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REVIEW AND SYNTHESIS

Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms

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Abstract

Ocean acidification is a pervasive stressor that could affect many marine organisms and cause profound ecological shifts. A variety of biological responses to ocean acidification have been measured across a range of taxa, but this information exists as case studies and has not been synthesized into meaningful comparisons amongst response variables and functional groups. We used meta-analytic techniques to explore the biological responses to ocean acidification, and found negative effects on survival, calcification, growth and reproduction. However, there was significant variation in the sensitivity of marine organisms. Calcifying organisms generally exhibited larger negative responses than non-calcifying organisms across numerous response variables, with the exception of crustaceans, which calcify but were not negatively affected. Calcification responses varied significantly amongst organisms using different mineral forms of calcium carbonate. Organisms using one of the more soluble forms of calcium carbonate (high-magnesium calcite) can be more resilient to ocean acidification than less soluble forms (calcite and aragonite). Additionally, there was variation in the sensitivities of different developmental stages, but this variation was dependent on the taxonomic group. Our analyses suggest that the biological effects of ocean acidification are generally large and negative, but the variation in sensitivity amongst organisms has important implications for ecosystem responses.

Keywords

Calcification, carbonate chemistry, climate change, CO_2, growth, meta-analysis, ocean acidification, ontogeny, pH, photosynthesis, reproduction.

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INTRODUCTION

Ocean acidification is considered as a global threat to marine ecosystems (Doney *et al.* 2009a; Fabry *et al.* 2009; Kleypas & Yates 2009). It is caused by rising atmospheric carbon dioxide (CO_2) concentrations, which drive changes in seawater carbonate chemistry and reduce pH (Gattuso & Buddemeier 2000). This process of ocean acidification is underway (Solomon *et al.* 2007) and will accelerate with increasing CO_2 emissions over the course of the current century (Caldeira & Wickett 2003; Meehl *et al.* 2007). Many marine organisms, from phytoplankton to fish, are sensitive to changes in carbonate chemistry, and their responses to the predicted changes could lead to profound ecological shifts in marine ecosystems (reviewed by Doney *et al.* 2009b). As such, ocean acidification has become a priority area for research, and the number of experiments examining its effects on marine organisms has grown exponentially. Marine organisms vary broadly in their responses to ocean acidification, in part due to the wide variety of processes affected (e.g., dissolution and calcification rates, growth rates, development and survival), making it challenging to predict how marine ecosystems will respond to ocean acidification.

Several hypotheses have been proposed to explain the variation in biological responses, including: (1) organisms that have a calcium carbonate ($CaCO_3$) structure will be more sensitive to ocean acidification than organisms that do not, (2) organisms with more soluble mineral forms of $CaCO_3$ in their structure (e.g., aragonite) will be more sensitive than organisms with less soluble mineral forms (e.g., calcite), (3) early life history stages will be more

sensitive than later life history stages, (4) highly mobile organisms with high metabolic rates may be more capable of compensating for changes in carbonate chemistry than sessile organisms with low metabolic rates and (5) autotrophs with less efficient or absent carbon-concentrating mechanisms (CCMs) will be more responsive than those with efficient CCMs. Below, we briefly review the leading hypotheses for variation in sensitivity to ocean acidification.

One of the primary hypotheses for variation in the biological responses to ocean acidification concerns the susceptibility of calcification. Calcification may be especially sensitive because altered carbonate chemistry directly affects the deposition and dissolution rates of the $CaCO_3$ used for structures (Gattuso & Buddemeier 2000). Reduced calcification rates or increased dissolution rates have been measured in tropical corals (Kleypas *et al.* 1999; Marubini *et al.* 2003; Hoegh-Guldberg *et al.* 2007), planktonic organisms (Riebesell *et al.* 2000; Orr *et al.* 2005), bivalves (Michaelidis *et al.* 2005; Gazeau *et al.* 2007) and echinoderms (Kurihara & Shirayama 2004; Shirayama & Thornton 2005) amongst others in response to ocean acidification. The impacts on calcification could then result in altered energy allocation (Wood *et al.* 2008), lower growth rates, reduced reproductive output and decreased survival amongst calcifying organisms under conditions of ocean acidification.

The sensitivity of calcification to ocean acidification may vary amongst calcifying organisms. Sensitivity may depend on the mineral form of $CaCO_3$ used by the organism, with the solubility and susceptibility increasing from low-magnesium calcite to aragonite and high-magnesium calcite (Morse *et al.* 2006; Ries *et al.* 2009). However, some species may be better able to control pH near calcification sites under differing external conditions, and thereby may be better equipped to cope with ocean acidification (Berry *et al.* 2002; Cohen & McConnaughey 2003; Taylor *et al.* 2007). Additionally, some organisms may be able to compensate for changes in carbonate chemistry by increasing calcification rates (Gutowska *et al.* 2008). Finally, the sensitivity of calcification processes may be buffered for calcifying algae and corals with symbiotic autotrophs due to interactions between photosynthesis and calcification. It is known that photosynthesis can stimulate calcification across numerous taxa (Borowitzka 1982; Gattuso *et al.* 1999, 2000; Rost & Riebesell 2004). If photosynthesis increases in these organisms under ocean acidification, it could potentially buffer the negative effects on calcification (Ries *et al.* 2009).

In addition, early life history stages may be more vulnerable to ocean acidification than adults. The larval and juvenile stages of marine organisms are typically more sensitive to environmental conditions (Pechenik 1987), and can suffer extremely high mortality (Gosselin & Qian 1997). Additionally, some invertebrates begin calcifying during the larval (echinoderms and molluscs) or juvenile (corals and crustaceans) phases (Kurihara 2008). During these phases of calcification, they may rely on a more soluble mineral form of $CaCO_3$ than the mineral used as an adult (Weiss *et al.* 2002; Addadi *et al.* 2003). Indeed, some echinoderms have shown delayed development or high mortality during larval stages when exposed to ocean acidification (Dupont *et al.* 2008).

Organisms may also vary in their sensitivity to ocean acidification in other physiological processes. Reduced seawater pH can disrupt the acid–base status of extracellular body fluid (e.g., blood or hemolymph). Highly mobile organisms such as fish, cephalopods and some crustaceans that are capable of controlling extracellular pH through active ion transport are predicted to be more tolerant of acidification (Gutowska *et al.* 2008; Pörtner 2008; Melzner *et al.* 2009). In turn, organisms unable to compensate for the reductions in extracellular pH have shown depressed metabolism, growth and fitness (Pörtner *et al.* 2004; Michaelidis *et al.* 2005; Siikavuopio *et al.* 2007). Higher maintenance costs in stressful abiotic environments could cause changes in energy allocation to reproduction and somatic growth.

The ability of marine autotrophs to increase photosynthetic rates under ocean acidification could also contribute to variation in organismal responses. Marine autotrophs rely on $CO_{2(aq)}$ or the bicarbonte ion (HCO_3^-) for photosynthesis, which will both increase in concentration with ocean acidification. However, many marine autotrophs utilize CCMs and do not appear carbon limited under current conditions (Raven & Beardall 2003). Yet there is variation in the efficiency of CCMs, and marine phytoplankton with less efficient CCMs have shown the capacity to increase photosynthetic rates in carbon and nutrient replete conditions (Rost & Riebesell 2004; Engel *et al.* 2005; Riebesell *et al.* 2007). In addition, seagrasses primarily rely on $CO_{2(aq)}$ and have shown increased photosynthesis and growth under conditions of ocean acidification (Zimmerman *et al.* 1997; Palacios & Zimmerman 2007; Hall-Spencer *et al.* 2008).

A recent quantitative review concluded that most biological processes are not significantly affected by near-future ocean acidification (Hendriks *et al.* 2010). However, that review did not use the standard methods for meta-analyses – quantitative methods for combining the results of several studies into an overall mean effect – which standardize studies for precision, account for variation between studies, and test for heterogeneity in effect sizes. Significant heterogeneity suggests there may be differences in responses between groups of studies, which can have ecologically important implications and guide the interpretation of the overall results of the meta-analyses (Hedges & Olkin 1985; Gurevitch & Hedges 1999; Rosenberg *et al.* 2000). For these reasons, heterogeneity is of primary interest when trying to quantify the variability or draw general

conclusions, and we focus our current analysis on describing the variation in responses to ocean acidification.

A better understanding of the variability or generality of the biological responses is necessary to provide support for emerging hypotheses and highlight areas where further research is necessary. We quantified variation in biological responses to ocean acidification using meta-analyses. Specifically, we tested whether ocean acidification had a negative effect on survival, calcification, growth and reproduction, and a positive effect on photosynthesis. Within these categories, we tested the following hypotheses: Ocean acidification has a larger effect on (1) calcifying organisms vs. non-calcifying organisms, (2) highly soluble mineral forms of $CaCO_3$ vs. less soluble forms and (3) early vs. later life history stages. To examine variability associated with other life history characteristics (e.g., mobility, metabolism and photosynthesis), we tested for differences between broad taxonomic groups. Finally, we tested whether the experimental methods (carbonate chemistry manipulation, magnitude of pH manipulation, duration of experiment) caused systematic biases in the results. As predicted, we found ocean acidification had negative effects across numerous processes, and that organisms with calcified structures were generally more sensitive. Results of this synthesis indicate that responses differ amongst taxonomic groups, regardless of the methodology used in experiments, and that some organisms appeared more resilient to acidification changes than others.

MATERIAL AND METHODS

Data selection

We searched the biological literature for studies that reported the effects of altered seawater carbonate chemistry on marine organisms. Literature searches were conducted using ISI Web of Science database for the relevant keywords: ocean acidification, carbon dioxide, CO_2, carbonate chemistry and pH. We also checked the history of the European Project on Ocean Acidification (EPOCA) blog (http://oceanacidification.wordpress.com/) for primary studies, and searched the literature cited of all relevant studies. Finally, we cross-checked our database with the database of ocean acidification experiments compiled by EPOCA (http://www.epoca-project.eu/). Studies were collected for analysis until 1 January 2010.

To explore the variety of biological effects of ocean acidification, we selected survival, calcification, growth, photosynthesis and reproduction response variables. Other response variables were not available in enough quantity to include in meaningful quantitative analyses (see Table S1 in Supporting Information). We selected studies that reported these responses amongst populations of a single species as well as responses in multiple species assemblages.

We collected experiments that reported the mean response, error, and sample size in a control and a carbonate chemistry manipulation treatment. We then restricted our dataset to those studies reporting pH values for the given manipulation. We further restricted our analyses to experiments with pH manipulations that reflected the predicted ocean acidification for the Intergovernmental Panel on Climate Change (IPCC) IS92a 'business as usual' emission scenario for the year 2100 (i.e., less than a 0.5-unit reduction in pH; Caldeira & Wickett 2003; IPCC 2007). Seawater pH is measured on several different scales, which can differ by as much as 0.14 units (Zeebe & Wolf-Gladrow 2001). Although it would be ideal to convert all pH measurements to the same scale before comparison, many authors do not report the data necessary to make these conversions. In addition, the value for an appropriate control pH can vary widely depending on the geographic location and ecosystem being studied. Thus, we compared responses to a similar unit change in pH from a given control pH, rather than the absolute values of a specific pH regime (e.g., we did not specify a control pH of 8.1 pH_T). We used the control pH value designated by the author(s) and the experimental pH treatment manipulation closest to a 0.4-unit decrease in pH, on whichever scale the author used for our comparisons.

A reduction in seawater pH can be produced by two different approaches: (1) increasing the dissolved inorganic carbon (DIC) while holding the total alkalinity (TA) constant or (2) decreasing the TA while holding the DIC constant. Increasing the DIC at constant TA is most commonly achieved by bubbling the experimental seawater with CO_2 enriched gas, while lowering the TA at a constant DIC is most commonly achieved by adding acid to the seawater. Thus, the same reduction in seawater pH can represent two different carbonate chemistry regimes depending on the experimental manipulation (Hurd *et al.* 2009). However, many studies do not report the values for all of the carbonate chemistry parameters, and it is difficult to standardize comparisons across all of the carbonate chemistry parameters. Therefore, we chose to standardize the study by unit change in pH, and noted the method of manipulation (altered DIC/constant TA or constant DIC/altered TA). We then tested for variations in responses between these methodological approaches, and examined the relationship between the magnitude of pH difference amongst experimental units and effects sizes.

Many studies included more than one experiment or more than one species in a given experiment. All separate experiments from a given study were included as long as they met the overall criteria for inclusion (see above). If the responses of multiple species were tested in the same experiment (i.e., within the same experimental tank) the

responses of both species were included. Although the inclusion of all species and experiments from the same study could decrease the independence of some data points, it allowed us to explore responses across a broader range of species. If the experiment reported the response over time, the final time point was used in the analyses. If an experiment reported more than one of the chosen response variables, all responses were used in the separate analyses. If an experiment reported more than one response that fell within a chosen response variable (e.g., both total length and biomass were measured, but both are considered growth responses), then only one response was used. In this case, the most inclusive response variable was chosen for inclusion (e.g., biomass was included over length). If a study manipulated more than one factor (e.g., temperature and pH were manipulated factorially) then the response to only altered carbonate chemistry was used. We included this response at the 'ambient' level of the additional factor as designated by the primary author. When the designation of ambient was not appropriate, the response to the mid-range manipulation of the second factor was included (e.g., if photon flux density (PFD) was manipulated at levels of 30, 50 and 150, we chose to include PFD 50).

Data were mined from the primary literature using software programs such as Data Thief III (v. 1.5) (Amsterdam, Netherlands) and GraphClick (v. 3.0) (Neuchatel, Switzerland). We recorded all information about the organisms including their developmental stage, as well as the methodological factors such as location of collected organisms, duration of experiment and the method of carbonate chemistry manipulation for each experiment.

Data analysis

The effect of ocean acidification was measured for each experiment as the ln-transformed response ratio,

$$LnRR = \ln(R) = \ln(\overline{X}_E) - \ln(\overline{X}_C),$$

where $\overline{X}_E$ and $\overline{X}_C$ are the mean response in the experimental and control treatments, respectively. Response ratios quantify the proportional change resulting from experimental manipulations and ln-transformed response ratios are commonly used because of their robust statistical properties and ease of biological interpretation (Hedges *et al.* 1999). A ln-transformed response ratio of zero is interpreted as the experimental treatment having no effect on the response variable, while a positive value indicates a positive effect and a negative value indicates a negative effect.

Traditional meta-analyses weight the individual effect sizes by the inverse of the effect size variance to account for the precision of each study (Hedges & Olkin 1985). The variance of the ln-transformed response ratio (L) for each study was calculated as

$$v = \frac{(S_E)^2}{n_E \overline{X}_E^2} + \frac{(S_C)^2}{n_C \overline{X}_C^2},$$

where s and n are the standard deviation and the sample size for the treatment denoted in the subscript. Thus, studies that have higher replication and lower variance are weighted more heavily because they are predicted to provide a more precise estimate of the population effect size (Hedges & Olkin 1985). Recent studies have advocated the use of unweighted meta-analyses to increase sample size and avoid underestimation of effect sizes (Arnqvist & Wooster 1995; Englund *et al.* 1999). We analysed our data using both weighted and unweighted analysis and did not find different results, and present the results from the weighted analyses.

We used a random effects model to calculate the overall mean effect for each response variable. We chose to use a random effects model to account for biological variation in responses due to the broad range of taxonomic groups included in each analysis. Random effects models account for this true variation in effect size by calculating the between-study variance, σ^2_{pooled}, and weighting each study by the inverse of sum of the individual study variance (v) and the between-study variance (σ^2_{pooled}). The statistical significance of mean effect sizes is based on bias-corrected bootstrapped 95% confidence intervals. When these 95% confidence intervals do not overlap zero, the effect size is considered significant ($\alpha = 0.05$). Because most survival (or mortality) responses are reported as percentages, most authors do not report any error estimates for survival. For this reason, we performed unweighted, fixed effects meta-analyses for survival.

Examining variation

To examine the variation in the responses to ocean acidification amongst different biological and experimental variables, we first calculated the heterogeneity in effect sizes for each response variable using the test statistic Q_T. A significant Q_T statistic indicates there is heterogeneity within the mean effect size. The studies were then separated to test for differences in effect sizes between *a priori* defined groups of studies. We assumed heterogeneity in mean effect size could be due to biological differences, and compared the mean effect size between calcifying organisms and non-calcifying organisms, different taxonomic groups (calcifying algae, corals, coccolithophores, molluscs, echinoderms, crustaceans, fish, fleshy algae and seagrasses) and developmental stages (adult, juvenile and larval). In addition, we compared the mean effect size amongst the different mineral forms of calcifying organisms (aragonite, low-Mg calcite and high-Mg calcite) for the calcification responses. To test for differences amongst these *a priori* defined groups, we performed separate categorical random effects meta-

analyses for each hypothesis for each response variable. A categorical meta-analysis calculates a new overall mean effect size for the experiments included in the analysis, and a mean effect size for each group. The total heterogeneity explained by the categorical model is estimated by Q_M. A significant Q_M indicates there are differences amongst the groups. The significance of mean effect sizes for each group is determined by bias-corrected bootstrapped 95% confidence intervals, and the significance of Q_M is tested by a randomization procedure that randomly reassigns the effect sizes to the groups to create a probability distribution using 9999 iterations. As repeated testing of the same data can result in an increased probability of type I error, we limited the number of categorical analyses to reflect our *a priori* hypotheses.

For the taxonomic categorical analyses, we did not include multi-species assemblage responses, and we discarded any groups when there were fewer than $n = 4$ experiments in the group. For the developmental stage analysis, we did not include experiments that were run for multiple generations of the experimental organism, and discarded any groups with fewer than $n = 3$ responses. Furthermore, we do not report the results for categorical meta-analyses on response variables when the effect sizes could not be appropriately distributed between the chosen categories (e.g., the effect sizes for reproduction could not be distributed amongst taxonomic groups because most data points are for echinoderms). Because of this, the categorical analyses do not include all the data points from the overall mean effect size analyses for response variables (above). Therefore, a restricted overall mean effect size was calculated for the subset of all experiments included in the respective categorical analysis for comparison purposes.

Finally, we examined the relationship between effect sizes and methodological factors. We quantified the differences in effect size between studies: (1) varying TA at constant DIC and those, (2) maintaining constant TA at varying DIC using a categorical random effects model. In addition, we tested for a linear relationship between the unit change in pH within a study and effect size, as well as the duration of the experiment and effect size using continuous random effects models. Continuous model meta-analyses, like categorical models, also estimate the variation explained by the model (Q_M), which can be interpreted in the same fashion. We predicted the duration of the experiment would have an effect in relative proportion to the life span of the organism being tested. For example, an organism with a 4-year life span might be less sensitive to a 10-day experiment than an organism with a 4-day life span, or *vice versa* if the organisms acclimate or adapt to the experimental conditions. Therefore, to examine the relationship between effect size and duration of experiment, we split the studies into groups of organisms with similar ages of first reproduction. These categories were: (1) short – up to 10 days, (2) medium – 10 days to 1 year and (3) long – over 1 year. We then ran separate continuous random effects meta-analyses with the duration of the experiment as the independent factor for each reproductive category (short, medium and long) for each response variable.

Sensitivity analyses

We performed several analyses to determine the sensitivity of our meta-analyses. To identify potential publication biases, we used qualitative analyses (funnel plots, normal quantile plots and weighted frequency histograms of effect sizes; Rosenberg *et al.* 2000). We also calculated Rosenthal's fail-safe number to determine the number of experiments with no significant effect that are needed to change the significance of the meta-analysis. Normal quantile plots and weighted histograms revealed non-normal distributions for all response variables. To deal with the issues of non-normality, we tested for significance in our statistics with randomization tests generated from 9999 iterations and used bootstrapped bias-corrected 95% confidence intervals for our effect sizes.

Given the high variation in responses to ocean acidification, we also wanted to examine the robustness of our results and the relative contribution of studies with particularly large effect sizes (Bancroft *et al.* 2007). For each meta-analysis, we ranked the data points by magnitude of effect size, and systematically removed the largest magnitude data point (regardless of the direction of the effect) in a stepwise fashion, and reran the meta-analyses to determine how many studies would need to be removed to change the significance of the results. When the removal of the largest data point changed the significance of the results, indicating it was driving the results, that data point was omitted from the analysis and the results. Next, we examined potential biases in effect size caused by particular studies. When a single study contributed five or more experiments to an analysis, we removed all experiments contributed by that study and reran the analysis.

Data selection criteria can have considerable influence on the results of meta-analyses (Englund *et al.* 1999). Given the number of studies on ocean acidification excluded from our analyses, we also tested whether the results of our weighted, random effects meta-analyses were robust to the addition of the studies that: (1) did not report variation and (2) studies with large manipulations of the carbonate chemistry. We ran unweighted, fixed effects meta-analyses on a larger dataset that included all studies with experimental and control responses to seawater carbonate chemistry manipulation, regardless of the magnitude of pH manipulation or whether variance was reported in the study. We report the results

from the overall and taxonomic categorical analyses for the unweighted, fixed effects models in the Supporting Information.

RESULTS

Effect of ocean acidification on different response variables

We found 139 studies that quantified the biological responses to ocean acidification. Seventy-three studies met our criteria, representing 251 unique experiments (see Table S2). Meta-analysis of these data revealed that ocean acidification had a significant negative effect on survival, calcification, growth and reproduction in marine organisms, but no significant effect on photosynthesis (Fig. 1). The negative effect of ocean acidification was most pronounced for calcification and survival. There was significant heterogeneity in the calcification (Q_T = 116.33, d.f. = 62, $P < 0.0001$) and growth responses (Q_T = 224.76, d.f. = 85, $P < 0.0001$), but not for the other response variables.

Biological variation in effects of ocean acidification

Survival

A comparison could not be made between the effect of ocean acidification on survival between calcifiers and non-calcifiers (Fig. 2) because the experiments were dominated by those examining the responses of calcifiers. Additionally, we could not detect a difference amongst taxonomic groups (Fig. 3). However, the effect of ocean acidification on survival varied amongst developmental stages (Q_M = 7.81, d.f. = 2, P = 0.015; Fig. 4). The effect size for larvae was the most negative, but this effect was not significant (*LnRR* = −1.24, 95% bias-corrected confidence interval = −3.4 to 0.01).

Calcification

The effect of ocean acidification on calcification did not differ significantly amongst taxonomic groups (Q_M = 16.24, d.f. = 5, P = 0.1; Fig. 3). Ocean acidification had significant negative mean effects on calcification in corals, and similar magnitude but non-significant negative mean effects on calcifying algae, coccolithophores and molluscs. Ocean acidification had a significant positive mean effect on calcification on crustaceans, and a non-significant positive effect on calcification on echinoderms (Fig. 3). The mean effect of ocean acidification on calcification varied amongst organisms with different mineral forms of $CaCO_3$ (Q_M = 9.91, d.f. = 2, P = 0.05; Fig. 5). Organisms using aragonite and low-magnesium calcite were negatively affected by ocean acidification, whereas organisms utilizing high-magnesium calcite were not significantly affected.

Growth

The effect of ocean acidification on growth varied between calcifying organisms and non-calcifying organisms (Q_M = 14.5, d.f. = 1, P = 0.004; Fig. 2) as well as amongst taxonomic groups (Q_M = 56.09, d.f. = 7, $P < 0.001$; Fig. 3). Ocean acidification had a significant negative mean

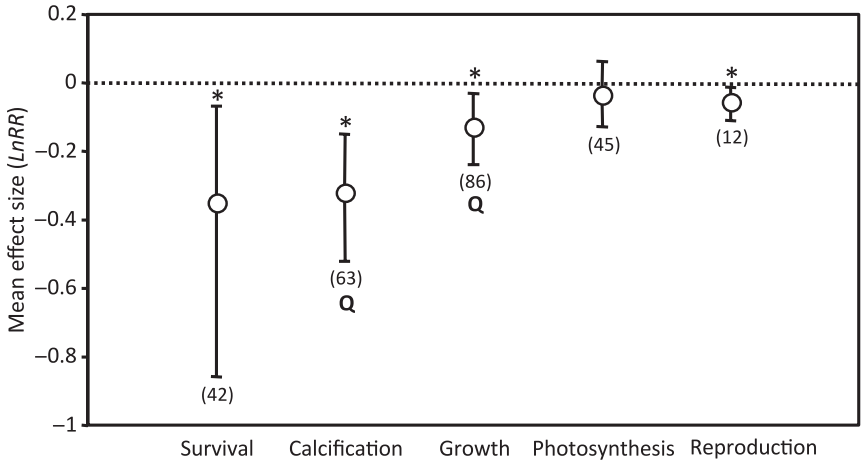

Figure 1 The effect of near-future (2100) ocean acidification on different response variables of marine organisms from weighted, random effects meta-analyses. The mean and bias-corrected bootstrapped 95% confidence interval are shown for separate analyses of survival, calcification, growth, photosynthesis and reproduction. The number of experiments in each analysis is shown in parentheses. The zero line indicates no effect, and significance of mean effects is determined when the 95% confidence interval does not overlap zero. All responses are significantly negative (*) except for photosynthesis, which shows no effect. There is significant heterogeneity (underlying data structure, denoted with *Q*) within the mean effect for calcification and growth.

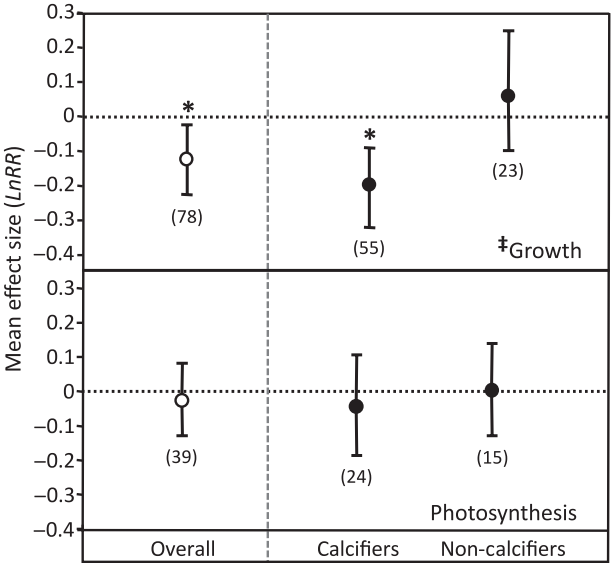

Figure 2 Mean effect of near-future ocean acidification on calcifying organisms and non-calcifying organisms. Mean effect size and 95% bias-corrected bootstrapped confidence interval are shown for all organisms combined (overall), calcifiers and non-calcifiers. The number of experiments used to calculate mean effect sizes are shown in parentheses. The mean effect size is significant when the 95% confidence interval does not overlap zero (*). ‡Indicates significant differences between calcifiers and non-calcifiers, based on the Q_M statistic.

effect on the growth of calcifiers, but we did not detect a significant effect on non-calcifiers. Within calcifiers, ocean acidification had a significant negative mean effect on calcifying algae and corals, and a non-significant negative mean effect on coccolithophores, molluscs and echinoderms. There was a significant positive mean effect on fish and fleshy algae, and a non-significant positive effect on crustaceans.

Photosynthesis

Ocean acidification did not have a significant overall mean effect on photosynthesis. Although calcifying organisms had a more negative mean effect than non-calcifying organisms, the difference was not significant ($Q_M = 0.30$, d.f. = 1, $P = 0.59$; Fig. 2). The mean effect was different amongst taxonomic groups ($Q_M = 12.03$, d.f. = 3, $P = 0.02$; Fig. 3), with a significant negative mean effect on calcifying algae (*LnRR* = −0.33, 95% bias-corrected confidence interval = −0.39 to −0.22).

Developmental stages

We did not detect differences amongst developmental stages in any of the response variables besides survival (Fig. 4). However, we did detect significant differences amongst developmental stages within specific taxonomic groups (molluscs, echinoderms and crustaceans; Fig. 6). For molluscs, there was a larger negative effect for larvae than adults regarding survival ($Q_M = 2.92$, d.f. = 2, $P = 0.05$). For echinoderms, there was a larger negative effect for juveniles than larvae in growth responses ($Q_M = 8.03$, d.f. = 1, $P = 0.05$). For crustaceans, there was a larger negative effect for adults than juveniles in survival ($Q_M = 0.36$, d.f. = 1, $P = 0.01$).

Methodological variation in responses to ocean acidification

Across all analyses, the methodological factors did not have consistent effects on mean effect size. The mean effect of ocean acidification differed amongst carbonate chemistry manipulation methods for the growth and photosynthesis analyses (see Figure S1). For the growth analysis, varying the TA at a constant DIC caused a more negative response, while for photosynthesis increasing DIC at a constant TA caused a more negative effect. We did not detect a significant relationship between the unit change in pH and effect size across any response variable for the extended data sets. For the restricted data sets (pH < 0.5 unit change), we did not find a significant linear relationship of unit change of pH on effect size for any response variable (see Table S3). We detected a significant linear relationship between the duration of the experiment and the mean effect size for organisms with 'short' times to reproductive maturity in the growth analysis ($Q_M = 0.95$, d.f. = 1, $P = 0.02$; Table 1), and for organisms with 'medium' and 'long' times to reproductive maturity in the calcification analyses (medium: $Q_M = 4.49$, d.f. = 1, $P = 0.01$; long: $Q_M = 6.10$, d.f. = 1, $P = 0.005$; Table 1). The duration of the experiment did not explain heterogeneity in any of the other response variables.

Sensitivity analyses

Rosenthal's fail-safe numbers were high for all response variables (ranging from 256 to 8805) except survival, which could not be accurately quantified due to the lack of reported error in the primary studies. We found mean effect sizes and heterogeneity statistics were robust to the removal of large effect sizes across all response variables except photosynthesis. The removal of the largest magnitude effect size (Zimmerman *et al.* 1997) from the overall photosynthesis analysis changed the heterogeneity from significant to non-significant. This study was therefore removed from all subsequent photosynthesis analyses, and is not included in the results. Eight to ten data points were removed stepwise before the significance of the effect size or heterogeneity changed in the survival, growth and calcification overall

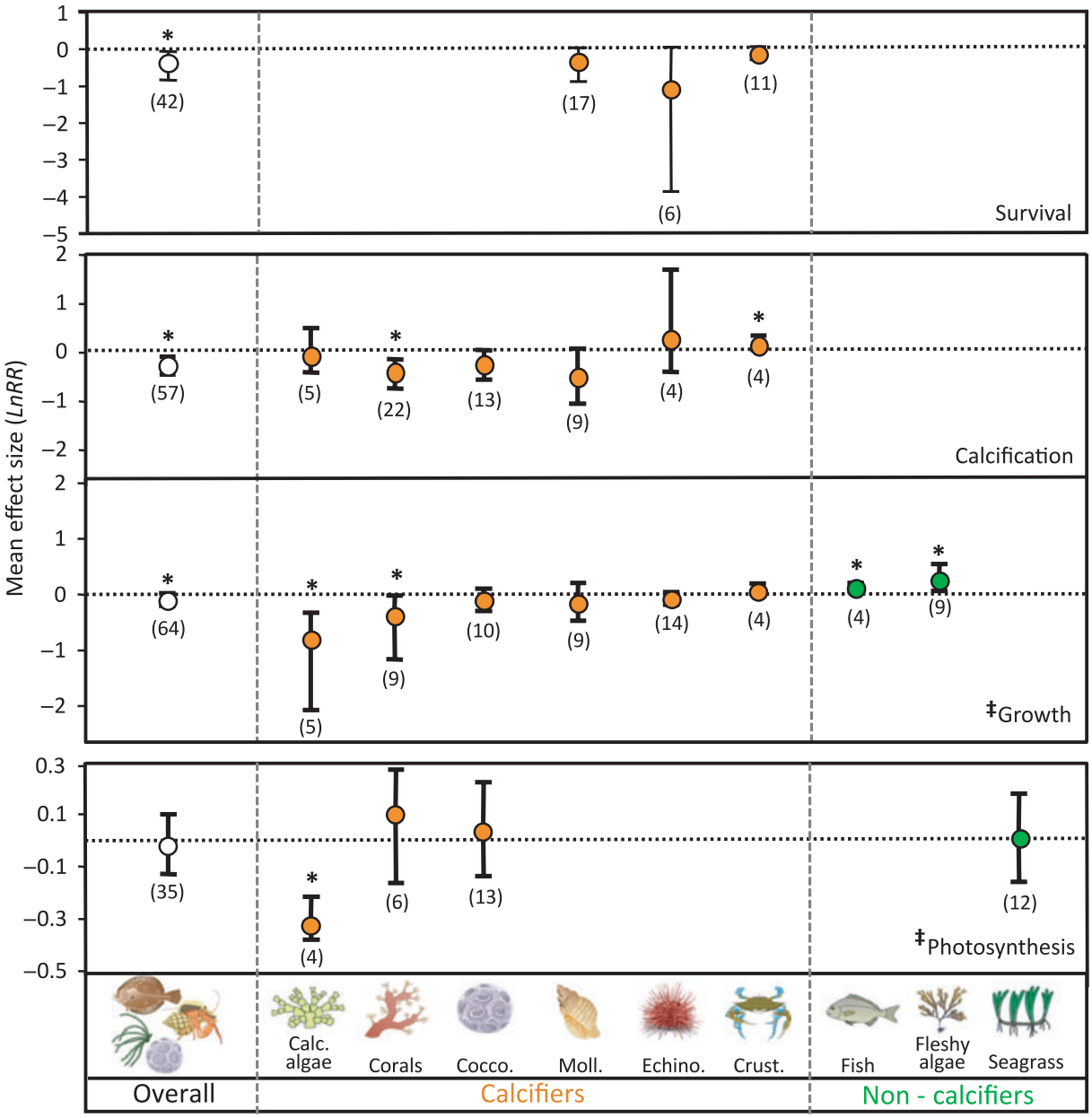

Figure 3 Taxonomic variation in effects of ocean acidification. Note the different *y*-axis scale for survival and photosynthesis. Mean effect size and 95% bias-corrected bootstrapped confidence interval are shown for all organisms combined (overall), calcifiers (orange) and non-calcifiers (green). The calcifiers category includes: calcifying algae, corals, coccolithophores, molluscs, echinoderms and crustaceans. The non-calcifiers category includes: fish, fleshy algae and seagrasses. The number of experiments used to calculate mean effect sizes are shown in parentheses. No mean effect size indicates there were too few studies for a comparison ($n < 4$). The mean effect size is significant when the 95% confidence interval does not overlap zero (*). ‡Indicates significant differences amongst the taxonomic groups tested, based on the Q_M statistic.

analyses. Four data points (representing 33% of total data points) were removed from the reproduction analysis before the significance of the effect size changed. The removal of all data points from a single study that contributed more than five data points to an analysis did not change the significance of the effect size or heterogeneity in any analysis.

An additional 83 experiments were included in the unweighted, fixed effects meta-analyses. The significance of the results of the overall analyses did not differ between the

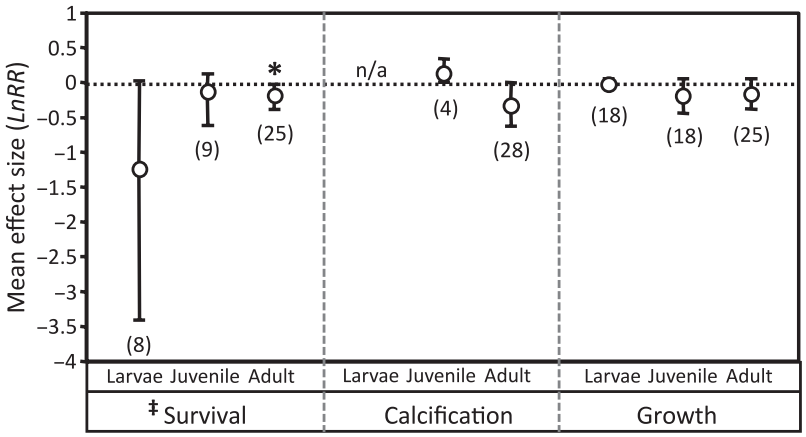

Figure 4 Variation in response to ocean acidification amongst developmental stages (larvae, juvenile and adult) across response variables (survival, calcification and growth). Analyses were not conducted for categories with fewer than three experiments. Mean effect size and 95% bias-corrected bootstrapped confidence interval are shown. The number of experiments used to calculate mean effect sizes are shown in parentheses. The mean effect size is significant when the 95% confidence interval does not overlap zero (*). ‡Indicates significant differences amongst the developmental stages tested, based on the Q_M statistic.

weighted, random effects analyses (Fig. 1) and the unweighted, fixed effects analyses of the larger dataset, with the exception of photosynthesis (see Figure S2). The overall photosynthetic response was significantly positive in the larger, unweighted fixed effects analysis. While the significance of the effect sizes of some taxonomic groups shifted in the taxonomic categorical, unweighted fixed effects analyses of the larger dataset (growth: corals, fish and fleshy algae; calcification: coccolithophores, molluscs; photosynthesis: all taxonomic groups), the results are qualitatively similar to the weighted, random effects models with the exception of photosynthesis (see Figure S3). Additionally, we were able to calculate a mean effect size for diatoms and bacteria. Ocean acidification had a significant positive mean effect on all four taxonomic groups included in the photosynthesis analysis (calcifying algae, corals, coccolithophores and fleshy algae).

DISCUSSION

The meta-analyses revealed significant negative effects on survival, growth, calcification and reproduction, suggesting predicted ocean acidification conditions will have negative consequences for many marine organisms by the end of this century. Calcification was the most sensitive process, and our analyses suggest calcifying organisms are more susceptible to ocean acidification across other response variables. This pattern was also highlighted in the differences in taxonomic groups, where survival and growth were negatively affected across most calcifiers. Our analyses also revealed additional variation in responses to ocean acidification that was driven by differences amongst taxonomic

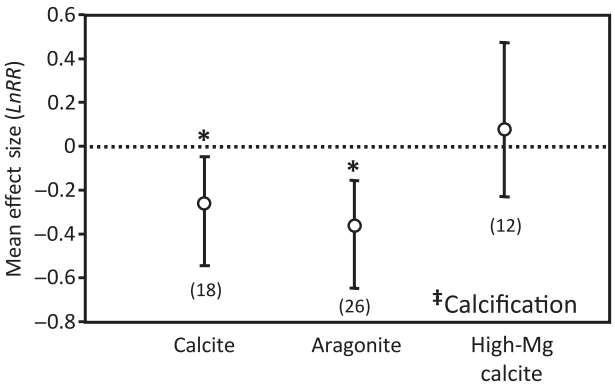

Figure 5 Variation in calcification response to ocean acidification amongst different polymorphs of calcium carbonate. Mean effect size and 95% bias-corrected bootstrapped confidence interval are shown for organisms utilizing calcite, aragonite and high-Mg calcite. Data included in this analysis are from studies that measured a calcification response only. The number of experiments used to calculate mean effect sizes are shown in parentheses. The mean effect size is significant when the 95% confidence interval does not overlap zero (*). Significant differences among the mineral forms based on the Q_M statistic is indicated by (‡).

groups, with organisms such as crustaceans, fish and fleshy algae responding positively to changes in carbonate chemistry. In addition, we found that the differences in effect sizes between developmental stages were specific to taxonomic groups. With the exception of crustaceans, these results suggest the effects of ocean acidification will be negative for most calcifying organisms, but that variation in life history characteristics will prove some organisms more resilient than others.

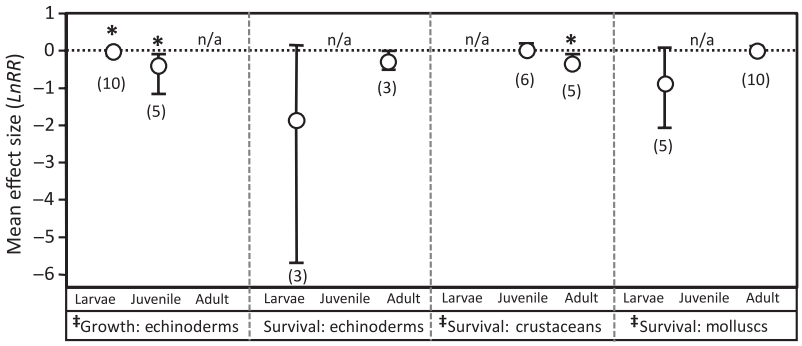

Figure 6 Variation in response to ocean acidification amongst developmental stages (larvae, juvenile and adult) for specific taxonomic groups (echinoderms, crustaceans and molluscs) across response variables (growth and survival). Analyses were not conducted for categories with fewer than three experiments. Mean effect size and 95% bias-corrected bootstrapped confidence interval are shown. The number of data points used to calculate mean effect sizes are shown in parentheses. The mean effect size is significant when the 95% confidence interval does not overlap zero (*). ‡Indicates significant differences amongst the developmental stages tested, based on the Q_M statistic.

Table 1 Relationship between duration of experiment and effect size (*LnRR*) for reproductive maturity ages (short, medium and long) for each response (survival, calcification, growth, photosynthesis and reproduction)

Response	Age to reproductive maturity	*N*	Slope	*P*-value
Survival	Short	1	n/a	n/a
	Medium	8	−0.001	0.785
	Long	30	0.011	0.083
Calcification	Short	14	−0.214	0.441
	Medium	6	0.011	0.010*
	Long	37	−0.001	0.005*
Growth	Short	24	0.002	0.023*
	Medium	11	−0.001	0.771
	Long	35	0	0.098
Photosynthesis	Short	15	−0.005	0.184
	Medium	12	−0.003	0.956
	Long	14	−0.002	0.775
Reproduction	Short	0	n/a	n/a
	Medium	0	n/a	n/a
	Long	11	−0.001	0.858

Short: < 10 days; medium: 10 days – 1 year; long: > 1 year. Analyses were not conducted for categories with fewer than four experiments. *P*-values < 0.05 indicate a significant linear effect of experiment duration on effect size (*).

Our results are in contrast to a previous meta-analysis, which concluded there was no consistent evidence for negative impacts on biological rates (with the exception of calcification rates for bivalves) under near-future ocean acidification scenarios (Hendriks *et al.* 2010). The differences in our conclusions can be explained by several factors. First, there was little overlap in the studies included in both analyses (i.e., 17 studies were shared by both analyses, and we included the results from 56 additional studies). Thus, the results from our meta-analyses represented a different dataset. Next, Hendriks *et al.* (2010) included multiple responses from the same experiments in the meta-analyses, which weighted the experiments reporting multiple responses more heavily than others. Additionally, Hendriks *et al.* (2010) included the response(s) of *Zostera marina* reported by Zimmerman *et al.* (1997), which was excluded from our analyses because of its disproportionate influence on the significance of our results, as indicated by the sensitivity analyses. There are also methodological differences between the studies, including our choice of an effect size metric, *LnRR*, and the model used for meta-analysis (i.e., random effects), and use of resampling statistics for determination of significance. Finally, our interpretation of the overall effects of ocean acidification differs from the previous analysis due to the significant heterogeneity highlighted in the categorical and continuous analyses.

The magnitude of the effect of ocean acidification on calcification was similar amongst calcifiers, with the exception of crustaceans and calcifying algae. This is somewhat surprising given the different calcification strategies (i.e., intracellular vesicles in coccolithophores vs. extracellular compartments in scleractinian corals). The negative responses of corals and coccolithophores to ocean acidification could have profound repercussions for marine ecosystems, with scleractinian corals serving as habitat for coral reef ecosystems and coccolithophores serving as the foundation of its food web. Crustaceans, however, appeared unaffected under conditions of ocean acidification, and the responses of calcifying algae were highly variable. These results are in contrast to the hypothesis that organisms utilizing high-Mg calcite will be more sensitive to ocean

acidification because both crustaceans and coralline algae (which made up most of the calcifying algae category) utilize high-Mg calcite for calcified structures. This hypothesis, based on the solubility of the pure mineral forms in seawater, may fail to predict the sensitivity of marine organisms to ocean acidification because it does not account for biogenic calcification processes (Pörtner 2008). Crustaceans maintain a high control of their intracellular pH through ion-transport regulation (Wheatly & Henry 1992), suggesting they may have the ability to optimize environmental conditions at the calcification site. Additionally, the exoskeleton of most crustaceans is covered by an extensive biogenic covering, which can buffer their $CaCO_3$ structures from direct dissolution in acidified seawater (Ries *et al.* 2009). Finally, unlike the other calcifiers included in our analysis, crustaceans moult their shells regularly and have less $CaCO_3$ in their structures. As such, our results do not support the hypothesis that more soluble forms of $CaCO_3$ will be more sensitive to ocean acidification, and the resilience of crustaceans and coralline algae requires further experimentation to understand the mechanisms for their responses.

Our results do not support the hypothesis that increased photosynthetic carbon assimilation amongst photosynthesis utilizing organisms in response to ocean acidification can buffer its effects on calcification. Coccolithophores have less efficient CCMs than many marine autotrophs, and can increase carbon assimilation under conditions of ocean acidification (Rost & Riebesell 2004). Despite this experimental evidence, we found an overall reduction in calcification. In addition, we did not detect an effect of ocean acidification on symbiotic coral zooxanthellae photosynthesis, and calcification in the host corals was reduced. For coral symbionts, photosynthesis is limited by inorganic nutrient supply, and experimental work suggests that calcification rates can be maintained under conditions of ocean acidification if the nutrient supply and consequently the primary and secondary production are increased (Langdon & Atkinson 2005; Cohen & Holcomb 2009; Ries *et al.* 2009).

Our results support the hypothesis that highly mobile organisms with developed intracellular/extracellular pH regulatory mechanisms may be more resilient to ocean acidification. Both fish and brachyuran crustaceans have well-developed pH regulation and were not negatively affected by the changes in carbonate chemistry. Interestingly, even though we detected significant increases in calcification amongst crustaceans, we were not able to detect significant effects on their growth. The decoupling of calcification and growth for these organisms may be explained by energy allocation strategies. If crustaceans use more energy to maintain calcification, this could result in reduced energy allocation to growth. This hypothesis is in contrast to the results of Arnold *et al.* (2009) where the European lobster *Homarus gammarus* (L.) larvae developed less calcified exoskeletons but maintained their growth rates under high CO_2/low pH conditions. In this instance, decreased calcification paired with increased growth could signal an opposing energy allocation strategy. However, these hypotheses assume that calcification processes are growth limiting, which may not be true for organisms that do not have extensive calcified structures or fast growth rates.

We did not detect significant effects of ocean acidification on photosynthesis in the overall weighted, random effects analysis. Although seagrasses use $CO_{2(aq)}$ for photosynthesis and the dominant coccolithophore species *Emiliania huxleyi* has a relatively inefficient CCM, neither group showed significant effects. However, these results differed in the unweighted, fixed effects analyses that included more experiments. In these analyses, all taxonomic groups responded positively to ocean acidification. The switch from non-significant to positive effects in the unweighted, fixed effects analysis was not driven by studies with especially large pH manipulations (data not shown), because most of the additional studies had experimental pH reductions < 0.5 units. Although photosynthesis was positively affected in the unweighted, fixed effects analysis, the magnitude of the effect sizes remained very small compared to other response variables, suggesting the photosynthetic responses of marine autotrophs are more subtle and less variable than the other response variables.

We did not detect variation amongst the mean effects of differing developmental stages across most response variables. However, this does not necessarily indicate differential sensitivities do not exist amongst life stages. Instead, these differences may be swamped by more pronounced causes of variation, such as taxonomic differences. While experiments examining larval responses were generally lacking, the categorical weighted meta-analyses amongst developmental stages within taxonomic groups showed significant differences amongst life stages (see also Dupont *et al.* 2010), but the patterns varied depending on the taxonomic group in question. These results indicate differential sensitivities amongst life stages is small compared to the degree of variation caused by differing life histories, and the most sensitive life stage may differ amongst taxonomic groups (Kurihara 2008).

Although our analyses highlighted several biological factors that explain variation in responses to ocean acidification, there are still likely to be species-specific sources of heterogeneity (Dupont *et al.* 2008; Fabry 2008; Kurihara 2008). Experimental work has shown that closely related species can respond very differently to the same conditions. For example, the echinoderm *Echinus esculentus* larvae had high mortality under ocean acidification, while the echinoderm *Strongylocentrotus droebachiensis* larvae showed

increased developmental success (Dupont & Thorndyke 2009). These species-specific differences may be more pronounced within certain taxonomic groups, and are likely responsible for our inability to detect strong effects of ocean acidification on calcification in coccolithophores or photosynthesis in seagrasses. For example, it appears that strains of the coccolithophore species *E. huxleyi* have different responses to ocean acidification (Langer *et al.* 2009; Ridgewell *et al.* 2009); and some species of seagrass significantly increase photosynthesis under reduced pH, whereas other species are relatively immune to the changes due to differences in their CCMs (Invers *et al.* 1997). The sources of variation between closely related species (or strains) remain a fruitful area of research for examining mechanisms for resiliency. However, we were able to detect significant negative effects of ocean acidification across all response variables except photosynthesis despite differing species, life histories and developmental stages indicating the differences caused by species-specific variation are minor compared to the overall effect of ocean acidification.

It is important to note that ocean acidification will occur in concert with other environmental changes, and the biological responses we have highlighted may differ with the presence of additional stressors. Several studies have considered the combined effects of ocean acidification and increased temperature on marine organisms. Although not included in our analyses, increased temperatures both increased and decreased the severity of the effect size of ocean acidification (Reynaud *et al.* 2003, 2004; Hare *et al.* 2007; Anthony *et al.* 2008; Findlay *et al.* 2008; Byrne *et al.* 2009; Martin & Gattuso 2009; Munday *et al.* 2009). However, it is not apparent what drives the direction of the response (i.e., increasing or decreasing in severity). Organisms differ in their thermal tolerance, and within their range of tolerance, increased temperatures can increase physiological rates and may buffer negative effects of ocean acidification. Outside their range of tolerance, increased temperatures are detrimental and could compound any stress caused by acidification (Pörtner 2008). Additionally, the responses of marine autotrophs are dependent on light intensity and nutrient concentrations, with increased light and nutrient supply potentially buffering any negative impacts of ocean acidification (Zondervan *et al.* 2001, 2002; Rost *et al.* 2002). Accurate forecasting of ocean acidification's impacts on marine organisms will require additional studies examining these interactions between multiple environmental stressors.

We did not detect strong effects of methodological factors on effect size. The method of carbonate chemistry manipulation did not consistently explain heterogeneity in effect sizes. This is consistent with reviews of methodology that indicate the speciation of carbonate parameters is very similar between both DIC addition at constant TA and manipulation of TA at constant DIC for moderate pCO_2 levels (*c.* 700 p.p.m.) (Ridgewell *et al.* 2009; Schulz *et al.* 2009; Shi *et al.* 2009; Gattuso *et al.* 2010). In addition, the magnitude of the unit change of pH did not predict the effect size for most of the analyses. The significant relationship between unit change in pH and the effect size in the growth analysis was driven by a few very negative effect sizes amongst echinoderms, and in combination with the other analyses, does not provide strong support for a linear relationship. This could be indicative of nonlinear relationships between pH and effect sizes, including the possibility of thresholds or tipping points (Scheffer *et al.* 2001; Ries *et al.* 2009). This also suggests the selection criteria did not have a systematic bias on the effect size (e.g., by only including studies with less than a 0.5-unit pH reduction our effect size estimates were not consistently underestimating the true effect size). More experiments that examine the relationship between unit change in pH and effect size will shed light on how the magnitude of the carbonate chemistry manipulation drives biological responses.

Additionally, we did not find strong support for a linear relationship between the duration of the experiment and the effect size. There were a few significant comparisons, however, the slopes of the fitted lines were nearly zero in all cases, and there was no trend in the direction of the slopes (Table 1). These correlations are dependent on the studies and species included in the analyses, and significant correlations could be unrelated to the methodological factors being tested. Overall, our results suggest methodological factors did not explain the variation in responses to ocean acidification.

The results of the sensitivity analyses suggest that the patterns highlighted in our meta-analyses are a robust representation of the current literature on ocean acidification. The results from the unweighted, fixed effects analyses of the larger dataset are very similar to the main results, suggesting our selection criteria for studies did not bias the results. However, the significance of the photosynthetic responses of marine autotrophs in the unweighted, fixed effects analysis suggests the power of the main photosynthesis analysis may have suffered from low sample size. Unfortunately, we did not include several studies because they did not report enough information to ensure their comparability (e.g., seawater pH or two parameters of the carbonate chemistry). The inclusion of all applicable studies is always preferable in meta-analysis (Englund *et al.* 1999) and this problem highlights the importance of data reporting in future ocean acidification research. Finally, it is important to consider that the published literature is probably biased towards studies that find significant effects.

Even though we included as many studies as possible to examine the biological responses to ocean acidification, the

studies were limited to a few taxonomic groups and even fewer ecosystems. Although ocean acidification is predicted to be a global stressor, the literature is dominated by studies in coral reef and pelagic ecosystems. Studies are needed in other coastal environments, including variable pH environments (e.g., upwelling zones, CO_2 vent systems and estuarine areas) to explore whether history of exposure to a more variable pH environment confers adaptation potential. More physiological studies are needed to understand how these individual responses combine to affect performance and fitness of marine organisms, and long-term studies are needed to address possible acclimation or adaptive responses. Additionally, studies are needed that examine the ecological consequences of reduced performance/fitness in marine communities or ecosystems.

In conclusion, our analyses revealed a strong negative effect of ocean acidification on marine organisms despite the variation in the sensitivity of taxonomic groups and developmental stages. However, differential sensitivities still have important implications for marine ecosystems where individual species often play disproportionately strong roles in structuring communities (Shurin *et al.* 2002; Borer *et al.* 2005). Additionally, differential sensitivities will influence species interactions and could drive unforeseen impacts on marine communities and ecosystems. However, this synthesis did not support all the leading hypotheses for variation in response to ocean acidification (i.e., early developmental stages and more soluble $CaCO_3$ polymorphs are more susceptible to ocean acidification). But instead, we found the explanatory power of these hypotheses was specific to organisms within taxonomic groups. This research has important implications because a clear understanding of the magnitude and the sources of the variation in the responses to ocean acidification will allow managers and policy makers to make more accurate generalizations, and improve the accuracy of models and forecasts for ocean acidification's impacts. Further progress in explaining the variation in biological responses to ocean acidification will require studies targeting the biological mechanisms for variation.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 Variation in effect sizes between experimental methods.
Figure S2 Overall results of unweighted, fixed effects meta-analyses on different response variables of marine organisms.
Figure S3 Taxonomic variation in response to ocean acidification from unweighted, fixed effects meta-analyses.
Table S1 Selection criteria for inclusion in meta-analyses.
Table S2 Studies used in meta-analyses.
Table S3 Relationship between unit change in pH and effect size.

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