Vol. 700: 79–93, 2022
https://doi.org/10.3354/meps14166

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published November 10[§]



# Effects of temperature and pH on the growth, calcification, and biomechanics of two species of articulated coralline algae

**Rebecca Guenther[1,2], Elliot M. A. Porcher[1], Emily Carrington[2,3], Patrick T. Martone[1,*]**

[1]Botany Department and Biodiversity Research Centre, University of British Columbia, Vancouver, BC V6T 1Z4, Canada
[2]Friday Harbor Laboratories, University of Washington, Friday Harbor, WA 98250, USA
[3]Department of Biology, University of Washington, Seattle, WA 98195, USA

ABSTRACT: Ocean warming and acidification are predicted to impact the physiology of marine organisms, especially marine calcifiers that must deposit calcium carbonate and resist dissolution. Of particular concern are articulated coralline algae, which must maintain both calcified segments (intergenicula) and uncalcified joints (genicula) in order to thrive along wave-swept rocky coastlines. We examined the effect of pH and temperature, both individually and in combination, on the growth, calcification, and biomechanical properties of 2 species of articulated coralline algae, *Corallina vancouveriensis* and *Calliarthron tuberculosum*, common on wave-exposed shores in the NE Pacific. Increased temperature and reduced pH were found to reduce growth rates in both species (30–89 % lower) but had little influence on the amount of intergenicular calcium carbonate or on the genicular biomechanical properties of these species. Results suggest that although growth rates may decline, these 2 coralline species will maintain the integrity of their tissues and continue to persist under future climate stress.

KEY WORDS: Climate change · Ocean acidification · Macroalgae · Interactive effects · *Corallina* · *Calliarthron*

## 1. INTRODUCTION

Anthropogenic climate change caused by increased $CO_2$ emissions is one of the major threats currently facing marine ecosystems (Bindoff et al. 2019). Elevated atmospheric $CO_2$ causes ocean temperature to rise and dissolves in seawater causing pH to fall—2 drivers that can impact marine organisms, including macroalgae, which provide essential food and habitat in marine communities. Though increasing ocean temperature may initially elicit a positive response until some optimum is reached, further warming quickly becomes detrimental (Schulte et al. 2011, Vasseur et al. 2014, Wernberg et al. 2016, Graba-Landry et al. 2018). Ocean warming (OW) has been shown to have a strong negative impact on many metabolic processes in algae, including photosynthesis, growth, and calcification (Graba-Landry et al. 2018, Piñeiro-Corbeira et al. 2018, Cornwall et al. 2019, Smale et al. 2019). Likewise, while increases in the partial pressure of $CO_2$ ($pCO_2$) may provide benefits to photosynthesis in some algae (Koch et al. 2013), the accompanying decrease in seawater pH and saturation states of carbonate minerals can negatively affect the growth, survival, and reproduction of many calcifying algae (Sabine et al. 2004, Hofmann & Bischof 2014, Meyer & Riebesell 2015).

*Corresponding author: pmartone@mail.ubc.ca

[§]Data archive section inserted on p. 91 after publication
This corrected version: November 21, 2022



Publisher: Inter-Research · www.int-res.com

Coralline algae (orders Corallinales, Sporolithales, and Hapalidiales) are predicted to be particularly sensitive to shifts in pH, as their biomineralized cell walls are composed mainly of Mg-calcite, a highly soluble polymorph of calcium carbonate ($CaCO_3$) (McCoy & Kamenos 2015, Nash et al. 2019). However, the effects of ocean acidification (OA) and OW studied in isolation may not accurately represent reality; *in situ*, corallines may be exposed simultaneously to multiple stressors that interact antagonistically, additively, or synergistically to affect organismal physiology (Gunderson et al. 2016, Kroeker et al. 2017, Bindoff et al. 2019). For example, responses to either elevated $pCO_2$ or temperature alone may be neutral or positive in some species (e.g. *Lithophyllum cabiochae*; Martin et al. 2013) but generally become strongly negative when experienced in combination (Martin & Gattuso 2009, Diaz-Pulido et al. 2012, Johnson & Carpenter 2012, Martin et al. 2013, Nash et al. 2016, Kim et al. 2018, Muñoz et al. 2018). Moreover, the impacts of climate stressors can be multifaceted, and maintenance of one physiological function may come at the expense of another. For example, some corallines may compensate for the effects of OA by regulating pH at the site of calcification, but active mechanisms of pH modification (such as H+ pumping or increased carbon fixation in the dark) necessarily divert energy from other functions such as growth or reproduction (Cornwall et al. 2017, Hofmann et al. 2018). Thus, characterizing several organismal responses to OW and OA may help identify physiological tradeoffs and provide a more holistic view of climate change impacts.

Under OA conditions, many coralline species experience reductions in net calcification and growth rates (Büdenbender et al. 2011, Egilsdottir et al. 2013, Porzio et al. 2018), with net calcification reflecting both decreased calcification and increased dissolution (Comeau et al. 2019). Changes in calcification can cause significant thinning and weakening of coralline thalli (Ragazzola et al. 2012, McCoy & Ragazzola 2014) and potentially make them more vulnerable to grazers (Johnson & Carpenter 2012, McCoy & Kamenos 2018, but see Martone et al. 2021) or wave impacts (Padilla 1993). In addition, articulated corallines specifically must develop and maintain uncalcified joints (genicula), which are remarkably strong, extensible, and resistant to fatigue (Martone 2006, Denny & King 2016, Janot & Martone 2016, Martone et al. 2019), in order to bend — but not break — under crashing waves (Martone & Denny 2008a,b, Janot & Martone 2018). Although changes in temperature and pH may impact the mechanical properties of uncalcified macroalgae (e.g. Simonson et al. 2015, Guenther et al. 2018), the effects of OW and OA on the mechanical properties of genicula are unknown, limiting our insight into how changes in ocean conditions may influence the ability of articulated thalli to survive in wave-swept habitats.

In this study, we examined the effects of reduced pH and warming, in isolation and in combination, on 2 species of articulated coralline algae, *Corallina vancouveriensis* Yendo (hereafter *Corallina*) and *Calliarthron tuberculosum* (Postels & Ruprecht) E.Y. Dawson (hereafter *Calliarthron*), commonly found on wave-exposed shores of the NE Pacific (Fig. 1). *Corallina* occurs from the Aleutian Islands to the Galapagos Islands (Abbott & Hollenberg 1976) and is found strictly in the intertidal zone, where it frequently grows out of or near the surface of tidepools (Padilla 1984). It has been shown to be highly resistant to increasing temperature and desiccation stress, likely due to its ability to retain water within its finely branched thalli during low tide (Guenther & Martone 2014). In contrast, *Calliarthron* occurs commonly from British Columbia, Canada, to Los Angeles County, California (Gabrielson et al. 2011) and is most abundant subtidally (Konar & Foster 1992) or at the bottom of large tidepools (Barner et al. 2018), suggesting that it is adapted to cooler temperatures and may be more sensitive to warming or decreased pH. We measured growth, calcification, and tissue


Fig. 1. (A) *Corallina vancouveriensis* and (B) *Calliarthron tuberculosum*, illustrating morphological differences between the 2 species. Scale bar = 10 mm

material properties to explore multi-faceted impacts of climate change in these 2 species. We hypothesized that *Corallina* would experience less reduction in growth and calcification than *Calliarthron* when exposed to temperature and pH stress, and that *Corallina* would maintain biomechanical properties under stressful temperature and pH treatments while *Calliarthron* would be more sensitive.

## 2. MATERIALS AND METHODS

### 2.1. Experimental design and specimen collection

We conducted 3 experiments, 2 with single factors (pH and temperature, in December 2013 and May 2015, respectively) and one with 2 factors (pH × temperature, in August 2014). For each experiment, specimens of *Corallina vancouveriensis* and *Calliarthron tuberculosum* were identified morphologically (Gabrielson et al. 2012) and collected from the intertidal zone at Deadman Bay (48° 30' 48.09'' N, 123° 8' 49.38'' W), San Juan Island, Washington. At this site, intertidal temperatures average ~10°C in winter, ranging from ~4 to 14°C, and average ~12°C in summer, ranging from ~10 to 30°C. The pH of seawater in Deadman Bay averages 7.9 in winter and 7.7 in summer (Guenther 2016). Specimens (thalli) were immediately transported to Friday Harbor Laboratories and held in outdoor flow-through seawater tanks (12–14°C) for up to 1 wk prior to beginning experimental treatments in mesocosms. The tanks were exposed to indirect sunlight and gently mixed by the seawater inflow. Each experiment followed a repeated measures design, with thalli subdivided into fronds that were distributed among all treatments (Fig. 2). Sample size was therefore 3–8 thalli, depending on the experimental design (e.g. single factor or 2 factors, described in more detail below), with 3 replicate fronds per thallus per treatment.

### 2.2. Mesocosm experiments

#### 2.2.1. Single-factor pH

Thalli of *Corallina* and *Calliarthron* (n = 6 for each species) were subdivided into fronds assigned to 6 different pH treatments (8.0, 7.8, 7.6, 7.4, 7.2, 7.0) for 5 wk, following the methods of O'Donnell et al. (2013) (Fig. 2). Briefly, flowing seawater was serially filtered (final pore diameter: 0.2 µm), scrubbed of $CO_2$, and sterilized with UV before entering each mesocosm (one for each pH treatment). Mesocosms were flow-through systems in which treatment water was first established in a mixing reservoir and then pumped into 6 replicate 4 l chambers where experimental organisms were held. Each chamber held 3 fronds from one individual of *Calliarthron* and 3 fronds from one individual of *Corallina*. Chamber inflow replenished the water every 2–3 h and outflow drained directly out of the mesocosm, ensuring water from different chambers did not mix. Approximately once per week, treatments were rotated among mesocosms to control for mesocosm effects, and fronds were scrubbed with a soft brush to prevent diatom fouling.

The temperature and pH of each mesocosm were continuously monitored using a Honeywell Durafet III pH electrode (Fig. S1, Table S1 in the Supplement at www.int-res.com/articles/suppl/m700p079_supp.pdf), calibrated weekly using a water sample analyzed for spectrophotometric pH using m-cresol purple dye. Carbonate chemistry was manipulated by bubbling a $CO_2$:$O_2$ gas mixture into each mesocosm through a Venturi injector. Control of pH was achieved by using a feedback loop between a Honeywell UDA2182 pH controller and the pH electrodes to control the amount of $CO_2$:$O_2$ gas mixture pumped into the mesocosm. Temperature was maintained at 12–13°C by pumping water through a heat exchanger and chiller system and fine-tuned with aquarium heaters. Light was supplied by Chroma 50 full-spectrum fluorescent lights (2 fixtures of 2 lights mesocosm$^{-1}$, 52.8 ± 0.8 µmol photons m$^{-2}$ s$^{-1}$).

Weekly water samples from mesocosm seawater were collected for carbonate chemistry analysis (spec-

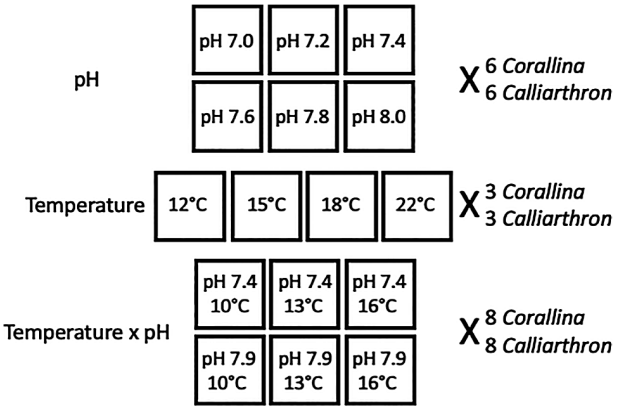


Fig. 2. Experimental design for each of the 3 mesocosm experiments (pH, temperature, and temperature × pH). Each square represents a 4 l specimen chamber which held 3 replicate fronds from an individual thallus of *Calliarthron tuberculosum* and *Corallina vancouveriensis*. Replication for each experiment is indicated on the right

trophotometric pH, total alkalinity) and measured within 24 h (Table S1). Total alkalinity was measured using an open cell titrator following SOP3b from Dickson et al. (2007). Using the program CO2calc version 1.3.0 (Robbins et al. 2010), $pCO_2$ and carbonate saturation states were calculated from measured temperature, salinity, pH, and total alkalinity values. The following CO2calc constants were used: $CO_2$ constants ($K1$, $K2$ from Mehrbach et al. 1973), $KHSO_4$ (Dickson 1990), pH scale (total scale; mol $kg^{-1}$ seawater), total boron (Lee et al. 2010), and air–sea flux (Wanninkhof 1992). After 5 wk, linear growth, calcification, and biomechanical assays were conducted on fronds from each treatment, as described below (Section 2.3).

#### 2.2.2. Single-factor temperature

Thalli of *Calliarthron* and *Corallina* (n = 3 for each species) were subdivided into fronds assigned to 4 different temperature treatments (12, 15, 18, and 22°C), representing a gradient from cool to warm tidepool temperatures in spring/summer (Guenther 2016), and were maintained for 4 wk under controlled temperature conditions in specimen chambers housed in an empty seawater table (Fig. 2); pH was not manipulated and was approximately 7.8. Each treatment had 3 replicate chambers, each containing 3 fronds from one individual of *Calliarthron* and 3 fronds from one individual of *Corallina*.

Temperature in each chamber was controlled by heat exchangers placed in three 5 gallon (~19 l) buckets of freshwater, with the exception of the ~12°C treatment, where ambient flowing seawater was used. The heat exchange buckets were either maintained at room temperature (15°C treatment) or warmed to a target temperature using an aquarium heater and a temperature controller (18 and 22°C treatments). Seawater was pumped slowly through the heat exchanger before entering into the specimen chambers through sprinkler heads, ensuring that temperature and flow within the specimen chambers remained stable. Each specimen chamber had a 12 V aquarium pump to provide sufficient water circulation and ibutton temperature loggers to monitor temperature (Fig. S1). Light was supplied by the same Chroma 50 full-spectrum fluorescent lights described in Section 2.2.1. Approximately once per week, treatments were rotated among mesocosms to control for mesocosm effects and fronds were scrubbed with a soft brush to prevent diatom fouling.

After 4 wk, linear growth, calcification, and biomechanical assays were conducted on fronds from each treatment, as described below (Section 2.3). Biomechanical data for *Calliarthron* were not collected in the single-factor temperature experiment due to equipment malfunction.

#### 2.2.3. Temperature and pH combined

Thalli of *Corallina* and *Calliarthron* (n = 8 for each species) were subdivided into fronds assigned to 6 temperature × pH treatment combinations consisting of 3 temperature levels (10, 13, 16°C) and 2 pH levels (7.4, 7.9) (Fig. 2). Fronds were maintained for 6 wk in mesocosms in each treatment combination established using the same procedures as the single-factor pH experiment described in Section 2.2.1. In this case, each temperature × pH treatment contained 8 replicate chambers, each with 3 fronds from one individual of *Calliarthron* and 3 fronds from one individual of *Corallina*.

Temperature and pH were continuously monitored in specimen chambers (Fig. S1, Table S1) and mixing reservoirs (Table S2) throughout the experiment. Seawater samples were collected from mesocosms once per week for carbonate chemistry analysis (spectrophotometric pH, total alkalinity) and measured within 24 h following the same methods described above in the single-factor pH experiment (see Table S1). Light was supplied by the same Chroma 50 full-spectrum fluorescent lights described in Section 2.2.1. Approximately once per week, treatments were rotated between mesocosms to control for mesocosm effects and fronds were scrubbed with a soft brush to prevent diatom fouling. After 6 wk, linear growth, calcification, and biomechanical assays were conducted on fronds from each treatment, as described below (Section 2.3).

### 2.3. Response assays

#### 2.3.1. Growth

Linear growth was assessed using the Calcofluor White method described in Martone (2010). Prior to each experiment, fronds were stained with a 0.05% solution of Calcofluor white (Sigma-Aldrich, Fluorescent Brightener 28) for approximately 1 h under natural lab lighting conditions. Post-experiment, the stained tissue (representing the previous position of the apical meristem) was visualized by exposing

fronds to black light and taking long exposure photographs (~10–13 s). Total linear growth was evaluated by measuring the distance from the distal edge of the Calcofluor stain mark to the apical tip of the frond in ImageJ (Fig. S2). Growth rate (mm $d^{-1}$) of each frond was estimated as the average of 5–10 tip linear growth measurements divided by the number of days since the stain was applied. Frond growth rates were then averaged for each replicate (specimen chamber).

#### 2.3.2. Calcification

The amount of $CaCO_3$ in calcified tissues (intergenicula) was determined by comparing dry weights of segments before and after decalcification in 1 N HCl. Intergenicula were categorized as either 'new tissue' (intergenicula which formed during the course of the experiment above the Calcofluor white stain as shown in Fig. S2; i.e. a proxy for the net sum of calcification and dissolution) or 'old tissue' (intergenicula which were present prior to the start of the experiment below the Calcofluor white stain; i.e. a relative proxy for dissolution given the lower rates of calcification in older intergenicula) (Pearse 1972, McCoy et al. 2016). Approximately 10–30 intergenicula were harvested per frond per individual for each measurement. Harvested segments were dried overnight at 68°C, weighed (g), and then decalcified in 1 N HCl for at least 12 h. Following decalcification, segments were rinsed with distilled water, dried, and re-weighed. Percent $CaCO_3$ was then calculated as follows:

$$\% \ CaCO_3 = (\text{oven dry weight} - \text{decalcified oven dry weight}) / (\text{oven dry weight}) \times 100$$

Percent $CaCO_3$ measurements of new and old tissue were averaged for each replicate (specimen chamber).

#### 2.3.3. Biomechanical properties

Breaking stress, breaking strain, and modulus (stiffness) of uncalcified tissues (genicula) were measured in tension using an Instron 5565 tensometer fitted with a temperature-controlled water bath (12°C) and submersible pneumatic grips (40 psi). Measurements of genicular cross-sectional area and genicular length were used to standardize force and length measurements generated by the tensometer.

Due to the small size of *Corallina*, it was not possible to test individual apical genicula. Instead, apical portions (~10–20 genicula long) were mounted in the grips with thin foam (2–3 mm) and fine sandpaper to reduce slippage. The sample was then extended at 0.2 mm $s^{-1}$ until the frond failed at one geniculum. The cross-sectional area of the geniculum that broke and the length of a neighboring genicula were measured under a dissecting microscope (40×). The number of genicula between the tensometer grips was then multiplied by the length of the measured geniculum to yield the total genicular length tested.

As *Calliarthron* has larger genicula, it was feasible to test only the first apical geniculum. The calcified intergeniculum on each side of the apical geniculum was glued into a small divot drilled into aluminum T-bars using Zap-a-Gap glue and kicker accelerator (Pacer Technology). The T-bars were then mounted into the pneumatic grips for tensile testing.

The extension and load values provided by the tensometer were used to construct stress versus strain curves for each frond from each treatment, from which individual breaking stress (MPa), breaking strain (mm $mm^{-1}$), and modulus (MPa) were calculated. Breaking stress (strength) of the material was calculated as the maximum force applied at breakage divided by the cross-sectional area of the geniculum; breaking strain (extensibility) was the maximum extension of the genicula at breakage divided by the initial length of the genicula. Tensile modulus (stiffness) of the genicula was quantified as the initial slope of the stress–strain curve. As all biomechanical parameters were normalized to the size and shape of the sample, these tests evaluated the properties of the genicular material regardless of thallus morphology. Biomechanical properties were averaged for each replicate (specimen chamber).

### 2.4. Statistical analyses

All statistical analyses were performed in R version 4.0.0 (R Core Team 2020). Separate analyses were conducted for each dependent variable (growth, % $CaCO_3$ new tissue, % $CaCO_3$ old tissue, modulus, breaking stress, and breaking strain) for each species in each experiment. The single-factor temperature and pH experiments were analyzed with a 2-factor linear mixed effects model (LME) using the 'lmer()' function ('lme4' version 1.1-23, Bates et al. 2015) with type III sums of squares (SS) using the 'Anova()' function ('car' version 3.0-8, Fox & Weisberg 2019). Temperature and pH were each treated as a fixed factor and chamber was included as a random factor. The temperature × pH experiment was analyzed using the same methods; temperature and pH were treated

as fixed factors in a fully factorial model with chamber included as a random factor. Estimated marginal means (EMMs) were used for post hoc comparisons; contrasts were performed across all experimental treatments within each species using the 'emmeans()' function ('emmeans' version 1.4.7; Lenth et al. 2020).

Calcification data were arcsine transformed and biomechanics data were log transformed in order to meet assumptions of normality and heteroscedasticity. Even after transformation, several possible outliers were visually identified within the calcification data sets (e.g. % $CaCO_3$ < 70%), and data were subsequently screened for outliers using the 'identify outliers()' function ('rstatix' version 0.5.0, Kassambara 2020). Data points that were flagged as both outliers and extreme values were omitted from the calcification analyses (n = 3, pH experiment; n = 2, temperature × pH experiment); outliers were not exclusively associated with any one individual. For complete R scripts, see the Supplement.

## 3. RESULTS

### 3.1. Growth

In the single-factor pH experiment, both *Corallina* and *Calliarthron* grew significantly slower as pH declined (Fig. 3A, Table 1). *Corallina* fronds grew 0.07 ± 0.01 mm $d^{-1}$ in pH 8.0–7.4 but grew less when pH ≤ 7.2. For example, the growth rate of *Corallina* in the lowest pH treatment (pH 7.0) was only 0.03 ± 0.01 mm $d^{-1}$, a ~66% reduction compared to the highest pH treatment (pH 8) (EMM, p < 0.05; Fig. 3A, Table S3). *Calliarthron* fronds grew fastest in pH 8 (0.10 ± 0.01 mm $d^{-1}$) and significantly declined by 20% in pH 7.8 (EMM, p < 0.05; Fig. 3A, Table S3). Interestingly, there was no significant reduction in growth of *Calliarthron* from pH 7.8 to 7.0 (EMM, p > 0.05; Fig. 3A, Table S3).

In the single-factor temperature experiment, increased temperature significantly reduced the growth rate of both *Corallina* and *Calliarthron* (Fig. 3B, Table 1). *Corallina* fronds grew 0.09 ± 0.01 mm $d^{-1}$ in the 12°C treatment but slowed to 0.01 ± 0.01 mm $d^{-1}$ in the 22°C treatment — a reduction of ~91% (EMM, p < 0.05; Fig. 3B, Table S3). *Calliarthron* grew fastest in the 12 and 15°C treatments (0.11 ± 0.01 mm $d^{-1}$, EMM: p > 0.05; Fig. 3B, Table S3) but slowed by 55% in the 18°C treatment (EMM, p < 0.05; Fig. 3B, Table S3), and at 22°C no growth was detected (EMM, p < 0.05; Fig. 3B, Table S3). Additionally, many *Calliarthron* fronds in the 22°C treatment partially bleached and lost some pigmentation, which was not observed in *Corallina*.

In the temperature × pH experiment, reduced pH and increased temperature affected the growth of both *Corallina* and *Calliarthron* fronds differently (Fig. 3C, Table 1). In *Corallina*, growth rate was 11%

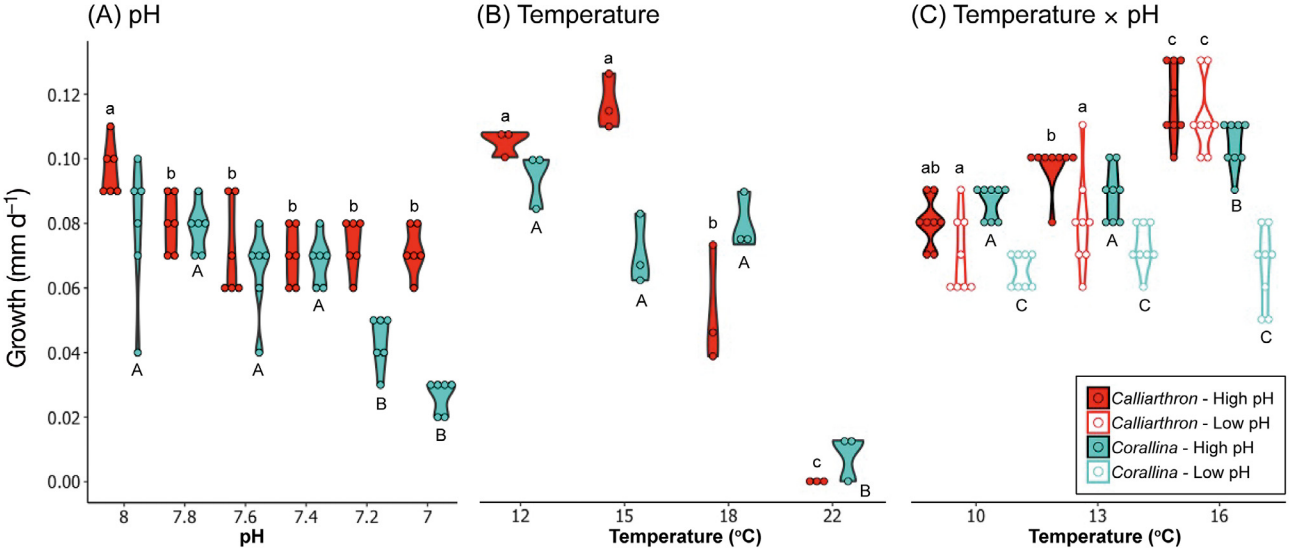


Fig. 3. Tissue growth rate in each of the 3 mesocosm experiments: (A) pH, (B) temperature, and (C) temperature × pH. Violin plots show data distribution over raw data (dots); red: *Calliarthron*; blue: *Corallina*. High pH and low pH are distinguished for each species in (C). Letters indicate results of separate post hoc estimated marginal means comparisons for each species (lowercase: *Calliarthron*; uppercase: *Corallina*)

Table 1. Linear mixed model estimation for tissue growth (mm) in *Calliarthron tuberculosum* and *Corallina vancouveriensis* in each of the 3 mesocosm experiments

| Experiment | Genus | Factor | *F* | df | df.res | Pr(>*F*) |
|---|---|---|---|---|---|---|
| pH | *Calliarthron* | Intercept | 326.2941 | 1 | 28.408 | <0.001 |
| | | pH | 7.3158 | 5 | 25.000 | <0.001 |
| | *Corallina* | Intercept | 911.250 | 1 | 5 | <0.001 |
| | | pH | 18.188 | 5 | 25 | <0.001 |
| Temperature | *Calliarthron* | Intercept | 252.47 | 1 | 2 | 0.004 |
| | | Temperature | 133.77 | 3 | 6 | <0.001 |
| | *Corallina* | Intercept | 603.110 | 1 | 2 | 0.002 |
| | | Temperature | 54.249 | 3 | 6 | <0.001 |
| Temperature × pH | *Calliarthron* | Intercept | 1953.436 | 1 | 6.99 | <0.001 |
| | | Temperature | 61.3418 | 2 | 34.148 | <0.001 |
| | | pH | 14.4864 | 1 | 34.152 | <0.001 |
| | | Temperature × pH | 2.1803 | 2 | 34.148 | 0.128 |
| | *Corallina* | Intercept | 2425.707 | 1 | 6.993 | <0.001 |
| | | Temperature | 3.2795 | 2 | 34.124 | 0.049 |
| | | pH | 143.8402 | 1 | 34.127 | <0.001 |
| | | Temperature × pH | 12.2905 | 2 | 34.124 | <0.001 |

higher at 16°C than in the 10 and 13°C treatments (EMM, $p < 0.05$; Fig. 3C, Table S3) but only at high pH (pH 7.9). In low pH (pH 7.4), growth rate of *Corallina* was unaffected by temperature but significantly reduced across all temperature groups compared to that in high pH (EMM, $p > 0.05$; Fig. 3C, Table S3). In *Calliarthron*, growth rates were 50% higher in the 16°C treatment than in the 10°C treatment, with no effect of pH at either temperature (EMM, $p < 0.05$; Fig. 3C, Table S3). A significant effect of pH was only detected at 13°C, where growth was reduced by 20% in the low pH treatment (EMM, $p < 0.05$; Fig. 3C, Table S3).

### 3.2. Calcification

In the single-factor pH experiment, decreased pH caused a significant reduction in % $CaCO_3$ of new tissue in fronds of both *Corallina* and *Calliarthron* (Fig. 4A, Table 2). As pH decreased, there was a greater decline in % $CaCO_3$ of new tissue deposited by *Corallina* than in new tissue deposited by *Calliarthron*; in *Corallina*, % $CaCO_3$ of new tissue declined by 12% at pH 7 compared to pH 8, while % $CaCO_3$ of new tissue in *Calliarthron* only declined by 4% in the same treatments (EMM, $p < 0.05$; Fig. 4A, Table S4). In *Corallina*, decreasing pH also had a significant effect on % $CaCO_3$ in old tissue, which declined by 9% at pH 7 compared to pH 8 (EMM, $p < 0.05$; Fig. 4D, Table S5); however, decreasing pH had no effect on % $CaCO_3$ of old tissue in *Calliarthron* (EMM, $p < 0.05$; Fig. 4D, Table S5).

In the single-factor temperature experiment, increased temperature alone had no effect on % $CaCO_3$ of new tissue (LME, temp, $p > 0.12$; Fig. 4B, Table 2) or old tissue (LME, temp, $p > 0.43$; Fig. 4E, Table 2) in either *Corallina* or *Calliarthron*.

In the temperature × pH experiment, the % $CaCO_3$ of new tissue in *Corallina* was negatively affected by both temperature (LME, temp, $p < 0.001$; Fig. 4C, Table 2) and pH (LME, pH, $p < 0.001$; Fig. 4C, Table 2) but not the interaction of the 2 factors (LME, temp × pH, $p = 0.17$; Fig. 4C, Table 2). In *Corallina*, % $CaCO_3$ of new tissue was ~6% lower in the 16°C temperature treatments compared to 13°C treatments at pH 7.4 and 7.9 (EMM, $p < 0.05$; Fig. 4C, Table S4), and % $CaCO_3$ was ~3% lower in pH 7.4 treatments compared to pH 7.9 treatments at both 13 and 16°C (EMM, $p < 0.05$; Fig. 4C, Table S4). In *Calliarthron*, % $CaCO_3$ of new tissue was unaffected by pH (LME, pH, $p = 0.73$; Fig. 4C, Table 2) but was slightly (3%) lower at 16°C than at 13°C in both pH treatments (EMM, $p < 0.05$; Fig. 4C, Table S4).

Increased temperature had no effect on % $CaCO_3$ of old tissue in *Corallina* at pH 7.9 (EMM, $p > 0.05$; Fig. 4F, Table S5), but at pH 7.4, % $CaCO_3$ of old tissue was slightly (3%) lower at both 10 and 16°C compared to 13°C (EMM, $p < 0.05$; Fig. 4F, Table S5). Temperature and pH did not significantly affect % $CaCO_3$ of old tissue in *Calliarthron* (LME, all factors, $p > 0.19$; Fig. 4F, Table 2).

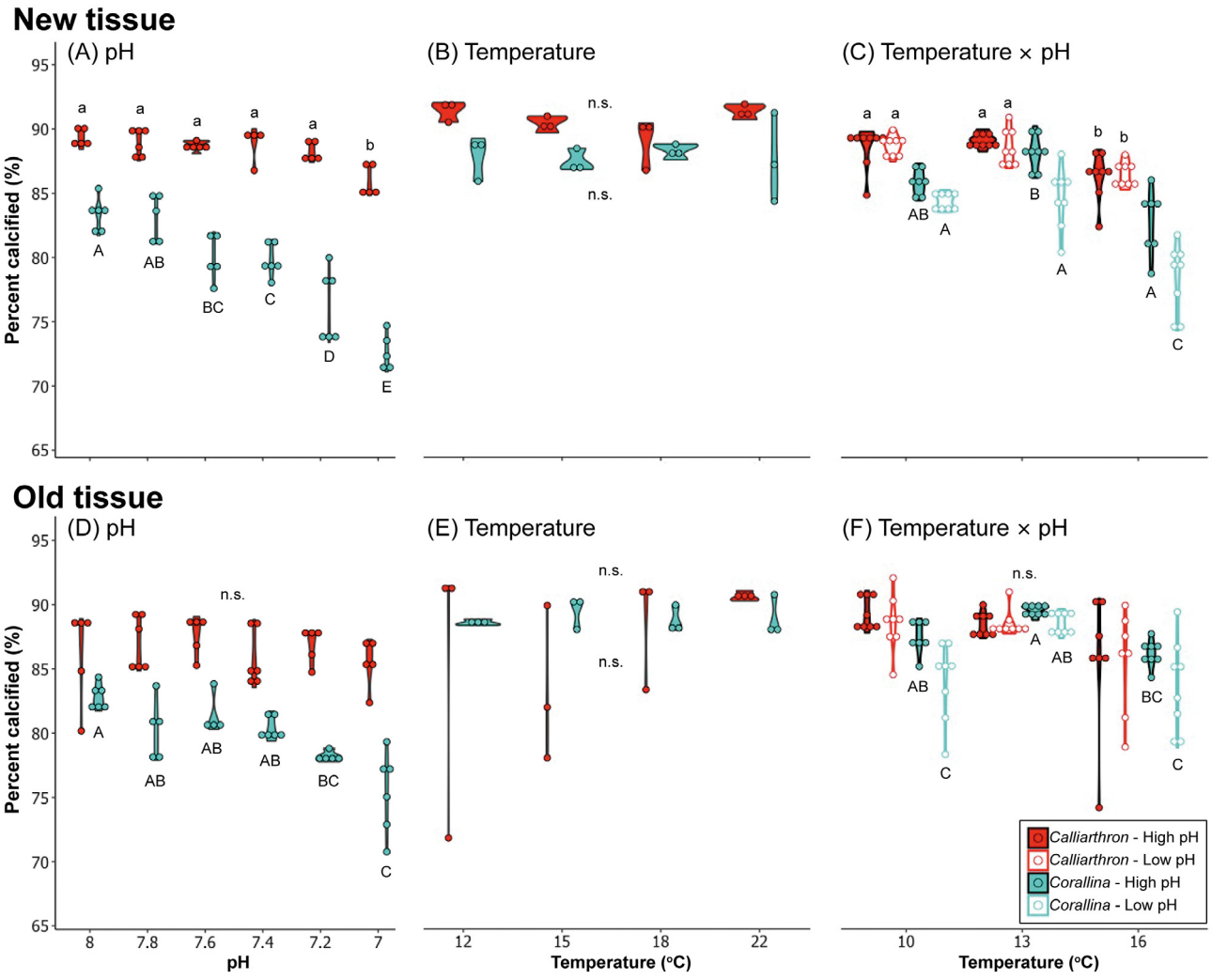


Fig. 4. Calcification (%) of (A–C) new tissue and (D–F) old tissue in each of the 3 mesocosm experiments: (A,D) pH, (B,E) temperature, and (C,F) temperature × pH. Violin plots show data distribution over raw data (dots); red: *Calliarthron*; blue: *Corallina*. High pH and low pH are distinguished for each species in (C) and (F). Letters indicate results of separate post hoc estimated marginal means comparisons for each species (lowercase: *Calliarthron*; uppercase: *Corallina*); n.s.: not significant

### 3.3. Biomechanical properties

In the single-factor pH experiment, reduced pH alone had no significant effects on the stiffness (LME, pH, $p > 0.17$), strength (LME, pH, $p > 0.09$), or extensibility (LME, pH, $p > 0.09$) of *Corallina* or *Calliarthron* genicula in any treatment (Fig. 5A,D,G, Table 3).

In the single-factor temperature experiment, increased temperature had no significant effects on the stiffness (LME, temp, $p = 0.12$), strength (LME, temp, $p = 0.05$), or extensibility (LME, temp, $p = 0.10$) of *Corallina* genicula (Fig. 5B,E,H, Table 3).

In the temperature × pH experiment, neither temperature nor pH had any significant effects on the stiffness (LME, all factors, $p > 0.06$) or the strength (LME, all factors, $p > 0.28$) of genicula in the 2 species (Fig. 5C,F, Table 3). In *Calliarthron*, there was no significant interaction between temperature and pH on extensibility (LME, temp × pH, $p = 0.05$; Fig. 5I, Table 3) and no effect of pH (LME, pH, $p = 0.14$) but a significant effect of temperature on extensibility (LME, temp, $p = 0.04$). However, post hoc comparisons failed to detect significant differences across any treatments (EMM, $p > 0.09$; Fig. 5I, Table S6), suggesting that any effect of temperature on extensibility was slight and not clearly demonstrated. In *Corallina*, temperature and pH had no significant effects on genicular extensibility (LME, all factors, $p > 0.05$; Fig. 5I, Table 3).

Table 2. Linear mixed model estimation for calcification (%) of new or old tissue in *Calliarthron tuberculosum* and *Corallina vancouveriensis* for each of the 3 mesocosm experiments

| Tissue type | Experiment | Genus | Factor | *F* | df | df.res | Pr>(*F*) |
|---|---|---|---|---|---|---|---|
| New | pH | *Calliarthron* | Intercept | 88988.3205 | 1 | 4.8262 | <0.001 |
| | | | pH | 9.5429 | 5 | 19.6380 | <0.001 |
| | | *Corallina* | Intercept | 79604.153 | 1 | 4.8513 | <0.001 |
| | | | pH | 26.664 | 5 | 22.7562 | <0.001 |
| | Temperature | *Calliarthron* | Intercept | 74216.1393 | 1 | 2 | <0.001 |
| | | | Temperature | 2.8914 | 3 | 6 | 0.124 |
| | | *Corallina* | Intercept | 23107.7357 | 1 | 2 | <0.001 |
| | | | Temperature | 0.1043 | 3 | 6 | 0.955 |
| | Temperature × pH | *Calliarthron* | Intercept | $1.8006 \times 10^5$ | 1 | 7 | <0.001 |
| | | | Temperature | 1.810 | 2 | 35 | <0.001 |
| | | | pH | 0.1220 | 1 | 35 | 0.729 |
| | | | Temperature × pH | 0.3516 | 2 | 35 | 0.706 |
| | | *Corallina* | Intercept | 91106.2739 | 1 | 6.922 | <0.001 |
| | | | Temperature | 38.0628 | 2 | 33.576 | <0.001 |
| | | | pH | 31.4921 | 1 | 33.477 | <0.001 |
| | | | Temperature × pH | 1.8982 | 2 | 33.576 | 0.166 |
| Old | pH | *Calliarthron* | Intercept | 16300.6087 | 1 | 4.9423 | <0.001 |
| | | | pH | 1.2312 | 5 | 21.2951 | 0.329 |
| | | *Corallina* | Intercept | 73548.359 | 1 | 4.8987 | <0.001 |
| | | | pH | 11.641 | 5 | 21.9853 | <0.001 |
| | Temperature | *Calliarthron* | Intercept | 1123.0573 | 1 | 2 | <0.001 |
| | | | Temperature | 1.0497 | 3 | 6 | 0.437 |
| | | *Corallina* | Intercept | 26155.8113 | 1 | 2 | <0.001 |
| | | | Temperature | 0.5707 | 3 | 6 | 0.655 |
| | Temperature × pH | *Calliarthron* | Intercept | 14697.0767 | 1 | 6.986 | <0.001 |
| | | | Temperature | 1.7255 | 2 | 34.175 | 0.193 |
| | | | pH | 1.1972 | 1 | 34.179 | 0.282 |
| | | | Temperature × pH | 0.5445 | 2 | 34.175 | 0.585 |
| | | *Corallina* | Intercept | 73576.2573 | 1 | 6.969 | <0.001 |
| | | | Temperature | 21.4837 | 2 | 34.257 | <0.001 |
| | | | pH | 14.5029 | 1 | 34.264 | <0.001 |
| | | | Temperature × pH | 1.3372 | 2 | 34.257 | 0.276 |

## 4. DISCUSSION

### 4.1. Growth

*Corallina vancouveriensis* is an intertidal species that is highly resistant to desiccation and heat stress (Guenther & Martone 2014); other *Corallina* species have demonstrated similar tolerances to temperature and pH stress, including short-term exposure to pH levels as low as 3 (Gao et al. 2016, Kim et al. 2018). It was therefore expected that *Corallina* would experience less reduction in growth when exposed to temperature and pH stress than *Calliarthron tuberculosum*, which is often restricted to subtidal and tidepool habitats (Martone et al. 2010). Nevertheless, species responses to environmental variation were difficult to predict and often contrary to expectations.

In both the single-factor temperature and the temperature × pH experiments, increasing temperature had a variable and sometimes positive effect on the growth of both species up to 16–18°C. However, in the single-factor temperature experiment, growth in both species dramatically declined at 22°C, presumably after some threshold had been surpassed. At this warmest temperature, growth in *Calliarthron* had stopped and tissues had partially bleached while growth in *Corallina* was still measurable, perhaps suggesting a greater sensitivity to high temperature in *Calliarthron*, partially consistent with our hypotheses.

The inverse was true for pH, where contrary to predictions, *Corallina* was more sensitive to reduced pH

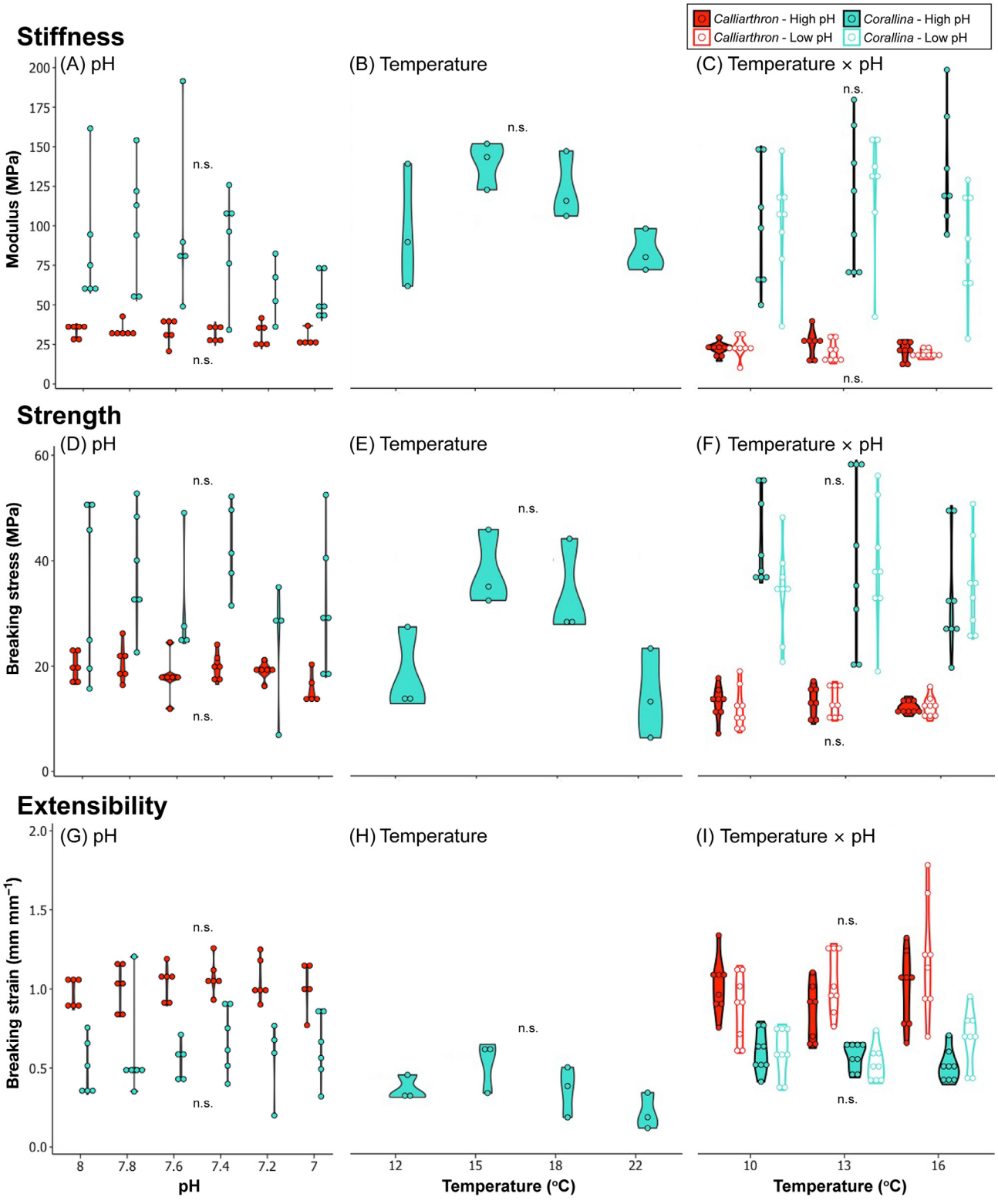


Fig. 5. Biomechanical properties (A–C) stiffness, (D–F) strength, and (G–I) extensibility of genicula in each of the 3 mesocosm experiments: (A,D,G) pH, (B,E,H) temperature, and (C,F,I) temperature × pH. Violin plots show data distribution over raw data (dots); red: *Calliarthron*; blue: *Corallina*. Note that material properties for the temperature experiment were not available for *Calliarthron.* High pH and low pH are distinguished for each species in (C), (F), and (I). No significant differences were detected across any treatments (denoted by n.s.)

Table 3. Linear mixed model estimation for the biomechanical properties (stiffness, strength or extensibility) of *Calliarthron tuberculosum* and *Corallina vancouveriensis* genicula in each of the 3 mesocosm experiments

| Property | Experiment | Genus | Factor | *F* | df | df.res | Pr>(*F*) |
|---|---|---|---|---|---|---|---|
| Stiffness | pH | *Calliarthron* | Intercept | 11952.6949 | 1 | 4.9574 | <0.001 |
| | | | pH | 0.6563 | 5 | 24.2516 | 0.660 |
| | | *Corallina* | Intercept | 3552.8489 | 1 | 4.9599 | <0.001 |
| | | | pH | 1.7376 | 5 | 22.7338 | 0.167 |
| | Temperature | *Corallina* | Temperature | 3.0123 | 3 | 6 | 0.116 |
| | Temperature × pH | *Calliarthron* | Intercept | 4057.1822 | 1 | 6.972 | <0.001 |
| | | | Temperature | 1.2164 | 2 | 34.247 | 0.309 |
| | | | pH | 1.4947 | 1 | 34.254 | 0.230 |
| | | | Temperature × pH | 0.5653 | 2 | 34.247 | 0.573 |
| | | *Corallina* | Intercept | 5275.5661 | 1 | 7 | <0.001 |
| | | | Temperature | 0.5543 | 2 | 35 | 0.579 |
| | | | pH | 1.6149 | 1 | 35 | 0.212 |
| | | | Temperature × pH | 3.0017 | 2 | 35 | 0.063 |
| Strength | pH | *Calliarthron* | Intercept | 11443.044 | 1 | 4.9574 | <0.001 |
| | | | pH | 2.165 | 5 | 24.2516 | 0.091 |
| | | *Corallina* | Intercept | 1755.6161 | 1 | 4.9742 | <0.001 |
| | | | pH | 1.5353 | 5 | 22.6751 | 0.217 |
| | Temperature | *Corallina* | Temperature | 4.7289 | 3 | 6 | 0.051 |
| | Temperature × pH | *Calliarthron* | Intercept | 5481.3610 | 1 | 6.962 | <0.001 |
| | | | Temperature | 0.3154 | 2 | 34.285 | 0.732 |
| | | | pH | 0.2512 | 1 | 34.293 | 0.620 |
| | | | Temperature × pH | 0.1831 | 2 | 34.285 | 0.834 |
| | | *Corallina* | Intercept | 6269.5243 | 1 | 7 | <0.001 |
| | | | Temperature | 0.9777 | 2 | 35 | 0.386 |
| | | | pH | 0.5011 | 1 | 35 | 0.484 |
| | | | Temperature × pH | 1.2893 | 2 | 35 | 0.288 |
| Extensibility | pH | *Calliarthron* | Intercept | 11443.044 | 1 | 4.9574 | <0.001 |
| | | | pH | 2.165 | 5 | 24.2516 | 0.092 |
| | | *Corallina* | Intercept | 1755.6161 | 1 | 4.9742 | <0.001 |
| | | | pH | 1.5353 | 5 | 22.6751 | 0.219 |
| | Temperature | *Corallina* | Temperature | 3.3497 | 3 | 6 | 0.097 |
| | Temperature × pH | *Calliarthron* | Intercept | 3.7684 | 1 | 36.628 | 0.060 |
| | | | Temperature | 3.6922 | 2 | 34.007 | 0.035 |
| | | | pH | 2.2443 | 1 | 34.007 | 0.143 |
| | | | Temperature × pH | 3.2472 | 2 | 34.153 | 0.051 |
| | | *Corallina* | Intercept | 202.8967 | 1 | 7 | <0.001 |
| | | | Temperature | 0.4469 | 2 | 35 | 0.643 |
| | | | pH | 0.6990 | 1 | 35 | 0.409 |
| | | | Temperature × pH | 3.2278 | 2 | 35 | 0.052 |

than *Calliarthron*, both in single-factor pH experiments and in combination with increased temperature. Growth in *Corallina* declined across single-factor pH treatments from pH 7.8 to 7.0, whereas growth in *Calliarthron* held steady and was relatively unchanged from pH 7.8 to 7.0. Past physiological studies of multiple stressors have often demonstrated a synergistic effect of temperature and pH, where high temperature–low pH in combination have a greater negative effect than either factor alone (Martin & Gattuso 2009, Diaz-Pulido et al. 2012, Johnson & Carpenter 2012, Martin et al. 2013). This was not the case in this study — in combination treatments, pH had little to no effect on growth of *Calliarthron* at any temperature, while in *Corallina*, low pH reduced growth uniformly, eliminating the effect of temperature completely.

### 4.2. Calcification

Calcification in *Corallina* was more sensitive to changes in pH than calcification in *Calliarthron*, contrary to our hypothesis. As pH was reduced in the

single-factor pH experiment, $CaCO_3$ content of *Corallina* tissues declined more rapidly than that of *Calliarthron* tissues. Yet both species showed surprising tolerance to reduced pH — even as low as pH 7.0, forming new calcified intergenicula with minimal reductions in total $CaCO_3$ content compared to higher pH conditions. In other systems, corallines are unable to survive pH 7.0 (e.g. Martin et al. 2008), and previous research on corallines has rarely included such low pH levels in their experiments (e.g. Martin & Gattuso 2009, Diaz-Pulido et al. 2012, Johnson & Carpenter 2012, Cornwall et al. 2017). Interestingly, no effect of temperature on the $CaCO_3$ content of new intergenicula was detected in single-factor temperature trials, but increased temperature negatively affected $CaCO_3$ of new tissue in both species in temperature × pH trials, even at high pH. This may have been due to the range of temperature and pH levels used or, more likely, an artefact of the greater sample size of the temperature × pH experiment compared to the single-factor temperature experiment.

The effect of pH and temperature on $CaCO_3$ content of intergenicula that were formed prior to the start of the experiment (old tissue) was not the same as the effect on new intergenicula. Notably, the % $CaCO_3$ of old tissue in *Calliarthron* was not affected by any combination of temperature or pH, despite changes to % $CaCO_3$ in new tissue under the same experimental conditions. The lack of change in the $CaCO_3$ content of old intergenicula in *Calliarthron* suggests that reductions in % $CaCO_3$ of new tissue under reduced pH/increased temperature may be due to impairments in calcification, more so than increased dissolution. This was less true in *Corallina*, where the effects of pH on % $CaCO_3$ were similar in both new and old tissue, suggesting that dissolution may play a more prominent role in affecting $CaCO_3$ content. It seems likely that resistance to dissolution may be a species-specific trait, perhaps linked to thallus thickness or intergeniculum diameter. Future studies should more carefully measure calcification and dissolution rates in these 2 species to see if our generalizations can be verified. In addition, because light plays an important role in coralline calcification rates (Chisholm 2000, McCoy et al. 2016), the incorporation of environmentally relevant light levels into future experiments will be particularly important if we hope to make predictions about calcification in the field.

Significant loss of $CaCO_3$ may increase the susceptibility of coralline algae to grazing and other damage (Johnson & Carpenter 2012, Manning et al. 2019). However, Martone et al. (2021) found that a complete loss of $CaCO_3$ had no effect on grazing rates of the urchin *Strongylocentrotus purpuratus* on *Corallina*, and a decrease of at least 20% $CaCO_3$ was necessary to significantly increase urchin grazing rates on *Calliarthron*. Because overall losses of $CaCO_3$ due to temperature and pH were quite low in this study (~13% in *Corallina*; ~4% in *Calliarthron*), neither species will likely experience increased susceptibility to urchin grazing in the future, although more data are needed to understand the relative importance of calcification in deterring other grazers.

### 4.3. Biomechanical properties

Increased temperature and low pH have been shown to negatively affect the structural integrity of calcified tissues in corallines (Ragazzola et al. 2012, Legrand et al. 2019), but this study is the first to examine the effects of pH and temperature on the biomechanical properties of non-calcified joint tissue (genicula) in articulated coralline algae. In contrast to the effects on calcification and growth, biomechanical properties of genicula were largely unaffected by changes in pH and temperature. In *Corallina*, genicular strength appeared to follow a non-linear trend with temperature alone, with a trend of both high and low temperatures causing slight weakening of genicula, although the effect was not significant. Genicular stiffness also showed a similar (and non-significant) response to temperature. It is possible that a larger sample size and/or more temperature treatments would provide better resolution of this potentially non-linear response.

Although genicular extensibility in *Calliarthron* was largely unaffected by changes in pH and temperature, tissues were most extensible (~35% higher on average) in combined warming and low pH treatments. Both low pH and reduced availability of $Ca^+$ ions can destabilize polysaccharide gels by inhibiting cross-linking (Li et al. 2013), and OA has been shown to negatively affect spore adhesion — a process which relies on hydrogel formation — in *Corallina* (Guenther et al. 2018). Additionally, many gel-forming polysaccharides known to occur in red algae (e.g. agarose) are temperature-sensitive, and only form gels below specific thermal thresholds (Graham et al. 2019). We speculate that degradation of polysaccharide linkages within the genicular cell wall may underlie trends in increased genicular extensibility observed at high temperature–reduced pH conditions.

Both flexibility and strength are vital for survival in wave-swept conditions. In kelps such as *Laminaria*

*digitata*, warming has been shown to decrease both the strength and extensibility of blade tissue, leading to increased breakage and mortality due to water motion (Simonson et al. 2015). In articulated corallines, flexibility allows blades to bend with water motion, which leads to decreased drag and reduced breakage; however, this comes at the cost of additional stress being placed on the bending genicula (Martone & Denny 2008a, Janot & Martone 2018). According to our results, genicular properties are largely maintained in the face of climate stresses, suggesting that the articulated fronds will continue to resist wave impacts along rocky coastlines in the future. Unfortunately, because experimental genicula were a mix of both new (formed during the course of the experiment) and old (formed prior to the experiment) tissue, understanding the long-term effects of OW and OA on genicular properties will require additional research.

### 4.4. Conclusions

Physiological tradeoffs under stress are well documented in coralline algae (Egilsdottir et al. 2013, Martin et al. 2013, McCoy & Ragazzola 2014, Kamenos et al. 2016). Organisms have a limited energetic budget; when exposed to stressful conditions, energy must often be diverted away from processes such as growth and reproduction in order to maintain those which are essential for immediate survival. Here, we observed significant decreases in growth rate in *Corallina* and *Calliarthron* under simulated OW and OA conditions while structural integrity was largely preserved (genicular mechanics) or only moderately affected (percent calcification). While these trends are consistent with a tradeoff between growth and structural integrity, the energetic costs associated with the production and maintenance of genicular and intergenicular material warrant further study.

Responses to OA and OW in corallines are highly variable, even among closely related species (McCoy & Kamenos 2015, Barner et al. 2018). The 2 species used in this study showed species-specific responses with clear differences in their respective tolerances to thermal and acidification stress. Reliable predictors of such interspecific variability are a topic of debate in the literature but may include habitat (Egilsdottir et al. 2013, Kwiatkowski et al. 2016), size (Barner et al. 2018), and skeletal mineralogy (Nash et al. 2013), among other factors. While we demonstrated that both *Calliarthron* and *Corallina* are likely to be resistant to future OW and OA, our results likely cannot be extrapolated to other coralline taxa. Rather, our results underscore the difficulties in predicting climate impacts, our inability to generalize, and the need to clarify underlying mechanisms of OA and thermal tolerance in corallines.

*Data archive*. Data are archived at https://digital.lib.washington.edu/researchworks/handle/1773/49470.

*Acknowledgements*. We thank Kindall Murie for providing the artwork in Fig. 1. This work was supported by National Science Foundation awards to E.C. (EF-1041213, OCE-2050273) and Natural Sciences and Engineering Research Council (NSERC) Discovery grants to P.T.M. (RGPIN-2014-06288, RGPIN-2019-06240).

LITERATURE CITED

Abbott IA, Hollenberg GJ (1976) Marine algae of California. Stanford University Press, Stanford, CA

Barner AK, Chan F, Hettinger A, Hacker SD, Marshall K, Menge BA (2018) Generality in multispecies responses to ocean acidification revealed through multiple hypothesis testing. Glob Change Biol 24:4464–4477

Bates D, Maechler M, Bolker B, Walker S (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67: 1–48

Bindoff NL, Cheung WWL, Kairo JG, Arístegui J, and others (2019) Changing ocean, marine ecosystems, and dependent communities. In: Pörtner HO, Roberts DC, Masson-Delmotte V, Zhai P and others (eds) IPCC Special Report on the Ocean and Cryosphere in a Changing Climate

Büdenbender J, Riebesell U, Form A (2011) Calcification of the Arctic coralline red algae *Lithothamnion glaciale* in response to elevated $CO_2$. Mar Ecol Prog Ser 441:79–87

Chisholm JRM (2000) Calcification by crustose coralline algae on the northern Great Barrier Reef, Australia. Limnol Oceanogr 45:1476–1484

Cornwall CE, Comeau S, McCulloch MT (2017) Coralline algae elevate pH at the site of calcification under ocean acidification. Glob Change Biol 23:4245–4256

Comeau S, Cornwall CE, Pupier CA, DeCarlo TM, Alessi C, Trehern R, McCulloch MT (2019) Flow-driven microscale pH variability affects the physiology of corals and coralline algae under ocean acidification. Sci Rep 9: 12829

Cornwall CE, Diaz-Pulido G, Comeau S (2019) Impacts of ocean warming on coralline algal calcification: meta-analysis, knowledge gaps, and key recommendations for future research. Front Mar Sci 6:186

Denny MW, King FA (2016) The extraordinary joint material of an articulated coralline alga. I. Mechanical characterization of a key adaptation. J Exp Biol 219:1833–1842

Diaz-Pulido G, Anthony KRN, Kline DI, Dove S, Hoegh-Guldberg O (2012) Interactions between ocean acidification and warming on the mortality and dissolution of coralline algae. J Phycol 48:32–39

Dickson AG (1990) Standard potential of the reaction AgCl (s) + 1/2 $H_2$(g) = Ag(s) + HCl(aq), and the standard acidity constant of the ion $HSO_4$- in synthetic seawater from

273.15 to 318.15 K. J Chem Thermodyn 22:113–127
Dickson AG, Sabine CL, Christian JR (eds) (2007) Guide to best practices for ocean $CO_2$ measurements. PISCES Special Publication 3, IPCC Report No. 8. North Pacific Marine Science Organization, Sidney
Egilsdottir H, Noisette F, Noël LMLJ, Olafsson J, Martin S (2013) Effects of $pCO_2$ on physiology and skeletal mineralogy in a tidal pool coralline alga *Corallina elongata.* Mar Biol 160:2103–2112
Fox J, Weisberg S (2019) An R companion to applied regression, 3rd edn. Sage Publications, Thousand Oaks, CA
Gabrielson PW, Miller KA, Martone PT (2011) Morphometric and molecular analyses confirm two distinct species of *Calliarthron* (Corallinales, Rhodophyta), a genus endemic to the northeast Pacific. Phycologia 50:298–316
Gabrielson P, Lindstrom S, O'Kelly C (2012) Keys to the seaweeds and seagrasses of southeast Alaska, British Columbia, Washington, and Oregon. Phycological Contribution No. 8. Printorium Bookworks, Victoria
Gao S, Sun Q, Tao Y, Wang X and others (2016) A decline in macro-algae species resulting in the overwhelming prevalence of *Corallina* species is caused by low-pH seawater induced by short-term acid rain. J Exp Mar Biol Ecol 475:144–153
Graba-Landry A, Hoey AS, Matley JK, Sheppard-Brennand H, Poore AGB, Byrne M, Dworjanyn SA (2018) Ocean warming has greater and more consistent negative effects than ocean acidification on the growth and health of subtropical macroalgae. Mar Ecol Prog Ser 595:55–69
Graham S, Marina PF, Blencowe A (2019) Thermoresponsive polysaccharides and their thermoreversible physical hydrogel networks. Carbohydr Polym 207:143–159
Guenther R (2016) The effect of temperature and pH on the growth and biomechanics of coralline algae. PhD thesis, University of British Columbia, Vancouver
Guenther RJ, Martone PT (2014) Physiological performance of intertidal coralline algae during a simulated tidal cycle. J Phycol 50:310–321
Guenther R, Miklasz K, Carrington E, Martone PT (2018) Macroalgal spore dysfunction: ocean acidification delays and weakens adhesion. J Phycol 54:153–158
Gunderson AR, Armstrong EJ, Stillman JH (2016) Multiple stressors in a changing world: the need for an improved perspective on physiological responses to the dynamic marine environment. Ann Rev Mar Sci 8:357–378
Hofmann LC, Bischof K (2014) Ocean acidification effects on calcifying macroalgae. Aquat Biol 22:261–279
Hofmann LC, Schoenrock K, de Beer D (2018) Arctic coralline algae elevate surface pH and carbonate in the dark. Front Plant Sci 9:1416
Janot K, Martone PT (2016) Convergence of joint mechanics in independently evolving, articulated coralline algae. J Exp Biol 219:383–391
Janot KG, Martone PT (2018) Bending strategies of convergently evolved, articulated coralline algae. J Phycol 54: 305–316
Johnson MD, Carpenter RC (2012) Ocean acidification and warming decrease calcification in the crustose coralline alga *Hydrolithon onkodes* and increase susceptibility to grazing. J Exp Mar Biol Ecol 434-435:94–101
Kamenos NA, Perna G, Gambi MC, Micheli F, Kroeker KJ (2016) Coralline algae in a naturally acidified ecosystem persist by maintaining control of skeletal mineralogy and size. Proc R Soc B 283:20161159
Kassambara A (2020) rstatix: pipe-friendly framework for basic statistical tests. R package version 0.5.0. https://CRAN.R-project.org/package=rstatix
Kim JH, Min J, Kang EJ, Kim KY (2018) Elevated temperature and changed carbonate chemistry: effects on calcification, photosynthesis, and growth of *Corallina officinalis* (Corallinales, Rhodophyta). Phycologia 57:280–286
Koch M, Bowes G, Ross C, Zhang XH (2013) Climate change and ocean acidification effects on seagrasses and marine macroalgae. Glob Change Biol 19:103–132
Konar B, Foster MS (1992) Distribution and recruitment of subtidal geniculate coralline algae. J Phycol 28:273–280
Kroeker KJ, Kordas RL, Harley CDG (2017) Embracing interactions in ocean acidification research: confronting multiple stressor scenarios and context dependence. Biol Lett 13:20160802
Kwiatkowski L, Gaylord B, Hill T, Hosfelt J and others (2016) Nighttime dissolution in a temperate coastal ocean ecosystem increases under acidification. Sci Rep 6: 22984
Lee K, Kim TW, Byrne RH, Millero FJ, Feely RA, Liu YM (2010) The universal ratio of boron to chlorinity for the North Pacific and North Atlantic oceans. Geochim Cosmochim Acta 74:1801–1811
Legrand E, Riera P, Lutier M, Coudret J, Grall J, Martin S (2019) Grazers increase the sensitivity of coralline algae to ocean acidification and warming. J Sea Res 148-149: 1–7
Lenth R, Buerkner P, Herve M, Jung M and others (2020) emmeans: estimated marginal means, aka least-squares means. R package version 1.4.7. www.CRAN.R-project.org/package=emmeans
Li X, Leck C, Sun L, Hede T, Tu Y, Ågren H (2013) Cross-linked polysaccharide assemblies in marine gels: an atomistic simulation. J Phys Chem Lett 16:2637–2642
Manning JC, Carpenter RC, Miranda EA (2019) Ocean acidification reduces net calcification and wound healing in the tropical crustose coralline alga, *Porolithon onkodes* (Corallinales, Rhodophyta). J Exp Mar Biol Ecol 520: 151225
Martin S, Gattuso JP (2009) Response of Mediterranean coralline algae to ocean acidification and elevated temperature. Glob Change Biol 15:2089–2100
Martin S, Rodolfo-Metalpa R, Ransome E, Rowley S, Buia MC, Gattuso JP, Hall-Spencer J (2008) Effects of naturally acidified seawater on seagrass calcareous epibionts. Biol Lett 4:689–692
Martin S, Cohu S, Vignot C, Zimmerman G, Gattuso JP (2013) One-year experiment on the physiological response of the Mediterranean crustose coralline alga, *Lithophyllum cabiochae*, to elevated $pCO_2$ and temperature. Ecol Evol 3:676–693
Martone PT (2006) Size, strength and allometry of joints in the articulated coralline *Calliarthron.* J Exp Biol 209: 1678–1689
Martone PT (2010) Quantifying growth and calcium carbonate deposition of *Calliarthron cheilosporioides* (Corallinales, Rhodophyta) in the field using a persistent vital stain. J Phycol 46:13–17
Martone PT, Denny MW (2008a) To bend a coralline: effect of joint morphology on flexibility and stress amplification in an articulated calcified seaweed. J Exp Biol 211: 3421–3432
Martone PT, Denny MW (2008b) To break a coralline: mechanical constraints on the size and survival of a

wave-swept seaweed. J Exp Biol 211:3433–3441
Martone PT, Alyono M, Stites S (2010) Bleaching of an intertidal coralline alga: untangling the effects of light, temperature, and desiccation. Mar Ecol Prog Ser 416:57–67
Martone PT, Janot K, Fujita M, Wasteneys G, Ruel K, Joseleau JP, Estevez JM (2019) Cellulose-rich secondary walls in wave-swept red macroalgae fortify flexible tissues. Planta 250:1867–1879
Martone PT, Schipper S, Froese T, Bretner J, DeMong A, Eastham T (2021) Calcification does not necessarily protect articulated coralline algae from urchin grazing. J Exp Mar Biol Ecol 537:151513
McCoy SJ, Kamenos NA (2015) Coralline algae (Rhodophyta) in a changing world: integrating ecological, physiological, and geochemical responses to global change. J Phycol 51:6–24
McCoy SJ, Kamenos NA (2018) Coralline algal skeletal mineralogy affects grazer impacts. Glob Chang Biol 24:775–4783
McCoy SJ, Ragazzola F (2014) Skeletal trade-offs in coralline algae in response to ocean acidification. Nat Clim Chang 4:719–723
McCoy SJ, Pfister CA, Olack G, Colman AS (2016) Diurnal and tidal patterns of carbon uptake and calcification in geniculate intertidal coralline algae. PSZNI:Mar Ecol 37:553–564
Mehrbach C, Culberson CH, Hawley JE, Pytkowicz RM (1973) Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol Oceanogr 18:897–907
Meyer J, Riebesell U (2015) Reviews and syntheses: responses of coccolithophores to ocean acidification: a meta-analysis. Biogeosciences 12:1671–1682
Muñoz PT, Sáez CA, Martínez-Callejas MB, Flores-Molina MR and others (2018) Short-term interactive effects of increased temperatures and acidification on the calcifying macroalgae *Lithothamnion crispatum* and *Sonderophycus capensis.* Aquat Bot 148:46–52
Nash MC, Opdyke BN, Troitzsch U, Russell BD and others (2013) Dolomite-rich coralline algae in reefs resist dissolution in acidified conditions. Nat Clim Chang 3:268–272
Nash MC, Martin S, Gattuso JP (2016) Mineralogical response of the Mediterranean crustose coralline alga *Lithophyllum cabiochae* to near-future ocean acidification and warming. Biogeosciences 13:5937–5945
Nash MC, Diaz-Pulido G, Harvey AS, Adey W (2019) Coralline algal calcification: a morphological and process-based understanding. PLOS ONE 14:e0221396
O'Donnell M, George M, Carrington E (2013) Mussel byssus attachment weakened by ocean acidification. Nat Clim Chang 3:587–590
Padilla D (1984) The importance of form: differences in competitive ability, resistance to consumers and environmental stress in an assemblage of coralline algae. J Exp Mar Biol Ecol 79:105–127
Padilla D (1993) Rip stop in marine algae: minimizing the consequences of herbivore damage. Evol Ecol 7:634–644
Pearse VB (1972) Radioisotopic study of calcification in the articulated coralline alga *Bossiella orbigniana*. J Phycol 8:88–97
Piñeiro-Corbeira C, Barreiro R, Cremades J, Arenas F (2018) Seaweed assemblages under a climate change scenario: functional responses to temperature of eight intertidal seaweeds match recent abundance shifts. Sci Rep 8: 12978
Porzio L, Buia MC, Ferretti V, Lorenti M and others (2018) Photosynthesis and mineralogy of *Jania rubens* at low pH/high $pCO_2$: a future perspective. Sci Total Environ 628-629:375–383
R Core Team (2020) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org/
Ragazzola F, Foster LC, Form A, Anderson PSL, Hansteen TH, Fietzke J (2012) Ocean acidification weakens the structural integrity of coralline algae. Glob Change Biol 18:2804–2812
Robbins LL, Hansen ME, Kleypas JA, Meylan SC (2010) CO2calc—A user-friendly seawater carbon calculator for Windows, Max OS X, and iOS (iPhone): U.S. Geological Survey Open-File Report 2010–1280
Sabine CL, Feely RA, Gruber N, Key RM and others (2004) The oceanic sink for anthropogenic $CO_2$. Science 305: 367–371
Schulte PM, Healy TM, Fangue NA (2011) Thermal performance curves, phenotypic plasticity, and the time scales of temperature exposure. Integr Comp Biol 51: 691–702
Simonson EJ, Scheibling RE, Metaxas A (2015) Kelp in hot water. I. Warming seawater temperature induces weakening and loss of kelp tissue. Mar Ecol Prog Ser 537: 89–104
Smale DA, Wernberg T, Oliver ECJ, Thomsen M, and others (2019) Marine heatwaves threaten global biodiversity and the provision of ecosystem services. Nat Clim Chang 9:306–312
Vasseur DA, DeLong JP, Gilbert B, Greig HS and others (2014) Increased temperature variation poses a greater risk to species than climate warming. Proc R Soc B 281: 20132612
Wanninkhof R (1992) Relationship between wind speed and gas exchange over the ocean. J Geophys Res: Oceans 97:7373–7382
Wernberg T, Bettignies T, Joy BA, Finnegan PM (2016) Physiological responses of habitat-forming seaweeds to increasing temperatures. Limnol Oceanogr 61: 2180–2190

*Editorial responsibility: Lisandro Benedetti-Cecchi, Pisa, Italy*
*Reviewed by: 3 anonymous referees*

*Submitted: December 17, 2021*
*Accepted: August 26, 2022*
*Proofs received from author(s): October 15, 2022*