



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

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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 CO₂ emissions is one of the major threats currently facing marine ecosystems (Bindoff et al. 2019). Elevated atmospheric CO₂ 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 CO₂ (pCO₂) 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).

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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 $p\text{CO}_2$ 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 $\times$ temperature, in August 2014). For each experiment, specimens of *Corallina vancouveriensis* and *Calliarthron tuberosum* 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 $\sim 10^{\circ}\text{C}$ in winter, ranging from ~ 4 to 14°C , and average $\sim 12^{\circ}\text{C}$ 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 ($12\text{--}14^{\circ}\text{C}$) 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: $0.2\text{ }\mu\text{m}$), 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 $\text{CO}_2\text{:O}_2$ 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 $\text{CO}_2\text{:O}_2$ gas mixture pumped into the mesocosm. Temperature was maintained at $12\text{--}13^{\circ}\text{C}$ 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} , $52.8 \pm 0.8\text{ }\mu\text{mol photons m}^{-2}\text{ s}^{-1}$).

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

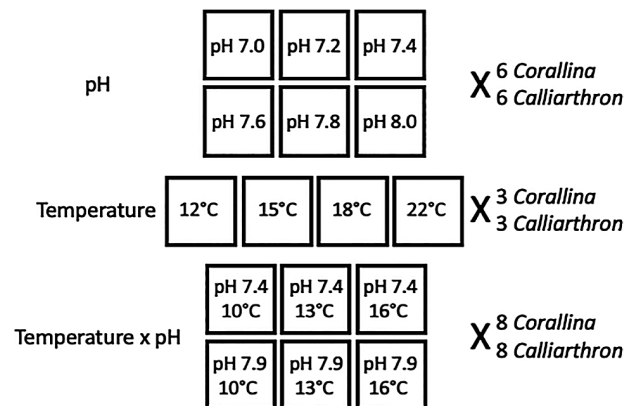


Fig. 2. Experimental design for each of the 3 mesocosm experiments (pH, temperature, and temperature $\times$ pH). Each square represents a 4 l specimen chamber which held 3 replicate fronds from an individual thallus of *Calliarthron tuberosum* and *Corallina vancouveriensis*. Replication for each experiment 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), $p\text{CO}_2$ and carbonate saturation states were calculated from measured temperature, salinity, pH, and total alkalinity values. The following CO2calc constants were used: CO_2 constants (K_1 , K_2 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 $\times$ 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 $\times$ 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:

$$\% \text{CaCO}_3 = (\text{oven dry weight} - \text{decalcified oven dry weight}) / (\text{oven dry weight}) \times 100$$

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 ($40\times$). 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 $\times$ 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. % $\text{CaCO}_3 < 70\%$), 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 $\times$ 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

$0.07 \pm 0.01 \text{ mm d}^{-1}$ in pH 8.0–7.4 but grew less when $\text{pH} \leq 7.2$. For example, the growth rate of *Corallina* in the lowest pH treatment (pH 7.0) was only $0.03 \pm 0.01 \text{ mm d}^{-1}$, 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 ($0.10 \pm 0.01 \text{ mm d}^{-1}$) 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 $0.09 \pm 0.01 \text{ mm d}^{-1}$ in the 12°C treatment but slowed to $0.01 \pm 0.01 \text{ mm d}^{-1}$ 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 ($0.11 \pm 0.01 \text{ mm d}^{-1}$, 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 $\times$ 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%

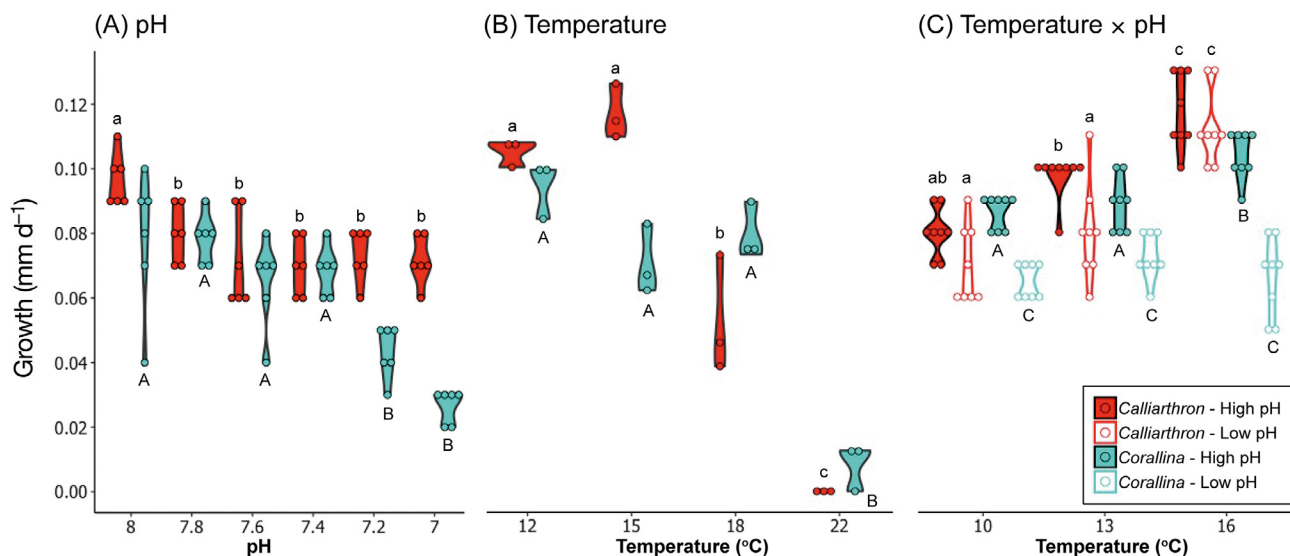


Fig. 3. Tissue growth rate in each of the 3 mesocosm experiments: (A) pH, (B) temperature, and (C) temperature $\times$ pH. Violin plots show data distribution over raw data (dots); red: *Calliarthron*; blue: *Corallina*. High pH and low pH are distinguished for each species in (C). Letters indicate 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	<i>Calliarthron</i>	Intercept	326.2941	1	28.408	<0.001
		pH	7.3158	5	25.000	<0.001
	<i>Corallina</i>	Intercept	911.250	1	5	<0.001
		pH	18.188	5	25	<0.001
Temperature	<i>Calliarthron</i>	Intercept	252.47	1	2	0.004
		Temperature	133.77	3	6	<0.001
	<i>Corallina</i>	Intercept	603.110	1	2	0.002
		Temperature	54.249	3	6	<0.001
Temperature × pH	<i>Calliarthron</i>	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
	<i>Corallina</i>	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).

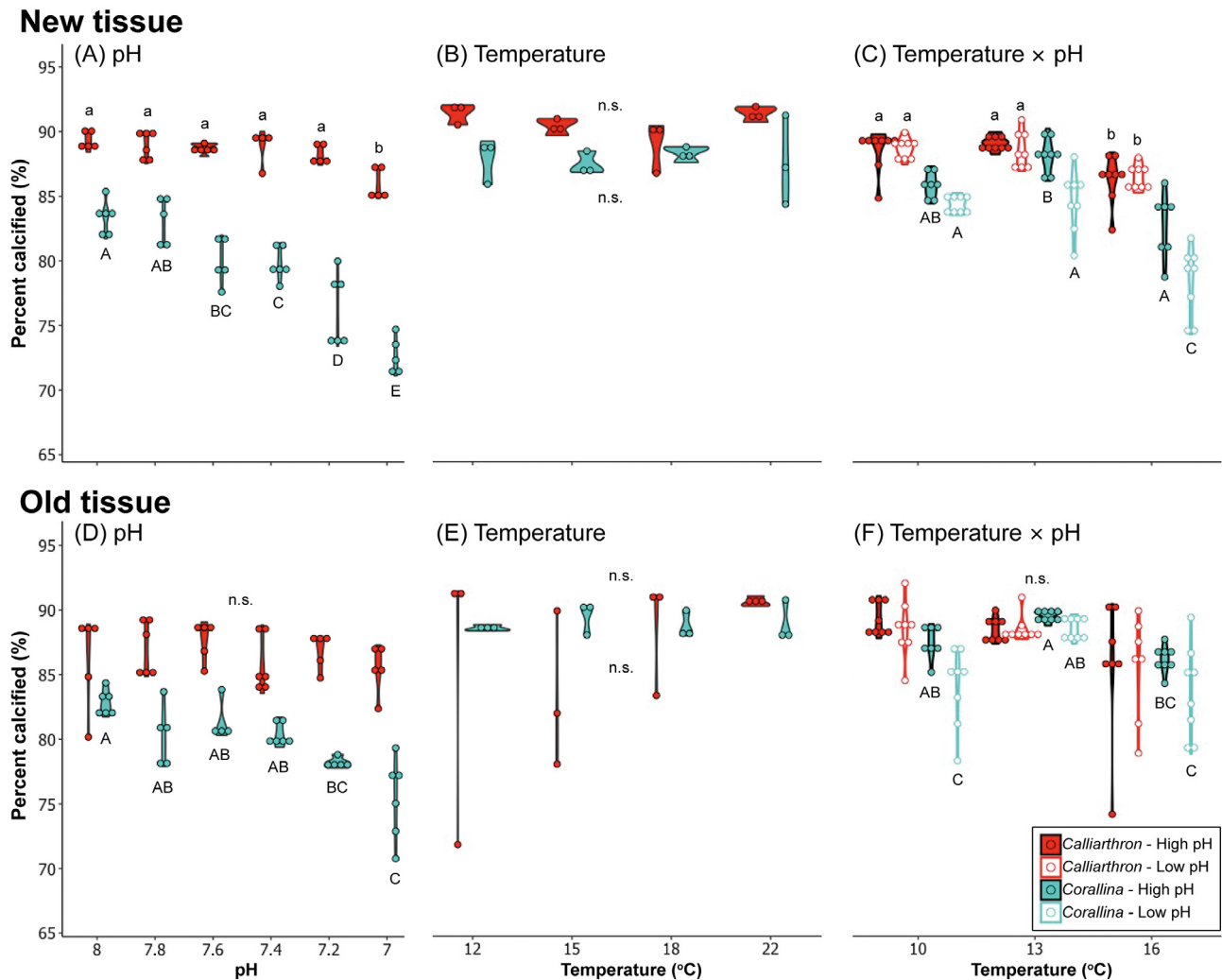


Fig. 4. Calcification (%) of (A–C) new tissue and (D–F) old tissue in each of the 3 mesocosm experiments: (A,D) pH, (B,E) temperature, and (C,F) temperature × pH. Violin plots show data distribution over raw data (dots); red: *Calliarthron*; blue: *Corallina*. High pH and low pH are distinguished for 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	<i>F</i>	df	df.res	Pr>(F)
New	pH	<i>Calliarthron</i>	Intercept	88988.3205	1	4.8262	<0.001
			pH	9.5429	5	19.6380	<0.001
		<i>Corallina</i>	Intercept	79604.153	1	4.8513	<0.001
			pH	26.664	5	22.7562	<0.001
	Temperature	<i>Calliarthron</i>	Intercept	74216.1393	1	2	<0.001
			Temperature	2.8914	3	6	0.124
		<i>Corallina</i>	Intercept	23107.7357	1	2	<0.001
			Temperature	0.1043	3	6	0.955
	Temperature × pH	<i>Calliarthron</i>	Intercept	1.8006×10^5	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
		<i>Corallina</i>	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	<i>Calliarthron</i>	Intercept	16300.6087	1	4.9423	<0.001
			pH	1.2312	5	21.2951	0.329
		<i>Corallina</i>	Intercept	73548.359	1	4.8987	<0.001
			pH	11.641	5	21.9853	<0.001
	Temperature	<i>Calliarthron</i>	Intercept	1123.0573	1	2	<0.001
			Temperature	1.0497	3	6	0.437
		<i>Corallina</i>	Intercept	26155.8113	1	2	<0.001
			Temperature	0.5707	3	6	0.655
	Temperature × pH	<i>Calliarthron</i>	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
		<i>Corallina</i>	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 environ-

mental 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

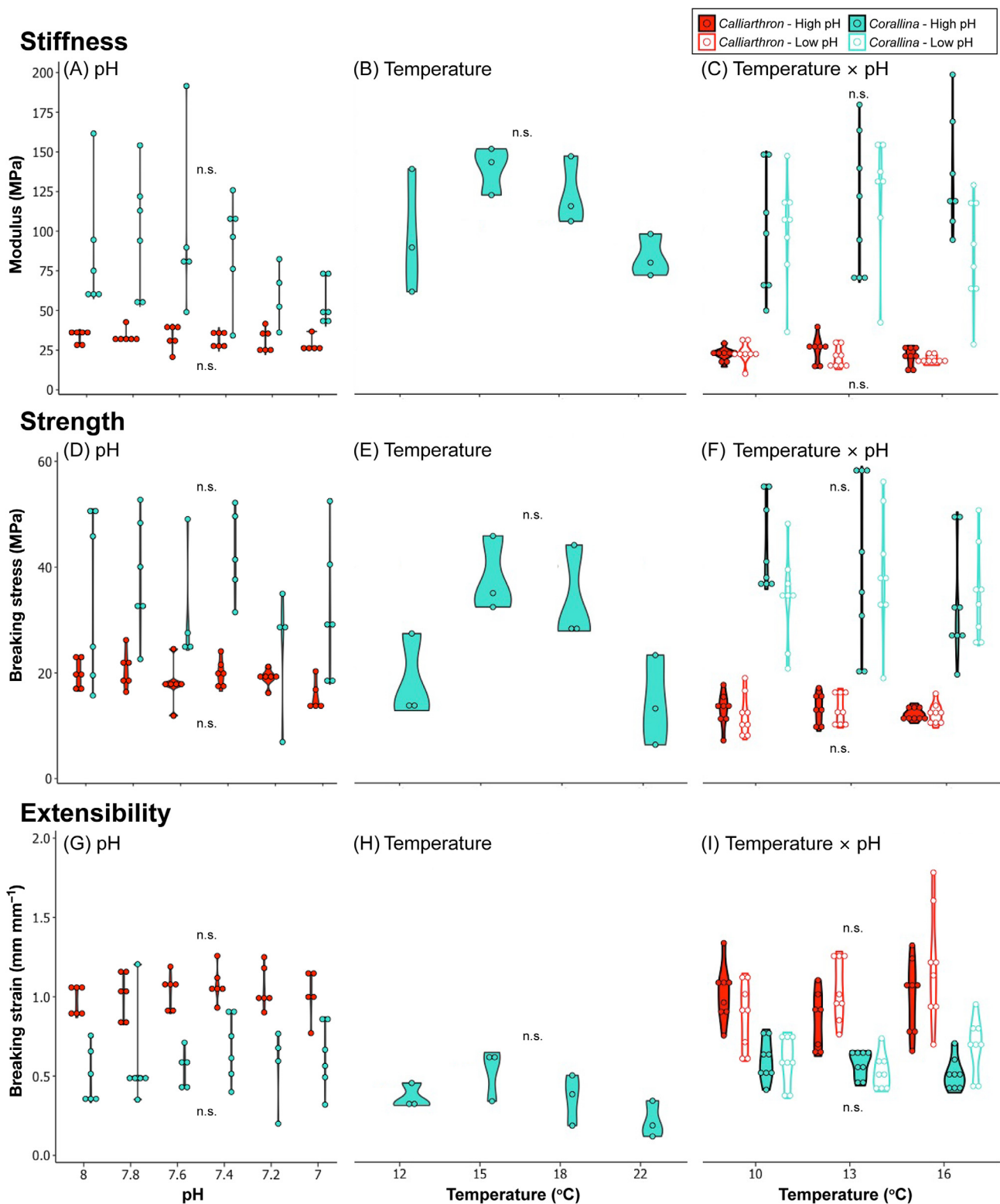


Fig. 5. Biomechanical properties (A–C) stiffness, (D–F) strength, and (G–I) extensibility of genicula in each of the 3 mesocosm experiments: (A,D,G) pH, (B,E,H) temperature, and (C,F,I) temperature × pH. Violin plots show data distribution over raw data (dots); red: *Calliarthron*; blue: *Corallina*. 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 tuberculatum* and *Corallina vancouveriensis* genicula in each of the 3 mesocosm experiments

Property	Experiment	Genus	Factor	<i>F</i>	df	df.res	Pr>(F)
Stiffness	pH	<i>Calliarthron</i>	Intercept	11952.6949	1	4.9574	<0.001
			pH	0.6563	5	24.2516	0.660
		<i>Corallina</i>	Intercept	3552.8489	1	4.9599	<0.001
			pH	1.7376	5	22.7338	0.167
	Temperature	<i>Corallina</i>	Temperature	3.0123	3	6	0.116
	Temperature × pH	<i>Calliarthron</i>	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
		<i>Corallina</i>	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	<i>Calliarthron</i>	Intercept	11443.044	1	4.9574	<0.001
			pH	2.165	5	24.2516	0.091
		<i>Corallina</i>	Intercept	1755.6161	1	4.9742	<0.001
			pH	1.5353	5	22.6751	0.217
	Temperature	<i>Corallina</i>	Temperature	4.7289	3	6	0.051
	Temperature × pH	<i>Calliarthron</i>	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
		<i>Corallina</i>	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	<i>Calliarthron</i>	Intercept	11443.044	1	4.9574	<0.001
			pH	2.165	5	24.2516	0.092
		<i>Corallina</i>	Intercept	1755.6161	1	4.9742	<0.001
			pH	1.5353	5	22.6751	0.219
	Temperature	<i>Corallina</i>	Temperature	3.3497	3	6	0.097
	Temperature × pH	<i>Calliarthron</i>	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
		<i>Corallina</i>	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 $\times$ 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 $\times$ 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^{2+} 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>.

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