zero indicates that there is no effect and that the responses in the control and treatment group are the same. Since not all studies are equally precise, meaning that they are based on different numbers of replicates and variable standard deviations, the simple computation of the mean effect sizes is not to be recommended. Instead, a weighted mean is computed where more precise studies are given more weight.

This meta-analysis of the response ratios follows the approach of Hedges et al. (1999) with a few variations when weighting the effect sizes. A random effects model was used where the assumption is made that the effect of ocean acidification varies between studies (Borenstein et al., 2010). For example, the effect size might differ between strains or it might turn out significant if the response was measured more reliably or if the incubation time was longer. The random effects model accounts for this variation and includes the within-study variance (ν_i) as well as the between study variance (σ^2_λ) when calculating the mean effect for the response variables. Statistical significance for all effect sizes is displayed by the 95% confidence interval. The effect size is considered to be significant ($\alpha = 0.05$), when the confidence intervals do not overlap zero.

Traditionally, when studies report means, standard deviation, and sample size for both the control and treatment groups, a weighted meta-analysis is possible and the variance (ν_i) within the experiment *i* can be calculated (v) can be calculated. . Consequently, studies with a higher number of replicates and lower variance are weighted more heavily, which results in a more robust meta-analysis where the estimate of the effect size is more precise than in unweighted meta-analyses (Hedges & Olkin, 1985). Some of the data required for a weighted meta-analysis, however, were not available for some studies. In those cases v_i was estimated as the average of the computed variances from those experiments where v_i was calculable. In this way it was possible to include all studies in the meta-analysis. Using the variance v_i and the mean of the response ratio $\overline{L_i}$,for each experiment *i*, Cochran's Q (Cochran, 1954) was computed. With the help of Q an estimate of the between experiment variance (σ^2_λ) was obtained (Hedges et al., 1999). The weighted mean of the log response ratio $\overline{L^*}$ is given by:

$$\overline{L^*} = \frac{\sum_{i=1}^{k} w_i^* L_i}{\sum_{i=1}^{k} w_i^*} \qquad (2)$$

where *k* is the number of studies and $w_i = 1/(v_i + \sigma^2_\lambda)$. With the help of Q an estimate of the between experiment variance (σ^2_λ) was obtained (Hedges et al., 1999).

Subsequently, the standard error of the weighted mean was estimated (see Eq. 7 in Hedges et al., 1999) and the confidence intervals were calculated. For all calculations Microsoft Excel ® 2008 was used.

A normal distribution of the mean response ratio was assumed. As described in Hedges et al. (1999), this assumption can be made, because the single response ratios are normally distributed as well.

2.3.1 Identifying heterogeneity

A test for heterogeneity in effect sizes was performed based on the Q-statistic. Q approximately follows the chi-squared distribution with k degrees of freedom. The Null hypothesis of homogeneity among the effects of different experiments is rejected if Q exceeds the 95 % quantile of the distribution. Heterogeneity results in a positive estimate for the between experiments variance σ^2_λ, which leads to a larger total variation, that is the sum of the within and between experiment variance. Consequently, larger standard errors as well as wider confidence intervals for the effect size are computed from the weighted variances.

3. Results

23 studies were obtained from the literature, summarized in Tables 1 and 2. A total of 48 single experiments, which met the above-mentioned criteria, were extracted from these studies to be included in this meta-analysis.

The carbonate chemistry perturbation experiments examining responses of *Emiliania huxleyi* are depicted in Table 1. A total of 19 studies dealt with the responses of 14 different strains to ocean acidification. In most experiments, strains of *Emiliania huxleyi* showed reduced calcification rates with increased pCO_2 concentrations (Barcelos e Ramos et al., 2010; De Bodt et al., 2010; Delille et al., 2005; Engel et al., 2005; Feng et al., 2008; Gao et al., 2009; Hoppe et al., 2011; Langer et al., 2009; Müller et al., 2010; Riebesell et al., 2000; Rokitta & Rost, 2012; Sciandra et al., 2003; Shi et al., 2009; Wuori, 2012; Zondervan et al., 2002). In other experiments some strains showed an optimum curve in response to increasing pCO_2 (Bach et al., 2011; Langer et al., 2009), no significant response (Langer et al., 2009; Richier et al., 2011) or increased calcification rates (Fiorini et al., 2011; Iglesias-Rodriguez et al., 2008; Shi et al., 2009).

Photosynthetic responses were more diverse. In six experiments no response was observed (De Bodt et al., 2010; Delille et al., 2005; Engel et al., 2005; Feng et al., 2008; Fiorini et al., 2011; Hoppe et al., 2011; Müller et al., 2010; Richier et al., 2011), while in another six experiments the POC production increased in response to elevated pCO_2 (Barcelos e Ramos et al., 2010; Hoppe et al., 2011; Iglesias-Rodriguez et al., 2008; Riebesell et al., 2000; Rokitta & Rost, 2012; Shi et al., 2009; Wuori, 2012; Zondervan et al., 2002). Five experiments showed decreasing photosynthesis rates (Bach et al., 2011; Langer et al., 2009; Sciandra et al., 2003; Shi et al., 2009), whereas in three experiments an optimum curve was obtained (Gao et al., 2009, Langer et al., 2009).

The observed PIC/POC ratios are more homogeneous across experiments with most of them decreasing with increased pCO_2 (Bach et al., 2011; Barcelos e Ramos et al., 2010; De Bodt et al., 2010; Delille et al., 2005; Engel et al., 2005; Feng et al., 2008; Gao et al., 2009; Hoppe et al., 2011; Langer et al., 2009; Müller et al., 2010; Riebesell et al., 2000; Rokitta & Rost et al., 2012; Shi et al., 2009; Wuori, 2012; Zondervan et al., 2002). Only in four experiments the PIC/POC ratio did not change with increasing pCO_2 (Iglesias-Rodriguez et al., 2008; Langer et al., 2009; Richier et al., 2011; Sciandra et al., 2003) and in one an increase was observed (Fiorini et al., 2011)

Experiments with other coccolithophore species also revealed varying responses (Table 2). Of the four experiments with *Coccolithus braarudii*, two observed a decrease in PIC production with increased CO_2 levels (Krug et al., 2011; Müller et al., 2010), whereas one observed no response (Langer et al., 2006) and the other a slight increase in the calcification rate (Rickaby et al., 2010). The POC production rates varied just as much and increased in two experiments (Rickaby et al., 2010; Müller et al., 2010), while they did not change significantly in another experiment (Langer et al., 2006). In a fourth experiment a non-linear response was observed (Krug et al., 2011).

In two experiments conducted with *Gephyrocapsa oceanica*, the calcification rates decreased (Riebesell et al., 2000) or did not change significantly (Rickaby et al., 2010) with increasing pCO_2, whereas photosynthetic carbon fixation increased in one experiment (Riebesell et al., 2000) and showed an optimum curve in the other one (Rickaby et al., 2010). The PIC/POC ratio declined in both experiments.

In a fourth coccolithophore species, *Calcidiscus leptoporus*, the calcification response was non-linear, while the photosynthesis rate remained constant over the tested CO_2 range (Langer et al., 2006, Langer & Bode, 2011).

3.1 Effect of ocean acidification on calcification responses

The meta-analysis of calcification responses to elevated CO_2 concentrations revealed different results between the examined species (Figure 1). Increasing CO_2 concentrations from pre-industrial to present day levels had no significant effect on calcification in *Emiliania huxleyi* (lnRR = -0.004). In contrast, the effect of near future CO_2 concentrations under both the 'business as usual' and the 'worst case' scenario had significant negative effects on calcification in this species. This negative effect was more pronounced at 1000 ppm compared to 780 ppm (780 ppm: lnRR = -0.19, confidence interval = -0.07 to -0.30; 1000 ppm: lnRR = -0.38, confidence interval = -0,08 to -0,67).

In *Gephyrocapsa oceanica* an increase from preindustrial to present day CO_2 concentrations had a slightly negative but non-significant effect on calcification. Projected future ocean acidification had a negative mean effect on calcification greater than in *Emiliania huxleyi*, but it was not significant (lnRR = -0.79, confidence interval = 0.61 to -2.19). In contrast, no significant effect of ocean acidification was detected in *Coccolithus braarudii*, where the mean effect sizes were slightly positive at both pCO_2 concentrations. Significant heterogeneity was detected for all calcification responses.

3.2 Effect of ocean acidification on photosynthetic responses

A significant effect of ocean acidification on photosynthesis was observed in *Gephyrocapsa oceanica* for the present-day as well as the high CO_2 concentration, with the mean response at 1000 ppm being more than twice as high (lnRR = 0.57) as the mean response at 380 ppm (lnRR = 0.24; Figure 2). For *Coccolithus braarudii,* a significant positive effect was observed at 380 ppm and a similar but non-significant positive effect at 1000 ppm. No effect of ocean acidification on photosynthesis was observed for *Emiliania huxleyi* at 380 ppm and 1000 ppm. Only at 780 ppm was the mean effect size slightly positive (lnRR = 0.044), but this effect was non-significant. A significant Q-statistic was calculated for all effect sizes.

3.3 Effect of ocean acidification on PIC/POC responses

The observed PIC/POC responses to an increased CO_2 concentration are similar to those observed for the calcification responses (Figure 3). For *Emiliania huxleyi*, there was a larger negative effect on PIC/POC at 1000 ppm (lnRR = -0.39) than at 780 ppm (lnRR = -0.22), but both responses were significantly negative. No effect was observed at present day CO_2 concentrations.

At both CO_2 concentrations a small, non-significant negative effect of a similar magnitude (380 ppm: lnRR = 0.05, 1000 ppm: lnRR = 0.07) was observed for *Coccolithus braarudii*. The strongest effect of ocean acidification on the PIC/POC ratio was observed for *Gephyrocapsa oceanica*. The mean effect size was significantly negative at both $p$$CO_2$ levels, with the negative mean effect size at 1000 ppm (lnRR = 1.37) being more than three times lower than at 380 ppm (lnRR = 0.36). There was significant heterogeneity in all PIC/POC responses.

3.4 Relationship between effect sizes and methodological factors

For the three response variables (PIC, POC and PIC/POC) a further meta-analysis was conducted in order to test whether they varied between the two different carbonate chemistry manipulation methods (constant TA vs. constant DIC) used in the experiments.

This meta-analysis revealed that the mean effects of ocean acidification were not consistent between the two methods (Figure 4). Keeping TA constant and changing DIC resulted in a more negative mean effect size for calcification and photosynthesis as compared to constant DIC and variable TA. However, the observed difference between the mean effect sizes for calcification was not significant ($p = 0.07$) and the overall effect of ocean acidification on calcification was negative, regardless of the manipulation method. In contrast, the mean effect sizes for photosynthesis differed substantially. While no significant effect was observed at constant TA, the effect size at constant DIC was significantly positive. There was significant difference between the mean effect sizes ($p = 0.0001$). The difference between the effect sizes for PIC/POC was only small. Here, ocean acidification had a slightly more negative effect when keeping DIC constant and changing TA. Both effect sizes were, however, significantly negative.

Interestingly, all experiments using *Coccolithus braarudii* and *Gephyrocapsa oceanica* manipulated the pCO_2 in the culture medium by adding acid, i.e. changing TA while keeping DIC constant. Thus, all these experiments were included in the constant DIC treatments, while only experiments with *Emiliania huxleyi* were included in the constant TA treatments. In order to eliminate a possible bias due to the unequal distribution of coccolithophore species across carbonate chemistry manipulation methods, a separate meta-analysis was conducted. This analysis only included experiments of *Emiliania huxleyi* and determined the variation of effect sizes between carbonate chemistry manipulations (Figure S1, supplement). The results of this analysis were not significantly different from those obtained from the analysis performed on the full data set. A bias due to the unequal distribution of species between treatments can therefore be ruled out.

4. Discussion

The difference in variance between single studies is statistically described as heterogeneity. The term indicates that there is more variability in results than would be expected from the sampling distribution. Differences in the experimental setup, deviations in the measuring method and biological differences between the examined organisms can generally explain the existence of heterogeneity.

Heterogeneity in effect size was detected in all analyses in the present study. In retrospect, this finding justifies the use of a random-effect model in this meta-analysis. In contrast to the fixed effect model that only includes variance within the studies, the random effects model accounts for the variance between and within single studies.

Our study revealed that heterogeneity in mean effect sizes is not due to different carbonate chemistry perturbation methods. The differences between TA and DIC manipulations in the carbonate chemistry were shown not to cause strong variations in biological responses in coccolithophores - with a possible exception in photosynthetic responses. Another proposed explanation for the high difference in variance between studies could be the morphological and genetic differences of single coccolithophore strains. A high physiological variability was already shown to exist in the coccolithophore *Emiliania huxleyi* (Iglesias-Rodriguez et al., 2006; Cubillos et al., 2007), with different strains and ecotypes exhibiting diverse responses to ocean acidification (Langer et al., 2009; Hoppe et al., 2011). Moreover, adaption processes of clones that are kept in culture over years could further result in variable responses in CO_2 perturbation experiments (Ridgwell et al., 2009). Thus, a large part of the variance between the analyzed studies is most likely due to intra-species variability of coccolithophore species, especially in *Emiliania huxleyi*. A further reason for heterogeneity in mean effect size could be discrepancies in calculating the carbonate system from measured parameters. As mentioned earlier in this study, all components of the carbonate system can be calculated if two variables, e.g. *p*H and DIC, are known. A recently published study suggests, that the *p*CO_2 concentration measured in CO_2 perturbation experiments differs strongly between calculations (up to 30%), when the input parameters for these calculations were different (Hoppe et al., 2012). The authors state that some publications may not be comparable with each other, as *p*CO_2 values might have been underestimated when they were calculated from TA and DIC, influencing the interpretation of coccolithophore responses. This finding also has implications for the present study, as some heterogeneity in mean effect size might be due to inconsistencies in calculating *p*CO_2.

The aim of this study was to synthesize the available data of coccolithophores biological responses to ocean acidification in order to more robustly estimate the actual effect of a lowered seawater *p*H on those calcifying organism. Despite known intra-specific variability, a negative effect of ocean acidification on calcification as well as on the cellular PIC/POC ratio was observed for the dominant and cosmopolitan species *Emiliania huxleyi.* Our results are in accordance with findings from a meta-analysis conducted by Findlay et al. (2011), who also identified a negative correlation between the cellular PIC/POC ratio in *Emiliania huxleyi* and the *p*CO_2 concentration in the culture medium. ~~The observations from the present study suggest that a~~Although some strains of ~~*Emiliania*~~ *E. huxleyi* ~~might be~~appear to be less sensitive to ocean acidification (Langer et al., 2009), the species shows a negative response towards reduced *p*CO_2 levels in our meta-analysis, suggesting that strain-specific variations are small compared to~~will be~~ the generally negative effect of ocean acidification on this species.~~small compared to the generally negative effect of ocean acidification on this species, which results in decreased calcification rates.~~

Calcification and PIC/POC in the coccolithophore *Gephyrocapsa oceanica* was even more negatively affected by future ocean acidification than in *Emiliania huxleyi*, indicating that *G. oceanica* is even more sensitive to changes in *p*CO_2 and *p*H. Although the meta-analysis with this species was based on only two studies and a significant effect on the calcification response was not observed, the mean effect sizes were even more negative than those observed for *Emiliania huxleyi* at 1000 ppm. We assume that the inclusion of more studies to the meta-analysis would likely minimize decrease the confidence interval of the mean effect size, resulting in a significantly negative effect of ocean acidification on calcification in *Gephyrocapsa oceanica*. The strong negative effect of ocean acidification on the PIC/POC ratio in this species was not only due to the strong decrease in calcification, but also a consequence of an increase in the photosynthesis rate with increasing pCO_2. Apparently, this species profits more from high pCO_2 levels during photosynthesis than the others. This might - at least for *Gephyrocapsa oceanica* - confirm the hypothesis that some coccolithophores might benefit from higher CO_2 concentrations, since their rate of carbon fixation is below CO_2 saturation at pre-industrial CO_2 levels (Riebesell et al., 2000, 2004; Rost et al., 2003; Nimer & Merrett, 1996). Higher CO_2 concentrations in the water would thus allow them to more efficiently assimilate and fix carbon during photosynthesis and thus increase their photosynthesis rate (Rost et al., 2008). It is further suggested that an increase in the photosynthesis rate might buffer a possible negative effect of ocean acidification on calcification (Ries et al., 2009). When photosynthesis becomes more efficient and additional energy is provided due to enhanced photosynthetic activity, the building and maintenance of coccoliths could be facilitated. This hypothesis, however, was not confirmed by the present analysis, since the species that showed the most positive effect on photosynthesis, *Gephyrocapsa oceanica*, was also the one where the effect of ocean acidification on calcification was most negative.

For *Coccolithus braarudii* the results from the present study confirm the hypothesis that this species is insensitive to elevated pCO_2 levels within the tested range (Langer et al., 2006). To some extent, i~~I~~t might even ~~seems to~~ benefit ~~to some extent~~ from higher CO_2 concentrations, as it ~~by~~ exhibits~~showing~~ a slightly positive photosynthesis response.

The results for the effect of ocean acidification on calcification gained by the present study are consistent with the observations by Kroeker et al. (2010, 2013) (Figure 5). These authors included responses of all coccolithophore species in one meta-analysis without distinguishing between species, and found a negative but non-significant effect of ocean acidification on calcification. They state that the absence of a significantly negative result might be due to the species-specific responses of coccolithophores, which can be confirmed by our study.

With some coccolithophore species being generally more sensitive with regard to ocean acidification than others, a replacement of sensitive strains by more tolerant strains of the same species or a shift in species composition is probable. It cannot be assessed if a general decline in the abundance of coccolithophores with a replacement by other photoautotrophic organism is possible, as long as the role of calcification in coccolithophores is not completely understood. What implications a reduced calcium carbonate production has on the physiological performance and ecological fitness of coccolithophores therefore needs to be further evaluated. Considering that the more prevalent coccolithophore species appear to be ~~most~~ vulnerable to ocean acidification, a local or global shift in the species composition or a replacement by other photoautotrophic organisms may occur and could affect higher trophic levels and ocean biogeochemical cycling.

Differences between TA and DIC manipulations were not the cause of variable calcification and PIC/POC responses between experiments, confirming earlier results by Kroeker et al. (2009), Findlay et al. (2011) and Hoppe et al. (2011) and following the reviews of Schulz et al. (2009) and Ridgwell et al. (2009). In contrast, mean effect sizes on photosynthetic rates were significantly different between the two manipulation methods. Whereas no effect of ocean acidification on photosynthesis was observed for the constant TA manipulations, the effect in the constant DIC manipulations was significantly positive. This finding is surprising, as the modifications of the carbonate system induced by the different manipulation methods are very similar, particularly in the range of carbonate chemistry changes projected to occur until the end of this century (Schulz et al., 2009). Although bubbling with CO_2 more closely resembles predicted changes in the oceans carbonate chemistry, because dissolved inorganic carbon increases while total alkalinity remains unchanged, the modification of each carbonate system parameter (pH, [CO_2], [CO_3^{2-}] and ΩCa) is rather similar. An exception is the concentration of HCO_3^-, which increases slightly more in experiments where the pCO_2 concentration is altered by CO_2 bubbling (constant TA manipulation). As not only CO_2, but also HCO_3^- is known to be a carbon source for photosynthesis in most phytoplankton species (Riebesell, 2004), one could assume that the higher HCO_3^- concentration in the constant TA manipulations was responsible for the observed difference in photosynthetic responses between manipulation methods. However, a higher rather than a lower photosynthesis rate would be expected in the constant TA manipulations compared to the constant DIC manipulations, as more inorganic carbon in the form of HCO_3^- would be available for photosynthesis. Thus, it does not seem likely that the slight deviation in the HCO_3^- concentration is responsible for the difference in mean effect sizes between manipulation methods. Nevertheless, ~~the~~ discrepancies between the two methods of CO_2 manipulation observed in the present study are consistent with findings of Kroeker et al. (20<u>10</u>~~09~~). In their meta-analysis a comparison of photosynthetic responses between manipulation methods also showed that keeping TA constant while increasing DIC caused a more negative effect. The deviation between the mean effect sizes was also significant in their study.

~~Despite deviating~~<u>Although variable</u> photosynthetic responses ~~observed~~ <u>have been observed</u> in different carbonate chemistry perturbation experiments, <u>it remains to be clarified what causes</u>

these differences. To date, s~~s~~tudies and reviews have mainly focused on revealing the reason for diverse calcification responses in coccolithophores (Ridgwell et al., 2009; Schulz et al., 2009). This is probably because ocean acidification is regarded to have a greater impact on calcification in those species than on photosynthesis. While the present study shows that this assumption holds true, a clear understanding of all physiological processes and their relevance for coccolithophore ecological fitness is necessary to realistically assess the influence of future ocean acidification on these organisms.

A limitation of the carbonate chemistry manipulation experiments included in this meta-analysis is the short duration of the experiments. As a result, they do not account for possible adaptation processes of coccolithophores that might occur over a longer time-period, and only test for non-adaptive responses. A recent study investigated evolutionary adaptation in *E. huxleyi* in a long-term experiment (Lohbeck et al., 2012). In this study a population adapted to higher pCO_2 levels showed significantly higher calcification rates than the control population. Although adaptation did not restore calcification rates under elevated pCO_2 to those measured under ambient pCO_2 levels, this observation highlights the possibility of adaptive evolution in coccolithophores. ~~It remains speculative, however, whether those results can be extrapolated from culture experiment to the natural environment (Lohbeck et al., 2012).~~ If species like *Emiliania huxleyi* and *Gephyrocapsa oceanica* ~~remain competitive despite being less calcified~~can adapt to decreased *p*H levels, consequences for the whole ecosystem might be averted. It remains speculative, however, whether results from monocultural experiments can be extrapolated to the natural environment. This also has to be acknowledged when interpreting results of the present study. Generalizations from laboratory observations must be drawn with great care and it has ~~Nevertheless, the strength of the carbonate pump could decline, thereby reducing its ballasting potential and diminishing the ability of the ocean to sequester inorganic carbon. Whether this effect can be counteracted by~~

an increased CO_2 sequestration owing to a decreased strength of the carbonate counter pump, remains to be ascertained.

It has to be kept in mind that ocean acidification is not the only consequence of anthropogenic carbon emissions. Global warming and increased surface ocean stratification as well as changes in nutrient availability will further affect the physiological responses of marine organisms, including coccolithophores. Therefore, the effects of ocean acidification might differ when other potential stressors are included. Some studies have already examined the interactive effects of multiple stress factors on coccolithophore responses (e.g. Zondervan et al., 2002; Feng et al., 2008; De Bodt et al., 2010; Sett et al., 2014). However, more studies are required that analyze responses of coccolithophores to multiple stressor within the marine ecosystem in order to better quantify community and ecosystem responses to ocean acidification and global warming.

References

Armstrong RA, Lee C, Hedges JI, Honjo S, Wakeham SG (2002) A new, mechanistic model for organic carbon fluxes in the ocean based on the quantitative association of POC with ballast minerals. Deep Sea Research Part II, **49**, 219–236.

Bach LT, Riebesell U, Schulz K (2011) Distinguishing between the effects of ocean acidification and ocean carbonation in the coccolithophore *Emiliania huxleyi*. Limnology and Oceanography, **56**, 2040–2050.

Barcelos e Ramos J, Müller MN, Riebesell U (2010) Short-term response of the coccolithophore *Emiliania huxleyi* to an abrupt change in seawater carbon dioxide concentrations. Biogeosciences, **7**, 177–186.

Borenstein M, Hedges LV, Higgins J, Rothstein HR (2010) A basic introduction to fixed-effect and random-effects models for meta-analysis. Research Synthesis Methods, **1**, 97–111.

Brownlee C, Taylor A (2004) Calcification in coccolithophores: A cellular perspective. In: Coccolithophores – From Molecular Processes to Global Impact (eds Thierstein HR, Young JR), pp- 99 – 125, Springer, Berlin, Germany.

Caldeira K, Wickett ME (2003) Anthropogenic carbon and ocean pH. Nature, **425**, 365.

Klaas C, Archer DE (2002) Association of sinking organic matter with various types of mineral ballast in the deep sea: Implications for the rain ratio. Global Biogeochemical Cycles. **16**, 63-1–63-14.

Cochran W (1954) The contribution of estimates from different experiments. Biometrics, **10**, 101–129.

Conway T, Tans P, NOAA/ESRL (www.esrl.noaa.gov/gmd/ccgg/trends/).

Cubillos JC, Wright SW, Nash G, de Salas MF, Griffiths B, Tilbrook B, Poisson A, Hallegraeff GM (2007) Calcification morphotypes of the coccolithophorid *Emiliania huxleyi* in the Southern Ocean: changes in 2001 to 2006 compared to historical data. Marine Ecology Progress Series, **348**, 47–54.

De Bodt C, Van Oostende N, Harlay J, Sabbe K, Chou L (2010) Individual and interacting effects of pCO_2 and temperature on *Emiliania huxleyi* calcification: study of the calcite production, the coccolith morphology and the coccosphere size. Biogeosciences, **7**, 1401–1412.

Delille B, Harlay J, Zondervan I, Jacquet S, Chou L, Wollast R, Bellerby RGJ, Frankignoulle M, Borges AV, Riebesell U, Gattuso JP (2005) Response of primary production and calcification to changes of pCO_2 during experimental blooms of the coccolithophorid *Emiliania huxleyi*. Global Biogeochemical Cycles, **19**, 1–14.

Engel A, Zondervan I, Aerts K et al. (2005) Testing the direct effect of CO_2 concentration on a bloom of the coccolithophorid *Emiliania huxleyi* in mesocosm experiments. Limnology and Oceanography, **50**, 493–507.

Feng Y, Warner ME, Zhang Y, Sun J, Fu FX, Rose JM, Hutchins DA (2008) Interactive effects of increased pCO_2, temperature and irradiance on the marine coccolithophore *Emiliania huxleyi* (Prymnesiophyceae). European Journal of Phycology, **43**, 87–98.

Findlay HS, Calosi P, Crawfurd, K (2011) Determinants of the PIC:POC response in the coccolithophore *Emiliania huxleyi* under future ocean acidification scenarios. Limnology and Oceanography, **56**, 1168–1178.

Fiorini S, Middelburg JJ, Gattuso JP (2011) Testing the effects of elevated pCO_2 on coccolithophores (Prymnesiophyceae): comparison between haploid and diploid life stages. Journal of Phycology, **47**, 1281–1291.

Gao K, Ruan Z, Villafañe VE, Gattuso JP, Helbling EW (2009) Ocean acidification exacerbates the effect of UV radiation on the calcifying phytoplankter *Emiliania huxleyi*. Limnology and Oceanography, **54**, 1855–1862.

Hedges LV, Olkin I (1985) Statistical Methods for Meta-Analysis. Academic Press, London, New York

Hedges LV, Gurevitch J, Curtis PS (1999) The meta-analysis of response ratios in experimental ecology. Ecology, **80**, 1150–1156.

Hendriks IE, Duarte CM, Álvarez M (2010) Vulnerability of marine biodiversity to ocean acidification: A meta-analysis. Estuarine, Coastal and Shelf Science, **86**, 157–164.

Hoppe CJM, Langer G, Rost B (2011) *Emiliania huxleyi* shows identical responses to elevated pCO_2 in TA and DIC manipulations. Journal of Experimental Marine Biology and Ecology, **406**, 54–62.

Hoppe CJM, Langer G, Rokitta SD, Wolf-Gladrow DA, Rost B (2012) Implications of observed inconsistencies in carbonate chemistry measurements for ocean acidification studies. Biogeosciences, **9**, 2401–2405.

Iglesias-Rodriguez MD, Schofield OM, Batley J, Medlin LK, Hayes PK (2006) Intraspecific genetic diversity in the marine coccolithophore *Emiliania huxleyi* (Prymnesiophyceae): the use of microsatellite analysis in marine phytoplankton population studies. Journal of Phycology, **42**, 526–536.

Iglesias-Rodriguez MD, Halloran PR, Rickaby RE et al. (2008) Phytoplankton Calcification in a High-CO_2 World. Science, **320**, 336–340.

Jones BM, Iglesias-Rodriguez MD, Skipp PJ et al. (2013) Responses of the *Emiliania huxleyi* Proteome to Ocean Acidification. PLoS ONE, **8**, e61868, 1–13.

Kroeker KJ, Kordas RL, Crim RN, Singh GG (2010) Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecology Letters, **13**, 1419–1434.

Kroeker KJ, Kordas RL, Crim R et al. (2013) Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Global Change Biology, **19**, 1884–1896.

Krug S, Schulz K, Riebesell U (2011) Effects of changes in carbonate chemistry speciation on *Coccolithus braarudii*: a discussion of coccolithophorid sensitivities. Biogeosciences, **8**, 771–777.

Langer G, Geisen M, Baumann KH, Kläs J, Riebesell U, Thoms S, Young JR (2006) Species-specific responses of calcifying algae to changing seawater carbonate chemistry. Geochemistry, Geophysics, Geosystems, **7**, 1–12.

Langer G, Nehrke G, Probert I, Ly J, Ziveri P (2009) Strain-specific responses of *Emiliania huxleyi* to changing seawater carbonate chemistry. Biogeosciences, **6**, 2637–2646.

Langer G, Bode M (2011) CO_2 mediation of adverse effects of seawater acidification in *Calcidiscus leptoporus*. Geochemistry, Geophysics, Geosystems, **12**, 1–8.

Lefebvre SC, Benner I, Stillman JH et al. (2012) Nitrogen source and pCO_2 synergistically affect carbon allocation, growth and morphology of the coccolithophore *Emiliania huxleyi*: potential implications of ocean acidification for the carbon cycle. Global Change Biology, **18**, 493–503.

Lohbeck KT, Riebesell U, Reusch TB (2012) Adaptive evolution of a key phytoplankton species to ocean acidification. Nature Geosciences, **5**, 346–351.

Müller MN, Schulz KG, Riebesell U (2010) Effects of long-term high CO_2 exposure on two species of coccolithophores. Biogeosciences, **7**, 1109–1116.

Nakicenovic N, Alcamo J, Davis G et al. (2000) IPCC 2000: Special Report on Emissions Scenarios: A Special Report of Working Group III of the Intergovernmental Panel on Climate Change (eds Nakicenovic N, Swart R) Cambridge University Press, Cambridge, UK and New York, NY, USA.

Nimer NA, Merrett MJ (1996) The development of a CO_2-concentrating mechanism in *Emiliania huxleyi*. New Phytologist, **133**, 383–389.

Richier S, Fiorini S, Kerros ME, Von Dassow P, Gattuso JP (2011) Response of the calcifying coccolithophore *Emiliania huxleyi* to low *p*H/high pCO_2: from physiology to molecular level. Marine Biology, **158**, 551–560.

Rickaby RE, Henderiks J, Young JN (2010) Perturbing phytoplankton: response and isotopic fractionation with changing carbonate chemistry in two coccolithophore species. Climate of the Past, **6**, 771–785.

Ridgwell A, Schmidt DN, Turley C, Brownlee C, Maldonado MT, Tortell P, Young JR (2009) From laboratory manipulations to Earth system models: scaling calcification impacts of ocean acidification. Biogeosciences, **6**, 2611–2623.

Riebesell U, Zondervan I, Rost B, Tortell PD, Zeebe RE, Morel FM (2000) Reduced calcification of marine plankton in response to increased atmospheric CO_2. Nature, **407**, 364–367.

Riebesell U (2004) Effects of CO_2 Enrichment on Marine Phytoplankton. Journal of Oceanography, **60**, 719–729.

Riebesell U, Bellerby RG, Engel A et al. (2008) Comment on "Phytoplankton Calcification in a High-CO_2 World". Science, **322**, 1466b .

Riebesell U, Körtzinger A, Oschlies A (2009) Sensitivities of marine carbon fluxes to ocean change. Proceedings of the National Academy of Sciences USA, **106**, 20602–20609.

Riebesell U, Tortell PD (2011) Effects of ocean acidification on pelagic organisms and ecosystems. In: Ocean Acidification. (eds Gattuso JP, Hansson L), pp. 99–121, Oxford University Press, Oxford, UK.

Ries JB, Cohen AL, McCorkle DC (2009) Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. Geology, **37**, 1131–1134.

Rokitta SD, Rost B (2012) Effects of CO_2 and their modulation by light in the life-cycle stages of the coccolithophore *Emiliania huxleyi*. Limnology and Oceanography, **57**, 607–618.

Rost B, Riebesell U, Burkhardt S, Sültemeyer D (2003) Carbon acquisition of bloom-forming marine phytoplankton. Limnology and Oceanography, **48**, 55–67.

Rost B, Riebesell U (2004) Coccolithophores and the biological pump: responses to environmental changes. In: Coccolithophores — From Molecular Processes to Global Impact (eds Thierstein HR, Young, JR), pp. 99-125, Springer, Berlin, Germany.

Rost B, Zondervan I, Wolf-Gladrow D (2008) Sensitivity of phytoplankton to future changes in ocean carbonate chemistry: current knowledge, contradictions and research directions. Marine Ecology Progress Series, **373**, 227–237.

Sabine CL, Feely RA, Gruber N et al. (2004) The oceanic sink for anthropogenic CO_2. Science, **305**, 367–371.

Sciandra A, Harlay J, Lefèvre D, Lemée R, Rimmelin P, Denis M, Gattuso JP (2003) Response of coccolithophorid *Emiliania huxleyi* to elevated partial pressure of CO_2 under nitrogen limitation. Marine Ecology Progress Series, **261**, 111–122.

Sett S, Bach LT, Schulz KG, Koch-Klavsen S, Lebrato M, Riebesell U (2014) Temperature modulates coccolithophorid sensitivity of growth, photosynthesis and calcification to increasing seawater pCO_2. PLoS ONE , **9**, e88308.

Shi D, Xu Y, Morel FMM (2009) Effects of the pH/$p$$CO_2$ control method on medium chemistry and phytoplankton growth. Biogeosciences, **6**, 1199–1207.

Shutler JD, Grant MG, Miller PI, Rushton E, Anderson K (2010) Coccolithophore bloom detection in the northeast Atlantic using SeaWiFS: Algorithm description, application and sensitivity analysis. Remote Sensing of Environment, **114**, 1008–1016.

Wolf-Gladrow D, Riebesell U, Burkhardt S, Bijma J (1999) Direct effects of CO_2 concentration on growth and isotopic composition of marine plankton. Tellus B, **51**, 461–476.

Wuori T (2012) Effects of elevated pCO_2 on the physiology of *Emiliania huxleyi*. M.Sc. Thesis, Western Washington University, USA.

Ziveri P, de Bernardi B, Baumann KH, Stoll HM, Mortyn PG (2007) Sinking of coccolith carbonate and potential contribution to organic carbon ballasting in the deep ocean. Deep Sea Research Part II, **54**, 659–675.

Zondervan I, Rost B, Riebesell U (2002) Effect of CO_2 concentration on the PIC/POC ratio in the coccolithophore *Emiliania huxleyi* grown under light-limiting conditions and different daylengths. Journal of Experimental Marine Biology and Ecology, **272**, 55–70.

Table 1. Summary of the available carbonate chemistry manipulation experiments and the responses of *Emiliania huxleyi* as reported by the authors of those studies~~found in those studies~~.

Symbols indicate: — no response, ╱ increased response, ⌒ non-linear response , ╲ decreased response

Reference	*E.huxlexi* strain	~~E~~experiment type	CO_2 manipulation	PIC production	POC production	PIC/POC	~~S~~specifics
Bach at al. (2011)	PML B92/11A	laboratory	constant DIC	⌒	╲	╲	large pCO_2 range
Barcelos e Ramos et al. (2010)	Raune Fjord, Norway 2005	laboratory	constant DIC	╲	╱	╲	short-term incubation
De Bodt et al. (2010)	AC481	laboratory	constant TA	╲	—	╲	variable temperatures
Delille et al. (2005)	Raune Fjord, Norway 2001	mesocosm	constant TA	╲	—	╲	
Engel et al. (2005)	Raune Fjord, Norway 2001	mesocosm	constant TA	╲	—	╲	
Feng et al. (2008)	CCMP 371	laboratory	constant TA	╲	—	╲	~~varibale~~variable light & temperature
Fiorini et al. (2011)	AC472	laboratory	constant TA	╱	—	╱	
Gao et al. (2009)	CS369	laboratory	constant TA	╲	⌒	╲	PAR & UVR
Hoppe et al. (2011)	RCC1256	laboratory	constant DIC and constant TA	╲	—	╲	
Hoppe et al. (2011)	NZEH	laboratory	constant DIC and constant TA	╲	╱	╲	
Iglesias-Rodriguez et al. (2008)	NZEH	laboratory	constant TA	╱	╱	—	
Langer et al. (2009)	RCC1212	laboratory	constant TA	╲	╲	╲	
Langer et al. (2009)	RCC1216	laboratory	constant TA	╲	╲	╲	
Langer et al. (2009)	RCC1238	laboratory	constant TA	—	⌒	—	
Langer et al. (2009)	RCC1256	laboratory	constant TA	⌒	⌒	—	
Müller et al. (2010)	Raune Fjord, Norway 2005	laboratory	constant DIC	╲	—	╲	long-term incubation
Riebesell et al. (2000)	PML B92/11A	laboratory	constant DIC	╲	╱	╲	variable day-~~lenghts~~lengths & ~~lightintensity~~light intensity
Richier et al. (2011)	RCC1216	laboratory	constant TA	—	—	—	
Rokitta and Rost et al. (2012)	RCC1216	laboratory	constant TA	╲	╱	╲	low and high light conditions
Sciandra et al. (2003)	TW1	laboratory	constant TA	╲	╲	—	chemostat
Shi et al. (2009)	NZEH	laboratory	constant TA	╲	╲	╲	
Shi et al. (2009)	NZEH	laboratory	constant DIC	╱	╱	╲	
Zondervan et al. (2002)	PML B92/11A	laboratory	constant DIC	╲	╱	╲	variable day-~~lenghts~~lengths & ~~lightintensity~~light intensity
Wuori et al. (2012)	CCMP 2668	laboratory	constant TA	╲	╱	╲	

Table 2. Summary of the available carbonate chemistry manipulation experiments and the responses of *Coccolithus braarudii*, *Gephyrocapsa oceanica* and *Calcidiscus leptoporus* found in those studies.

Reference	**Species**	**Strain**	**Eexperiment type**	**CO_2 manipulation**	**PIC production**	**POC production**	**PIC/POC**
Krug et al. (2011)	*Coccolithus braarudii*	RCC 1200	laboratory	constant DIC	↘	∩	↘
Langer et al. (2006)		AC400	laboratory	constant DIC	—	—	—
Müller et al. (2010)		RCC 1200	laboratory	constant DIC	↘	↗	↘
Rickaby et al. (2010)		4762	laboratory	constant DIC	↗	↗	—
Riebesell et al. (2000)	*Gephyro-capsa oceanica*	PC7/1	laboratory	constant DIC	↘	↗	↘
Rickaby et al. (2010)		PZ 3.1	laboratory	constant DIC	—	∩	↘
Langer et al. (2006)	*Calcidiscus leptoporus*	AC365	laboratory	constant DIC	∩	—	∩
Langer and Bode (2011)		AC365	laboratory	constant DIC	∩	—	∩

Figure 1. The effect of elevated CO_2 concentrations on the calcification rates of the three coccolithophore species *Emiliania huxleyi*, *Coccolithus braarudii* and *Gephyrocapsa oceanica* [mean effect size and 95% confidence interval]. Responses are relative to 280 ppm. * indicates a significant response, which is given when the confidence interval does not overlap zero. The number of experiments used to calculate mean effect sizes are shown in parentheses. The zero line indicates no effect.

Figure 2. Mean effect of elevated CO_2 concentrations (relative to 280 ppm) on the photosynthesis rates of three coccolithophore species, *Emiliania huxleyi*, *Coccolithus braarudii* and *Gephyrocapsa oceanica.* Error bars denote the 95% confidence intervals. * indicates a significant response, which is given when the confidence interval does not overlap zero. The number of experiments included in the meta-analysis is shown in parentheses. The zero line indicates no effect.

Figure 3. The effect of elevated CO_2 concentrations on the inorganic to organic carbon ratio of three coccolithophore species: *Emiliania huxleyi*, *Coccolithus braarudii* and *Gephyrocapsa oceanica* [mean effect size and 95% confidence interval]. Responses are relative to 280 ppm. * indicates a significant response, which is given when the confidence interval does not overlap zero. The number of experiments included in the meta-analysis is shown in parentheses. The zero line indicates no effect.

Figure 4. Comparison of effect sizes between the methods of carbonate chemistry manipulation. White diamonds symbolize treatments where total alkalinity [TA] was kept constant while dissolved inorganic carbon [DIC] changed. Black diamonds symbolize treatments where DIC was kept constant and TA varied. The number of experiments included

in the meta-analysis are shown in parentheses. The mean effect size is significant when the 95% confidence interval does not overlap zero [*].

Figure 5. Comparison of effect sizes from PIC and POC analyses derived from the study by Kroeker et al. (2010) [circles], Kroeker et al. (2013) [triangles] and the present study [diamonds]. Data from Kroeker et al. (2010 and 2013) were extracted directly out of the study with the help of the Web Plot Digitizer Software [www.arohatgi.info/WebPlotDigitizer/]. The meta-analysis from the present study contains experiments of all coccolithophore species, including those of *Calcidiscus leptoporus* [see Table 2]. Error bars denote the 95% confidence intervals. * indicates a significant response, which is given when the confidence interval does not overlap zero. The number of experiments included in the meta-analysis is shown in parentheses. The zero line indicates no effect.

Figure S1. Comparison of effect sizes between the methods of carbonate chemistry manipulation in~~for~~ experiments with *Emiliania huxleyi*. White diamonds symbolize treatments where total alkalinity [TA] was kept constant while dissolved inorganic carbon [DIC] changed. Black diamonds symbolize treatments where DIC was kept constant and TA varied. The ~~number of experiments included in the meta-analysis are~~number of experiments included in the meta-analysis is shown in parentheses. The mean effect size is significant when the 95% confidence interval does not overlap zero [*].