Received: 17 November 2017 | Revised: 29 May 2018 | Accepted: 12 June 2018

DOI: 10.1111/gcb.14372

PRIMARY RESEARCH ARTICLE

WILEY Global Change Biology

Generality in multispecies responses to ocean acidification revealed through multiple hypothesis testing

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Funding information
U.S. Environmental Protection Agency, Grant/Award Number: FP917429; Oregon State University Hatfield Marine Science Center Mamie Markham Research Grant; David and Lucile Packard Foundation; Kingfisher Foundation; Wayne and Gladys Valley Foundation; National Science Foundation, Grant/Award Number: OCE-1061233, OCE-1521633; Oregon State University Integrative Biology Research Fund

Abstract

Decades of research have demonstrated that many calcifying species are negatively affected by ocean acidification, a major anthropogenic threat in marine ecosystems. However, even closely related species may exhibit different responses to ocean acidification and less is known about the drivers that shape such variation in different species. Here, we examine the drivers of physiological performance under ocean acidification in a group of five species of turf-forming coralline algae. Specifically, quantitating the relative weight of evidence for each of ten hypotheses, we show that variation in coralline calcification and photosynthesis was best explained by allometric traits. Across ocean acidification conditions, larger individuals (measured as noncalcified mass) had higher net calcification and photosynthesis rates. Importantly, our approach was able to not only identify the aspect of size that drove the performance of coralline algae, but also determined that responses to ocean acidification were not dependent on species identity, evolutionary relatedness, habitat, shape, or structural composition. In fact, we found that failure to test multiple, alternative hypotheses would underestimate the generality of physiological performances, leading to the conclusion that each species had different baseline performance under ocean acidification. Testing among alternative hypotheses is an essential step toward determining the generalizability of experiments across taxa and identifying common drivers of species responses to global change.

KEYWORDS
allometry, calcification, coralline algae, eco-physiology, functional groups, functional traits, global change, rocky intertidal

1 | INTRODUCTION

Ocean acidification (OA) has been identified as a key but understudied stressor for marine organisms, leading to rapid growth in efforts to test its effects on a wide range of potentially susceptible species (Kroeker, Kordas, Crim, & Singh, 2010; Kroeker et al., 2013a). Although experiments have revealed that many species are negatively impacted by OA, some organisms exhibit mixed responses and we know less about the variation in OA response among closely related taxa or among taxa with similar physiological traits (e.g., Calosi et al., 2013; Dupont, Ortega-Martínez, & Thorndyke, 2010; Gooding, Harley, & Tang, 2009; Noisette, Egilsdottir, Davoult, & Martin, 2013; Okazaki et al., 2017; Ries, Cohen, & McCorkle, 2009). As a consequence, it is unclear if variation in the response of species to OA is a result of inherent physiological differences among species or a result of infrequent efforts to test for generalities across taxa (Gaylord et al., 2015; O'Connor, Selig, Pinsky, & Altermatt, 2012; O'Connor et al., 2015; Wernberg, Smale, & Thomsen, 2012). This is an important issue because if each species is differentially sensitive to OA, the number of experiments needed to quantitate multispecies

response to OA is greatly increased, likely making such an approach infeasible.

Ecologists have long sought to explain variation in species responses to the environment by grouping species according to shared evolutionary histories and/or traits (e.g., Raunkiaer, 1934). For example, species can be categorized into functional groups that respond similarly to climate change (Smith, Shugart, & Woodward, 1997). Previous studies have examined OA sensitivity using various grouping methods, including by whether organisms produce a calcified structure (Kroeker et al., 2010), the relative solubility of their shell and skeletal minerals (Kroeker et al., 2010; Ries et al., 2009), their carbon use strategy (Koch, Bowes, Ross, & Zhang, 2013), or their evolutionary history (Schaum, Rost, Millar, & Collins, 2013). However, few studies have tested among a broad array of potential alternative hypotheses that could explain organismal performance under OA (Betini, Avgar, & Fryxell, 2017; Brown et al., 2011; O'Connor et al., 2015). A quantitative, statistical approach is critical for assessing the relative importance of the many factors that may directly or indirectly influence performance under stressors such as OA.

In this study, we tested the role of evolutionary relatedness, functional traits, and habitat in shaping multispecies performance in the context of OA. We developed hypotheses relevant to a system of co-occurring coralline algae and tested them in a series of OA experiments. Focal species were five species of turf-forming coralline algae (Corallinales, Rhodophyta) of the Northeast Pacific: *Corallina officinalis*, *Corallina vancouveriensis*, *Calliarthron tuberculosum*, *Bossiella orbigniana*, and *Bossiella plumosa*. Coralline algae are among the most vulnerable calcifying species to an acidifying ocean (Koch et al., 2013) and the ecological consequences of coralline algae decline or extinction are likely to be high, given their important role as facilitators of foundation species in this system (Barner, Hacker, Menge, & Nielsen, 2016). The concepts and data behind each hypothesis are detailed below.

1.1 | From ecological theory to quantitative hypotheses

We developed ten hypotheses that consider aspects of species identity, evolutionary relationship, size, morphology, and habitat to explain variation in physiological performance under OA among multiple species of coralline algae (Figure 1). Beyond the simple hypothesis that species may not respond to OA (hypothesis H_0), we considered whether physiological performance under OA differs among species (hypothesis H_1). Differences in physiological performance may be manifested in two ways. In the context of OA, species may have different baseline physiological rates that are testable as differences in the intercepts of each species' performance (Figure 1, an "additive" effect). Alternatively, OA effects may differ among species, translated as differences in the slopes of the effect of OA among species (Figure 1, an "interactive" effect). In this study, we tested for both additive and interactive effects (see Methods), as both are of interest. For example, an organism with a low baseline physiological rate may fare poorly under OA compared to organisms with higher baseline physiological rates (an additive effect) or organisms may have similar baseline rates in current conditions, but OA may differentially influence their physiological performance (an interactive effect).

Next, we hypothesize that all individuals in our study have similar performance under OA, with no other predictors of performance than OA itself (hypothesis H_2). If species respond similarly to OA, we may classify them as a functional group, defined as a set of species defined by shared responses to environmental conditions (Grime, 1973; Raunkiaer, 1934; Smith et al., 1997). Among seaweeds, the turf-forming articulated coralline algae in this study all fall within a previously assigned functional group based on similar morphological traits and similar responses to physical disturbance (Steneck & Dethier, 1994). Across this group, responses to OA have been qualitatively similar (Koch et al., 2013; Kroeker et al., 2013a; McCoy & Kamenos, 2015): calcification decreases with OA (as a function of saturation state, Ω) while photosynthesis increases with OA (as a function of carbon availability, pCO_2). However, because some previous studies have found differences in response to OA among coralline algae (e.g., Doropoulos, Ward, Diaz-Pulido, Hoegh-Guldberg, & Mumby, 2012; Noisette et al., 2013), the shared characteristics previously used to group turf-forming coralline algae may not produce similar responses to OA. Functional groups may vary depending on the focal global change stressor, and groupings may be inappropriate if group membership is not explicitly linked to the environmental change in interest (Dormann & Woodin, 2002).

A third hypothesis (hypothesis H_3) is that multispecies physiological performance under OA could map onto evolutionary relationships (Figure 1). In this study, species in the genus *Bossiella* are more closely related to *Calliarthron* than to species in the genus *Corallina* (Figure 2; Hind & Saunders, 2013). If physiological responses to environmental conditions are evolutionarily conserved, more closely related species may have more similar physiological performance under global change (Buckley & Kingsolver, 2012; Marchin, Salk, Hoffmann, & Dunn, 2015).

Further hypotheses (hypotheses H_4–H_8) are that multispecies physiology under OA could depend on specific functional traits. As functional traits are not always reflected in evolutionary relationships, they may be a key predictive link between physiology, morphology, and ecosystem function under climate change (Guittar, Goldberg, Klanderud, Telford, & Vandvik, 2016). Here, we focus on a selected set of traits, primarily related to organismal size and shape (Figure 2) that are shared across species and can be hypothesized a priori to link physiology and performance under OA.

As background, variation in metabolic rates across taxa is strongly constrained by variation in size (Brown, Gillooly, Allen, Savage, & West, 2004; Robinson, Peters, & Zimmerman, 1983). Size has been shown to interact with OA such that smaller individuals were less affected by OA than larger individuals (Carey & Sigwart, 2014; an interactive effect in Figure 1). In calcifying species, size could be measured in a number of ways including mass, surface area, and shape. For example, by mass, the largest individuals or the largest species may have the highest net calcification and photosynthesis

Groups	Hypotheses and predictions
Species identity	H_1: Physiological performance is species-specific. *Interactive* P_1: Each species will have a different net physiological response to OA.
Evolutionary relatedness	H_3: Closely related species will have similar physiological performances. *Interactive* P_3: Physiological performance of *Bossiella* and *Calliarthron* species under OA will be more similar to each other than to performance of *Corallina*.
Traits	$H_{4\text{-}6}$: Physiological performance depends on… H_4: individual total mass H_5: individual non-calcified mass H_6: individual surface area *Interactive* $P_{4\text{-}6}$: Larger individuals will have higher net physiological rates than smaller individuals and will be less affected by OA than smaller individuals.
	H_7: Physiological performance depends on percent non-calcified mass. *Interactive* P_7: After accounting for individual size, species with higher percent non-calcified mass will have higher net physiological rates and be less affected by OA than species with lower percent non-calcified mass. Thus, *Corallina officinalis* > *Corallina vancouveriensis* = *Bossiella plumosa* > *B. orbiginiana* > *Calliarthron tuberculosum.*
	H_8: Physiological performance depends on surface area to volume (SAV) ratio. *Interactive* P_8: After accounting for individual size, species with higher SAV ratios will have higher net physiological rates and be less affected by OA than species with higher SAV ratios. Thus, *Corallina vancouveriensis* = *Bossiella plumosa* = *B. orbiginiana* > *Corallina officinalis* = *Calliarthron tuberculosum.*
Habitat	H_9: Physiological performance depends on a species' current habitat. *Interactive* P_9: Tide pool species *Corallina officinalis* and *Calliarthron tuberculosum* will be less sensitive to OA than low-flow species (*Bossiella orbiginiana*), while intertidal high-flow species (*Corallina vancouveriensis, B. plumosa*) should have an intermediate response.

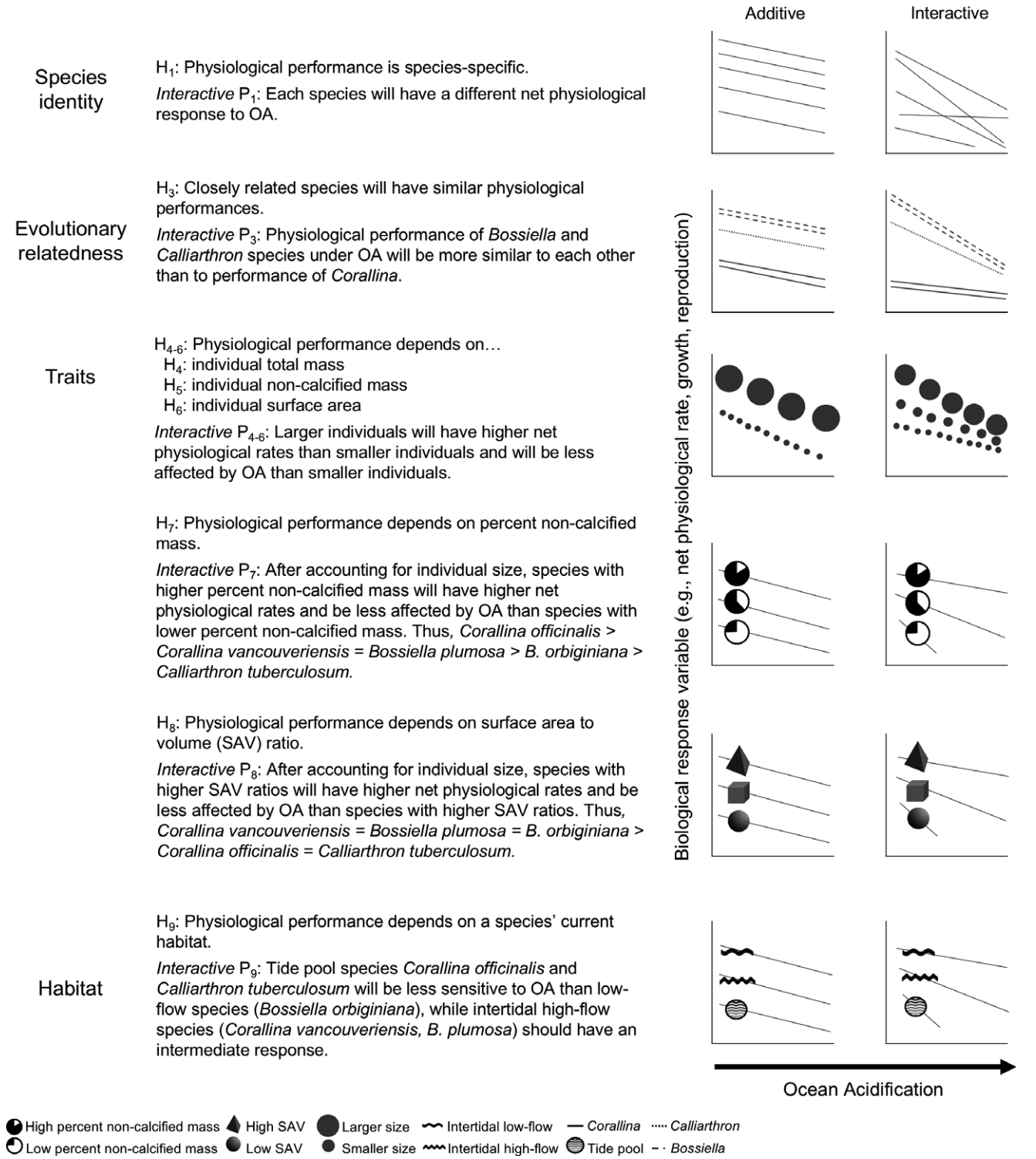

FIGURE 1 Hypotheses and predictions tested in this study. Each nonclimate driver was tested for an additive and interactive effect with OA, shown as visual predictions with species hypothetical fitted responses to OA. If OA acts independently from the driver, these effects are modeled with additive predictors (see Appendix S2 for explicit additive predictions). However, if the effect of OA on the biological response depends on the driver, this is modeled as an interactive effect. The expected effect of OA will depend on the specific biological response of interest. In these hypothetical visualizations, the overall effect of OA is primarily negative, modeling the expected effect of OA on calcification. Data to support each prediction can be found in Appendix S1

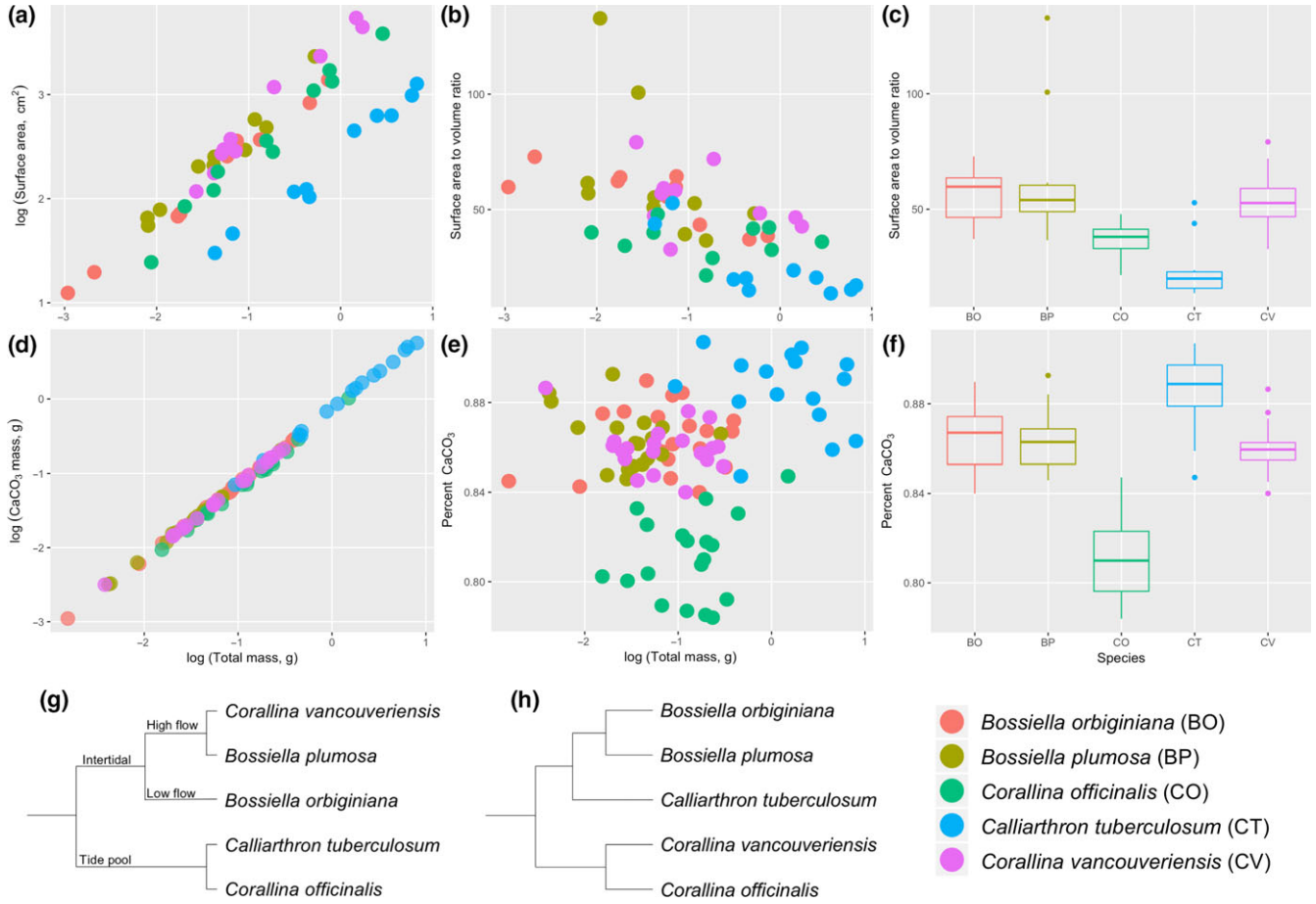

FIGURE 2 Data underlying each hypothesis. Relationships between total mass and (a) surface area, (b) surface area to volume (SAV) ratio, (d) calcified mass, and (e) percent calcified mass. While total mass, calcified mass, and surface area vary among individuals, and were thus treated as continuous predictors. SAV ratio and percent calcified mass vary among species (c, f), and were thus treated as categorical predictors. (g) Primary habitats of taxa. (h) Evolutionary relationships among taxa, from Hind and Saunders (2013). Additional details may be found in Appendix S1

rates for any given OA condition. Measurements of mass can include both living and calcified components (total mass; hypothesis H_4) or just living components (noncalcified mass; hypothesis H_5). H_4 predicts that calcified parts are vulnerable to dissolution under OA, and thus this measure of mass may be the best metric to describe whole-organism performance under OA. Alternatively, H_5 predicts that because noncalcified mass is the living component of the organism, it will best reflect the ability of the organism to perform physiologically under OA.

Other size or shape-related alternatives are also possible. For example, because algae derive carbon and nutrients through direct surface contact with water, net photosynthesis and calcification may be proportional to surface area (hypothesis H_6—*individual surface area*). Furthermore, after accounting for differences in mass among individuals, differences occur among species in their percent noncalcified composition, which may drive differences in performance under OA (H_7—*species percent noncalcified composition*). Specifically, organisms with higher percent noncalcified mass may have lower physiological rates and be more affected by OA. Also, vulnerability to OA may be a function of shape, as measured by surface area to volume (SAV) ratio (Smith, 2009). That is, a "rounder" individual (smaller SAV ratio) will have lower surface area exposure to surrounding water than a same-sized individual of another species with a larger SAV ratio. This lower exposure may limit physiological capacity to photosynthesize and calcify as a function of lower effective surface area (hypothesis H_8—*species surface area to volume ratio*). In this study, size did not differ among species but it did among individuals, and SAV ratio and percent noncalcified composition varied among species but not individuals.

Finally, habitat differences could explain physiological performance under OA (hypothesis H_9). If species ranges are determined by climate-sensitive environmental conditions, then current species habitat distributions may predict future sensitivity to global change (i.e., the principle employed in climate envelope modeling; Buckley & Kingsolver, 2012). For example, in this study, all five species co-occur but are distributed across three habitats potentially differing in pH regime (Hurd et al., 2011; Kwiatkowski et al., 2016). Tide pools in the NE Pacific experience more acidified pH regimes than occurs outside pools (Chan et al., 2017; Kwiatkowski et al., 2016). Thus, tide pool species may be less sensitive to OA than species that occur

on emergent rock. Furthermore, among emergent intertidal taxa, species in low-flow areas may be more sensitive to OA than those in high-flow areas. Low flow promotes formation of a diffusion boundary layer ameliorating low-pH conditions during active photosynthesis (Comeau, Edmunds, Lantz, & Carpenter, 2014; Hurd et al., 2011), thereby buffering low-flow species from OA exposure. Thus, hypothesis H_9 states that when placed in common conditions, tide pool species should be least sensitive under OA, followed by taxa from turbulent intertidal locations (high flow), then those from low-flow intertidal locations (Figures 1 and 2).

To test our hypotheses, we assayed the physiology of species under short-term experimental OA. Using a regression design, we tested OA effects on net calcification and photosynthesis rates across a wide range of saturation state values that corresponded to levels currently experienced and predicted for coastal waters in the region (see Materials and Methods; Reum et al., 2014; Kwiatkowski et al., 2016; Chan et al., 2017). We asked: (a) which model best describes multispecies coralline physiology under OA, and (b) does the consideration of nonclimate drivers change the conclusion about coralline performance under OA?

2 | MATERIALS AND METHODS

2.1 | Experiments

2.1.1 | Overview of experiments

Short-term experiments were repeated five times to assemble a continuous range of OA exposure treatments (Table 1). Multiple trials of each experiment were conducted over 1–2 days. Net photosynthesis and calcification rates in response to short-term OA exposure were assayed using the alkalinity anomaly method (Smith & Key, 1975). Briefly, the alkalinity anomaly method exploits the fixed stoichiometric relationship between calcium carbonate ($CaCO_3$) precipitation, declines in total alkalinity (TA), and dissolved inorganic carbon (DIC), which proceeds in molar ratios of 2:1 for every mole of $CaCO_3$ produced. Reductions in DIC deviating from the 2:1 TA:DIC ratio reflect carbon fixation in photosynthesis. By precisely measuring changes in TA and DIC, this technique allows quantitation of net calcification and net photosynthesis rates in rapid assays (Chisholm & Gattuso, 1991). Because net rates of calcification and photosynthesis are directly equivalent to growth, the alkalinity anomaly method yields metrics that can be readily interpreted in terms of individual performance in our system, where competition for space is strong (Dayton, 1971). Short duration experiments are particularly advantageous with slow-growing organisms like coralline algae, where the common method of net calcification estimation requires organisms to be held in long-term laboratory culture, posing logistical and interpretive challenges (Maier et al., 2013). Furthermore, the alkalinity anomaly method correlates with long-term buoyant weight estimators of calcification (Schoepf et al., 2017) and meta-analysis suggests that experiment duration does not dramatically alter the conclusions of the effect of OA on calcifying algae (Kroeker et al., 2013a).

Each experiment followed the same overall design and protocol (Table 1). In each trial, algae were placed in chambers with water of known carbonate chemistry, across a range of OA treatments (pH: 7.11–8.22, pCO_2: 343.6–4590.6 μatm, Ω_{arag}, 0.38–2.4; Appendix S3). Current observed values of pH, pCO_2, Ω_{arag} for coastal waters in our region are among the most acidified regimes in the world, ranging from 7.22 to 9.0, 10 to 3,276 μatm, and 0.2 to 2.8, respectively (Chan et al., 2017; Kwiatkowski et al., 2016; Reum et al., 2014). Intertidal zones in our region also commonly experience rapid shifts in pH, with hourly shifts in pH of ~0.3 pH units per hour (Chan et al., 2017; Kwiatkowski et al., 2016). Overall, compared to 1990s values, surface pH is expected to decline globally by 0.07–0.3 units by 2100 (IPCC scenario RCP 2.6 vs. RCP 8.5; Bopp et al., 2013). In our region saturation state is expected to decline by ~0.5–1.5 units by 2100 (RCP 8.5 vs. 2.6; Kwiatkowski et al., 2016). These values correspond to ranges used in our experiments.

Due to constraints of laboratory access and the occasional inaccessibility of field sites where algae were collected, experiments were conducted over a period of eight months. Each experiment varied little in design, with two exceptions. First, experiment 5 was designed to provide a wider range of individual sizes for testing

TABLE 1 Details of the experiments. "Type" of experiment refers to whether the OA treatment water was generated using water mixing or a flow-through mesocosm. Standard errors for means are in parentheses

Date	8, 10 July 2014	6 August 2014	21 August 2014	23 February 2015	28, 29 April 2015
Experiment number	1	2	3	4	5
Water type	MW	MGW	MGW	MW	MW
Range pH treatments	7.11–8.03	7.40–8.02	7.27–8.04	7.57–8.11	7.13–8.06
Number of trials	5	2	2	1	4
Number of species	5	5	5	2[a]	5
Samples per species, across treatments	14	5	5	5	20
Mean temperature, °C	9.88 (0.22)	14.56 (0.07)	14.38 (0.04)	11.17 (0.32)	10.61 (0.14)
Mean irradiance, μmol s^{-1} m^{-2}	454.1 (14.2)	527.8 (42.9)	539.6 (42.1)	417.3 (8.3)	352.0 (9.6)

Notes. M: mixed; MG: mesocosm-generated.
[a]*Bossiella plumosa*, *Corallina vancouveriensis*.

hypotheses $H_{4–5}$, so smaller sized algal fronds were collected than in experiments 1–4. Second, mean light conditions and temperatures varied among our experiments (Table 1, Appendix S3) but were within the range of light (Close, 2014) and temperature (Helmuth et al., 2016) variation measured in the field. We accounted for this variation in experimental conditions in our statistical analysis with mixed effects models (see Materials and Methods: Statistical Analysis).

2.1.2 | Experimental procedure

For each experiment, we first chiseled rock chunks with attached algal fronds from the intertidal low zone substrate at Fogarty Creek near Depoe Bay, Oregon, USA. (44°84′N, 124°06′W). Taxa found primarily in tide pools (*Calliarthron tuberculosum*, *Corallina officinalis*) were collected from low zone tide pools and the other species were collected outside of tide pools. Samples were transported to Hatfield Marine Science Center (HMSC) in Newport, Oregon and acclimated to laboratory conditions in ambient flow-through seawater under a 12-hr light cycle for 4–6 days prior to assays. On the day of the experiment, we removed any attached invertebrates or nontarget algal epibionts and transferred the samples from HMSC to our laboratory at Oregon State University (Corvallis, Oregon).

Treatment water was generated for these assays in two similar ways, depending on whether the OA laboratory at HMSC was being used for experiments independent of this one (Table 1). Each method is fundamentally similar, in that pure CO_2 is bubbled into seawater drawn from the same source to reach a target pCO_2 value. In the first method, we used water generated at specified pCO_2 levels in our OA mesocosm system at HMSC, thus simplifying the process of generating target treatment water. Briefly, pure CO_2 and CO_2-free air was mixed and regulated electronically by mass-flow controllers to generate air of a specific composition, which was then injected into ambient flowing seawater to alter seawater chemistry in three reservoir tanks to reach three target pCO_2 values (Fangue et al., 2010). Water was drawn from these reservoir tanks for each trial. In the second method, when the mesocosm was not in operation, instead of generating treatment water with mass-flow controllers, we generated treatment water "by hand". Seawater was collected in 20 L carboys from the sand-filtered seawater line at HMSC and stored temporarily in Corvallis at −20°C to cool to in situ conditions. DIC content was either reduced by bubbling with N_2 or elevated by bubbling with pure CO_2. Dissolved oxygen was monitored during this process and increased through brief aeration with room air to bring levels to near saturation. To prepare the individual treatments, high and low DIC water was mixed to achieve the desired treatment level. In both methods, we measured pH spectrophotometrically using an autonomous unit (SAMI Ocean pH Sensor, Sunburst Sensors) configured to run as a benchtop unit (Martz, Carr, French, & DeGrandpre, 2003).

To run the experiment, chambers (5.5 L or 4 L plastic food containers) were filled with treated water and placed under two grow lights (Sunlight Supply, Inc.; Sun Blaze T5 HO 36W Fluorescent Type Lamp). Algae were assigned to chambers using a stratified random design, such that each row and column of chambers contained each of the five species and a mix of treatment levels (Appendix S4). To maintain water motion, an aquarium pump (Hydor Pico 400 centrifugal pump) was added to each chamber. The assay began when the light environment stabilized (as measured by LI-COR LI-250 Light Meter with a LI-190SA Quantum Sensor) and all algal samples were dropped simultaneously into their respective chambers. Algae remained in the chambers for 30–45 min, then were removed from the chambers, dried, and weighed (Appendix S1). Temperature was recorded for every chamber. To regulate temperature under the lights, cool water was placed externally around the experimental chambers.

2.1.3 | Experimental controls

In experiment 5, we added a control (i.e., no algae present) low and high Ω_{arag} treatment for each trial (n = 6 per OA level). We used these results to determine if either alkalinity or DIC changed in the controls during the experiment. Using linear regression, for both alkalinity and DIC, the slope of the relationship between starting and ending conditions for controls did not differ from 1 (alkalinity slope 95% CI: −0.6520 to 1.776, DIC slope 95% CI: 0.8964–1.5646). In addition, we regressed starting against final saturation state to determine if the relationship differed from the expected 1:1 (see Figure S7 for $\Omega_{end} \sim \Omega_{start}$ with algae present). Unlike the slope of the line for the same relationship when algae were present (95% CI: 0.7983–0.8831), the slope of the line when algae were absent did not differ from 1.00 (95% CI: 0.7141–1.012). This suggests that the change in saturation state over the course of the algal assays was not due to water equilibrating with air.

2.1.4 | Seawater sample analysis

To characterize starting treatments, water samples were collected in acid-washed glass bottles, treated with 50 μl mercuric chloride to stop biological activity, and capped for later pH and alkalinity analysis. Water was collected from each chamber at the end of each trial in the same way to characterize pH and alkalinity changes. Measuring pH and alkalinity of each water sample enabled parameterization of the entire carbonate system. We measured pH spectrophotometrically as above. Alkalinity was determined by spectrophotometric titration (Yao & Byrne, 1998). The quality of our alkalinity measurements was assessed at the beginning of each day of sample analysis by comparison of analytical precision to certified seawater standards from Andrew Dickson (Scripps Institute of Oceanography, La Jolla, CA), as recommended by community best practices standards (Dickson, Sabine, & Christian, 2007). Our analytical precision was 2236.99 ± 2.39 μmol/kg for alkalinity reference Batch 130 (in mean ± standard deviation; reference = 2238.04 ± 0.53) and 2224.87 ± 3.60 for Batch 145 (reference = 2226.16 ± 0.71, information on each reference batch is available at http://cdiac.ornl.gov/oceans/Dickson_CRM/batches.html). Using measured pH, alkalinity, and temperature of each sample, aragonite saturation state

(hereafter, "saturation state"), DIC and pCO_2 were calculated with the program CO2calc (Robbins, Hansen, Kleypas, & Meylan, 2010) using carbonate constants of Lueker, Dickson, and Keeling (2000) and constant salinity (31 $_{o/oo}$ for experiments 1–3, 33 $_{o/oo}$ for experiment 4, 34 $_{o/oo}$ for experiment 5), measured using laboratory salinometers (Autosal Guideline Instruments).

With the alkalinity anomaly method, net calcification rate (in μmol Carbon hr^{-1}) was then calculated following Smith and Key (1975):

$$\frac{\frac{\Delta Alk}{2} \times V}{time/60}$$

Where ΔAlk is the difference in alkalinity from the start to end of the assay (Alk_{start}–Alk_{final}), V is the volume of saltwater for each assay, and "time" is the duration of the assay in minutes. Net photosynthesis rate (μmol Carbon hr^{-1}) was calculated as:

$$\frac{\left(\Delta DIC - \frac{\Delta Alk}{2}\right) \times V}{time/60}$$

Where ΔDIC is the difference in dissolved inorganic carbon from the start to end of the assay (DIC_{start}–DIC_{final}).

2.2 | Statistical analysis

We tested among our alternative OA hypotheses using mixed effects models to account for random variation among trials and experiments. Analyses were conducted in R using the "lme4", "MuMIn", and "AICcmodavg" packages for mixed effects modeling and model selection (Bartón, 2017; Bates et al., 2017; Mazerolle, 2017; R Core Team, 2017). Data are available in the Pangaea database (Pangaea.de, doi: 10.1111/gcb.14372).

2.2.1 | Fixed effects structure

Each hypothesis was mapped to a corresponding fixed effects model and support for each hypothesis was evaluated using model selection. Fixed effects depended on the hypothesis being tested, with net calcification and photosynthesis rate as dependent variables. The OA fixed effect was saturation state (Ω) in the calcification models and pCO_2 in the photosynthesis models, treated as continuous due to the regression experimental design. We first fit and tested among several relationships between pCO_2 and photosynthesis (linear and saturating), and saturation state and calcification (linear, saturating, unimodal). We compared AICc (Akaike's Information Criterion corrected for small sample size; see Materials and Methods: Model Selection Procedure) among models with a linear relationship between OA and physiological rate or a saturating relationship between OA and physiological rate. In addition, for calcification and saturation state, we tested the fit of a unimodal relationship using a quadratic term, as previous studies have suggested that calcification is highest at intermediate saturation states (e.g., Ries et al., 2009). The saturating relationship of calcification with OA was also tested using a simple model with a binary term allowing the slope of the effect of saturation state on net calcification to vary depending on whether conditions thermodynamically favored calcification ($\Omega > 1$) or dissolution ($\Omega < 1$) (e.g., binary term = 0 when saturation state < 1, = 1 when saturation state > 1). The best fit calcification OA model included a linear effect of saturation state on calcification and this simple binary term, while the best fit photosynthesis OA model included a linear relationship with pCO_2. The relationships between OA and physiology for these best fit models were then implemented, with the nonclimate drivers, for hypotheses 1–9 (below).

To test among ecological hypotheses, in addition to the null hypothesis (H_0) that OA has no impact on calcification and photosynthesis rates, each rate was modeled as either a function of (a) OA alone (H_2: equivalent performance); (b) OA with dummy variables for species identity (H_1), genus (H_3: evolutionary constraints), or habitat (H_9); (c) OA with the continuous predictors of total mass (H_4), noncalcified mass (H_5), and surface area (H_6); and (d) OA with a continuous predictor of size (tested with total mass, noncalcified mass, and surface area) with a dummy variable for rank SAV ratio (H_8) or rank percent noncalcified composition (H_7). No models included interactions among nonclimate drivers (e.g., habitat × size). Of the predictors, total mass was measured for each sample in our experiments. Surface area and noncalcified mass required destructive sampling and thus was instead calculated from total mass using conversion equations (Appendix S1). Percent noncalcified composition was determined to vary more among species than among individuals (Appendix S1), so samples were assigned a rank by species. Analysis of statistical power is in Appendix S4.

As with other calcifying species, calcification and photosynthesis was highly correlated across all species (Appendix S5). This correlation is important in the context of OA because photosynthesis directly influences OA water chemistry. Specifically, carbon uptake during photosynthesis increased the saturation state of the water surrounding the coralline algae (Cornwall et al., 2012; De Beer & Larkum, 2001; Kwiatkowski et al., 2016). Furthermore, in some species, photosynthesis itself may stimulate calcification (Borowitzka & Larkum, 1976). Thus, to comprehensively evaluate whether calcification was a function of saturation state, we re-tested all calcification models with an additive or interactive fixed effect of photosynthesis and OA.

2.2.2 | Candidate set and subset

One of the most difficult and important steps in the model selection process is building the "candidate set" of possible models for inference (Burnham & Anderson, 2002; Johnson & Omland, 2004). Best practices for building the candidate set emphasize the importance of using a priori hypotheses to build ecologically relevant and interpretable statistical models, restricting the number of models in the candidate set to minimize spurious results (Burnham & Anderson, 2002; Johnson & Omland, 2004), and avoiding correlated biological predictors (Symonds & Moussalli, 2011). In this study, we built our set of statistical fixed effects models based on a priori hypothesized relationships among the predictors and response variables (see

Introduction, Appendix S1). Another approach to model-building is "all-subsets selection" or "dredging", where all possible models are included in the candidate set (Burnham & Anderson, 2002; Johnson & Omland, 2004). While all-subsets is a legitimate form of model selection, it is primarily used for exploratory analyses and can lead to high Type I error rates if the all-subsets models are used for inference (Harrison et al., 2018). As such, because we did not design the experiment to test for interactive effects among our hypothesized ecological predictors (e.g., habitat × size), we did not include such models in our candidate set. The only included interactions were for size-related categorical variables (e.g., individual mass × SAV, individual mass × percent calcium carbonate), to disentangle the role of mass from the role of shape or composition. However, implications of other possible interactions can be found in the Discussion.

To compare our method of multiple alternative hypotheses testing to methods that omit nonclimate drivers of physiology or only test for differences among species, we reduced our candidate set. This reduced set included only models that either accounted for OA or for OA and species (both OA + species and OA × species). The specific models included in the reduced set are indicated in the list of all models in Appendix S6. From among these models, we then followed the same model selection procedure described below in Materials and Methods: Fixed effects structure.

2.2.3 | Random effects structure

To determine random effect structure, we tested the fit of several random effects structures for both calcification and photosynthesis models using AIC (see Methods: Model selection procedure) and a maximal fixed effects structure (Barr, Levy, Scheepers, & Tily, 2013; Zuur, Ieno, Walker, Saveliev, & Smith, 2009). We evaluated the fit of random intercept models that varied by experiment or by trial nested in experiment, and random slopes models that accounting for variation in the effect of temperature and light either among experiments or trials. The best random effect structure for calcification models that did not include a fixed effect of photosynthesis accounted for random variation in the effect of temperature on calcification among experiments. The best structure for calcification models that included a fixed effect of photosynthesis accounted for random variation in the effect of photosynthesis among experiments. The best structure for photosynthesis models accounted for random variation in the effect of light among experiments. See Appendix S4 for the fitted random effects for each model. Our results did not qualitatively change if only a random intercept model was used or no random effect structure was specified (e.g., model selection consistently identified the same top models).

2.2.4 | Model selection procedure

For each hypothesis, we tested among a no-OA (Y = X), an additive (Y = OA + X), and an interactive (Y = OA × X) model. Overall, we selected among a total of 84 competing calcification models and 38 photosynthesis models, each corresponding to a hypothesis (Appendix S6). The model with the lowest AICc was considered the "best" model in the set of models, as the difference in AICc (ΔAICc) between the best model and a model of interest is a measure of information lost between the two models (Burnham & Anderson, 2002). Generally, any model with $\Delta AICc < 2$ is considered a top model (Burnham & Anderson, 2002). In this study, there were two top calcification models and two top photosynthesis models. All continuous data were centered and rescaled before analysis to allow for comparison among disparate units on a common scale (Schielzeth, 2010). Assumptions of normality were checked and met for all models. Effect sizes of top models are thus presented in rescaled and centered units, with parameter estimates ± standard error. Size variables (total mass, noncalcified mass, surface area) were log transformed to meet assumptions of normality before centering and rescaling. For all models, we calculated Akaike weights, which are a measure of the weight of evidence for each model, considering the full model set (Burnham & Anderson, 2002). Higher Akaike weights correspond to models with higher probability of being the best models for the data and the Akaike weights of all models in the set sum to 1.

3 | RESULTS

Overall, models without an effect of OA both on calcification and photosynthesis were not supported (H_0; Figure 3). The best models of physiological performance under OA supported the same hypothesis for calcification and photosynthesis, that variation in performance under OA was driven by OA and individual noncalcified mass (H_5). Similar hypotheses related to individual size hypotheses had high support (H_4—total mass, H_6—surface area; Figure 3), although support for the next-highest ranked hypothesis was at least an order of magnitude lower than support for the top hypothesis. Among the models with lowest support for both calcification and photosynthesis were those corresponding to hypotheses H_1 (species-specific response), H_8 (SAV ratio), and H_9 (habitat) (Appendix S6).

According to the top models, variation in photosynthesis rates was explained by pCO_2 and individual noncalcified mass (Figure 4). One top model included additive effects of pCO_2 and size, such that photosynthesis increased with OA (higher pCO_2; $\beta_{pCO_2} = 0.501 \pm 0.045$) and was higher across all OA levels for larger individuals ($\beta_{\text{noncalcified mass}} = 0.446 \pm 0.055$). The other top model included a weak interactive effect of size and OA ($\beta_{\text{noncalcified mass} \times pCO_2} = -0.032 \pm 0.046$), such that at high pCO_2, larger individuals photosynthesized less than smaller individuals. The random effect of light on photosynthesis varied among experiments (mean β_{light} across experiments = 0.020; Supporting Information Appendices S4 and S7).

Calcification scaled additively with individual noncalcified mass ($\beta_{\text{noncalcified mass}} = 0.395 \pm 0.045$, Figure 4). The effect of OA on net calcification depended on saturation state: when the saturation state thermodynamically favored dissolution ($\Omega < 1$), OA had a strong negative effect on net calcification rate ($\beta_{\Omega < 1} = 0.492 \pm 0.147$).

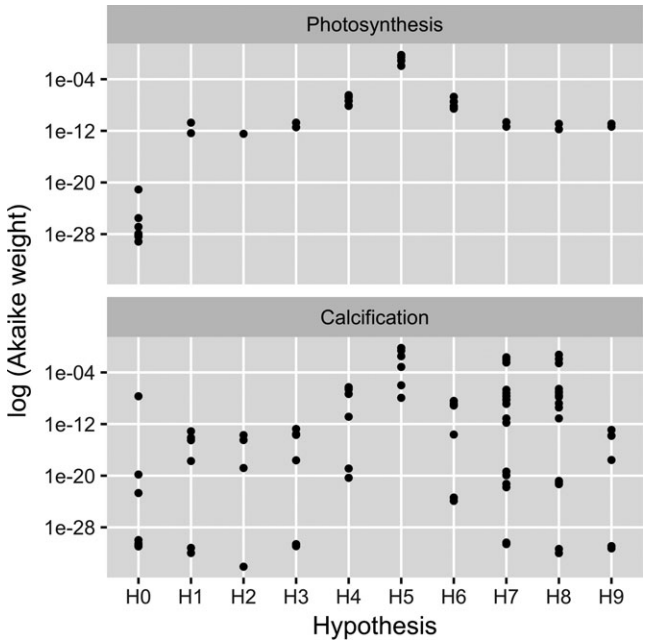

FIGURE 3 Weights of evidence for each statistical model, by hypothesis and response variable. Akaike weights represent the probability that the given model is the best model for the data. Thus, higher Akaike weights correspond with a higher weight of evidence for a model. Note the log scale of the *y*-axis

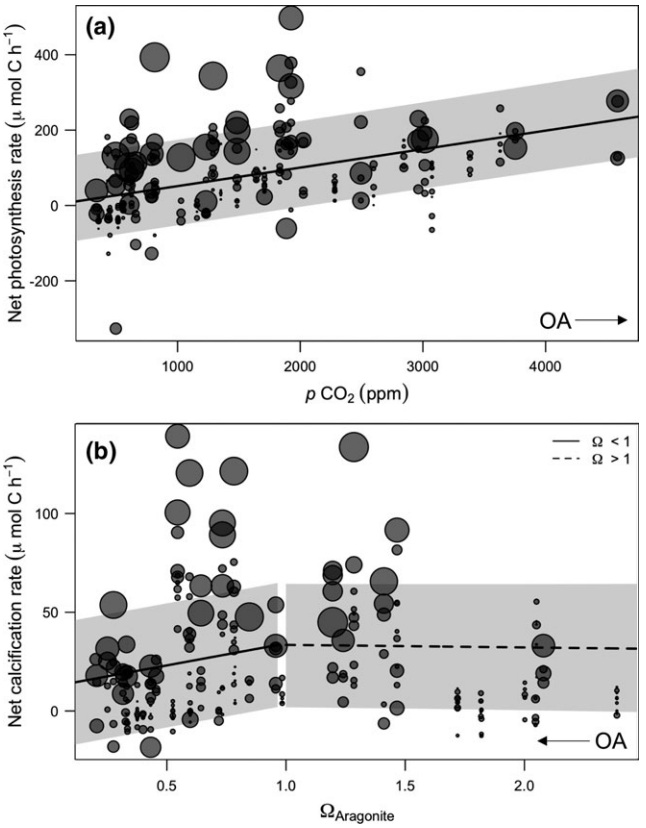

FIGURE 4 Fitted response of coralline algae to ocean acidification. Both photosynthesis (a) and calcification (b) show additive functions of OA (pCO_2 and saturation state [Ω], respectively) and log noncalcified mass. Higher pCO_2 and lower saturation state are expected under OA. Points represent the raw data. Larger point size corresponds to higher individual noncalcified mass. Fitted regression lines show the mean response to ocean acidification when all other predictors are set to the mean condition (see Appendix S7 for variation in fitted regression lines for the top additive model), and the different line types represent the effect of thermodynamically favorable (Ω > 1) or unfavorable (Ω < 1) saturation state on calcification

However, when calcification is favored (Ω > 1), the effect of saturation state on net calcification rate was neutral ($\beta_{\Omega>1}$ = −0.022 ± 0.176). The effect of OA on net calcification rate was amplified through an indirect additive effect on individual photosynthesis (β_{photo} = 0.303 ± 0.075), which varied randomly among experiments (Supporting Information Appendices S4 and S7). One top calcification model included only additive effects of OA and size, while, like the photosynthesis models, the other top model included a weak interactive effect of size and OA ($\beta_{noncalcified\ mass \times pCO_2}$ = −0.026 ± 0.045). Overall, the top models explained 70% and 61% of the total variation in multispecies calcification and photosynthesis rates, respectively (adjusted R^2).

Finally, because many marine global change studies do not include nonclimate drivers (Brown et al., 2011; O'Connor et al., 2015), we evaluated the relative performance of our alternative hypothesis approach by re-analyzing our data without trait, habitat, or phylogenetic predictors. Thus, we reduced our model set to include only models corresponding to hypotheses H_0 (no effect of OA), H_1 (species differ in responses to OA), and H_2 (species have the same responses to OA). Given this set of models, the top models of calcification and photosynthesis included a term for species identity, supporting hypothesis H_1 (ΔAICc ≤ 2; Appendix S6).

4 | DISCUSSION

In this study, five species of turf-forming coralline algae had declining net calcification rates and increasing net photosynthesis rates with short-term increases in OA. Previous studies of coralline algae have demonstrated a primarily negative effect of experimental acidification on calcification rates, growth, recruitment, and skeletal structure (Koch et al., 2013; Kroeker et al., 2010; McCoy & Kamenos, 2015). Such responses are not universal: the experimental effect of OA on coralline algae sometimes can be neutral (Doropoulos et al., 2012; Dutra, Koch, Peach, & Manfrino, 2016; Noisette et al., 2013), may depend on source location (Padilla-Gamiño, Gaitán-Espitia, Kelly, & Hofmann, 2016), and may be modulated by the organisms themselves (Cornwall, Comeau, & McCulloch, 2017). In the field, coralline algae are absent from, or rare, near low-pH seeps (Baggini et al., 2014; Kroeker, Gambi, & Micheli, 2013b), suggesting a long-term effect of OA on coralline algal abundance and distribution.

The hypothesis that organismal size was the best predictor of an individual's physiological performance under OA was supported. However, unlike previous studies, we have only weak evidence that size influences the fundamental relationship between OA and

physiology (Carey & Sigwart, 2014; Kroeker et al., 2013a; Thomsen, Haynert, Wegner, & Melzner, 2015). We found that species with higher noncalcified mass had higher baseline photosynthesis and calcification rates, similar to a study by Jensen, Gibson, Littler, and Littler (1985) of the genus *Halimeda* (calcifying crustose algae). The ultimate cause of the scaling of physiology with organismal size has been attributed to a variety of factors (reviewed in LaBarbera, 1989), including (a) surface area, and (b) geometry of internal transport networks (West, Brown, & Enquist, 1997). However, coralline algae do not have the internal venation networks that would support (b) and neither surface area nor SAV ratio were supported as predictors of OA response in our study (H_6, H_8). In a previous study, SAV ratio was found to be an important predictor of dissolution for dead calcareous bryozoans under OA conditions (rounder taxa dissolved more slowly; Smith, Nelson, & Danaher, 1992). However, live photosynthesizing calcifiers like the coralline algae in this study, can mitigate local OA conditions through photosynthesis (Comeau et al., 2014; Hurd et al., 2011) suggesting that more living biomass simply results in more metabolic "machinery".

We found the lowest support for hypotheses H_2 and H_8, which stated that performance under OA was species-specific or dictated by SAV ratio, respectively. We also found low support for evolutionary relatedness (hypothesis H_3), similar to a study of picoplankton OA response (Schaum et al., 2013). This is not surprising given that both studies use closely related organisms (present study: within one subfamily; Schaum et al., 2013: ecotypes in a genus) and thus may not represent a wide enough range of trait differences that might predict response to OA (Kroeker et al., 2010). A taxonomically broad experimental OA study with a paired quantitative phylogeny would allow a more nuanced analysis and contribute to a better understanding of the relationship between evolution and the effect of OA on organisms (Widdicombe & Spicer, 2008).

Similarly, habitat (H_9) was not an important predictor of sensitivity to OA in this study. Pacifici et al. (2015) differentiate *sensitivity* and *vulnerability* to global change: sensitivity is the intrinsic ability of a species to tolerate changes in climate while vulnerability to global change is a combination of species' sensitivity, their exposure to global change, and their capacity to adapt to change (Pacifici et al., 2015). In this study, while habitat did not predict species *sensitivity* to OA, a species' ultimate *vulnerability* to OA may be a function of habitat, as our five coralline species live in different habitats with differing present pH conditions. Additional work is needed to quantitate the relationship between physiology and habitat under present and future OA. Habitat might also indirectly influence sensitivity to OA via the influence of habitat on size. Species in this study that were primarily found in tide pools (*Calliarthron tuberculosum*, *Corallina officinalis*) were larger in tide pools than on emergent rock (Appendix S1). Species were collected from their dominant habitat type, so further work to disentangle size and habitat would be an important experiment.

In a systematic review of experiments from 2000 to 2009, 77% of OA experiments included only a single species (Wernberg et al., 2012). By conducting replicate experiments with five species (including coralline species, *Bossiella orbiginiana*, *Bossiella plumosa*, and *Calliarthron tuberculosum*, which have not previously been included in OA experiments), we were able to detect general responses across five abundant coralline species in the NE Pacific intertidal ecosystem. However, there may be additional reasons why these species would perform similarly under OA, beyond that tested in this study. For example, all the species in this study use the same calcium carbonate polymorph to calcify (Koch et al., 2013). Furthermore, all species in our experiments were collected from the same site. Because there is evidence of local and regional adaptation in the physiological response of some species to OA (Calosi et al., 2017; Padilla-Gamiño et al., 2016), a follow-up study testing spatial variation in response seems warranted.

4.1 | Linking experimental physiology to global change ecology

Ultimately, physiological responses to global change must be integrated with population and community-level consequences to make predictions about how such changes will impact species or ecosystems of interest (e.g., economically important target species, ecosystems providing specific services). Of course, long-term OA physiology studies are necessary next steps, given the possibility for acclimation and adaptation (Kelly & Hofmann, 2013), or the long-term upregulation of coralline calcification in response to experimental OA (Cornwall et al., 2017). To move from physiological response to population response to global change requires a mechanistic link between individual physiology (Pörtner & Farrell, 2008) and population growth (McLean, Lawson, Leech, & van de Pol, 2016). For example, although experiments have linked calcification responses to OA in short-term (hours) to longer experiments (weeks; Schoepf et al., 2017), future work could explore the link between individual calcification and population responses to OA.

Based on our findings, a focus on organismal size may be a useful link between individual and population response to OA. While it is already standard practice to include size in physiological analyses, a feasible next step would be to design OA metabolic studies comparable to the predictions of metabolic scaling theory (Brown et al., 2004). Over longer periods of acidification, mean population body size may decrease in response to the energetic demands of OA (Sheridan & Bickford, 2011), which could have downstream community-level impacts. In crustose coralline algae, size determines the outcome of competition for space (McCoy & Pfister, 2014; Steneck, Hacker, & Dethier, 1991). If, as we found, smaller species are the most sensitive to OA due to low baseline physiological rates or if coralline size decreases with OA (McCoy & Ragazzola, 2014), OA may drive observed decadal changes to dominance hierarchies in these species (McCoy & Pfister, 2014).

Linking experimental results to population-level response to OA may be limited by the nature of laboratory experimentation. As a function of the common OA experimental design involving mesocosm containers (Fangue et al., 2010), over the time course of our experiments, the respiration that resulted from low carbon

availability drove the saturation state from favorable for calcification to unfavorable (Appendix S5). On the other hand, when starting saturation state was low, net calcification was equally low across all individuals, but the high availability of carbon for photosynthesis likely buoyed saturation state to prevent dissolution. This effect of photosynthesis on calcification was double the magnitude of the direct effect of saturation state on calcification. Our experimental approach likely underestimated the effect of photosynthesis on calcification, given the water flow in our experiment, which may have broken up diffusion boundary layers that buffer the impact of OA on calcification (Hurd et al., 2011). Although it remains to be seen whether photosynthesis has scalable impacts on calcification in the field, these results are consistent with in situ observations from tide pools (Kwiatkowski et al., 2016) and in low-flow water regimes (Hurd, 2015).

Moving from population trends to community response requires understanding of the factors controlling community composition and range limits (e.g., OA influences on crust thickness in coralline algae which decreases competitive ability; McCoy & Pfister, 2014 and McCoy & Ragazzola, 2014). Given data constraints and the limitations of current community-level climate change prediction methods (Pacifici et al., 2015), our approach tests alternative hypotheses of species responses to global change. The goal is to move beyond a case-by-case evaluation of the impact of global change on individual species and toward a synthesis that informs forecasts.

4.2 | Ways forward for ocean acidification ecology

A major frontier for ecology is moving from heuristic models to *explanatory* predictions derived from conceptual models, and ultimately toward *anticipatory* predictions that forecast the likely future structure and function of ecological communities under global change (Mouquet et al., 2015). Our study represents a step in this direction by identifying key drivers that shape multispecies physiological responses to global change, an important component of mechanistically scaling between short-term experiments and ecosystem-level climate change predictions. Ecologists have long been interested in grouping species by shared environmental responses (Grime, 1973; Raunkiaer, 1934), and in applying such groupings to climate change predictions (Smith et al., 1997). Physiological experiments have the capacity to be a major tool for assessment and generalization of species responses to global change (Pörtner & Farrell, 2008). To date, the focus of multispecies global change research has been on alternative drivers of observational population-level responses to climate change (Angert et al., 2011; McLean et al., 2016; Pacifici et al., 2017). We instead examine the alternative drivers of physiological responses to experimental global change.

Focusing on OA, a major threat to marine life that has relatively few conceptual syntheses (Gaylord et al., 2015), we suggest that previous research likely underestimates the generality of OA responses if nonclimate drivers are not included in multispecies analyses. When we did not include nonclimate drivers such as evolutionary relatedness, functional traits or habitat in our models, we concluded that each species had a different baseline physiological performance under OA (e.g., supported hypothesis H_1). However, when we included a full suite of drivers, hypothesis H_1 was consistently among the hypotheses with the lowest support (Figure 3). By testing multiple, alternative hypotheses based on ecological first principles, we found that individual size predicted physiology, influencing response to OA, explaining up to 70% of the total variation in multispecies calcification rates.

Going forward, multispecies OA experiments that test ecologically, evolutionarily, and physiologically relevant alternative hypotheses could reveal insights into broad constraints on species response to global change. Promisingly, global change biologists have the tools and information to formulate such system-specific hypotheses, from natural history knowledge and increasingly available phylogenetic and trait databases. Such alternative hypotheses should include nonOA predictors of physiology, an important step toward characterizing the influence of OA relative to other global change factors, such as temperature or eutrophication in multi-stressor scenarios. Overall, our approach provides tools to move toward predicting organismal responses to global change using both existing theory and data.

ACKNOWLEDGEMENTS

We are grateful to the people, and especially the facilities staff, of Oregon State University's Hatfield Marine Science Center. For their help throughout this project, we thank E. Cerny-Chipman, C. Shen, T. Rohrer, J. Sullivan, J. Sapp, and A. Olson. Funding was provided by a Mamie Markham Research Grant from HMSC (AB), the National Science Foundation (OCE-1061233: FC, SH, BAM; OCE-1521633: AH), an EPA STAR Graduate Research Fellowship (FP917429) (AB), the Oregon State University Integrative Biology Research Fund (AB), the Kingfisher Foundation (BAM), the Wayne and Gladys Valley Foundation (BAM), and the David and Lucile Packard Foundation (BAM, FC). This is publication 485 from PISCO, the Partnership for Interdisciplinary Studies of Coastal Oceans.

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How to cite this article: Barner AK, Chan F, Hettinger A, Hacker SD, Marshall K, Menge BA. Generality in multispecies responses to ocean acidification revealed through multiple hypothesis testing. *Glob Change Biol*. 2018;24:4464–4477. https://doi.org/10.1111/gcb.14372