

PHYSIOLOGICAL PERFORMANCE OF INTERTIDAL CORALLINE ALGAE DURING A SIMULATED TIDAL CYCLE¹

Rebecca J. Guenther² and Patrick T. Martone

Department of Botany and Biodiversity Research Centre, University of British Columbia, 6270 University Blvd., Vancouver, British Columbia V6T 1Z4, Canada

Intertidal macroalgae endure light, desiccation, and temperature variation associated with submerged and emerged conditions on a daily basis. Physiological stresses exist over the course of the entire tidal cycle, and physiological differences in response to these stresses likely contribute to spatial separation of species along the shore. For example, marine species that have a high stress tolerance can live higher on the shore and are able to recover when the tide returns, whereas species with a lower stress tolerance may be relegated to living lower on the shore or in tidepools, where low tide stresses are buffered. In this study, we monitored the physiological responses of the tidepool coralline *Calliarthron tuberosum* (Postels and Ruprecht) E.Y. Dawson and the nontidepool coralline *Corallina vancouveriensis* Yendo during simulated tidal conditions to identify differences in physiology that might underlie differences in habitat. During high tide, *Corallina* was more photosynthetically active than *Calliarthron* as light levels increased. During low tide, *Corallina* continued to out-perform *Calliarthron* when submerged in warming tidepools, but photosynthesis abruptly halted for both species when emerged in air. Surprisingly, pigment composition did not differ, suggesting that light harvesting does not account for this difference. Additionally, *Corallina* was more effective at resisting desiccation by retaining water in its branches. When the tide returned, only *Corallina* recovered from combined temperature and desiccation stresses associated with emergence. This study broadens our understanding of intertidal algal physiology and provides a new perspective on the physiological and morphological underpinnings of habitat partitioning.

Key index words: *Calliarthron*; *Corallina*; desiccation; intertidal; light; photosynthesis; physiology; recovery; seaweed; stress; temperature; tidepool

As the tide rises and falls, intertidal macroalgae must contend with both marine and terrestrial conditions on a daily basis. Such alternations of submergence and emergence bring extreme swings in physiological challenges (Davison and Pearson 1996, Lobban and Harrison 1997, Helmuth and Hofmann 2001). During high tide, intertidal algae are underwater and generally experience reduced light levels and cool water temperatures, while during low tides, intertidal algae may be emerged and exposed to increased light stress, elevated air temperatures, and increased desiccation stress. When the tide returns, intertidal organisms are rapidly submerged, rehydrated and exposed to cool water temperatures and reduced light conditions once more. All phases of the tidal cycle impact the physiology and survival of marine algae. Yet, few studies have tracked changes in algal performance throughout a tidal cycle.

The vertical distributions of marine macroalgae are correlated with physiological limits to abiotic stressors that occur during low tide (Madsen and Maberly 1990, Bell 1993, Harley and Paine 2009, Lamote et al. 2012). For example, abiotic stressors may limit the upshore growth of intertidal algae (Schonbeck and Norton 1979, 1980, Madsen and Maberly 1990): macroalgal species that live higher in the intertidal zone are generally more tolerant of light, temperature and desiccation stresses during low tide (Quadir et al. 1979, Oates and Murray 1983, Davison 1991, Bell 1993, Scrosati and DeWreede 1998, Häder et al. 2003, Sampath-Wiley et al. 2008). When the tide returns, intertidal algae recover from stresses incurred during the low tide and the rate and extent of photosynthetic recovery after re-immersion is one way to quantify stress tolerance (Smith and Berry 1986, Lüning 1990, Davison and Pearson 1996). High intertidal species are more likely to recover rapidly upon re-immersion (Smith and Berry 1986, Dring 1987, Lipkin et al. 1993). Macroalgae that are unable to recover from acute stresses of excess light, temperature, and desiccation at low tide may be physiologically compromised when the tide returns, potentially impacting growth and reproduction (Sudatti et al. 2011). Thus, the immediate response to and the recovery from low tide stresses may affect zonation patterns and spatial segregation of species along the shore

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²Author for correspondence: e-mail rebecca.guenther@botany.ubc.ca.

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(Lubchenco and Menge 1978, Abe et al. 2005, Schagerl and Möstl 2011, Lamote et al. 2012).

Understanding the impact of environmental stresses on intertidal macroalgae throughout all phases of the tidal cycle may help resolve patterns of habitat selection. For example, intertidal macroalgae living in tidepools may be able to circumvent stresses experienced when the tide recedes. In tidepools, algae avoid desiccation (Dethier 1980) and are exposed to less variable temperature and light regimes (Metaxas and Scheibling 1993). Thus, macroalgae living in tidepools may be found higher on the shore than would be predicted by tide height alone (Underwood and Jernakoff 1984). What physiological stresses constrain tidepool and nontidepool macroalgae living at approximately the same tidal elevation?

In this study, we quantify the physiological performance of two species of intertidal algae over the course of a simulated tidal cycle to explore the range of responses to environmental stress and to determine which abiotic factors are most important in habitat partitioning. Two species of coralline algae were examined: *Calliarthron tuberculosum* and *Corallina vancouveriensis*. Both species are commonly found in the intertidal zone throughout the Northeast Pacific (Foster 1975, Padilla 1984), but their position on the shore differs. *Corallina* is strictly found in the intertidal zone growing emergently or at the rims of tidepools (Padilla 1984, Van Tamelen 1996), where it experiences high light and temperature stresses. Furthermore, it is the only articulated coralline species that can survive desiccation stresses associated with emergence (Abbott and Hollenberg 1976), a likely consequence of its delicate branches and thick, bush-like form, which allow it to hold water like a paintbrush (Padilla 1984). *Calliarthron*, on the other hand, is abundant subtidally (Konar and Foster 1992) suggesting that it is well-adapted to low light, cooler water temperatures, and is highly susceptible to desiccation (Padilla 1984, Martone et al. 2010a), although it can be found in some intertidal tidepools.

To explore differences in algal physiology, we first assessed baseline physiological performance under a simulated high tide. Next, we explored how these intertidal algae respond to light, temperature, and desiccation stresses at low tide. Lastly, we quantified the ability of these species to recover photosynthesis when the tide returns. Results from these experiments lend insight into the physiological shifts that occur during different phases of the tide and help to explain the physiological patterns that underlie habitat differences between these two species.

METHODS

Specimen collection and laboratory conditions. Specimens were collected from a variety of locations on Vancouver Island, British Columbia in the summer and fall of 2010. Specimens

were collected in July 2010 (Botanical Beach: 48°31' 03.18" N, 124°26' 03.19" W) for light response curves and the submerged photosynthesis experiment, and in August 2010 (Sombrio Beach: 48°30' 04.19" N, 124°17' 53.67" W) for pigment analyses and the emerged photosynthesis experiment, and in September 2010 (Prasiola Point: 48°49' 02" N, 125°10' 06" W) for the desiccation tolerance experiment and the recovery experiment. All collection sites are located on the west coast of Vancouver Island, with Prasiola Point ~60 km north of Sombrio Beach and Botanical Beach; all sites likely experience similar wave exposures, temperature, and light regimes.

Individual fronds were collected haphazardly from the lower middle intertidal zone. Fronds of *Calliarthron* were collected from within shallow tidepools (<0.5 m depth), and fronds of *Corallina* were collected from outside the same tidepools. Specimens for all experiments except the recovery experiments and desiccation tolerance experiment were transported to the lab at the University of British Columbia within 12 h of collection and were maintained in the dark, submerged in cooled seawater during transport. Specimens for the recovery experiments and the desiccation tolerance experiment were transported to Bamfield Marine Sciences Centre (Bamfield, BC, Canada) within 2 h of collection and were maintained in the dark, submerged in cooled seawater during transport.

Once in the lab, specimens were maintained at a 12:12 light:dark photoperiod in a recirculating seawater table at 12°C, and dim light (5–10 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). Specimens were acclimated to lab conditions for ~2 d before beginning experiments (Hanelt et al. 1993, Johansson and Snoeijs 2002). All experiments were completed within 1 week of collection.

Simulating tidal conditions. Experimental parameters were selected according to environmental conditions along the Pacific Northwest Coast. At a nearby site in Washington, intertidal organisms experience low water temperatures (11°C–15°C), and low light levels (200–500 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) during high tide, and higher air temperatures (12°C–25°C), and higher light levels (up to 2000 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) during low tide (FHL Weather station, <http://depts.washington.edu/fhl/wx.html>). Low tides may last as long as 6 h, depending on shore height. Water temperatures in tidepools are typically 10°C–15°C, but can reach 30°C as they heat up during summer daytime low tides (B. Helmuth, personal communication). Light levels at the bottom of tidepools are also reduced, and are typically in the range of 200–400 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (Van Tamelen 1996). To test the performance of *Calliarthron* and *Corallina* during a tidal cycle, experimental parameters were selected to simulate the observed range of environmental variation in the field (Table 1).

Physiological differences at high tide. Submerged photosynthetic rates were determined by oxygen concentration changes over a period of 5 min in filtered natural seawater (12°C). Oxygen and temperature probes (Neofox, Ocean Optics, Dunedin, FL, USA) and a stirbar were placed in a 25 mL glass sample vial with a coralline sample, and filled with seawater. The oxygen and temperature probes were secured to the sample vial with a rubber stopper. Putty was used to seal the sample vial and ensure the system was air tight. Light was provided by a full spectrum slide projector and was manipulated with a series of wire screens. A Li-Cor 250A light meter with a Li-Cor 190 quantum sensor (LI-COR Biosciences, Lincoln, NE, USA) was used to take an average of five light measurements for each irradiance level. Water temperature was controlled with a recirculating water chiller. A stir plate was placed beneath the water bath to

TABLE 1. Environmental parameters used to simulate tidal conditions in experiments.

Treatment	Temperature	Light	Desiccation
High tide	Water: 12°C	300 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$	None
Low tide: submerged in tidepools	Water: 12°C, 16°C, and 20°C	0, 50, & 300 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$	None
Low tide: emerged in air	Air: 18°C	300 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$	Wet (>100% RWC), Blotted (~100% RWC), or Desiccated (~50% RWC)
Return of the tide: submerged recovery	Water: 12°C and 20°C	300 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$	None
Return of the tide: emerged recovery	Air: 16°C and 25°C	300 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$	High (50% RWC), Low (75% RWC)

maintain water motion in the sample vials and to ensure an even distribution of dissolved oxygen concentration via the stir bar.

Apical tissue of coralline fronds were tested in all experiments (~2 cm long, 100–300 mg). Photosynthetic measurements of whole thalli may have yielded different results (Binzer and Middelboe 2005), but given the large discrepancy in thallus size, measuring whole thalli may have misrepresented the meristematic activity in each species and was therefore avoided. Specimens were cleaned with a soft brush to remove epiphytes and invertebrates before physiological measurements were taken and were then secured to the temperature/oxygen probe in the sample vial. Two samples were run simultaneously in separate vials with separate oxygen probes (one *Corallina* and one *Calliarthron*). Species were alternated between the vials to ensure that there was no effect of the individual oxygen probes. The above methods were used for all measurements of submerged net photosynthetic rates.

To identify saturating and subsaturating irradiances of both species, light response curves were constructed by measuring oxygen evolution or consumption at increasing irradiances (0, 12, 20, 31, 52, 89, 144, 245, 415, and 716 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) at 12°C in submerged conditions. Several low level irradiances were chosen to accurately resolve the photosynthetic efficiency (α). Since both species reached photo-saturation by 716 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, higher light levels were not generated in the lab. Thus, photoinhibition could not be accurately resolved.

Before generating light response curves, respiration was measured in darkness (0 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) by covering the entire water bath with black plastic. For each species, light response curves were constructed for five replicate fronds by measuring the rate of change in oxygen concentration over a 5 min sampling period at each irradiance level. Photosynthetic rate was calculated in $\mu\text{mol O}_2$ g per oven dry weight $\cdot \text{min}^{-1}$ ($\mu\text{mol O}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$). Oven dry weight of each sample was measured after drying the sample for 48 h at 68°C, which proved to be an adequate drying time to achieve a constant mass. The proportion of calcium carbonate within these coralline species (*Calliarthron*: $84 \pm 0.3\%$ CaCO_3 and *Corallina*: $64 \pm 0.7\%$ CaCO_3) was then used to calculate the mass of noncalcified tissue.

To analyze light response data, Table Curve 2D v 5.01 (Systat Software Inc., San Jose, CA, USA) was used to fit a curve to the photosynthesis versus irradiance data to estimate parameters α and P_{max} according to Webb et al. (1974):

$$P_{\text{net}} = (P_{\text{max}} + P_0)(1 - \exp^{-\alpha I / P_{\text{max}}}) - P_0 \quad (1)$$

where P_{net} is the net photosynthetic rate ($\mu\text{mol O}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$), P_{max} is the maximum photosynthetic rate

($\mu\text{mol O}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$), P_0 is the respiration rate ($\mu\text{mol O}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$), α is the photosynthetic efficiency, and I is the irradiance ($\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). Mean α , mean P_0 and mean P_{max} were used to construct one average light response curve for each species. The compensation (I_c) and saturation irradiances (I_k) were calculated for each light response curve according to Henley (1993):

$$I_c = P_0 \alpha^{-1} \quad (2)$$

$$I_k = (P_{\text{max}} + P_0) \alpha^{-1} \quad (3)$$

where P_0 is the respiration rate ($\mu\text{mol O}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$), α is the photosynthetic efficiency, and P_{max} is the maximum photosynthetic rate ($\mu\text{mol O}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$). One-way ANOVAs were performed in SPSS 17.0 (SPSS Inc., Chicago, IL, USA) on the raw data ($n = 5$) to test for differences in I_c , I_k , α and P_{max} among species.

Stress resistance during low tide. To explore the effect of temperature and light on submerged photosynthesis in tidepools, rates of oxygen production were measured as described previously. This experiment was constructed with a factorial design with three levels of temperature (12°C, 16°C, and 20°C) and three levels of light (darkness (0 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$), subsaturating (50 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) and saturating (300 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). Subsaturating and saturating light levels were selected by examining previously determined light response curves (Fig. 1). In darkness, 14 replicate samples were taken for each level of temperature. For each treatment combination of light and temperature, five to seven replicate measurements of *Calliarthron* and *Corallina* were taken. Sample sizes were slightly unequal, due to infrequent equipment failure.

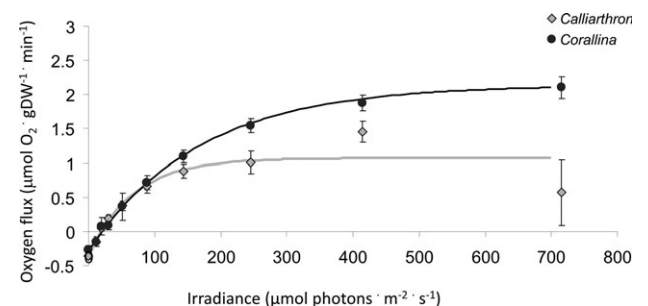


FIG. 1. Net photosynthetic rates as measured by oxygen evolution by irradiance level. Smoothed lines are photosynthetic model (Webb et al. 1974), using estimated parameters P_{max} and α ; means \pm SE, $n = 5$.

To test the effect of light and temperature levels, a two-way ANOVA was performed in SPSS 17.0 (SPSS Inc.) on photosynthesis and respiration data for each species separately with temperature and light as fixed factors and net photosynthesis or respiration as the response variable. Normality was confirmed with Shapiro-Wilk test and equal variance was confirmed with Levene's test.

To measure desiccation resistance, the amount of water lost through time was measured for *Calliarthron* and *Corallina* at two hydration levels ('wet' and 'blotted'). In the 'wet' treatment ($n = 5$), fronds were allowed to retain water within their branches. This treatment was standardized by shaking fronds twice after removing them from seawater (Padilla 1984). In the 'blotted' treatment ($n = 5$), excess water was removed from fronds by gently blotting their branches with paper towels. Each apical frond was placed separately in a tin weighing dish and a constant low humidity was maintained by placing the weighing dishes in a bin with the desiccant, Drierite. Desiccation experiments were performed at room temperature ($18 \pm 3^\circ\text{C}$). Two hydrochrons (iButtons; Maxim Integrated Products, Sunnyvale, CA, USA) were placed at opposite ends of the bin to measure relative humidity and temperature throughout the sampling period. Weight measurements were then taken every 15 min for 90 min to get a measure of relative water content (RWC) of thalli over time. Specimens were handled with forceps to reduce any accidental 'blotting' during the weighing process. After the 90 min period, specimens were dried in a 68°C oven for 48 h and oven dry weight measurements were taken. In the field, *Corallina* may experience desiccation for several hours, but for this experiment, we only explored water loss over the first 90 min. RWC of the fronds was calculated for each 15 min time point (Slayter 1967):

Relative Water Content (RWC)

$$= \frac{\text{Desiccated weight} - \text{Oven dry weight}}{\text{Initial fresh weight} - \text{Oven dry weight}} \quad (4)$$

To analyze the effect of hydration ('wet' and 'blotted') and species (two levels: *Calliarthron* and *Corallina*) on RWC loss through time, a repeated-measures ANOVA (RM-ANOVA) was performed in SPSS 17.0 (SPSS Inc.). Homogeneity of variance was tested with Levene's test and normality was tested with the Shapiro-Wilk test. Sphericity was tested with Mauchly's test. Corrections for sphericity were performed when necessary by correcting degrees of freedom using Greenhouse Geisser estimates of sphericity.

An infrared gas analyzer was used (Qubit Systems Inc., Kingston, ON, Canada) to measure CO_2 consumption or production in air to estimate photosynthesis out of tidepools. All photosynthetic measurements were taken at ambient CO_2 conditions (400–500 ppm CO_2), and the sample chamber was flushed with CO_2 between photosynthetic measurements. The sample chamber was constructed of two pieces of plexiglass with a rubber seal and a nut/bolt system to ensure an airtight chamber. A hydrochron (iButton; Maxim Integrated Products) was placed in the sample chamber to measure temperature and relative humidity throughout photosynthetic measurements. Light was provided with a slide projector at a saturating irradiance ($300 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) and measurements were taken at room temperature ($18 \pm 0.3^\circ\text{C}$). As with measurements in water, the rate of change in CO_2 concentration was measured for 5 min to obtain photosynthetic rates. Dark respiration rate was taken by covering the entire sampling chamber with dark plastic. To prevent desiccation within the sample chamber during the measurement period, a moistened filter paper was placed at the bottom of the chamber. Fronds were cleaned with a soft brush to

remove epiphytes and invertebrates before beginning photosynthetic measurements.

Emerged photosynthetic rates were tested at three different hydration levels (wet, blotted, and desiccated) at room temperature ($18 \pm 0.3^\circ\text{C}$). For all hydration levels, six replicate samples were taken for each species. In the 'wet' hydration treatment, fronds were allowed to retain water within their branches as described above. In the 'blotted' hydration treatment excess water was removed by blotting the fronds with a paper towel to remove excess water from the branches. In the 'desiccated' treatment fronds were desiccated to $\sim 50\%$ RWC according to previously determined fresh weight to oven dry weight ratios. Actual RWC of fronds were quantified after the experiment by drying at 68°C for 24 h. The actual RWC of fronds was $55 \pm 2\%$ RWC. Saturating light levels ($300 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) were provided during the desiccation period, and all samples desiccated for less than 30 min.

To analyze emerged photosynthetic data, a two-way ANOVA was performed in SPSS 17.0 (SPSS Inc.) with species (two levels; *Calliarthron* and *Corallina*) and hydration level (three levels; 'wet', 'blotted', 'desiccated') as fixed factors, and net photosynthesis or respiration as the response variable. Normality was confirmed with Shapiro-Wilk test and equal variance was confirmed with Levene's test.

Subsamples for baseline pigment analysis were taken before applying any desiccation treatment. Additionally, pigment samples were taken after applying the desiccation treatments in the emerged photosynthesis experiment. All samples were frozen in liquid nitrogen and stored at -80°C until pigment analyses were performed.

Phycobilin pigments were extracted first, followed by chl *a* and carotenoids. Samples were ground with a super-cooled mortar and pestle, and the sample was extracted in sodium phosphate buffer (0.1 M, 6.8 pH) at 4°C for 2 h in darkness. The sample was then centrifuged (20,800 g, 3 min) and the phycobilin pigment concentration of the supernatant was determined with a spectrophotometer (Ultrospec 2100 UV/Visible Spectrophotometer; Biochrom Ltd., Cambridge, UK). Phycobilin pigments were determined according to the equations in Lüder et al. (2001). The pellet was resuspended with 90% acetone overnight in darkness at 4°C to extract chl *a* and carotenoid pigments. The sample was centrifuged as above and pigment concentrations of the supernatant were again determined with a spectrophotometer. Repeated extractions according to the above protocol were then performed to remove all chl *a* and carotenoid pigments from samples. Chl *a* concentrations were calculated according to the equations in Arnon (1949) and carotenoid pigments were determined according to the equations in Wellburn (1994).

Differences in baseline pigment composition between *Corallina* ($n = 13$) and *Calliarthron* ($n = 14$) were first tested using independent sample *t*-tests. Then, to analyze pigment data after desiccation, a two-way ANOVA was performed in SPSS 17.0 (SPSS Inc.) with hydration level (three levels: 'wet', 'blotted', and 'desiccated') and species (two levels: *Calliarthron* and *Corallina*). For each treatment and species combination, four to six replicate samples were taken. Normality and equal variance were tested for all statistical tests.

Recovery when the tide returns. To measure recovery after submerged temperature stress, initial submerged photosynthetic rates were measured before any treatment was applied. After initial photosynthetic measurements at 12°C , *Calliarthron* and *Corallina* fronds were placed into one of two submerged temperature treatments: 12°C , representing continued high tide (control), or 20°C , representing a warm tide-pool during low tide. For each temperature and species treatment, five to seven replicate photosynthetic

measurements were taken. In both treatments, temperature varied by $<1^{\circ}\text{C}$. In the 12°C treatment, fronds were placed in vials of 12°C seawater and were placed back into the circulating water bath under saturating irradiance ($\sim 300 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). In the 20°C treatment, fronds were placed into vials of 20°C seawater, and saturating light was provided ($\sim 300 \mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). Fronds were exposed to temperature treatments for 30 min. Although tidepools heat up slowly over time, this was a measure of net photosynthesis at an elevated tidepool temperature. After the 30 min treatment period, fronds were re-immersed into a new vial of pre-chilled seawater (12°C), mimicking the return of the tide. Photosynthetic and respiration rates were then measured starting at 20 min, and were recorded every 10 min for 1 h. After recovery measurements were taken, the specimens were dried in a 68°C oven for 48 h to obtain the oven dry weight. The amount of calcium carbonate was then accounted for to obtain the decalcified dry weight of the algae for photosynthetic calculations.

A RM-ANOVA design was used to analyze photosynthetic recovery data in SPSS 17.0 (SPSS Inc.). First, only photosynthetic data in the recovery period (not including initial photosynthetic measurements) was tested with a RM-ANOVA and no significant effect of time was found. Because of this, any significant effect of time is due to a difference between initial photosynthetic measurements and recovery measurements. Homogeneity of variance was tested with Levene's test. Sphericity was tested with Mauchly's test. Corrections for sphericity were performed when necessary by correcting degrees of freedom using Greenhouse Geisser estimates of sphericity. A RM-ANOVA was performed in SPSS 17.0 (SPSS Inc.), including the initial measurement and five recovery measurements.

To measure recovery after emergent stressors, initial photosynthetic measurements were taken in 12°C seawater, and then fronds were placed into temperature and desiccation treatments. Fully factorial manipulations of air temperature (two levels: 16°C and 25°C) and desiccation (two levels: 75% and 50% RWC) were used. In each combination of temperature and desiccation, 7–12 replicate measurements of photosynthesis were taken for both species. All fronds were desiccated under saturating light conditions. Fronds were not allowed to desiccate for more than 30 min to avoid differential exposure to light. Previously determined fresh to oven dry weight ratios were used to determine the RWC of desiccating fronds. Air temperature and relative humidity in treatments were measured with a hydrochron (iButton, Maxim Products). Since fronds of *Calliarthron* and *Corallina* desiccate at different rates, either two fronds of *Corallina* or two fronds of *Calliarthron* were treated and analyzed simultaneously. After the treatment period, fronds were placed into a new vial of fresh seawater at 12°C and photosynthetic and respiration rates were measured starting at 20 min, and measurements were recorded every 5 min for 1 h. The specimens were then dried in a 68°C oven for 48 h to obtain an oven dry weight. The amount of calcium carbonate was subtracted from this dry weight to obtain the oven dry weight of only algal material.

A RM-ANOVA design was used to analyze photosynthetic recovery data. First, only photosynthetic data in the recovery period (not including initial photosynthetic measurements) were tested with a RM-ANOVA in SPSS 17.0 (SPSS Inc.) and no significant effect of time was found. Because of this, any significant effect of time is due to a difference between initial photosynthetic measurements and recovery measurements. Homogeneity of variance was confirmed with Levene's test. Sphericity was tested with Mauchly's test. Data had equal variances but was not spherical and therefore degrees of freedom were corrected for using Greenhouse Geisser estimates of sphericity. A two way mixed RM-ANOVA was performed in

SPSS 17.0 (SPSS Inc.), including the initial measurement and five recovery measurements.

RESULTS

Physiological differences at high tide. The physiological performance of *Calliarthron* and *Corallina* were similar in low light, but differed in high light (Fig. 1). *Corallina* had approximately twice the maximum net photosynthetic rate as *Calliarthron*. With little or no light, both species had similar respiration rates, photosynthetic efficiencies, and compensation points (Table 2). However, in bright light, *Corallina* had a higher P_{max} and saturating irradiance (Table 2; Fig. 1). The *Calliarthron* curves suggest some decrease of photosynthetic rate in high light, but photoinhibition was not quantified.

Calliarthron and *Corallina* had similar pigment profiles. There were no significant differences between the two species in any of the light harvesting pigments or photoprotective pigments (Table S1 in the Supporting Information).

Stress resistance at low tide. Elevated temperature had a significant negative effect on the submerged net photosynthetic rate of *Calliarthron* whereas *Corallina* remained unaffected (Table 3; Fig. 2A). Elevated light had a significant positive effect on the submerged net photosynthetic rate of *Corallina* whereas *Calliarthron* was unaffected by increased light availability (Table 3; Fig. 2A).

Under subsaturating light, *Corallina* had positive net photosynthetic rates in the lowest two temperature treatments (12°C and 16°C) but when temperature was increased to 20°C , net photosynthetic rates approached zero (Fig. 2A). *Calliarthron* had very low net photosynthetic rates in the lowest temperature treatment (12°C) and as temperature was increased, the net photosynthetic rate dropped to zero at 16°C and at 20°C , *Calliarthron* was predominately respiring (Fig. 2A). For both species, net photosynthetic rates decreased as temperature was increased from 12°C to 20°C . Such an increase in temperature resulted in a one $\mu\text{mol O}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$ drop in net photosynthesis in *Calliarthron* but only a

TABLE 2. Photosynthetic efficiency (α), respiration (P_0), maximum photosynthetic rates (P_{max}), compensation irradiance (I_c), and saturation irradiance (I_k) of *Calliarthron* and *Corallina*, 12°C , submerged. One-way ANOVAs, $n = 5$.

	<i>Calliarthron</i>	<i>Corallina</i>	P-value
Respiration	-0.36 ± 0.05	-0.27 ± 0.07	0.36
Photosynthetic efficiency	0.02 ± 0.002	0.01 ± 0.002	0.41
Maximum photosynthetic rate	1.07 ± 0.19	2.14 ± 0.19	$<0.01^*$
Compensation irradiance	20.2 ± 1.7	24.4 ± 7.2	0.59
Saturation irradiance	83.9 ± 8.1	223.8 ± 41.6	0.01^*

*Significant ANOVA result (mean \pm SE).

TABLE 3. Results of two-way ANOVA for the effects of temperature and light on net photosynthesis and respiration of submerged fronds of *Calliarthron* and *Corallina*.

	Species	Source	Type III Sum of Squares	df	Mean Square	F	P-value
Photosynthesis	<i>Calliarthron</i>	Light	0.68	1	0.67	1.15	0.29
		Temperature	5.39	2	2.70	4.64	<0.05*
		Light × Temperature	0.47	2	0.23	0.40	0.67
	<i>Corallina</i>	Light	9.44	1	9.44	32.21	<0.001*
		Temperature	1.30	2	0.65	2.21	0.13
		Light × Temperature	0.15	2	0.07	0.25	0.78
Respiration	Species	2.69	1	2.69	7.25	<0.01*	
	Temperature	18.92	2	9.46	25.49	<0.001*	
	Species × Temperature	1.30	2	0.65	1.75	0.18	

*Significant result.

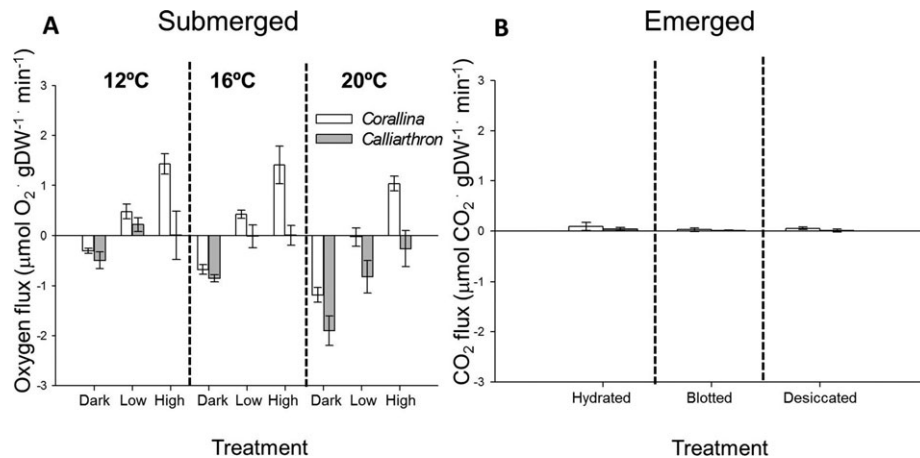


FIG. 2. (A) Net oxygen flux of *Calliarthron* (grey bars) and *Corallina* (white bars) in submerged conditions by temperature (12°C, 16°C, and 20°C). Dark = 0 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; subsaturating light = 50 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; saturating light = 300 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; darkness ($n = 14$ at all levels of temperature for both species); saturating light/high temperature ($n = 6$ for *Calliarthron*; $n = 7$ for *Corallina*); saturating light/medium temperature ($n = 5$ for *Calliarthron*; $n = 7$ for *Corallina*); subsaturating light/medium temperature ($n = 6$ for *Calliarthron*, $n = 6$ for *Corallina*); saturating light/low temperature ($n = 5$ for *Calliarthron*; $n = 6$ for *Corallina*); subsaturating light/low temperature ($n = 7$ for *Calliarthron*; $n = 7$ for *Corallina*), means \pm SE. (B) Net carbon dioxide flux of *Calliarthron* and *Corallina* in air (18°C \pm 0.3°C). Dark = 0 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; light = 300 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. 'Desiccated' is 55% RWC ($n = 6$ for *Calliarthron*; $n = 6$ for *Corallina*), 'Blotted' is with water removed from branches ($n = 6$ for *Calliarthron*; $n = 6$ for *Corallina*), and 'Wet' is with water in branches ($n = 6$ for *Calliarthron*; $n = 6$ for *Corallina*); means \pm SE.

0.5 $\mu\text{mol O}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$ drop in net photosynthesis in *Corallina*.

Under saturating light, *Corallina* had high net photosynthetic rates across all temperatures, with only a slight reduction (0.2 $\mu\text{mol O}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$) in the high temperature treatment (Fig. 2A). Net photosynthesis of *Calliarthron* at 12°C was approximately zero, and rates became increasingly negative as temperature got warmer. Overall, temperature did not affect net photosynthetic rates in *Corallina* (Table 3; Fig. 2) but net photosynthetic rates were higher in high light than in low light. Net photosynthetic rates of *Calliarthron* were low regardless of light level, but as temperature increased, net photosynthetic rates became negative.

At all temperatures, respiration was always higher in fronds of *Calliarthron* than in fronds of *Corallina* (Table 3; Fig. 2A); however, increases in tempera-

ture caused respiration rates to increase similarly in both species (Table 3; Fig. 2A). It is important to note that even when net photosynthetic rates were very low, photosynthesis was still occurring but was offset by respiration rates. For example, at 16°C and 20°C, it appears that *Calliarthron* is not photosynthesizing but these low rates of oxygen production are primarily due to high respiration rates.

When hydrated, fronds of *Calliarthron* and *Corallina* desiccated at different rates but when water was removed from fronds, both species desiccated similarly (Table 4; Fig. 3). *Corallina* was able to hold water within its branches and thereby delay desiccation (Table 4; Fig. 3). For example, at the beginning of the experiment, *Corallina* fronds held 150% more water than its thallus alone (250% RWC) while *Calliarthron* fronds only held 50% more water than its thallus alone (150% RWC; Fig. 3). As desic-

TABLE 4. Results of repeated measures ANOVA of the rate of desiccation of wet and blotted fronds of *Calliarthron* and *Corallina* at 55% relative humidity.

	Source	Type III sum of squares	df	Mean square	F	Pvalue
'Wet'	Species	87,792.48	1	87,792.48	35.80	<0.001*
	Time	182,530.11	1.61	113,174.49	648.83	<0.001*
	Time * Species	4,058.87	1.61	2,516.63	14.43	0.001*
'Blotted'	Species	255.91	1	255.91	1.23	0.30
	Time	64,916.13	1.82	35,717.79	871.37	<0.001*
	Time * Species	204.21	1.82	112.36	2.74	0.10

*Significant ANOVA result.

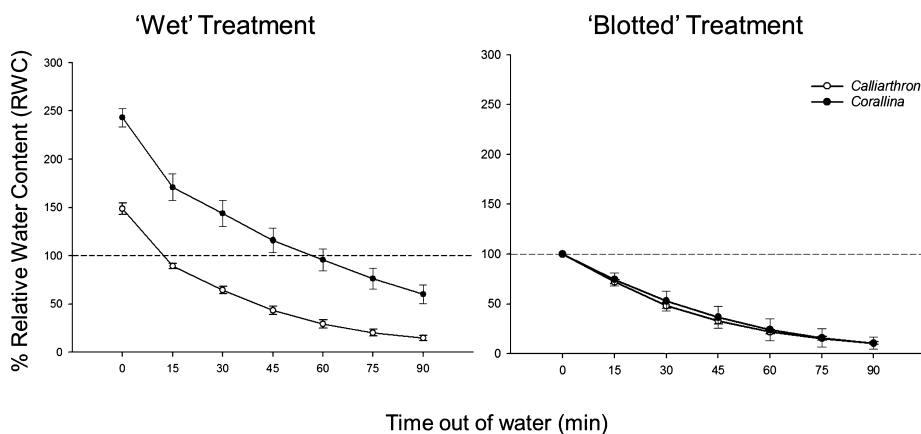


FIG. 3. The change in relative water content of fronds of *Calliarthron* (open symbols) and *Corallina* (black symbols) with increasing time out of water. Fronds were allowed to retain water in branches in 'wet' treatment ($n = 5$ for *Calliarthron*; $n = 5$ for *Corallina*), and water was removed from fronds in 'blotted' treatment ($n = 5$ for *Calliarthron*; $n = 5$ for *Corallina*). Experiment performed at 55% relative humidity; means \pm SE.

cation progressed, 'wet' fronds of *Calliarthron* delayed thallus desiccation by 15 min while *Corallina* fronds delayed thallus desiccation by ~60 min (Fig. 3; Table 4).

At the end of the sampling period, fronds of *Calliarthron* contained less water than *Corallina* (Fig. 3): 'wet' *Calliarthron* fronds had $14.4 \pm 5.5\%$ RWC (mean \pm SE), while 'wet' *Corallina* fronds still had $59.8 \pm 22.1\%$ RWC (Fig. 3). However, 'blotted' fronds of *Calliarthron* and *Corallina* had similar RWCs (*Calliarthron*: $10.72 \pm 3.72\%$; *Corallina*: $10.65 \pm 4.97\%$ RWC).

Independent of hydration level, both species exhibited similar net photosynthetic and respiration rates in the air, and these rates were approximately zero (Fig. 2B, Table S2 in the Supporting Information). *Calliarthron* had a mean photosynthetic rate of $0.02 \pm 0.06 \mu\text{mol O}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$ (mean \pm SE) and *Corallina* had a mean photosynthetic rate of $0.07 \pm 0.12 \mu\text{mol O}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$ in the air (Fig. 2B). *Calliarthron* and *Corallina* also demonstrated similarly low respiration rates in air, regardless of hydration level (Table S2). The mean respiration rate of *Calliarthron* was $0.01 \pm 0.05 \mu\text{mol CO}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$, and the mean respiration rate of *Corallina* was $0.04 \pm 0.08 \mu\text{mol CO}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$ (Fig. 2B).

Desiccation did not cause pigments to degrade in either *Calliarthron* or *Corallina*. Regardless of hydration level *Calliarthron* and *Corallina* (Table S3 in the Supporting Information) had similar final chl *a*

concentrations. Both species also had similar concentrations of phycoerythrin, phycocyanin, and allophycocyanin regardless of hydration level (Table S3). However, *Calliarthron* had less carotenoid pigment than *Corallina* (two-way ANOVA, $F_{1,2} = 16.01$, $P < 0.001$; Table S3) but desiccation did not affect carotenoid concentrations (Table S3).

Recovery when the tide returns. Both *Calliarthron* and *Corallina* recovered from warm tidepool conditions (Fig. 4, A and B). In all recovery experiments, fronds were considered to recover if net photosynthetic rates were positive upon re-immersion. Twenty min after re-immersion, net photosynthetic rates of both *Corallina* and *Calliarthron* were positive and not significantly different from initial rates. However, the two species exhibited significantly different net photosynthetic rates (RM-ANOVA, $F_{1,2,34} = 11$, $P < 0.01$; Table S4 in the Supporting Information; Fig. 4). In 12°C seawater, the mean photosynthetic rate was $1.40 \pm 0.29 \mu\text{mol O}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$ (mean \pm SEM) for *Calliarthron* (Fig. 4A) and $1.77 \pm 0.12 \mu\text{mol O}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$ for *Corallina* (Fig. 4B). After exposure to 20°C seawater, the mean photosynthetic rate for *Calliarthron* was $1.13 \pm 0.18 \mu\text{mol O}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$ (Fig. 4A) and $1.93 \pm 0.07 \mu\text{mol O}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$ for *Corallina* (Fig. 4B).

The interactive effect of time, temperature and species on respiration rates made it difficult to describe clear trends in respiration due to physical factors (RM-ANOVA, $F_{1,5} = 6.1$, $P < 0.001$; Table S4;

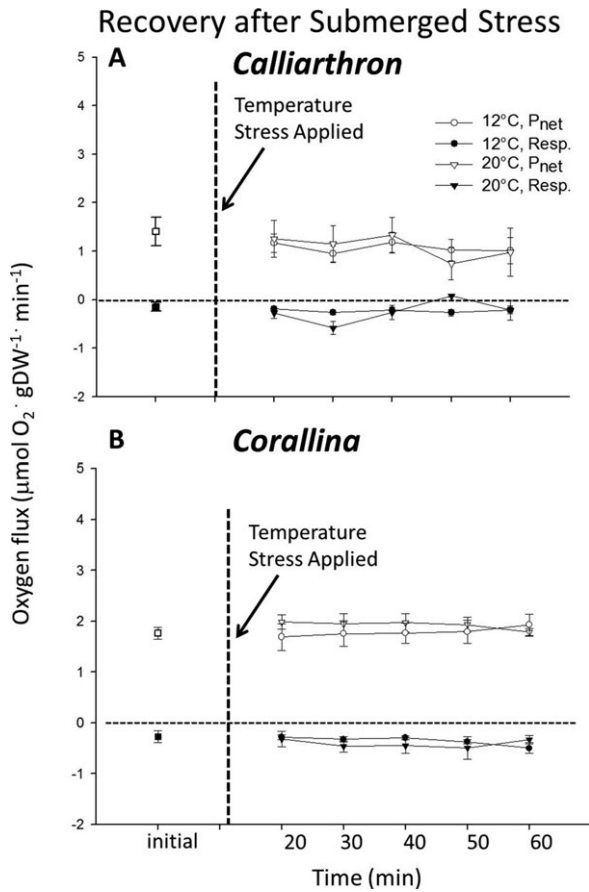


FIG. 4. Net photosynthetic and respiration rates of *Calliarthron* (A) and *Corallina* (B) before and after exposure to submerged temperature treatments. Initial rates are shown to the left of the vertical line ($n = 12$ for *Calliarthron*; $n = 13$ for *Corallina*) and recovery rates are to the right of this line (12°C: $n = 7$ for *Calliarthron*; $n = 6$ for *Corallina*; 20°C: $n = 5$ for *Calliarthron*; $n = 7$ for *Corallina*). Horizontal dashed line represents 0 net photosynthesis. Open symbols indicate net photosynthesis and closed symbols represent respiration rates. Circles are the 12°C treatment and triangles are the 20°C treatment; means \pm SE.

Fig. 4). In 12°C seawater, respiration rates for *Calliarthron* were $0.15 \pm 0.10 \mu\text{mol O}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$ (Fig. 4A) and $0.27 \pm 0.11 \mu\text{mol O}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$ for *Corallina* (Fig. 4B). After exposure to 20°C seawater, the mean respiration rate for *Calliarthron* was $0.24 \pm 0.07 \mu\text{mol O}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$ (Fig. 4A) and $0.41 \pm 0.07 \mu\text{mol O}_2 \cdot \text{gDW}^{-1} \cdot \text{min}^{-1}$ for *Corallina* (Fig. 4B).

Corallina recovered from the combined temperature and desiccation stress (Fig. 5), while *Calliarthron* did not. For all temperature and desiccation treatment combinations, fronds of *Calliarthron* only respired in the recovery period. The difference between initial photosynthetic measurements and the recovery measurements was reflected in the significant effect of time on the net photosynthesis of *Calliarthron* (RM-ANOVA, $F_{1,1.96} = 19.2$, $P < 0.01$;

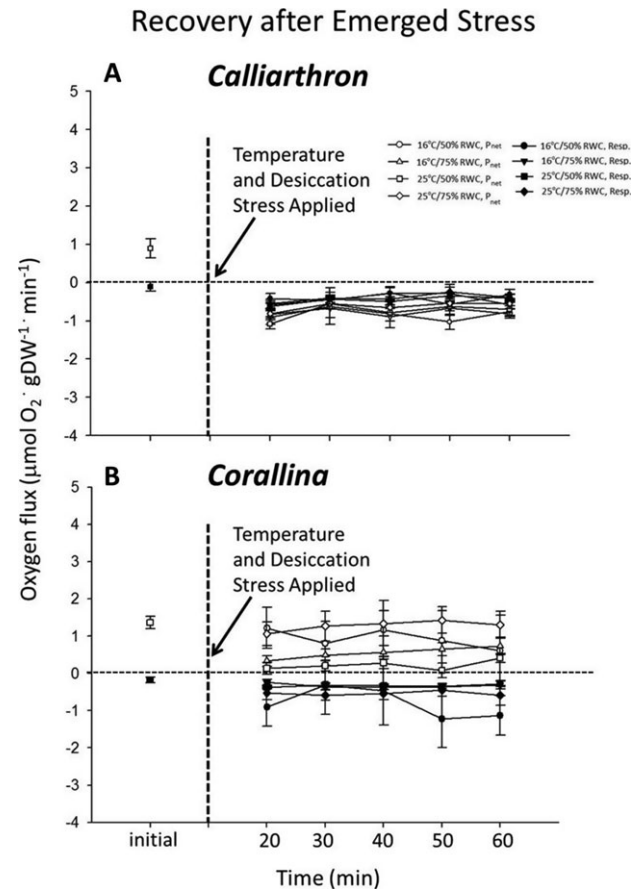


FIG. 5. Net photosynthetic and respiration rates of *Calliarthron* (A) and *Corallina* (B) before and after emergent stress. Initial rates are shown to the left of the vertical line and recovery rates are to the right of this line. Horizontal dashed line represents zero net photosynthesis. Open symbols indicate photosynthetic rates and solid symbols represent respiration rates. Circles are the low temperature (16°C) and high desiccation (50% RWC) treatment (*Calliarthron*: $n = 8$; *Corallina*: $n = 7$), triangles are low temperature (16°C) and low desiccation (75% RWC) treatment (*Calliarthron*: $n = 10$; *Corallina*: $n = 10$), squares are high temperature (25°C) and high desiccation (50% RWC) treatment (*Calliarthron*: $n = 13$; *Corallina*: $n = 12$), diamonds are high temperature (25°C) and low desiccation (75% RWC) treatment (*Calliarthron*: $n = 8$; *Corallina*: $n = 8$); means \pm SE.

Table S5 in the Supporting Information, Fig. 5A). Photosynthetic rates of fronds of *Calliarthron* were negatively affected by all temperature and desiccation treatments (Fig. 5A), and so, no significant differences among temperature or desiccation treatments were detected in the ANOVA analysis.

Respiration rates in *Calliarthron* were higher after temperature and desiccation treatments compared to initial photosynthetic rates, and this was reflected in the significant effect of time (RM-ANOVA, $F_{1,3.01} = 4.1$, $P < 0.01$; Table S5; Fig. 5A) on respiration rates. However, in the recovery phase, photosynthetic productivity was the same whether light was applied (net photosynthesis vs. respiration). Fronds of *Calliarthron* did not photosynthesize after

stress treatments, but continued to respire (Fig. 5A).

Unlike *Calliarthron*, *Corallina* fronds recovered after temperature and desiccation stress (Fig. 5B). Recovery measurements all exhibited a positive net photosynthetic rate after the temperature and desiccation stress treatments, which is in contrast with the trend seen in *Calliarthron* (Fig. 5A). In fronds of *Corallina*, there was a compounded effect of temperature and desiccation on net photosynthesis, such that greater temperatures exacerbated the negative effect of desiccation (RM-ANOVA, $F_{1,2,22} = 4.5$, $P < 0.05$; Table S5; Fig. 5B). Additionally, the initial and recovery measurements differed (RM-ANOVA, $F_{1,2,22} = 6.9$, $P < 0.01$; Table S5; Fig. 5B).

Respiration rates of *Corallina* fronds were unaffected by temperature and desiccation treatments (Table S5; Fig. 5B). There was no significant change in respiration rates between initial and recovery measurements nor after the temperature or desiccation stresses (Table S5; Fig. 5B).

DISCUSSION

Intertidal seaweeds resist a suite of abiotic stressors associated with submergence and emergence during the course of a tidal cycle. Differences in the ability of macroalgae to tolerate stressors contribute to habitat partitioning along the shore. It is well documented that tolerance to acute low tide stress is higher for seaweeds growing high on the shore (Oates and Murray 1983, Bell 1993, Lipkin et al. 1993, Matta and Chapman 1995, Hunt and Denny 2008), and results from this study suggests that seaweed growing outside of tidepools are more tolerant to environmental stress than those living in tidepools. Data presented here reveals that habitat partitioning of intertidal seaweeds likely stems not only from physiological differences during low tide, but also from differences during high tide and during recovery after low tide.

Physiological differences at high tide. During high tide, differences in light acclimation and temperature tolerance influence habitat separation. For example, the photosynthetic performance of *Corallina* decreases significantly with decreasing light levels, suggesting that photosynthesis declines when the tide is high and possibly explains the lack of *Corallina* in subtidal or in deep tidepool environments. *Calliarthron*, on the other hand, performs similarly to *Corallina* in low light and experiences a sharp decline in photosynthesis as temperatures increase, suggesting that *Calliarthron* fares best subtidally or in tidepools where temperature fluctuations are muted (Williams and Dethier 2005) and aerial exposure is avoided, which is consistent with its distribution in the field.

Frequently, macroalgae adapted to different light environments exhibit differences in photosynthetic

performance, reflecting differences in pigment concentrations (Larkum and Barrett 1983). Surprisingly, physiological differences between the two species in this study were not due to differences in pigments: Concentrations of light-harvesting pigments in *Calliarthron* and *Corallina* were similar. In low light, similar pigmentation lead to comparable performance of *Calliarthron* and *Corallina*, where photosynthetic rates are directly related to the ability of algae to harvest light (Henley 1993). However, in high light, physiology was significantly different, despite similarities in pigments. Differences in metabolic processes such as electron transport rate or RuBisCO activity may account for the difference in photosynthetic rates in high light (Taiz and Zeiger 2002), but this was not tested and requires further experimentation.

Stress resistance during low tide. When submerged, *Corallina* is tolerant of high temperatures and high light, making it well-adapted to shallow tidepool habitats. However, the photosynthetic performance of *Corallina* decreases significantly as light levels decrease, suggesting that photosynthesis may be compromised if fronds are deeply submerged or shaded in tidepools. As temperature increased, *Calliarthron's* net photosynthetic rate significantly decreased, suggesting that *Calliarthron* is ill suited to temperature stress (Fig. 2) and fares best subtidally and in deeper tidepools where temperature fluctuations are limited which is consistent with the typical habitat distribution of *Calliarthron*.

Photosynthetic rates measured in this portion of this study are conservative and field rates may actually be lower due to the limiting effects of still-water conditions at low tide. The effects of diffusive boundary layers and mass transfer limitation on seaweed production may be significant (Falco et al. 1975, Hurd et al. 1996) for seaweeds in tidepools during the low tide.

Corallina can resist desiccation for over an hour when the tide is out by holding water in its fine branches. *Calliarthron*, on the other hand, is very desiccation prone and dries out within 15 min (Fig. 3). This supports the results of Martone et al. (2010a), who found that desiccation was the primary environmental stress that limited the habitat range of intertidal *Calliarthron*. It is likely that intertidal *Calliarthron* is relegated to tidepools to avoid desiccation. Interestingly, when excess water was blotted from their branches, *Calliarthron* and *Corallina* desiccated at a similar rate, suggesting that calcified thalli produced by the two species are equally susceptible to desiccation, but that the arrangement of fine branches in *Corallina* provides a significant morphological advantage. This supports the work of Padilla (1984), who found that when branches of *Corallina* are thinned in the field, they bleach and die while control fronds remain healthy. In sum, both species are severely susceptible to desiccation stress; *Calliarthron* avoids drying out by staying

submerged and *Corallina* avoids drying out by retaining water.

When emerged during low tide, *Corallina* and *Calliarthron* do not photosynthesize in the air, and apparently 'shut down' respiration and photosynthesis regardless of hydration level (Fig. 2B). A decline in photosynthesis is not due to pigment degradation since severe desiccation did not degrade pigments in either *Calliarthron* or *Corallina*, even after desiccating to ~50% RWC. Thus, *Corallina*'s ability to live outside tidepools is not linked to enhanced aerial photosynthesis, as was found in other seaweeds (Johnson et al. 1974, Dring and Brown 1982), but is instead likely related to its unique morphological ability to delay desiccation.

Recovery when the tide returns. Both *Calliarthron* and *Corallina* were able to recover completely from being submerged in warming tidepool conditions (Fig. 4). Although *Calliarthron* is stressed by warm water temperatures (Fig. 2), it recovers rapidly when the tide returns and temperatures are reduced. *Corallina*, on the other hand, is not stressed by warm temperatures while submerged and so likely remains productive while the tidepool is heating up (Fig. 2). These results are consistent with the observation of *Corallina* growing at the rims of tidepools, and *Calliarthron* residing deeper in tidepools, where temperatures are greatly reduced as compared to the surface water.

Calliarthron and *Corallina* showed significant differences in their capacity to recover from aerial exposure. *Corallina* regained positive photosynthetic rates with the return of the tide, although photosynthetic rates during recovery were significantly less than initial photosynthetic rates (Fig. 5B). This suggests that *Corallina* is somewhat stressed by emergence, but recovers photosynthetic activity soon after resubmergence. *Calliarthron*, however, never recovered after any emergent low tide treatment (Fig. 5A). This finding supports the results of Martone et al. (2010a), confirming that *Calliarthron* is extremely sensitive to emergent stress and cannot recover from combined temperature and desiccation stresses.

It should be noted that stressors in both recovery experiments were applied for only 30 min, which is shorter than exposure during some tidal cycles. However, conclusions drawn from these experiments document relative physiological differences between the two species. Future experiments could explore longer desiccation times, or greater variation in temperature and humidity.

Neither *Corallina* nor *Calliarthron* recovered photosynthesis after emersion as rapidly as other intertidal algae, such as *Mastocarpus papillatus*, *Endocladia muricata*, *Fucus serratus*, and *Fucus spiralis* (Dring and Brown 1982, Madsen and Maberly 1990, Martínez et al. 2012), which can all completely recover within 10–35 min of re-immersion (Britting 1992, Bell 1993, Hunt and Denny 2008). However, to our

knowledge, this is the first study to investigate the recovery of coralline algae. Corallines may be less likely to resist and recover from desiccation stress because thalli contain high levels of calcium carbonate, but low levels of sulfated polysaccharides, which help fleshy macroalgae retain water (Kloareg and Quatrano 1988, Martone et al. 2010b). Although *Corallina* recovers from emergence stress, photosynthetic rates upon re-immersion are quite low and it isn't clear how long it would take for rates to fully recover. Differences in recovery rates may be linked to differences in cyclic electron flow, which can ameliorate environmental stress, such as desiccation (Canaani et al. 1989, Herbert et al. 1990, Golding and Johnson 2003, Gao et al. 2011). During stressful conditions, cyclic electron flow around PSI can provide ATP as a source for repair of PSII units (Canaani et al. 1989). Future studies should explore the differences in cyclic electron flow between *Calliarthron* and *Corallina*.

Although net photosynthetic rates in *Corallina* decrease as light levels decrease, *Corallina* photosynthesizes at the same or higher rate than *Calliarthron* at all temperatures and light levels. So why do we not observe *Corallina* living deep in tidepools in the *Calliarthron* zone? What defines the lower limit of *Corallina*? Van Tamelen (1996) documented zonation of coralline algae in tidepools and attributed species distributions to the effects of scouring. Scouring is the greatest at the bottoms of tidepools, and so only algae that can withstand such stress can flourish there. Robust thalli of *Calliarthron* may be able to resist scouring, whereas the fine branches of *Corallina* may be susceptible to such physical disturbance.

Herbivory may help also explain this pattern. Padilla (1984) found that the mean number of molluscan herbivores, such as the chiton *Katharina*, increased deeper in tidepools and that *Corallina* is particularly susceptible to herbivory. *Calliarthron*, on the other hand, is resistant to common molluscan herbivores, including limpets and chitons (Padilla 1984). This resistance is structural in nature – the large calcified segments and sparse branches produced by *Calliarthron* are difficult for herbivores to eat (Padilla 1984). *Calliarthron* has a higher percentage of calcium carbonate than *Corallina*, which could also deter herbivory. Since many herbivores also find refuge in tidepools during the low tide, *Corallina* may live outside of tidepools to avoid herbivory while *Calliarthron* persists. *Corallina*'s physiology perhaps compensates for its susceptibility for herbivory, while *Calliarthron* simply persists in tidepools without being eaten. This putative pattern of herbivory defining the lower limit of *Corallina* and environmental stress defining the upper limit of *Calliarthron* in the intertidal zone would be consistent with classic ecological theory (Doty 1946, Connell 1972, Paine 1994, Raffaelli and Hawkins 1996, Somero 2002).

Summary. In this study, we documented morphological and physiological differences during the course of the tidal cycle that help explain habitat partitioning of two intertidal coralline algae. *Corallina* performs well in high light, high temperature environments, resists desiccation when the tide is out, and recovers quickly when the tide returns. These adaptations permit *Corallina* to survive emergent conditions and to live out of tidepools. *Calliarthron*, on the other hand, is highly susceptible to desiccation stress, generally relegating *Calliarthron* to subtidal and deep tidepool habitats. Understanding physiological performance of seaweeds throughout the entire tide cycle helps clarify the spatial segregation of organisms inhabiting the intertidal zone.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Table S1. Baseline pigment concentrations of *Corallina* and *Calliarthron*, and results of independent sample *t*-tests; means \pm SE

Table S2. Two-way ANOVA results of net photosynthesis and respiration of *Calliarthron* and *Corallina* in air.

Table S3. Results of two-way ANOVA of light harvesting pigment concentrations in fronds of *Calliarthron* and *Corallina* in 'wet', 'blotted', and 'desiccated' treatments. * denotes significant ANOVA result.

Table S4. Repeated measures ANOVA results of recovery of net photosynthesis after submerged temperature treatments (12°C and 20°C). * denotes significant result.

Table S5. Repeated Measures ANOVA results for recovery of net photosynthesis and respiration after exposure to temperature and desiccation treatments. * indicates significant result.