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Quo Vadimus

Responses of benthic calcifying algae to ocean acidification differ between laboratory and field settings

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Accurately predicting the effects of ocean and coastal acidification on marine ecosystems requires understanding how responses scale from laboratory experiments to the natural world. Using benthic calcifying macroalgae as a model system, we performed a semi-quantitative synthesis to compare directional responses between laboratory experiments and field studies. Variability in ecological, spatial, and temporal scales across studies, and the disparity in the number of responses documented in laboratory and field settings, make direct comparisons difficult. Despite these differences, some responses, including community-level measurements, were consistent across laboratory and field studies. However, there were also mismatches in the directionality of many responses with more negative acidification impacts reported in laboratory experiments. Recommendations to improve our ability to scale responses include: (i) developing novel approaches to allow measurements of the same responses in laboratory and field settings, and (ii) researching understudied calcifying benthic macroalgal species and responses. Incorporating these guidelines into research programs will yield data more suitable for robust meta-analyses and will facilitate the development of ecosystem models that incorporate proper scaling of organismal responses to *in situ* acidification. This, in turn, will allow for more accurate predictions of future changes in ecosystem health and function in a rapidly changing natural climate.

Keywords: calcifying algae, coralline algae, environmental change, global change, pH, scaling responses.

Introduction

Ocean and coastal acidification (Caldeira and Wickett, 2003; Duarte *et al.*, 2013) impact marine life on a variety of ecological scales from individuals to ecosystems (Andersson *et al.*, 2015; Edmunds *et al.*, 2016). For example, direct and indirect effects of acidification on marine organisms, species interactions, and biogeochemical cycling have been documented (Hendriks *et al.*, 2010;

Kroeker *et al.*, 2010, 2013; Hoffmann *et al.*, 2012; Cyronak *et al.*, 2014; McCoy *et al.*, 2016a). Seawater carbonate chemistry also varies over shorter time scales in coastal regions (Hofmann *et al.*, 2011; Cyronak *et al.*, 2020), which may influence the resilience of marine organisms to long-term ocean acidification (Rivest *et al.*, 2017; Kapsenberg and Cyronak, 2018). Thus, predicting future marine biodiversity and ecosystem function in an era of climate change depends on understanding how responses scale across ecological,

spatial, and temporal scales (Levin, 1992; Thrush *et al.*, 1997; Chave, 2013).

Scientists currently use a variety of methodologies in laboratory and field settings to understand the effects of acidification on marine life (Wernberg *et al.*, 2012; Andersson *et al.*, 2015; Cornwall and Hurd, 2016). Most research, however, has focused on testing the responses of single species in small-scale, controlled experiments. Fewer studies have documented responses of individuals (i.e. species), populations, and communities to *in situ* changes and gradients in seawater pH. As a result of increasing CO₂ emissions and associated ocean acidification (Feely *et al.*, 2009; Bates *et al.*, 2014), there is an increasing recognition for the need to bridge results from laboratory experiments to natural environments. Because oceanographic processes act at a variety of scales, the responses of individuals in small-scale experiments may not directly reflect those at broader ecological, spatial, and temporal scales (Thrush *et al.*, 1997; Underwood *et al.*, 2005; Chave, 2013). Yet, very few studies have compared responses of marine organisms in laboratory versus field settings (McElhany, 2017) to understand how single-species experiments translate to population-wide effects. Therefore, within a growing body of acidification literature, we ask the question: *Can we utilize the existing wealth of laboratory experiments to predict the responses of marine organisms to acidification in the natural environment?*

Marine organisms and ecosystems that rely on calcification may be especially vulnerable to altered carbonate chemistry resulting from increased seawater CO₂ concentrations (Kleypas and Yates, 2009; Kroeker *et al.*, 2010). Thus, the impacts of acidification on calcifiers have been studied widely, making them an ideal functional group for examining how effects of acidification scale from individuals (i.e. species) to communities. Calcifying benthic macroalgae (red, green, and brown) have a cosmopolitan range (Nelson, 2009) and provide essential ecological functions including primary production, carbon storage, habitat formation, and physical habitat stabilization across a broad latitudinal range (Foster, 2001; McCoy and Kamenos, 2015). For example, the calcifying green alga *Halimeda* spp. forms habitat (Stoner and Lewis, 1985), shapes community structure through ecological interactions such as competition (Brown *et al.*, 2019), and plays a role in carbon sequestration in tropical habitats (Payri, 1988; Rees *et al.*, 2007; Krause-Jensen and Duarte, 2016). Non-geniculate coralline algae fill an additional suite of ecological roles in polar, temperate, and tropical systems by producing chemical cues that facilitate settlement of invertebrate larvae (Roberts *et al.*, 2004), cementing coral reef ecosystems (Adey, 1998), and providing three-dimensional habitat for economically valuable and diverse species assemblages (Chisholm, 2000; Foster, 2001; McCoy and Kamenos, 2015).

The impacts of acidification on calcifying benthic macroalgae are generally negative (Hofmann and Bischof, 2014; McCoy and Kamenos, 2015; McNicholl *et al.*, 2020). However, effects can vary depending on species, environmental history, and co-occurring stressors (Anthony *et al.*, 2008; Price *et al.*, 2011; Johnson *et al.*, 2014; Johnson and Carpenter, 2018; Johnson *et al.*, 2019). Previous reviews have described the physiological and ecological responses of calcifying macroalgae to acidification (Hofmann and Bischof, 2014; McCoy and Kamenos, 2015). We initially attempted to build on this work by conducting a meta-analysis to compare responses of tropical and temperate calcifying benthic macroalgae to acidification across laboratory ($n = 97$) and field ($n = 27$) studies. However, differences in sample sizes (i.e. lack of data for field settings) and methodological approaches between laboratory

and field studies did not allow for robust analyses. Therefore, we conducted a semi-quantitative synthesis to explore the collective knowledge regarding the directional responses of calcifying benthic macroalgae to acidification. We highlight the agreements and differences between effects observed in the laboratory and field. From this synthesis, we recommend future directions that will facilitate our ability to conduct robust meta-analyses and ecosystem models for laboratory evaluating how laboratory studies may translate to natural environments.

Methods

We created an initial database by using returns from a Web of Science search with the terms “ocean acid*,” “calcified alga*,” and “coralline alga*” (search run on 03 April 2020) compiled with entries from the Ocean Acidification International Coordination Centre Bibliographic Database (OA-ICC, 2020). There were 124 studies published between 1979 and 2020. They report the responses of 36 temperate and 32 tropical calcifying benthic macroalgae species to artificial (i.e. through laboratory experiments) and natural (i.e. across gradients at CO₂ seeps) changes in seawater carbonate chemistry (Figure 1).

Papers were then manually filtered for those that exposed calcareous brown, green, and red macroalgae to acidification through increased seawater pCO₂ (i.e. studies using HCl to manipulate seawater pH were excluded). Each response was categorized as a laboratory or field study. Any effects at acidification levels that are unrealistic by the year 2100 under current emission scenarios (i.e. pH < 7.2; IPCC, 2019) were not considered in this synthesis. Since anthropogenic ocean acidification results in multiple co-occurring changes in carbonate chemistry parameters, results within a study that decoupled carbonate chemistry parameters (e.g. elevated bicarbonate but no change in carbonate ion concentration) were also excluded from our database. Finally, for papers that used both static and varying pH treatments, we only compared static treatments to each other or varying treatments to each other because variability, or lack thereof, in seawater pH may influence how marine organisms respond to ocean acidification (Rivest *et al.*, 2017). Overall, there were less than five papers that used a diurnally fluctuating pH treatment. The bibliographic list of all studies used in this synthesis can be found in the Supplemental Information (Supplementary Table S1).

For each paper, the directional responses of calcifying macroalgae were noted as positive, neutral, or negative. Results that were not statistically significant from control pH/pCO₂ levels were scored as “neutral.” Positive responses indicate increased directional response relative to control pH/pCO₂ levels, regardless of effect on fitness and ecosystem function. Negative responses indicate decreased directional response relative to control pH/pCO₂ levels, regardless of effect on fitness and ecosystem function. For example, increased bleaching was scored as a positive response, despite a physiological interpretation of decreased fitness. The directional responses were noted because we do not have a full understanding of how changes in every parameter influences fitness or ecosystem function of calcifying benthic macroalgae.

When multiple species and/or response variables were tested within one study, each response was categorized separately for each species. When observations were reported for separate short-term experiments conducted in different seasons or years, responses were scored separately for each time frame. Each response was then further categorized as being at the individual (i.e. species)

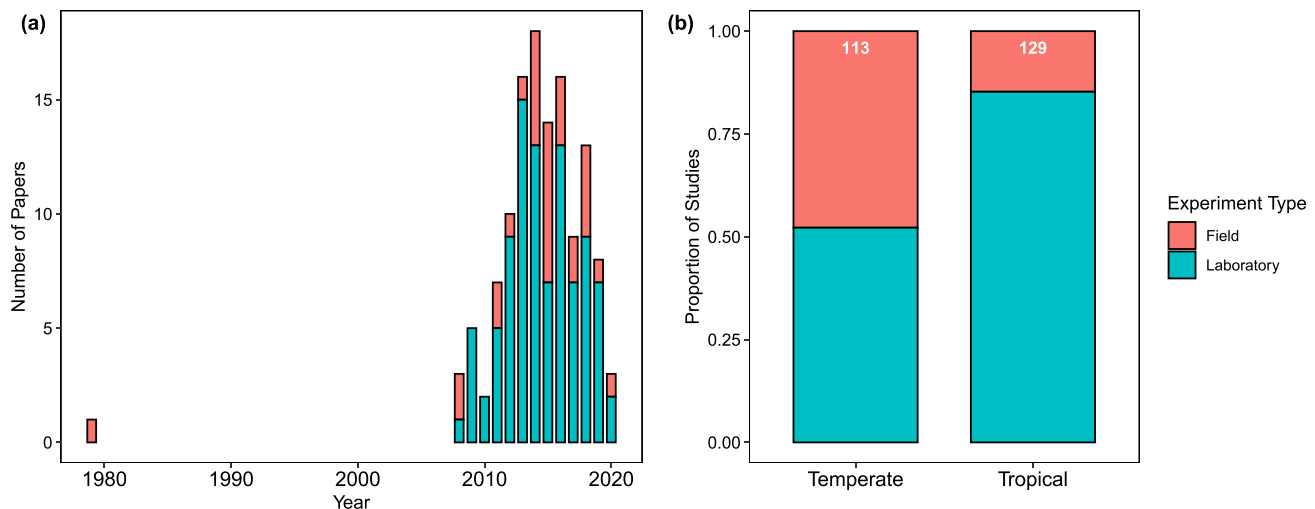


Figure 1. Summary of papers reporting responses of calcifying and calcareous benthic macroalgae to ocean acidification. (a) The number of papers published per year, as of April 2020. (b) The proportion of laboratory and field studies in tropical and temperate environments.

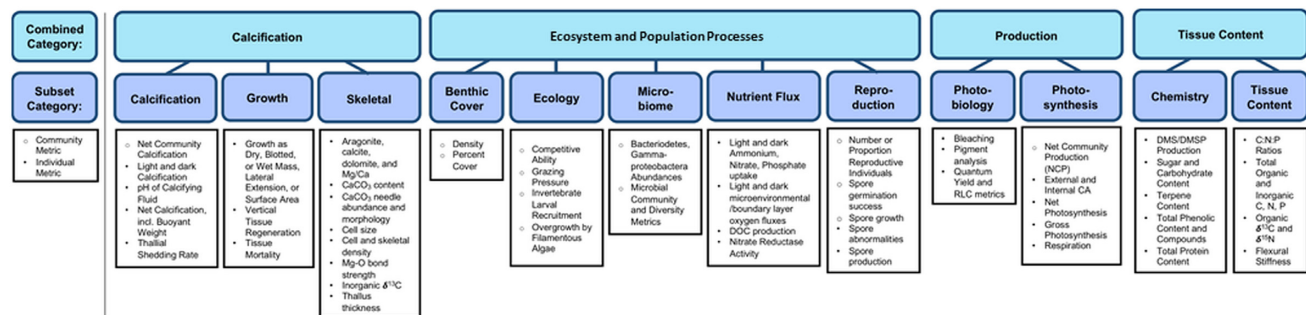


Figure 2. Flow chart of response categorization. Measured responses that were sorted into combined and subset categories are described under headings of 12 subset categories, boxes coloured purple. These subset categories are grouped into four combined categories, shown by boxes coloured blue. Response types are coded with open bullets to denote community-level responses and filled bullets to denote individual-level responses.

or community/ecosystem level (e.g. net community production). Most observations of microbial communities could not be categorized within this matrix but are discussed in more detail below.

The results were divided into broad categories for visualizing and synthesizing the proportion of positive (i.e. increased), neutral (i.e. no significant effect), and negative (i.e. decreased) responses across studies (Figure 2). The four broad categories were: (1) calcification, (2) production, (3) tissue content, and (4) ecosystem and population processes. Results within each of these broad categories were divided into smaller subsets (Figure 2) for synthesis and discussion.

Results

Overview of individual and community responses

Most studies (89%) reported responses of individual species, while only 11% focused on community-level metrics (Figure 3a). Although there were fewer community level responses reported overall compared to those of individual species, many, but not all, of the community metrics measured in the laboratory were also

measured in the field. In the laboratory experiments, 93% of measured responses were at the individual level ($n = 355$) and 7% were at the community or ecosystem level ($n = 27$). However, within field studies, community metrics were more common, making up 29% of responses ($n = 63$).

Overall, responses of individual organisms to acidification (including both laboratory and field studies) were primarily reported as neutral (56%), with 32% negative and only 12% positive (Figure 3b). This distribution was similar when laboratory and field studies were grouped separately indicating that there are few positive responses of eco-physiological processes at the individual scale. In laboratory experiments, 57% of responses were reported as neutral, 30% were reported as negative, and 12% were reported as positive. In the field, there was a more even split between documented neutral (44%) and negative (43%) responses, though positive responses remained low (13%).

In contrast to the individual species-level results, community metrics were dominated by negative responses to elevated seawater $p\text{CO}_2$ (Figure 3c). Overall, observed community responses in both laboratory and field studies were 30% neutral, 62% negative, and 8% positive. Neutral (44%) and negative (48%) responses were

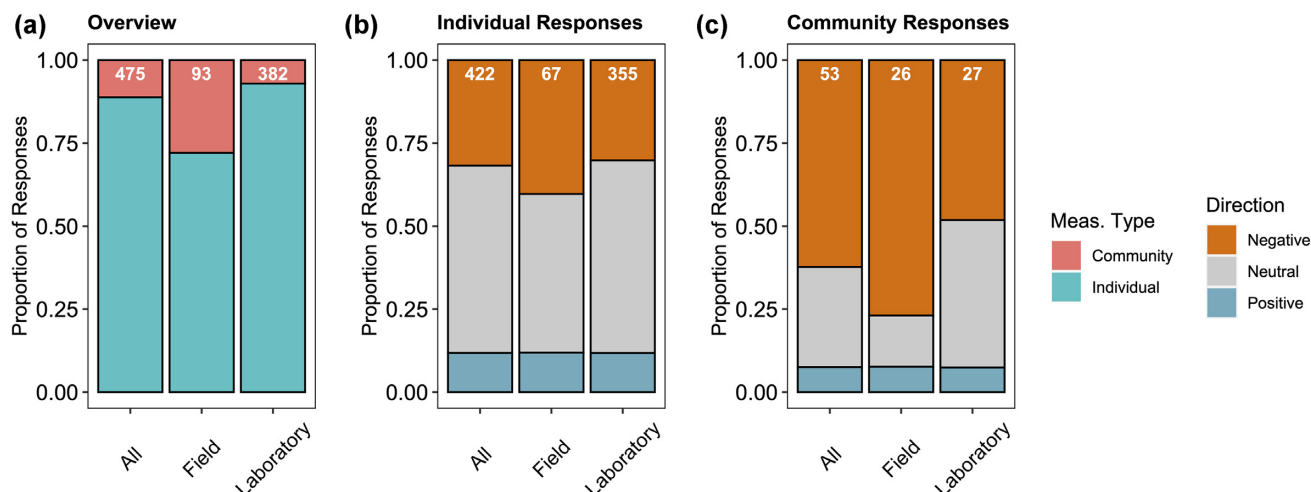


Figure 3. Overview of individual- vs. community-level responses measured for calcifying benthic macroalgae in response to acidification. (a) Measurement type plotted for all, field, and laboratory studies. Measurement type is shown by colour, with pink, the top portion of the stacked barplot, showing community-level responses, and teal, the bottom portion of the stacked barplot, depicting individual-level metrics. (b) Directionality of individual-level responses are grouped by all, field, and laboratory studies. (c) Directionality of individual-level responses are grouped by all, field, and laboratory studies. In panels (b) and (c) red, the top portion of the stacked barplot, depicts negative responses (decreased directional response). Blue, the bottom portion of the stacked barplot, depicts positive responses (increased directional response). Grey portions, in the middle, depict neutral responses. Total number of responses recorded is given in white numbers at the top of each bar.

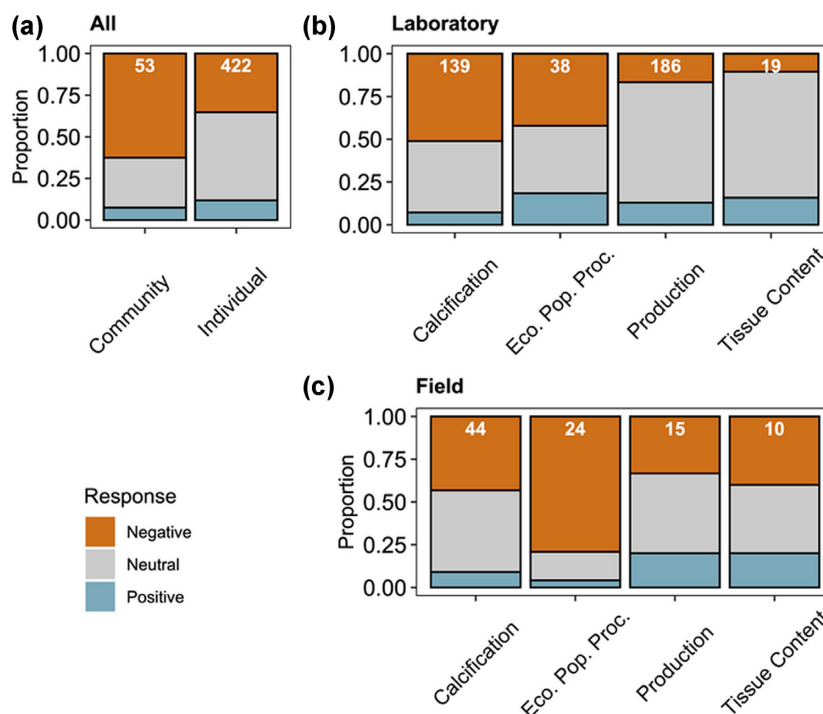


Figure 4. Directional responses of calcified algae in ocean acidification studies. (a) All responses grouped by community or individual measurements types. (b) Responses from laboratory studies grouped into four combined categories. (c) Responses from field studies grouped into the same four categories. Red, the top portion of the stacked barplot, depicts negative responses. Blue, the bottom portion of the stacked barplot, depicts positive responses. Grey portions, in the middle, depict neutral responses. Total number of responses recorded is given in white numbers at the top of each bar.

prevalent in those laboratory experiments, with only 7% of experiments demonstrating a positive response. In the field, community responses also were skewed towards negative responses (77%), with few being neutral (15%) or positive (8%).

Description of measured responses by categories

There was variation in the number of studies that measured variables within the four broad response categories (i.e. calcification, production, tissue content, and ecosystem and population

processes) for individuals and communities (Figure 4a; Table 1). Laboratory experiments largely focused on calcification ($n = 139$) and production ($n = 186$; Figure 4b; Table 1). In contrast, most field studies focused on calcification as well as ecosystem and population processes (e.g. nutrient uptake; Figure 4c; Table 1). Many subsets of responses within the broad categories had fewer than ten recorded instances, and some (i.e. microbiome, nutrient flux, and photosynthesis) were not represented in the field data at all. We discuss the responses of each broad category in detail below.

Calcification

Calcifying algae precipitate diverse polymorphs of calcium carbonate (CaCO_3) that have varying solubilities. For example, crustose coralline algae (CCA) precipitate the most soluble (high Mg-calcite) and least soluble (dolomite) forms of CaCO_3 (Nash *et al.*, 2013; Diaz-Pulido *et al.*, 2014). Other calcifying algae, such as *Halimeda* spp., produce aragonitic crystals (Macintyre and Reid, 1995). The diverse mineralogies of calcifying algae and crystals (Rude and Aller, 1991) and uncertainties surrounding the solubilities of biogenic high Mg-calcite make it difficult to predict calcification responses to acidification based on their mineralogies alone (Morse and Arvidson, 2002; Morse *et al.*, 2006; Kroeker *et al.*, 2010).

Although the impacts of acidification on calcification are diverse, likely due to differences in environmental conditions at collection sites (Johnson *et al.*, 2019) and species physiology and growth rates (Kroeker *et al.*, 2010; Comeau *et al.*, 2014; Johnson *et al.*, 2014), results from the laboratory and field match relatively well. Overall, calcification responses were 42% neutral, 51% negative, and 7% positive in laboratory experiments ($n = 139$), which compares well to observations in field studies with 48% neutral, 43% negative, and 9% positive ($n = 44$; (Figure 4; Table 1).

However, when calcification responses were broken down into sub-categories (i.e. calcification rate, growth rate, and skeletal characteristics) discrepancies between laboratory and field studies emerged (Table 1). Calcification rates showed more positive responses (i.e. rates increased under acidification) in the field (29%) compared to the laboratory (3%; Table 1). Conversely, 50% of laboratory observations showed decreased calcification rates, while only 29% of field studies were negative (Table 1). Growth rates (e.g. extension, changes in biomass) also were differentially impacted in the laboratory and field studies. Although there are limited growth measurements in the field ($n = 5$), responses were overwhelmingly neutral (80%). In contrast, 58% of growth responses in the laboratory were negative (Table 1). Unlike calcification and growth measurements, the impact of acidification on skeletal properties (e.g. mineralogy, crystal structure, and so on) was consistent between laboratory and field studies—approximately half of the responses were negative (Table 1).

While it is likely that CaCO_3 production by calcifying algae is sensitive to acidification, the discrepancies between the laboratory and field data make interpreting these impacts difficult. Specifically, laboratory experiments may be overestimating the negative impacts of acidification on algal calcification in the real world. The different responses in laboratory and field studies may be due to acclimatization, adaptation, and resilience of field specimens that are regularly exposed to low pH waters (Kapsenberg and Cyronak, 2018). Furthermore, non-ideal laboratory conditions for growth (e.g. inadequate light levels, changes in nutrient concentrations or flow regime, or unfavourable growth substrate) could impact how algae respond to ocean acidification treatments (Cornwall and Hurd, 2016).

Understanding the nuances underlying these results is essential because predictions based on the negative calcification responses from laboratory experiments may be inflated.

Production

Understanding how the primary production, photosynthesis, and respiration of calcifying algae respond to acidification is critical to predicting their future health. Growth responses of calcifying algae to acidification are likely related to mechanisms of inorganic carbon acquisition and photosynthesis (Hofmann *et al.*, 2016; McCoy *et al.*, 2016b). However, most of our understanding of algal production is derived from studies using non-calcified algae. Primary production begins with the fixation of dissolved inorganic carbon by RuBisCO enzymes at the site of photosynthesis. Species without carbon concentrating mechanisms (CCMs) or with low affinity for dissolved inorganic carbon could benefit from increased seawater $p\text{CO}_2$ (Giordano *et al.*, 2005). Likewise, species that downregulate CCMs can profit from energetic savings, thus leading to elevated growth. Macroalgae that solely rely on CCM activity, however, often experience no change in growth under acidification (Diaz-Pulido *et al.*, 2016; Comeau *et al.*, 2017; Cornwall and Hurd, 2020).

Overall, laboratory experiments ($n = 186$) compared to field studies ($n = 15$) reported a higher proportion of neutral responses related to production (70% vs. 47%, respectively), although the large discrepancy in the sample sizes must be considered (Figure 4; Table 1). Differences in photobiology responses likely drive the inconsistency between field and laboratory studies. Most laboratory experiments documented no significant changes in photobiology (72%) with fewer negative (19%) and positive (9%) responses (Table 1). Many field studies, in contrast, observed negative responses (63%) with fewer neutral (25%) and positive (13%) responses (Table 1). Photosynthetic pigment concentrations were maintained or depressed under elevated $p\text{CO}_2$ in most laboratory experiments and field studies. Most Pulse Amplitude Modulated (PAM) fluorometry measurements showed no change in photobiology under elevated seawater $p\text{CO}_2$.

Despite contrasting impacts of acidification on photobiology in the laboratory and field, rates of photosynthesis and respiration tend to be maintained (68% and 71% neutral responses in the laboratory and field, respectively; Table 1). However, several laboratory experiments documented decreased gross and net photosynthesis and respiration (13%), while field studies showed no negative responses in any photosynthesis parameters (Table 1). Like calcification responses, laboratory experiments may be overestimating negative impacts of acidification. Conversely, field studies may be underestimating the production responses to elevated seawater $p\text{CO}_2$ if the laboratory experiments are capturing real world responses that are not being captured in the field for some methodological reason.

Tissue content

The impacts of acidification on CaCO_3 production and photosynthesis of calcifying macroalgae may influence tissue chemical content, composition, and secondary metabolite production (Schupp and Paul, 1994). These changes, in turn, indirectly influence community-level interactions. For example, algal tissue CaCO_3 content regulates toughness and nutritional value to herbivores, thus acting as a defense mechanism by altering the effectiveness of digestion (Lobel, 1981; Hay *et al.*, 1994). Lower CaCO_3

Table 1. Total number of responses documented in laboratory experiments and field studies for each of the response categories and sub-categories. Positive responses indicate a response increased relative to control seawater pH/pCO₂ while negative responses indicate parameters that decreased relative to control seawater pH/pCO₂. Responses that did not statistically significantly differ from control treatments were scored as “neutral” responses.

	Total responses		Laboratory experiments			Field studies		
	Laboratory	Field	Positive	Neutral	Negative	Positive	Neutral	Negative
I. Calcification	139	44	10	58	71	4	21	19
a. Calcification			2	33	35	2	3	2
b. Growth			2	8	14	0	4	1
c. Skeletal			6	17	22	2	14	16
II. Production	186	15	38	253	64	4	10	12
a. Photo-biology			10	79	21	1	2	5
b. Photosynthesis			28	174	43	3	8	7
III. Tissue content	19	10	3	8	2	0	2	2
a. Chemistry			3	4	1	0	1	0
b. Tissue content			0	4	1	0	1	2
IV. Ecosystem and population processes	38	24	7	14	13	2	5	21
a. Benthic cover			0	3	6	0	2	18
b. Ecology			1	5	1	1	2	1
c. Microbiome			1	1	2	0	0	0
d. Nutrient flux			5	5	4	0	0	0
e. Reproduction			0	0	0	1	1	2

content may weaken the efficacy of herbivore defense in certain algal species (Campbell *et al.*, 2014). In addition, coralline algae also represent a significant stock of dimethylsulphoniopropionate (DMSP) (Burdett *et al.*, 2015), a major precursor of the climate-regulating gas dimethylsulphide (DMS). DMS has been documented to drive a range of community interactions including grazing behaviours (Lyons *et al.*, 2010). Since species interactions play vital roles in structuring the community composition of marine ecosystems (Lubchenco, 1978), studying changes in algal tissue content is critical to understanding how acidification may influence marine plant–herbivore interactions and, subsequently, community structure and ecosystem processes of reef habitats.

Several studies have focused on understanding the impact of ocean acidification on the tissue content of calcifying algae ($n = 19$ and 10 in the laboratory and field, respectively; Table 1). No significant changes in tissue content (74%) were most commonly observed in laboratory experiments (Figure 4; Table 1). However, field studies reported equal percentages of neutral (40%) and negative (40%) responses (Figure 4; Table 1). More specifically, the impact of acidification on tissue chemistry (DMS/DMSP production, sugar content, terpene content, etc.) is understudied in both the laboratory ($n = 8$) and field ($n = 1$). Most of these documented responses were neutral (Table 1).

Ecosystem and population processes

Benthic cover by calcifying macroalgae is an important community metric because it integrates processes such as propagule settlement and adult growth with other ecological interactions such as grazing and competition for space. In both laboratory and field experiments, benthic cover predominantly decreased as acidity increased. Benthic cover, reported as % cover on experimental tiles or natural substrates (e.g. seagrass blades), was more commonly reported in field studies ($n = 20$) relative to laboratory experiments ($n = 9$; Table 1). Field studies reported 90% negative and 10% neutral changes in benthic cover with acidification,

compared to 67% negative and 33% neutral responses in the laboratory (Table 1).

Ecological responses focus specifically on interactions with other species including algal–grazer interactions, facilitation of invertebrate larval recruitment or settlement, and competitive overgrowth. In the laboratory, responses were primarily neutral ($n = 5$) with respect to changes in ecological interactions, with only one study reporting a positive effect and one reporting a negative impact of acidification (Table 1). In the field ($n = 4$), two studies reported neutral responses. It is important to note that these four responses come from different species within a single study of competitive interactions among calcified macroalgae (McCoy and Pfister, 2014) and may not represent overall responses in the field. Further, this example illustrates that outcomes for a single species may not be representative of outcomes for calcified macroalgae as a whole. Shifts in the relative competitive abilities of multiple species may lead to a novel competitive hierarchy at equilibrium that is not easily quantified as a positive or negative change to ecosystem function (McCoy *et al.*, 2016a).

Changes to the microbiome of calcifying algae, reproductive metrics, and nutrient flux caused by ocean acidification were quantified only in the laboratory. Surprisingly, there were less than fifteen studies, despite the importance of these metrics to holobiont health, population dynamics, and attribution to community composition (via recruitment and species interaction) and biogeochemical cycling. Laboratory experiments ($n = 4$) quantified the abundance of specific bacterial groups and metrics of microbial community and diversity using molecular tools. Given the limited research, it was difficult to assess whether general microbiome changes contributed or detracted from ecosystem function. Therefore, those data are not included in our summary (Webster *et al.*, 2016; Huggett *et al.*, 2018). Likewise, few laboratory experiments ($n = 3$) measured changes in reproductive health. Measurements of nutrient flux are related to tissue content but focus on processes including nutrient uptake, enzyme activities, and micro-environment studies at the algal cell surface. Laboratory studies documenting nutrient fluxes by algae

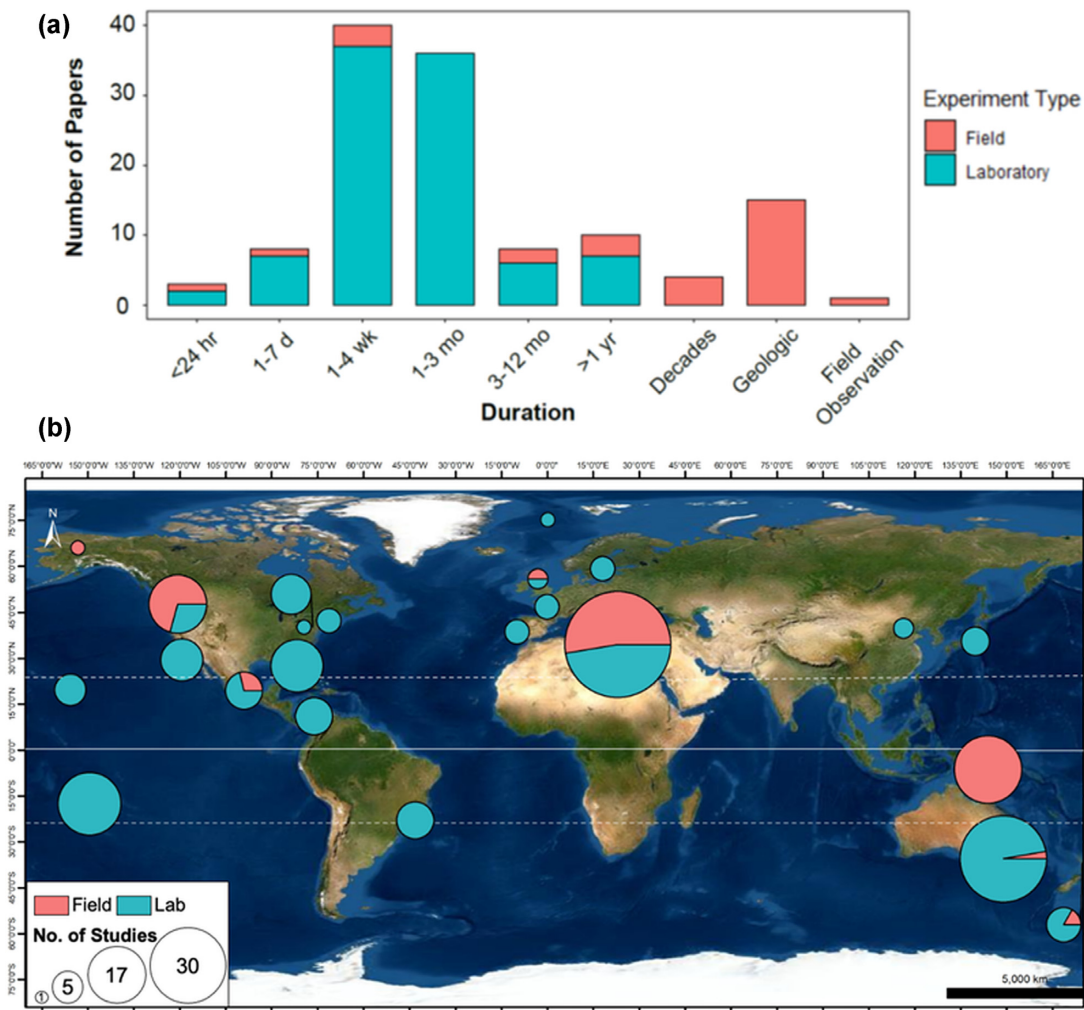


Figure 5. Exposure periods and locations of laboratory and field studies. (a) All studies grouped by categories based on different periods of exposure to acidification conditions. Decade refers to 10s of years, Geologic to 100s of years, and field observations are treated separately because observations have no specific time component (b) Map showing locations of laboratory and field studies with the circle sizes representing the relative number of studies and colour representing laboratory (aqua) or field (orange) studies. Equator (solid white line), Tropic of Cancer (dashed white line), and Tropic of Capricorn (dashed white line) are denoted on the map to distinguish studies conducted in the tropic and temperate regions.

($n = 14$) reported a nearly even division across neutral (36%), negative (28%), and positive (36%) responses (Table 1).

Discussion

Additional considerations for comparison of responses to acidification in laboratory experiments and field studies

Duration of exposure to acidification

Differences between laboratory and field responses to acidification may be related to differences in spatio-temporal scales. For instance, the lengths of exposure periods vary greatly between laboratory and field studies (Figure 5a), and methodological differences mean responses have been integrated over different time scales. Most laboratory experiments quantified acidification impacts over mid to long (8–90 d) time scales with no studies lasting longer than 2 years, likely due to the inherent challenges of maintaining laboratory conditions for prolonged periods. Field studies represent *in situ*

observations of communities that have developed in naturally lower pH environments over decades or centuries. Furthermore, long-term experiments and *in situ* observations represent integrated responses over diurnal and seasonal time scales, which may influence the direction and magnitude of biological responses to acidification (Godbold and Solan, 2013; Rivest *et al.*, 2017; Kapsenberg and Cyronak, 2018).

Spatial coverage of studies

We found an equal number of studies focused on the response of calcifying algae in tropical ($n = 129$) and temperate ($n = 113$) waters (Figure 5b). However, over half of these studies have taken place in coastal environments of just four regions: Mediterranean Sea, Australia, Papua New Guinea, and French Polynesia. Additionally, studies from each region tend to focus on just one approach (laboratory experiments or field studies). Acidification research conducted in the Mediterranean has close to an equal number of laboratory

and field studies due to the many studies analysing the natural CO₂ seep sites near Italy in addition to laboratory experiments. Overall, field studies are under-represented in tropical environments. Ocean acidification and associated carbonate chemistry changes vary across latitudes (Sutton *et al.*, 2019) with Earth System Model projections consistently showing elevated acidification in polar regions (Steinacher *et al.*, 2009; Joos *et al.*, 2011; Steiner *et al.*, 2014). Despite this expected latitudinal trend, there have only been two studies investigating responses of polar calcifying algae (Büdenbender *et al.*, 2011; Schoenrock *et al.*, 2016). If we want to synthesize the responses of marine taxa to future acidification, studies need to encompass all regions across latitudes, particularly in developing nations that rely economically on marine ecosystems dependent on calcifying algae (e.g. coral reefs).

Temporal variability in seawater carbonate chemistry

Targeted and static manipulations, which do not attempt to capture the natural variability of coastal waters, do not represent the dynamic environments these organisms experience. Incorporating natural diurnal and seasonal variability into future experiments may alter our understanding of how calcifying algae are impacted by acidification, particularly because variability could amplify these effects (Johnson *et al.*, 2019) or alter the direction of response. For example, one study showed coralline algae grown in fluctuating pH conditions had lower growth rates compared to constant levels of pH (Cornwall *et al.*, 2013). Other studies have suggested that calcifying organisms naturally exposed to higher fluctuations may be pre-adapted to tolerate future carbon dioxide levels (Cornwall *et al.*, 2013; Johnson *et al.*, 2014; Ruesink *et al.*, 2018) or potentially find refuge in the variability (Kapsenberg and Cyronak, 2018). In contrast, however, at least one study shows no differences in the sensitivity of calcifying algae from habitats with different variability regimes (Noisette *et al.*, 2013). Nonetheless, variability in carbonate chemistry is predicted to be amplified over time with ocean acidification, including higher highs and lower lows, depending on the local buffering capacity (Shaw *et al.*, 2013; Pacella *et al.*, 2018). These predictions may move beyond the bounds of the current extremes in some systems pushing organisms beyond their physiological thresholds (Gruber *et al.*, 2012).

Methodological approaches in physiological measurements

Methodological differences between physiological measurements made in the laboratory and field also may contribute to inconsistent responses since there are substantial differences in the time scales over which these measurements are integrated. For example, incubations provide instantaneous metabolic rates (e.g. calcification rate and photosynthetic rate) at set environmental conditions (e.g. temperature and light level) during the incubation. Incubations are commonly used in laboratory experiments, but they are very difficult to accomplish in the field. Changes in buoyant, dry, and wet weight—which are used in both laboratory and field studies—integrate calcification and growth over more extended periods (days to weeks) during which the algae may be exposed to a range of environmental conditions. Since common methodologies often differ between laboratory (e.g. incubations) and field (e.g. buoyant weight) settings, it can be challenging to determine how measurements compare or whether field responses are even consistent with the laboratory results. To accurately predict the impact of acidification on calcifying macroalgae, it will be necessary to model

effects across ecological scales, not just infer using laboratory-based responses, by incorporating measured variability from the field into the model.

Conclusions and future recommendations

This cross comparison of laboratory and field acidification studies highlighted the following issues that undermine our ability to reliably use experimental findings to predict future marine ecosystem health and function: (i) laboratory experiments most often measured calcification and production of individual organisms while most field studies evaluated community-level metrics, (ii) the directionality of responses often differed from laboratory to field, and (iii) laboratory findings more often documented larger negative effects of acidification.

In addition, we found it difficult to determine whether laboratory responses of calcifying benthic macroalgae match acidification effects in the field due to differences in ecological, spatial, and temporal scales in the methodologies. This challenge was particularly evident when we first attempted to conduct a meta-analysis. Thus, we recommend the following approaches for future research to leverage the abundance of laboratory experiments at the individual (i.e. species) scale to better explain observed responses in the field, and *vice versa*. This will subsequently increase our faith in laboratory findings as predictors of future species and community responses to acidification. Although our recommendations are based on a semi-quantitative analysis of response of calcifying benthic macroalgae to acidification, these recommendations and following conclusions are broadly applicable to other marine organisms and ecosystems.

1. Conduct additional field studies that expand the geographic coverage of studies and, whenever possible, employ consistent methodologies (e.g. PAM fluorometry, tissue sampling and analyses, buoyant weight technique, and so on) in both laboratory and field settings.
2. Develop novel instruments and approaches for field studies such as the Benthic Ecosystem and Acidification Measurement System (Takeshita *et al.*, 2016), Coral *in situ* Metabolism and Energetics instrument (Romanó de Orte *et al.*, 2021), and *in situ* Free Ocean Enrichment Experiments (Kline *et al.*, 2012; Stark *et al.*, 2019) that can extend measurements predominantly conducted in laboratory settings to the field.
3. Conduct additional population and community-scale experiments in laboratory settings (e.g. mesocosms) that measure responses across ecological scales to aid in our understanding of how these responses are linked and integrated (Edmunds *et al.*, 2016). Furthermore, these larger scale experiments can give insight into the mechanisms and feedbacks between ecological processes and biogeochemical cycling that may indirectly influence acidification responses.
4. Expand the ability to mimic current and future environmental variability in laboratory studies, basing the variability on actual observations from the field.
5. Pinpoint why calcifying algae appear to be more tolerant to acidification in field settings to understand why there is a mismatch between laboratory and field responses. This includes expanding our laboratory research efforts to include additional calcifying algae species and morphological groups beyond those commonly reported in the current literature (Supplementary Figure S1).

6. Use multi-stressor experiments to determine whether co-varying seawater parameters (e.g. temperature, food availability, light, and dissolved oxygen) influence the apparent tolerance of calcifying algae to acidification, potentially explaining why stronger negative impacts of acidification are documented in laboratory experiments
7. Expand the use of several understudied responses (e.g. tissue content, reproduction, and microbial communities), in both laboratory experiments and field settings, because they may have important implications for algal physiology and ecological roles.
8. Determine how to incorporate laboratory responses into species and ecosystem models (i.e. how to accurately model *in situ* responses from laboratory experiments) to improve our ability to predict the status of future marine ecosystems. Incorporating species interactions and multi-trophic studies (i.e. community-level metrics) are key to this effort.
9. Improve opportunities for multidisciplinary collaborations that include partnerships across institutions, countries, and regions—particularly under-studied regions (Indian Ocean, polar regions, and Southeast Asia) to strengthen our research effort (e.g. Global Ocean Acidification Observing Network). These opportunities should increase scientific capacity in developing nations and broaden spatial coverage of observations, thus allowing more accurate generalizations of how marine taxa respond to environmental changes.

This synthesis highlights the need for a more comprehensive understanding of how calcifying algae will respond to ongoing and future acidification in natural environments. Therefore, we strongly encourage collaborations across disciplines to bridge responses from laboratory to natural settings across ecological scales. Collaborative efforts focusing on the suggested research approaches will increase our predictive power and allow for more accurate projections of the fate of calcifying benthic macroalgae populations and the ecosystems they support.

Data availability statement

No new data were generated or analysed in support of this research.

Supplementary data

[Supplementary material](#) is available at the *ICESJMS* online version of the manuscript.

Author contributions

All authors contributed to the conceptualization and design and methodology of the study. All authors compiled literature for the semi-quantitative analysis. SJM, MDJ, and KDB created figures and tables. All authors contributed to analyses of findings as well as drafting and revising the manuscript.

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