

Responses of coccolithophores to ocean acidification: a meta-analysis

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1 **Abstract**

2 Concerning their sensitivity to ocean acidification, coccolithophores, a group of calcifying
3 single-celled phytoplankton, are one of the best-studied groups of marine organisms.
4 However, in spite of the large number of studies investigating coccolithophore physiological
5 responses to ocean acidification, uncertainties still remain due to variable and partly
6 contradictory results. In the present study we have used all existing data in a meta-analysis to
7 estimate the effect size of future $p\text{CO}_2$ changes on the rates of calcification and
8 photosynthesis and the ratio of particulate inorganic to organic carbon (PIC/POC) in different
9 coccolithophore species. Our results indicate that ocean acidification has a negative effect on
10 calcification and the cellular PIC/POC ratio in the most abundant coccolithophore species
11 *Emiliana huxleyi* and *Gephyrocapsa oceanica*. In contrast the more heavily calcified species
12 *Coccolithus braarudii* did not show a distinct response when exposed to elevated
13 $p\text{CO}_2$ /reduced $p\text{H}$. Photosynthesis in *Gephyrocapsa oceanica* was positively affected by high
14 CO_2 , while no effect was observed for the other coccolithophore species. There was no
15 indication that the method of carbonate chemistry manipulation was responsible for the
16 inconsistent results regarding observed responses in calcification and the PIC/POC ratio. The
17 perturbation method, however, appears to affect photosynthesis, as responses varied
18 significantly between total alkalinity (TA) and dissolved inorganic carbon (DIC)
19 manipulations. These results emphasize that coccolithophore species respond differently to
20 ocean acidification, both in terms of calcification and photosynthesis. Where negative effects
21 occur, they become evident at CO_2 levels in the range projected for this century in case of
22 unabated CO_2 emissions. As the data sets used in this meta-analysis do not account for
23 adaptive responses, ecological fitness and ecosystem interactions, the questions remains how
24 these physiological responses play out in the natural environment.

25

26 **1. Introduction**

27 Coccolithophores, a globally distributed group of marine haptophytes, are major primary
28 producers in the ocean and the most prolific calcifying organisms on our planet (Brownlee &
29 Taylor, 2004; Shutler et al., 2010). By performing photosynthesis and calcification, they
30 contribute to both biological carbon pumps, the soft tissue pump and the carbonate counter
31 pump. While the former supports carbon sequestration in the ocean through production and
32 sinking of organic matter to depth, the latter decreases the ocean's capacity to take up CO₂
33 due to the reduction of surface layer alkalinity. Moreover, by providing ballast material,
34 which accelerates sinking velocities of organic particles to depth, coccolithophore-derived
35 calcite contributes to enhancing carbon sequestration to depth (Klaas & Archer, 2002;
36 Armstrong et al., 2002; Ziveri et al., 2007). Thus, changes in the contribution of
37 coccolithophores to ocean primary production could potentially impact global carbon cycling
38 (Riebesell et al., 2009).

39
40 In the face of global change phytoplankton are subjected to rapid alterations in their
41 environmental conditions. Due to the sensitivity of calcification to ocean acidification,
42 coccolithophores are considered to be among those, which may be adversely affected in a
43 high CO₂ future ocean. While impacts of ocean acidification on coccolithophores have been
44 studied extensively (for review see e.g. Riebesell & Tortell, 2011), variable and partly
45 conflicting responses were observed in different perturbation studies (for a summary see
46 Tables 1 and 2). Differences in experimental conditions, such as in light intensity,
47 temperature, salinity, nutrient concentration and *p*CO₂ levels have been attributed as possible
48 causes for those variations. But even studies with comparable experimental conditions
49 provided deviating responses of coccolithophores. Some of this divergence was shown to be
50 related to species- and strain-specific differences (Langer et al., 2006, 2009). But also the

51 method of carbonate chemistry manipulation, whether through changes in total alkalinity
52 (TA) or dissolved inorganic carbon (DIC), was discussed as possible cause for some of the
53 observed discrepancies (Iglesias-Rodriguez et al., 2008; Shi et al., 2009).

54

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

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76 **2. Materials and Methods**

77 **2.1 Literature search**

78 A literature search was conducted to assemble all published data sets on CO₂/pH sensitivities
79 of coccolithophore calcification and photosynthesis. As a first step the ISI database Web of
80 Science (www.webofknowledge.com) was scanned for appropriate studies. Additional
81 literature was obtained from the EPOCA (European Project on Ocean Acidification) database
82 (www.epoca-project.eu) and from the associated blog
83 (www.oceanacidification.wordpress.com). Subsequently, the reference lists of all studies
84 identified by this approach were scanned for other relevant literature.

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

88

89 **2.2 Data selection**

90 All studies in which the carbonate system was altered and the effect on coccolithophores
91 reported, comprising both laboratory and field experiments, were selected for this meta-
92 analysis. Studies that varied other environmental factors in addition to seawater carbonate
93 chemistry, such as light intensity, day length, temperature or nutrient availability, were also
94 incorporated. Data of particulate inorganic (PIC) and organic carbon (POC) production rates,
95 pH values, carbonate system parameters and experimental conditions (light level, day length,
96 temperature, nutrients) were obtained for the control (ambient or pre-industrial pCO₂ level)
97 and the experimental treatments (elevated pCO₂ level). If PIC and POC were provided as
98 quota values on a per-cell basis, production rates were calculated by multiplying the growth
99 rates (μ) with the cell quota of organic or inorganic carbon.

100 The following $p\text{CO}_2$ levels were chosen to compare the responses of *Emiliana huxleyi* to pre-
101 industrial carbon dioxide concentrations of ~ 280 parts per million (ppm):

102 (1) ~ 380 ppm – reflecting the present day $p\text{CO}_2$ level,

103 (2) ~ 780 ppm – the $p\text{CO}_2$ level projected for the end of this century under the SRES A1B
104 scenario, IPCC Report 2000 (Nakicenovic et al., 2000), and

105 (3) ~ 1000 ppm – the $p\text{CO}_2$ level projected for the end of the century under the ‘worst case’
106 emission scenario A1FI, IPCC Report 2000 (Nakicenovic et al., 2000).

107 Since there was not a sufficient number of studies investigating the responses of *Coccolithus*
108 *braarudii* and *Gephyrocapsa oceanica* at $p\text{CO}_2$ levels around 780 ppm, only concentrations
109 of ~ 380 ppm and ~ 1000 ppm were used to compare the responses of these species. All
110 experiments where the $p\text{CO}_2$ levels deviated no more than ± 50 ppm from the targeted 380
111 ppm and no more than ± 100 ppm from the targeted 780 ppm and 1000 ppm were included in
112 the analysis. Since the studies by Lefebvre et al. (2012) and Jones et al. (2013) did not meet
113 these specifications, they were excluded from the meta-analysis.

114

115 Manipulation of the seawater carbonate chemistry can be achieved in various ways. First, the
116 carbonate system can be adjusted by bubbling with CO_2 . This approach increases $[\text{CO}_2]$,
117 $[\text{HCO}_3^-]$ and DIC, decreases $p\text{H}$ and $[\text{CO}_3^{2-}]$ and does not change the alkalinity. Second, acid
118 can be added, which increases $[\text{CO}_2]$ and $[\text{HCO}_3^-]$, decreases the alkalinity and $[\text{CO}_3^{2-}]$ and
119 does not change DIC. In both manipulations the saturation state (Ω) decreases as well.

120 Although there are other ways to adjust the carbonate system, the above-mentioned methods
121 are the ones most commonly used. It was noted which manipulation method was applied to
122 decrease the $p\text{H}$ in each study. Subsequently, a separate meta-analysis was conducted in order
123 to analyse whether responses of coccolithophores varied between the methods. Here, only

124 responses to a $p\text{CO}_2$ elevation from pre-industrial levels to 780 ppm and 1000 ppm were
125 included in the analysis. On this basis 22 experiments were excluded.

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

129 If not only the carbonate system, but also other factors such as light intensity or day length
130 were changed in a study, the approach of Kroeker et al. (2010) was adopted and the ambient
131 level of the factor, defined by the authors of the primary study, was used to ensure the
132 comparability between the experiments. If the observed responses of a study did not differ
133 significantly for the ambient and non-ambient levels of a given environmental factor (always
134 regarding the same $p\text{CO}_2$ value), both experiments were included.

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

142

143 **2.3 Data analysis**

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

148

149
$$L = \ln(RR) = \ln(\bar{X}_E) - \ln(\bar{X}_C) \quad (1)$$

150

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

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

171 Traditionally, when studies report means, standard deviation, and sample size for both the
172 control and treatment groups, a weighted meta-analysis is possible and the variance (v_i) within
173 the experiment i can be calculated. Consequently, studies with a higher number of replicates

174 and lower variance are weighted more heavily, which results in a more robust meta-analysis
175 where the estimate of the effect size is more precise than in unweighted meta-analyses
176 (Hedges & Olkin, 1985). Some of the data required for a weighted meta-analysis, however,
177 were not available for some studies. In those cases v_i was estimated as the average of the
178 computed variances from those experiments where v_i was calculable. In this way it was
179 possible to include all studies in the meta-analysis. Using the variance v_i and the mean of the
180 response ratio L_i for each experiment i , Cochran's Q (Cochran, 1954) was computed. With the
181 help of Q an estimate of the between experiment variance (σ^2_λ) was obtained (Hedges et al.,
182 1999). The weighted mean of the log response ratio \bar{L}^* is given by:

$$183 \quad \bar{L}^* = \frac{\sum_{i=1}^k w_i^* L_i}{\sum_{i=1}^k w_i^*} \quad (2)$$

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

185 Subsequently, the standard error of the weighted mean was estimated (see Eq. 7 in Hedges et
186 al., 1999) and the confidence intervals were calculated. For all calculations Microsoft Excel ®
187 2008 was used.

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

191

192 **2.3.1 Identifying heterogeneity**

193 A test for heterogeneity in effect sizes was performed based on the Q-statistic.
194 Q approximately follows the chi-squared distribution with k degrees of freedom. The Null
195 hypothesis of homogeneity among the effects of different experiments is rejected if Q exceeds
196 the 95 % quantile of the distribution. Heterogeneity results in a positive estimate for the
197 between experiments variance σ^2_λ , which leads to a larger total variation, that is the sum of

198 the within and between experiment variance. Consequently, larger standard errors as well as
199 wider confidence intervals for the effect size are computed from the weighted variances.

200

201 **3. Results**

202 23 studies were obtained from the literature, summarized in Tables 1 and 2. A total of 48
203 single experiments, which met the above-mentioned criteria, were extracted from these
204 studies to be included in this meta-analysis.

205 The carbonate chemistry perturbation experiments examining responses of *Emiliana huxleyi*
206 are depicted in Table 1. A total of 19 studies dealt with the responses of 14 different strains to
207 ocean acidification. In most experiments, strains of *Emiliana huxleyi* showed reduced
208 calcification rates with increased $p\text{CO}_2$ concentrations (Barcelos e Ramos et al., 2010; De
209 Bodt et al., 2010; Delille et al., 2005; Engel et al., 2005; Feng et al., 2008; Gao et al., 2009;
210 Hoppe et al., 2011; Langer et al., 2009; Müller et al., 2010; Riebesell et al., 2000; Rokitta &
211 Rost, 2012; Sciandra et al., 2003; Shi et al., 2009; Wuori, 2012; Zondervan et al., 2002). In
212 other experiments some strains showed an optimum curve in response to increasing $p\text{CO}_2$
213 (Bach et al., 2011; Langer et al., 2009), no significant response (Langer et al., 2009; Richier et
214 al., 2011) or increased calcification rates (Fiorini et al., 2011; Iglesias-Rodriguez et al., 2008;
215 Shi et al., 2009).

216 Photosynthetic responses were more diverse. In six experiments no response was observed
217 (De Bodt et al., 2010; Delille et al., 2005; Engel et al., 2005; Feng et al., 2008; Fiorini et
218 al., 2011; Hoppe et al., 2011; Müller et al., 2010; Richier et al., 2011), while in another six
219 experiments the POC production increased in response to elevated $p\text{CO}_2$ (Barcelos e Ramos
220 et al., 2010; Hoppe et al., 2011; Iglesias-Rodriguez et al., 2008; Riebesell et al., 2000; Rokitta
221 & Rost, 2012; Shi et al., 2009; Wuori, 2012; Zondervan et al., 2002). Five experiments
222 showed decreasing photosynthesis rates (Bach et al., 2011; Langer et al., 2009; Sciandra et al.,

223 2003; Shi et al., 2009), whereas in three experiments an optimum curve was obtained (Gao et
224 al., 2009, Langer et al., 2009).

225 The observed PIC/POC ratios are more homogeneous across experiments with most of them
226 decreasing with increased $p\text{CO}_2$ (Bach et al., 2011; Barcelos e Ramos et al., 2010; De Bodt et
227 al., 2010; Delille et al., 2005; Engel et al., 2005; Feng et al., 2008; Gao et al., 2009; Hoppe et
228 al., 2011; Langer et al., 2009; Müller et al., 2010; Riebesell et al., 2000; Rokitta & Rost et al.,
229 2012; Shi et al., 2009; Wuori, 2012; Zondervan et al., 2002). Only in four experiments the
230 PIC/POC ratio did not change with increasing $p\text{CO}_2$ (Iglesias-Rodriguez et al., 2008; Langer
231 et al., 2009; Richier et al., 2011; Sciandra et al., 2003) and in one an increase was observed
232 (Fiorini et al., 2011)

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

241 In two experiments conducted with *Gephyrocapsa oceanica*, the calcification rates decreased
242 (Riebesell et al., 2000) or did not change significantly (Rickaby et al., 2010) with increasing
243 $p\text{CO}_2$, whereas photosynthetic carbon fixation increased in one experiment (Riebesell
244 et al., 2000) and showed an optimum curve in the other one (Rickaby et al., 2010). The
245 PIC/POC ratio declined in both experiments.

246 In a fourth coccolithophore species, *Calcidiscus leptoporus*, the calcification response was
247 non-linear, while the photosynthesis rate remained constant over the tested CO₂ range (Langer
248 et al., 2006, Langer & Bode, 2011).

249

250 **3.1 Effect of ocean acidification on calcification responses**

251 The meta-analysis of calcification responses to elevated CO₂ concentrations revealed different
252 results between the examined species (Figure 1). Increasing CO₂ concentrations from pre-
253 industrial to present day levels had no significant effect on calcification in *Emiliana huxleyi*
254 (lnRR = -0.004). In contrast, the effect of near future CO₂ concentrations under both the
255 ‘business as usual’ and the ‘worst case’ scenario had significant negative effects on
256 calcification in this species. This negative effect was more pronounced at 1000 ppm compared
257 to 780 ppm (780 ppm: lnRR = -0.19, confidence interval = -0.07 to -0.30; 1000 ppm: lnRR = -
258 0.38, confidence interval = -0,08 to -0,67).

259 In *Gephyrocapsa oceanica* an increase from preindustrial to present day CO₂ concentrations
260 had a slightly negative but non-significant effect on calcification. Projected future ocean
261 acidification had a negative mean effect on calcification greater than in *Emiliana huxleyi*, but
262 it was not significant (lnRR = -0.79, confidence interval = 0.61 to -2.19). In contrast, no
263 significant effect of ocean acidification was detected in *Coccolithus braarudii*, where the
264 mean effect sizes were slightly positive at both pCO₂ concentrations. Significant
265 heterogeneity was detected for all calcification responses.

266

267 **3.2 Effect of ocean acidification on photosynthetic responses**

268 A significant effect of ocean acidification on photosynthesis was observed in *Gephyrocapsa*
269 *oceanica* for the present-day as well as the high CO₂ concentration, with the mean response at
270 1000 ppm being more than twice as high (lnRR = 0.57) as the mean response at 380 ppm

271 (lnRR = 0.24; Figure 2). For *Coccolithus braarudii*, a significant positive effect was observed
272 at 380 ppm and a similar but non-significant positive effect at 1000 ppm. No effect of ocean
273 acidification on photosynthesis was observed for *Emiliana huxleyi* at 380 ppm and 1000
274 ppm. Only at 780 ppm was the mean effect size slightly positive (lnRR = 0.044), but this
275 effect was non-significant. A significant Q-statistic was calculated for all effect sizes.

276

277 **3.3 Effect of ocean acidification on PIC/POC responses**

278 The observed PIC/POC responses to an increased CO₂ concentration are similar to those
279 observed for the calcification responses (Figure 3). For *Emiliana huxleyi*, there was a larger
280 negative effect on PIC/POC at 1000 ppm (lnRR = -0.39) than at 780 ppm (lnRR = -0.22), but
281 both responses were significantly negative. No effect was observed at present day CO₂
282 concentrations.

283 At both CO₂ concentrations a small, non-significant negative effect of a similar magnitude
284 (380 ppm: lnRR = 0.05, 1000 ppm: lnRR = 0.07) was observed for *Coccolithus braarudii*.
285 The strongest effect of ocean acidification on the PIC/POC ratio was observed for
286 *Gephyrocapsa oceanica*. The mean effect size was significantly negative at both pCO₂ levels,
287 with the negative mean effect size at 1000 ppm (lnRR = 1.37) being more than three times
288 lower than at 380 ppm (lnRR = 0.36). There was significant heterogeneity in all PIC/POC
289 responses.

290

291 **3.4 Relationship between effect sizes and methodological factors**

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

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

307 Interestingly, all experiments using *Coccolithus braarudii* and *Gephyrocapsa oceanica*
308 manipulated the $p\text{CO}_2$ in the culture medium by adding acid, i.e. changing TA while keeping
309 DIC constant. Thus, all these experiments were included in the constant DIC treatments,
310 while only experiments with *Emiliana huxleyi* were included in the constant TA treatments.
311 In order to eliminate a possible bias due to the unequal distribution of coccolithophore species
312 across carbonate chemistry manipulation methods, a separate meta-analysis was conducted.
313 This analysis only included experiments of *Emiliana huxleyi* and determined the variation of
314 effect sizes between carbonate chemistry manipulations (Figure S1, supplement). The results
315 of this analysis were not significantly different from those obtained from the analysis
316 performed on the full data set. A bias due to the unequal distribution of species between
317 treatments can therefore be ruled out.

318

319

320 **4. Discussion**

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

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

330 Our study revealed that heterogeneity in mean effect sizes is not due to different carbonate
331 chemistry perturbation methods. The differences between TA and DIC manipulations in the
332 carbonate chemistry were shown not to cause strong variations in biological responses in
333 coccolithophores - with a possible exception in photosynthetic responses. Another proposed
334 explanation for the high difference in variance between studies could be the morphological
335 and genetic differences of single coccolithophore strains. A high physiological variability was
336 already shown to exist in the coccolithophore *Emiliana huxleyi* (Iglesias-Rodriguez et al.,
337 2006; Cubillos et al., 2007), with different strains and ecotypes exhibiting diverse responses
338 to ocean acidification (Langer et al., 2009; Hoppe et al., 2011). Moreover, adaption processes
339 of clones that are kept in culture over years could further result in variable responses in CO₂
340 perturbation experiments (Ridgwell et al., 2009). Thus, a large part of the variance between
341 the analyzed studies is most likely due to intra-species variability of coccolithophore species,
342 especially in *Emiliana huxleyi*. A further reason for heterogeneity in mean effect size could
343 be discrepancies in calculating the carbonate system from measured parameters. As
344 mentioned earlier in this study, all components of the carbonate system can be calculated if

345 two variables, e.g. pH and DIC, are known. A recently published study suggests, that the
346 pCO_2 concentration measured in CO_2 perturbation experiments differs strongly between
347 calculations (up to 30%), when the input parameters for these calculations were different
348 (Hoppe et al., 2012). The authors state that some publications may not be comparable with
349 each other, as pCO_2 values might have been underestimated when they were calculated from
350 TA and DIC, influencing the interpretation of coccolithophore responses. This finding also
351 has implications for the present study, as some heterogeneity in mean effect size might be due
352 to inconsistencies in calculating pCO_2 .

353 The aim of this study was to synthesize the available data of coccolithophores biological
354 responses to ocean acidification in order to more robustly estimate the actual effect of a
355 lowered seawater pH on those calcifying organism. Despite known intra-specific variability, a
356 negative effect of ocean acidification on calcification as well as on the cellular PIC/POC ratio
357 was observed for the dominant and cosmopolitan species *Emiliana huxleyi*. Our results are in
358 accordance with findings from a meta-analysis conducted by Findlay et al. (2011), who also
359 identified a negative correlation between the cellular PIC/POC ratio in *Emiliana huxleyi* and
360 the pCO_2 concentration in the culture medium. Although some strains of *E. huxleyi* appear to
361 be less sensitive to ocean acidification (Langer et al., 2009), the species shows a negative
362 response towards reduced pCO_2 levels in our meta-analysis, suggesting that strain-specific
363 variations are small compared to the generally negative effect of ocean acidification on this
364 species.

365 Calcification and PIC/POC in the coccolithophore *Gephyrocapsa oceanica* was even more
366 negatively affected by future ocean acidification than in *Emiliana huxleyi*, indicating that *G.*
367 *oceanica* is even more sensitive to changes in pCO_2 and pH . Although the meta-analysis with
368 this species was based on only two studies and a significant effect on the calcification
369 response was not observed, the mean effect sizes were even more negative than those

370 observed for *Emiliana huxleyi* at 1000 ppm. We assume that the inclusion of more studies to
371 the meta-analysis would likely decrease the confidence interval of the mean effect size,
372 resulting in a significantly negative effect of ocean acidification on calcification in
373 *Gephyrocapsa oceanica*. The strong negative effect of ocean acidification on the PIC/POC
374 ratio in this species was not only due to the strong decrease in calcification, but also a
375 consequence of an increase in the photosynthesis rate with increasing $p\text{CO}_2$. Apparently, this
376 species profits more from high $p\text{CO}_2$ levels during photosynthesis than the others. This might
377 - at least for *Gephyrocapsa oceanica* - confirm the hypothesis that some coccolithophores
378 might benefit from higher CO_2 concentrations, since their rate of carbon fixation is below
379 CO_2 saturation at pre-industrial CO_2 levels (Riebesell et al., 2000, 2004; Rost et al., 2003;
380 Nimer & Merrett, 1996). Higher CO_2 concentrations in the water would thus allow them to
381 more efficiently assimilate and fix carbon during photosynthesis and thus increase their
382 photosynthesis rate (Rost et al., 2008). It is further suggested that an increase in the
383 photosynthesis rate might buffer a possible negative effect of ocean acidification on
384 calcification (Ries et al., 2009). When photosynthesis becomes more efficient and additional
385 energy is provided due to enhanced photosynthetic activity, the building and maintenance of
386 coccoliths could be facilitated. This hypothesis, however, was not confirmed by the present
387 analysis, since the species that showed the most positive effect on photosynthesis,
388 *Gephyrocapsa oceanica*, was also the one where the effect of ocean acidification on
389 calcification was most negative.

390 For *Coccolithus braarudii* the results from the present study confirm the hypothesis that this
391 species is insensitive to elevated $p\text{CO}_2$ levels within the tested range (Langer et al., 2006). To
392 some extent, it might even benefit from higher CO_2 concentrations, as it exhibits a slightly
393 positive photosynthesis response.

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

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

411
412 Differences between TA and DIC manipulations were not the cause of variable calcification
413 and PIC/POC responses between experiments, confirming earlier results by Kroeker et
414 al. (2009), Findlay et al. (2011) and Hoppe et al. (2011) and following the reviews of Schulz
415 et al. (2009) and Ridgwell et al. (2009). In contrast, mean effect sizes on photosynthetic rates
416 were significantly different between the two manipulation methods. Whereas no effect of
417 ocean acidification on photosynthesis was observed for the constant TA manipulations, the
418 effect in the constant DIC manipulations was significantly positive. This finding is surprising,

419 as the modifications of the carbonate system induced by the different manipulation methods
420 are very similar, particularly in the range of carbonate chemistry changes projected to occur
421 until the end of this century (Schulz et al., 2009). Although bubbling with CO₂ more closely
422 resembles predicted changes in the oceans carbonate chemistry, because dissolved inorganic
423 carbon increases while total alkalinity remains unchanged, the modification of each carbonate
424 system parameter (*pH*, [CO₂], [CO₃²⁻] and ΩCa) is rather similar. An exception is the
425 concentration of HCO₃⁻, which increases slightly more in experiments where the *pCO*₂
426 concentration is altered by CO₂ bubbling (constant TA manipulation). As not only CO₂, but
427 also HCO₃⁻ is known to be a carbon source for photosynthesis in most phytoplankton species
428 (Riebesell, 2004), one could assume that the higher HCO₃⁻ concentration in the constant TA
429 manipulations was responsible for the observed difference in photosynthetic responses
430 between manipulation methods. However, a higher rather than a lower photosynthesis rate
431 would be expected in the constant TA manipulations compared to the constant DIC
432 manipulations, as more inorganic carbon in the form of HCO₃⁻ would be available for
433 photosynthesis. Thus, it does not seem likely that the slight deviation in the HCO₃⁻
434 concentration is responsible for the difference in mean effect sizes between manipulation
435 methods. Nevertheless, discrepancies between the two methods of CO₂ manipulation
436 observed in the present study are consistent with findings of Kroeker et al. (2010). In their
437 meta-analysis a comparison of photosynthetic responses between manipulation methods also
438 showed that keeping TA constant while increasing DIC caused a more negative effect. The
439 deviation between the mean effect sizes was also significant in their study.

440 Although variable photosynthetic responses have been observed in different carbonate
441 chemistry perturbation experiments, it remains to be clarified what causes these differences.
442 To date, studies and reviews have mainly focused on revealing the reason for diverse
443 calcification responses in coccolithophores (Ridgwell et al., 2009; Schulz et al., 2009). This is

444 probably because ocean acidification is regarded to have a greater impact on calcification in
445 those species than on photosynthesis. While the present study shows that this assumption
446 holds true, a clear understanding of all physiological processes and their relevance for
447 coccolithophore ecological fitness is necessary to realistically assess the influence of future
448 ocean acidification on these organisms.

449 A limitation of the carbonate chemistry manipulation experiments included in this meta-
450 analysis is the short duration of the experiments. As a result, they do not account for possible
451 adaptation processes of coccolithophores that might occur over a longer time-period, and only
452 test for non-adaptive responses. A recent study investigated evolutionary adaptation in
453 *E. huxleyi* in a long-term experiment (Lohbeck et al., 2012). In this study a population
454 adapted to higher $p\text{CO}_2$ levels showed significantly higher calcification rates than the control
455 population. Although adaptation did not restore calcification rates under elevated $p\text{CO}_2$ to
456 those measured under ambient $p\text{CO}_2$ levels, this observation highlights the possibility of
457 adaptive evolution in coccolithophores. If species like *Emiliana huxleyi* and *Gephyrocapsa*
458 *oceanica* can adapt to decreased $p\text{H}$ levels, consequences for the whole ecosystem might be
459 averted. It remains speculative, however, whether results from monocultural experiments can
460 be extrapolated to the natural environment. This also has to be acknowledged when
461 interpreting results of the present study. Generalizations from laboratory observations must be
462 drawn with great care and it has to be kept in mind that ocean acidification is not the only
463 consequence of anthropogenic carbon emissions. Global warming and increased surface
464 ocean stratification as well as changes in nutrient availability will further affect the
465 physiological responses of marine organisms, including coccolithophores. Therefore, the
466 effects of ocean acidification might differ when other potential stressors are included. Some
467 studies have already examined the interactive effects of multiple stress factors on
468 coccolithophore responses (e.g. Zondervan et al., 2002; Feng et al., 2008; De Bodt et al.,

469 2010; Sett et al., 2014). However, more studies are required that analyze responses of
470 coccolithophores to multiple stressor within the marine ecosystem in order to better quantify
471 community and ecosystem responses to ocean acidification and global warming.

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

Symbols indicate: — no response, / increased response, ○ non-linear response, \ decreased response

Reference	<i>E. huxleyi</i> strain	Experiment type	CO ₂ manipulation	PIC production	POC production	PIC/POC	Specifics
Bach et 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	/	—	/	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-lengths & 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-lengths & 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	Experiment type	CO ₂ manipulation	PIC production	POC production	PIC/POC
Krug et al. (2011)	<i>Coccolithus braarudii</i>	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)	<i>Gephyrocapsa oceanica</i>	PC7/1	laboratory	constant DIC			
Rickaby et al. (2010)		PZ 3.1	laboratory	constant DIC			
Langer et al. (2006)	<i>Calcidiscus leptoporus</i>	AC365	laboratory	constant DIC			
Langer and Bode (2011)		AC365	laboratory	constant DIC			

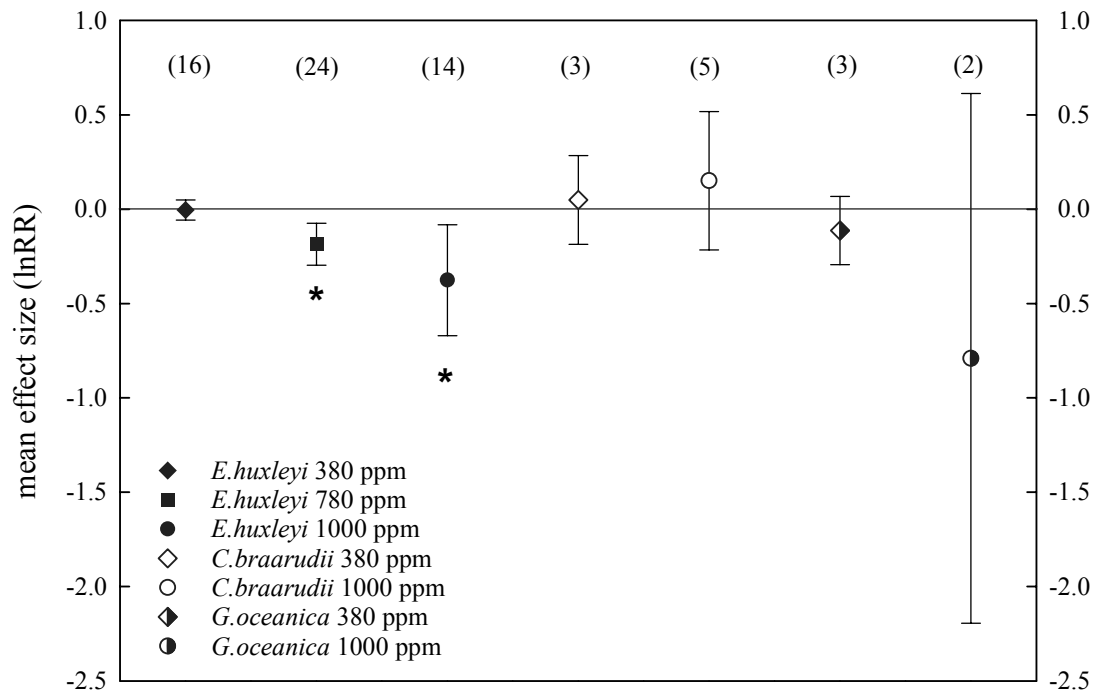


Figure 1. The effect of elevated CO₂ concentrations on the calcification rates of the three coccolithophore species *Emiliana huxleyi*, *Coccolithus braarudii* and *Gephyrocapsa oceanica* [mean effect size and 95% confidence interval]. Responses are relative to 280 ppm. * indicates a significant response, 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.

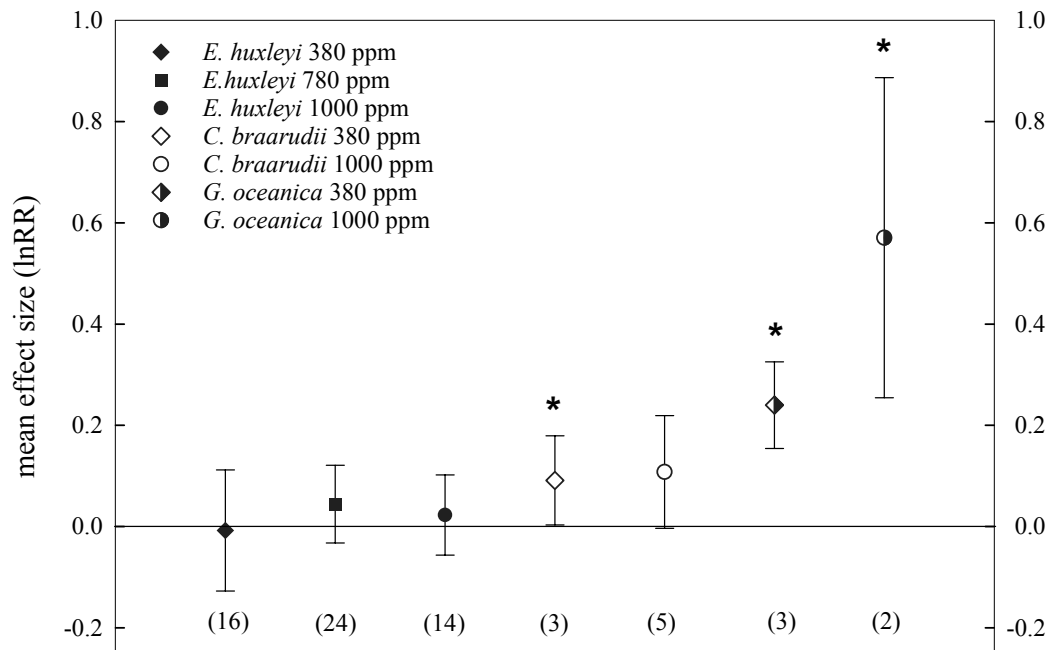


Figure 2. Mean effect of elevated CO₂ concentrations (relative to 280 ppm) on the photosynthesis rates of three coccolithophore species, *Emiliana huxleyi*, *Coccolithus braarudii* and *Gephyrocapsa oceanica*. Error bars denote the 95% confidence intervals. * indicates a significant response, which is 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.

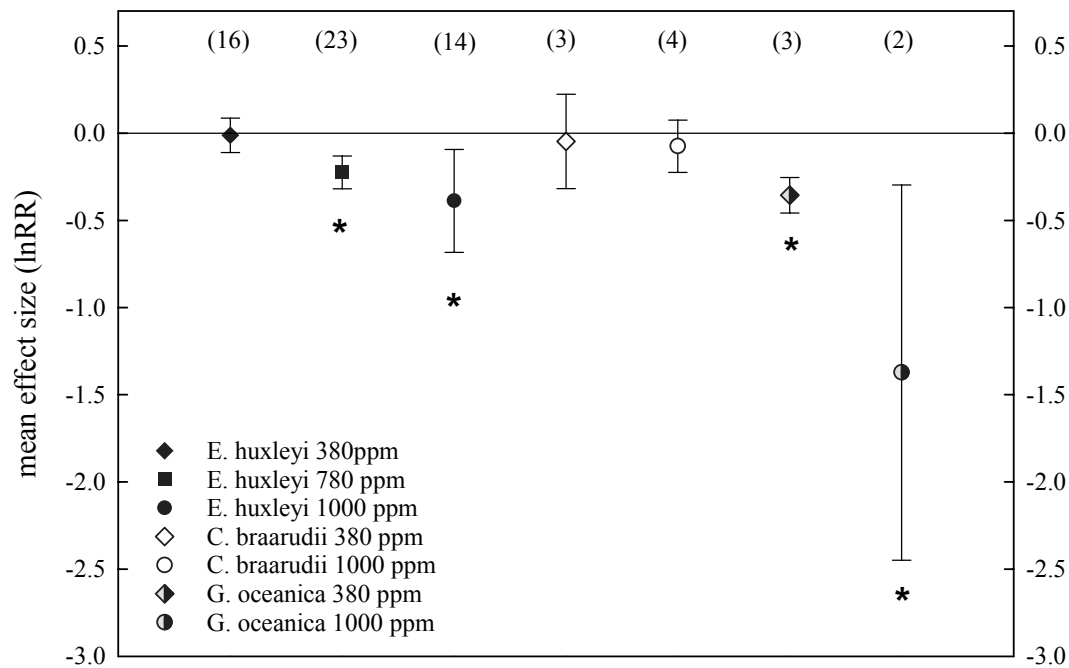


Figure 3. The effect of elevated CO₂ concentrations on the inorganic to organic carbon ratio of three coccolithophore species: *Emiliania huxleyi*, *Coccolithus braarudii* and *Gephyrocapsa oceanica* [mean effect size and 95% confidence interval]. Responses are relative to 280 ppm. * indicates a significant 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.

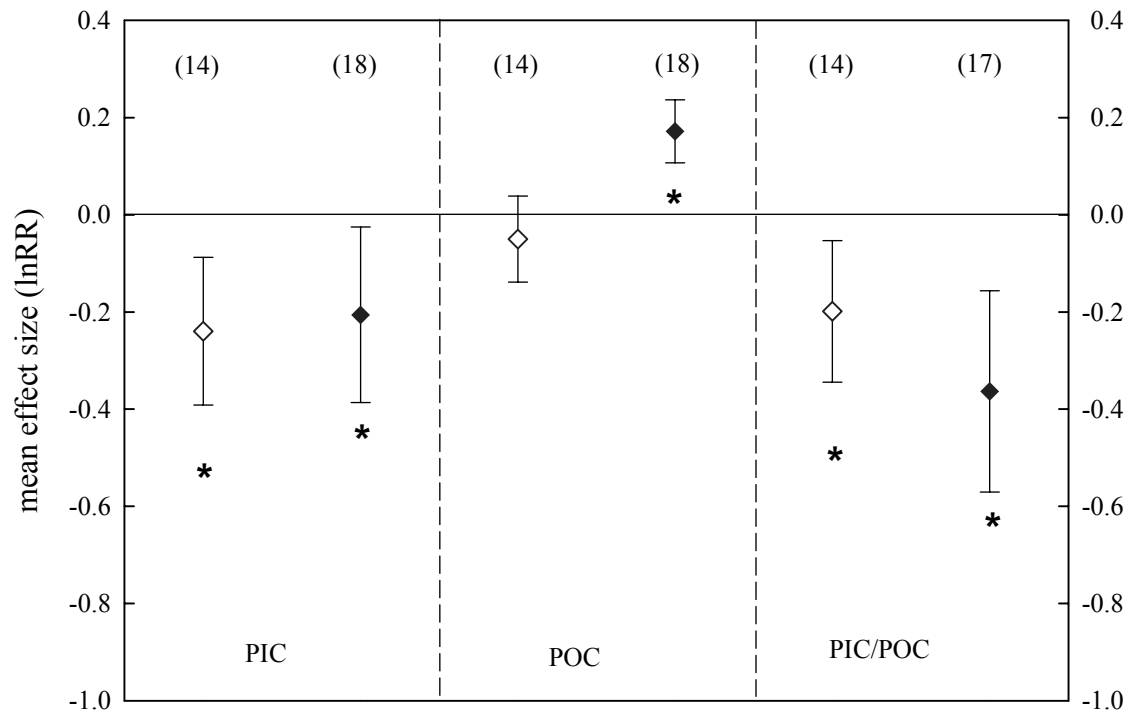


Figure 4. Comparison of effect sizes between the methods of carbonate chemistry manipulation. White diamonds symbolize treatments where total alkalinity [TA] was kept constant while dissolved inorganic carbon [DIC] changed. Black diamonds symbolize treatments where DIC was kept constant and TA varied.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

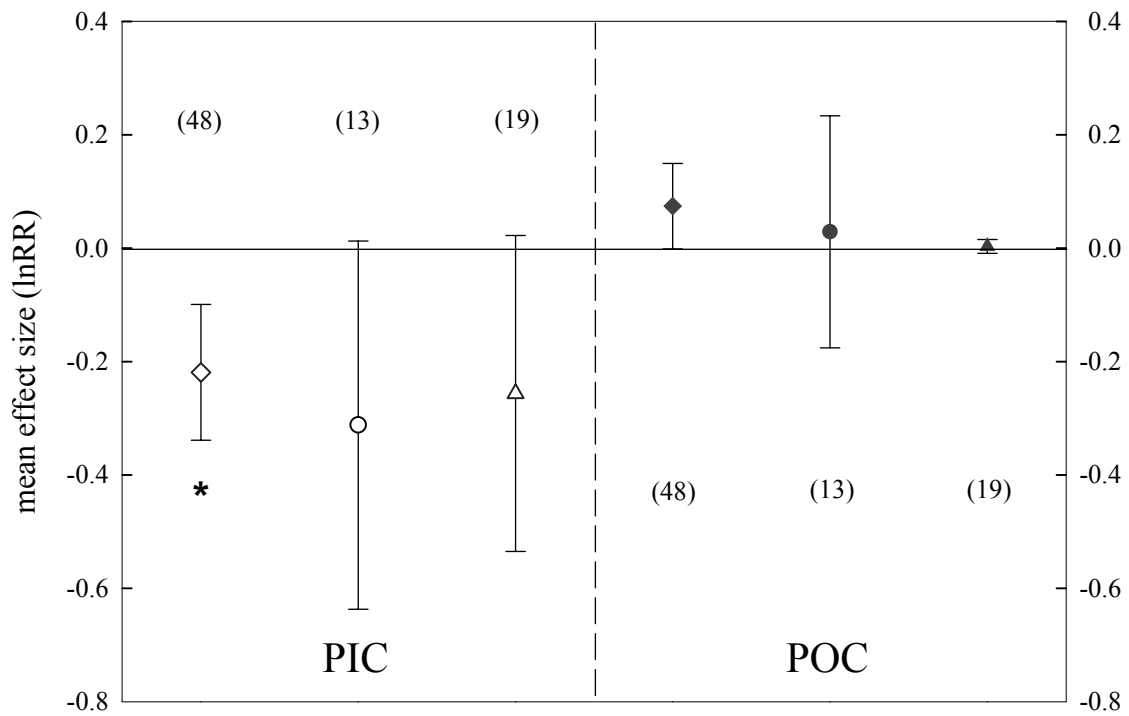


Figure 5. Comparison of effect sizes from PIC and POC analyses derived from the study by Kroeker et al. (2010) [circles], Kroeker et al. (2013) [triangles] and the present study [diamonds]. Data from Kroeker et al. (2010 and 2013) were extracted directly out of the study with the help of the Web Plot Digitizer Software [www.arohatgi.info/WebPlotDigitizer/]. The meta-analysis from the present study contains experiments of all coccolithophore species, including those of *Calcidiscus leptopus* [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.

