PHYSIOLOGICAL RESPONSES OF TWO SPECIES OF ARTICULATED CORALLINE ALGAE DURING A SIMULATED TIDAL CYCLE

by

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Abstract

Intertidal macroalgae endure stresses associated with submerged and emerged conditions on a daily basis. Differences in physiology at high tide, low tide, and during recovery underlie spatial separation of species along the shore. Tidepools provide refugia from physical stresses associated with the low tide, and species with low stress tolerance may be restricted to these habitats. Species that survive emergence employ physiological and morphological strategies to survive exposure to pseudo-terrestrial conditions. I explored how tidepool and non-tidepool macroalgae respond to and recover from intertidal stressors, such as light, temperature, and desiccation. I investigated whether differences in physiology could explain differences in habitat distributions.

To answer these questions, I explored the physiological responses of the coralline algae, *Calliarthron tuberculosum* (Postels and Ruprecht) E.Y. Dawson and *Corallina vancouveriensis* Yendo, to simulated tidal conditions. *Calliarthron* is restricted to tidepools, while *Corallina* can survive emersion during low tide.

First, I documented physiological differences between the two species at high tide. *Corallina* performed similar to a high light adapted plant, while *Calliarthron*'s performance resembled that of a low light adapted plant. Surprisingly, their pigment composition did not differ, suggesting that both species are able to harvest light similarly but that other metabolic processes are at play.

Second, I compared morphological and physiological strategies employed by *Calliarthron* and *Corallina* to resist stress during low tide. I found differences in the physiological responses of the two species to increased light and temperature, two chief stressors present in the tidepool microhabitat. Unlike *Calliarthron*, *Corallina* exhibited high

tolerance to increasing water temperatures and was more effective at resisting desiccation via morphology. However, neither species photosynthesized in the air, regardless of hydration level.

Finally, I quantified recovery upon the return of the tide. Both species recovered from warm tidepool temperatures. However, only *Corallina* recovered from the combination of temperature and desiccation stress associated with emergence.

This study describes the variation in physiological performance of two intertidal macroalgal species during the tidal cycle, and documents several morphological and physiological strategies employed by species to survive stresses associated with low tide. Results help to explain the habitat differences between the two species.

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Introduction

Marine intertidal organisms are subjected to an alternation of submergence and emergence on a daily basis. During daylight high tides in temperate waters, these organisms are submerged and exposed to aquatic conditions, including cool water temperatures and reduced light. At daylight low tides, intertidal organisms are emerged and exposed to terrestrial conditions, including high light, high temperature, and desiccation.

Vertical distributions of marine macroalgae have often been explained through the physiological tolerances and thresholds to abiotic stressors, such as light, temperature and desiccation. These abiotic stressors have been shown to limit the upshore growth of intertidal algae (Schonbeck and Norton 1979, 1980, Madsen and Maberly 1990) contributing to the pattern of zonation and habitat partitioning along the shore (Doty 1946, Dring and Brown 1982). Exposure to low tide conditions such as high light intensities and desiccation has been shown to damage the photosynthetic apparatus and negatively impact photosynthetic rates (Sampath-Wiley *et al.* 2008). Moreover, pigments degrade in response to excess light (Henley and Ramus 1989, Sampath-Wiley *et al.* 2008) and desiccation stress (Martone *et al.* 2010). With desiccation stress, photosynthesis and respiration decrease (Hodgeson 1981, Lipkin *et al.* 1993), and this reduction is more pronounced with extreme levels of desiccation (Scrosati and DeWreede 1998, Bell 1993, Hodgeson 1981).

Photosynthesis increases with increasing temperature to an optimum temperature, above which photosynthesis sharply declines (Davison 1991, Bell 1993). Optimum temperatures for photosynthesis are often correlated with the ambient temperatures in which the algae grow (Berry and Bjorkman 1980, Davison 1991). Algae grown at high temperatures in the lab exhibit higher photosynthetic rates in warm water while algae grown at low temperatures

exhibit higher photosynthetic rates in cool water (Berry and Bjorkman 1980). However, the potential for such temperature acclimation varies widely between species (Berry and Bjorkman 1980).

Many seaweeds possess mechanisms that provide protection from stressful low tide events. For example, algae synthesize heat shock proteins for protection against increasing temperatures (Li and Brawley 2004, Henkel and Hofmann 2008). To avoid damage to the photosynthetic apparatus from excess light, algae can increase protective pigment levels (Beach and Smith 1996). Some algae resist desiccation through morphology by growing as a densely-branched turf (Padilla 1984, Hay 1981), or as water-filled sacs (Oates 1985, Matta and Chapman 1995). It has also been demonstrated that some high intertidal algae maintain high photosynthetic rates in the air and reach maximum photosynthetic rates when slightly desiccated (Johnson *et al.* 1974, Quadir *et al.* 1979, Lipkin *et al.* 1993), suggesting a putative benefit to aerial emergence for high intertidal algae.

Tidepools are a prominent microhabitat feature of the intertidal zone that may mitigate the stresses associated with low tide. For example, intertidal organisms living in tidepools avoid desiccation (Dethier 1980) and are exposed to less variable temperature and light regimes (Metaxas and Scheibling 1993). Thus, tidepools generally are considered to be refugia for animals and algae from the stresses of low tide (Metaxas and Scheibling 1993), but the protective effect may be limited. Tidepools can increase in temperature during a low tide, and tidepool temperatures can reach 20-22°C during a mid-day low tide in temperate marine ecosystems (Johnson and Skutch 1928, Nakamura 1976, Helmuth *et al.* 2002). Yet, the degree to which tidepools increase in temperature is highly variable and is affected by a

number of factors, such as shading, aspect, shore height and depth of the tidepool. Thus, the protective effect of tidepools is variable and depends on many factors.

When the tide returns, organisms that have survived the low tide are submerged once again, exposed to cool water temperatures and reduced light conditions. Thus, the ability of intertidal algae to recover from the stress of the low tide is critical for survival. The tolerance of a species to the stresses of low tide can be determined by the extent to which photosynthesis recovers when algae are re-immersed in seawater (Davison and Pearson 1996). Generally, species that live higher in the intertidal zone are more tolerant to abiotic stress and more likely to recover rapidly upon re-immersion (Dring and Brown 1987).

In this study, I explore the physiological responses of two species of intertidal algae over the course of a tidal cycle. First, I assess baseline physiological performance under a simulated high tide condition. Next, I explore how these intertidal algae respond to the stresses associated with low tide. I investigate the effects of light, temperature, and desiccation in isolation and in combination to determine whether physiological differences underlie differences in habitat distributions (in tidepools vs. out of tidepools). Lastly, I quantify the ability of these species to recover photosynthetic rates after emergence during a low tide.

To investigate these patterns, I examine the link between physiology and habitat in two species of intertidal coralline red algae. Coralline red algae (Order Corallines, Phylum Rhodophyta) deposit calcium carbonate in the form of calcite in their cell walls. They are important components of the mid-low intertidal zone of exposed rocky coasts (Stephenson and Stephenson 1972, Johansen l98l, Stewart 1982, Grahame and Hanna 1989, Dye 1993, Benedetti-Cecchi and Cinelli 1994), and major competitors for space (McClanahan 1997).

They provide key ecological functions, such as cementing carbonate fragments into reef structures (Adey 1998, Björk *et al.* 1995). In addition, articulated coralline algae serve as refugia for invertebrates from predation (Coull and Wells 1983, Pawlak 2008), wave exposure (Dommasnes 1969), and desiccation (Gibbons 1988). Investigating the responses of coralline algae to environmental perturbations will help us understand the fundamental patterns and drivers of rocky intertidal community structure along the shore.

Two species of coralline algae are examined in this study: *Calliarthron tuberculosum* (Postels and Ruprecht) E.Y. Dawson and *Corallina vancouveriensis* Yendo, hereafter referred to as *Calliarthron* and *Corallina*. Both species are commonly found in the intertidal zone throughout the Northeast Pacific (Foster 1975, Padilla 1984). *Corallina vancouveriensis* has a wide distribution, from the Aleutian Islands to the Galapagos Islands (Abbott and Hollenberg 1976). Furthermore, the genus *Corallina* is found throughout the world, including warm waters, suggesting that at least some species are adapted to high light conditions and warm temperatures. The environmental tolerance of *Corallina* is reflected in its habitat; it is strictly found in the intertidal zone where it frequently grows out of tidepools or near the surface of tidepools (Padilla 1984). Furthermore, it is the only articulated coralline species that can survive emergence (Abbott and Hollenberg 1976). The genus *Calliarthron,* on the other hand, is endemic to the Northeast Pacific, occurring from Alaska to Mexico (Abbott and Hollenberg 1976) and is most abundant subtidally (Konar and Foster 1992), suggesting that it is well adapted to low light conditions and cooler temperatures. However, it extends into the low intertidal zone if deeply immersed in tidepools.

Padilla (1984) concluded that morphological differences between these two genera likely play a role in habitat separation. *Corallina* has delicate branches and a thick, bush-like form,

features which allow it to hold water like a paintbrush, thus avoiding desiccation (Figure 1). *Calliarthron,* on the other hand*,* has thick, open branches, making it susceptible to desiccation (Padilla 1984, Martone *et al.* 2010, Figure 1). Although morphological differences likely play a role in differentiating habitat distributions of *Calliarthron* and *Corallina*, it is unknown whether physiological differences also underlie their distributions within the intertidal zone.

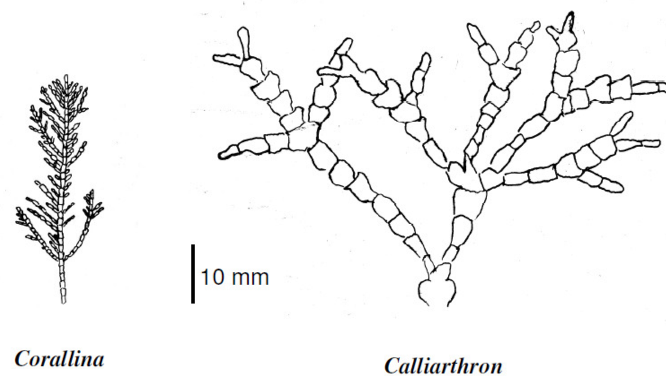


Figure 1: Line drawings of *Corallina* and *Calliarthron* showing morphological differences between the two genera.

In Chapter 1, I examine the physiological state of *Calliarthron* and *Corallina* during high tide using light response curves and pigment analyses. I test the hypothesis that *Corallina* is adapted to high light environments, given its propensity to live out of tidepools, whereas *Calliarthron* is adapted to low light conditions, given its tendency to remain submerged. I also quantify light harvesting pigments (chlorophyll *a* and phycobilins) and photoprotective pigments (carotenoids) to establish baseline differences in pigment profiles between the two species. Given the habitat distributions of *Calliarthron* and *Corallina*, I predict that *Corallina* has a lower concentration of light harvesting pigments and a higher concentration of photoprotective pigments than *Calliarthron*.

In Chapter 2, I examine both the morphological and physiological strategies for resisting stress in *Calliarthron* and *Corallina* during a simulated daytime low tide. First, I quantify the physiological response of these two species to increased light and temperature, as if submerged in a warm tidepool when the tide recedes. Second, I compare the physiological responses of *Calliarthron* and *Corallina* to aerial emersion during daytime low tide. I test the hypothesis that *Corallina* is able to briefly photosynthesize in air, but as hydration decreases, photosynthetic rates decrease. I also quantify light harvesting and photoprotective pigments to determine if desiccation results in pigment degradation. I test the hypothesis that with increasing desiccation, pigments degrade in both species, but that pigments degrade more rapidly in desiccation-susceptible *Calliarthron*. Finally, I compare the ability of these two species to delay desiccation morphologically. I hypothesize that *Corallina* retains water in its branches and resists desiccation for a longer period of time, whereas *Calliarthron* is not able to retain water in its branches and is more susceptible to desiccation.

In Chapter 3, I explore the ability of *Calliarthron* and *Corallina* to recover upon the return of the tide. In the first experiment, I test the ability of each species to recover after exposure to warm temperatures, simulating the flushing of cool water into a warm tidepool when the tide returns during a daytime tidal cycle. Next, I investigate the ability of *Calliarthron* and *Corallina* to recover from the combination of temperature and desiccation stresses resulting from emersion during low tide. I hypothesize that *Corallina* is able to recover rapidly from desiccation and temperature stresses, contributing to its ability to live outside tidepools, but that *Calliarthron* is not be able to recover from emergent stress, relegating intertidal populations to tidepools.

This study identifies physiological and morphological strategies employed by *Calliarthron* and *Corallina* to resist environmental stresses associated with the tidal cycle. 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 the two species.

Chapter 1

A Baseline Physiological Comparison of *Calliarthron* and *Corallina*

1.1 Introduction

High tide conditions are generally considered to be non-stressful for intertidal species, which despite sometimes having the ability to tolerate terrestrial conditions, have evolved in the marine environment. At high tide, intertidal algae experience low levels of light, cool water temperatures and no desiccation stress.

Marine algae are exposed to temporal and spatial variations in the light environment (Colombo-Pallotta *et al.* 2006). During high tide, the light environment changes drastically, namely, light intensity decreases and light quality changes (Jerlov 1976). Underwater, light levels are significantly reduced (0-800 $\mu mol/m^2/s$), compared to 1500-2500 $\mu mol/m^2/s$ when emerged at low tide in the summer months (Larkum and Barrett 1983). Additionally, red and blue wavelengths of light are reduced and blue-green light dominates (Ramus *et al.* 1976).

In response to the variation in the light environment, many algae can adjust their pigment profiles rapidly (Beach 1996, Colombo-Pallota *et al.* 2006). At high tide, algae have increased light harvesting accessory pigments to maximize light harvesting under a reduced light environment (Sagert *et al.* 1997). Accessory pigments, such as phycobilins, are also upregulated at high tide (Sagert *et al.* 1997), assisting algae in light harvesting under blue-green wavelengths that dominate at high tide.

Corallina and *Calliarthron* occupy different zones within the intertidal zone, and so, they likely are adapted to different light environments. I hypothesize that *Corallina* is adapted to high light environments and has lower photosynthetic rates than *Calliarthron* when submerged in low light conditions. *Calliarthron* likely is adapted to low light, so I

hypothesize that it has higher photosynthetic rates under low light conditions, and likely becomes photoinhibited at high irradiances.

In relation to pigments, I hypothesize that *Corallina* has a lower concentration of light harvesting pigments and a higher concentration of photoprotective pigments compared to *Calliarthron*. The differences in light acclimation and pigmentation between *Calliarthron* and *Corallina* explored in this study may be the first step in explaining habitat separation between the two species.

1.1.1 Light Response Curves

In plants, physiological health and localized adaptation is often evaluated by measuring O_2 consumption (respiration) and O_2 evolution (photosynthesis) by constructing light response curves (See Figure 2). Curves are generated by measuring O_2 consumed and produced by photosynthetic tissue at increasing light levels. In darkness, plants only respire, as there is no light for photosynthesis. However, when the plant is exposed to light, photosynthesis steadily increases until the tissue is maximally saturated and cannot utilize light at a higher rate.

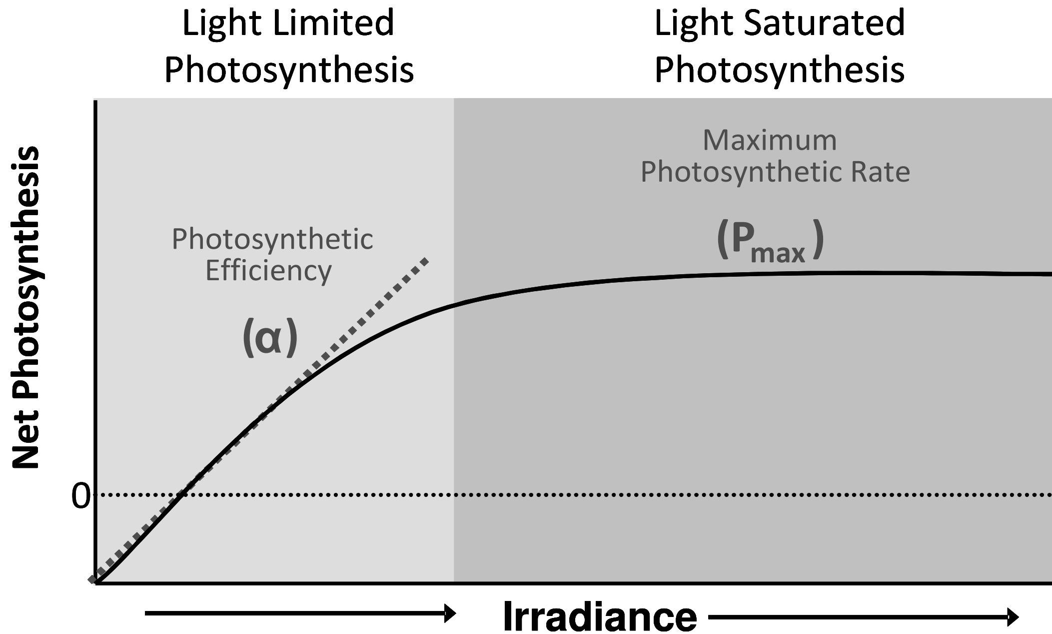


Figure 2: A typical light response curve showing light limited and light saturated regions of photosynthesis. The slope of photosynthesis within the light limiting region is the photosynthetic efficiency (α). Maximum photosynthetic rate (P_{max}) is shown within the light saturated region.

Several informative parameters can be inferred from a light response curve and can be used to evaluate physiological condition: compensation point (I_c), saturation point (I_k), photosynthetic efficiency (α) and maximum photosynthetic rate (P_{max}).

Initially, light response curves display a linear increase in photosynthetic rate with increasing light; this region of the curve is termed the "light limited region". In this region, light availability limits the photosynthetic rate. The slope of this line represents the efficiency (α) of the photosynthetic apparatus, and indicates how efficiently the plant converts incident light into energy. Therefore, a photosynthetic efficiency (α) of 0 corresponds to no light being used in photosynthesis, whereas a photosynthetic efficiency of 1 corresponds to all light being used for photosynthesis (Taiz and Zeiger 2002). Plants that are adapted to low light generally have higher photosynthetic efficiencies than high light adapted plants (Taiz and Zeiger 2002). Since *Calliarthron* is more abundant subtidally and

only lives in the intertidal zone if deeply submerged in tidepools, I expected *Calliarthron* to have a higher photosynthetic efficiency than *Corallina*.

The irradiance level at which photosynthesis equals respiration is termed the light compensation point (I_c). Plants that have a low compensation point require less light to overcome respiration and balance respiration and photosynthesis. The compensation point varies both intraspecifically and interspecifically. Intraspecific variation is often due to exposure to different light environments during development (Boardman 1977, Prezelin and Sweeney 1978, Taiz and Zeiger 2002), whereas interspecific variation is likely the result of different genotypes. In general, plants adapted to low light environments have lower compensation points than plants adapted to high light environments. Since *Calliarthron* lives in low light environments relative to *Corallina*, I predict that *Calliarthron* has a lower compensation point than *Corallina*.

The light response curve continues to increase linearly until further increases in light no longer result in higher photosynthetic rates and the curve levels off. The light level at this point is termed the saturation irradiance (I_k) and represents the beginning of the "carbon limited" or "light saturated" region. In this region, light availability no longer affects photosynthetic rates. Instead, other light saturated reactions, such as electron transport rate, RuBisCO (ribulose-1,5-bisphosphate carboxylase-oxygenase) activity, or the metabolism of triose phosphates limit photosynthesis (Taiz and Zeiger 2002). Plants adapted to high light environments generally have a higher saturation point than plants adapted to low light environments. And so, if *Corallina* is high light adapted, I predict *Corallina* to have a higher saturation point than *Calliarthron*.

The net photosynthetic rate at which photosynthesis becomes saturated and the curve levels off is termed the maximum photosynthetic rate (P_{max}). In this region of the light response curve, differences in enzymatic processes and metabolism of triose phosphates influence the maximum photosynthetic rate. This results in differences in maximum photosynthetic rate due to species specific differences. Differences in maximum photosynthetic rates can also result from differences in light adaptation; maximum photosynthetic rates may vary depending upon the irradiance levels experienced during development; the maximum photosynthetic rate tends to be higher in plants grown at higher irradiances (Boardman 1977, Prezelin and Sweeney 1978). High light adapted plants generally have more photosynthetic units (PSU) than low light adapted plants (Lobban and Harrison 1997). As more light is required to saturate the greater number of reaction centres, high-light adapted plants tend to have higher maximum photosynthetic rates relative to low light adapted plants (Taiz and Zeiger 2002). Thus, I hypothesize that *Corallina* has a higher maximum photosynthetic rate than *Calliarthron*.

Variation in the parameters, photosynthetic efficiency, compensation point, saturation point, and maximum photosynthetic rate, can affect the overall shape of a light response curve (Figure 3). Aside from species-specific variation, abiotic factors such as high light and high temperature are chief contributors to this variation. When a plant is exposed to very high light for a short period of time it may become dynamically photoinhibited (Figure 3A, Curve 2). Photoinhibition reduces photosynthetic efficiency (α) due to the diversion of absorbed light energy towards photoprotective processes, such as heat dissipation (Taiz and Zeiger 2002, Fork *et al.* 1986). As long as the high light exposure is brief, the chance of permanent damage to the photosynthetic tissue is low and the maximum photosynthetic rate

(P_{max}) remains unchanged (Henley 1993, Taiz and Zeiger 2002, Figure 3A, curve 2). If the exposure is long, however, the light response curve will show a reduction in both photosynthetic efficiency and maximum photosynthetic rates (Henley 1993, Figure 3A, curve 3). If photoinhibition occurs during the measurement of the light response curve, a downward trend at high light irradiances is seen (Henley 1993, Figure 3A, curve 4). Temperature fluctuations can cause P_{max} to change without changing photosynthetic efficiency (Henley 1993, Figure 3B). Although light is the primary factor dictating photosynthetic rates in the "light limited" region, rates in the "light saturated" region are affected by temperature sensitive enzymatic processes in the photosynthetic apparatus (Figure 3B). As temperatures increase, photosynthetic rates increase up to a point due to increased processing speeds of enzymes, after which high temperatures damage the photosynthetic apparatus and can cause proteins and enzymes to denature (Taiz and Zeiger 2002, Bidwell 1985).

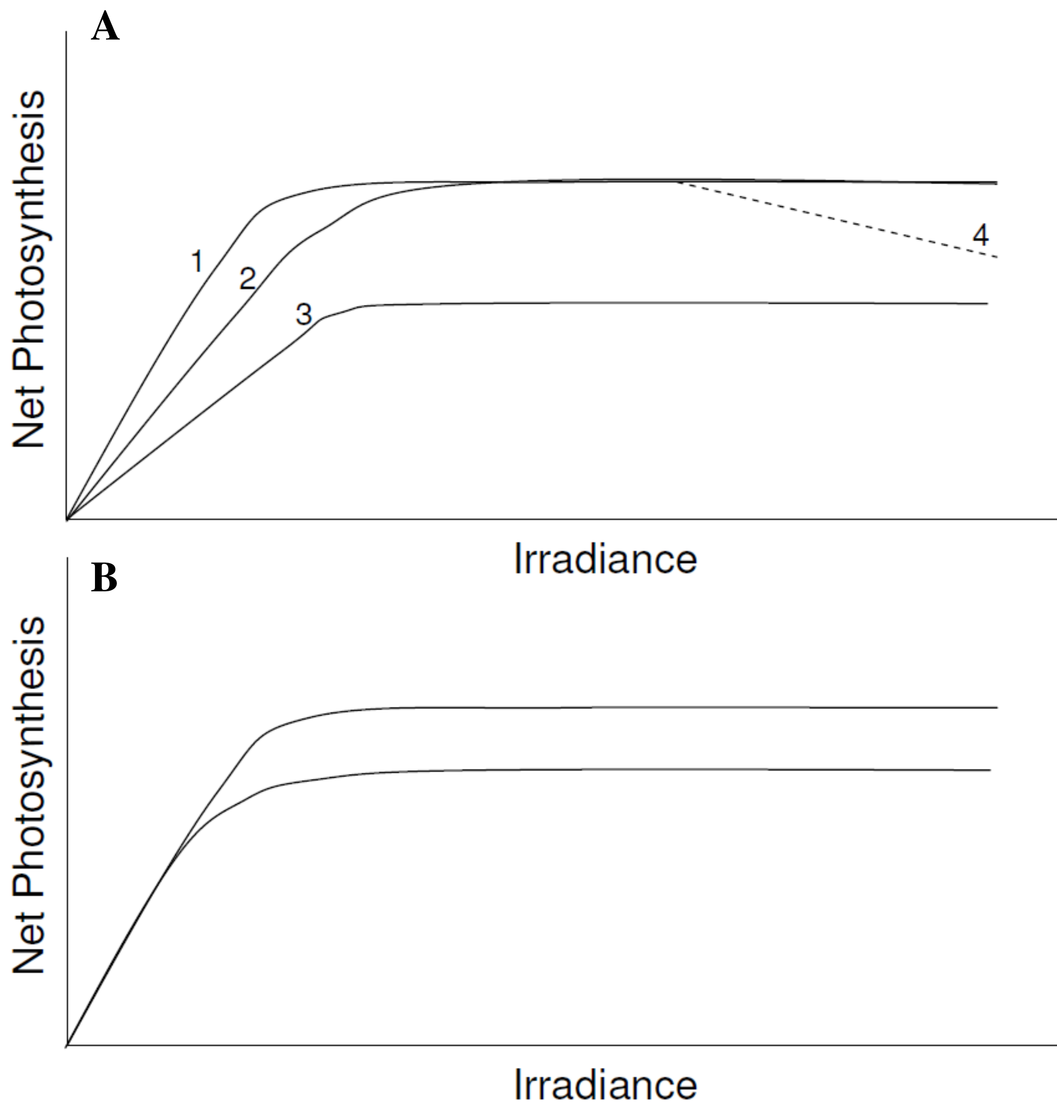


Figure 3 A: Curves are constructed from brief photosynthetic measurements (e.g. <5 min at each irradiance). Curve 1: unstressed alga (control curve); Curve 2: after brief exposure to inhibitory light, $I_{max,}$ showing a reduction in photosynthetic efficiency (α); Curve 3: after long exposure to inhibitory light, $I_{max,}$ showing a reduction in both photosynthetic efficiency (α) and maximum photosynthetic rate, $P_{max;}$ Curve 4: response for an unstressed alga if photoinhibition occurs during measurement of the curve. B: Light responses of two individuals having identical α but different P_{max} and the resultant differences in I_k. (Figure redrawn from Henley 1993).

Differences in light response curves also reflect adaptation to different light environments (Boardman 1977, Prezelin and Sweeney 1978). In this study, I consider sun

plants to be synonymous with high light adapted plants, and shade plants to be synonymous with low light adapted plants. Typically, sun plants have lower photosynthetic efficiencies (α) and higher maximum photosynthetic rates (P_{max}) than shade plants (Figure 4). Sun and shade plants compensate and saturate photosynthesis at different irradiances. For land plants adapted to high light environments (sun plants), the compensation point (I_c) typically ranges between 10 and 20 μmol/m^2/s, whereas low light adapted plants (shade plants) typically compensate between 1 and 5 μmol/m^2/s (Taiz and Zeiger 2002). Because light decreases with depth, subtidal marine macroalgae exhibit compensation and saturation points similar to shade plants, whereas intertidal macroalgae are similar to sun plants. For example, compensation points for intertidal algae have been documented in the range of 8-20 μmol/m^2/s (Arnold 1980, Eggert and Wiencke 2000), similar to sun plants. Markager and Sand-Jensen (1992) found compensation irradiances in the range of 0.3-2.7 μmol/m^2/s for five species of algae adapted to low light; these values are similar to shade plants. The saturation point often reflects the average light level a plant is exposed to during growth (Taiz and Zeiger 2002). Deep, subtidal species require less than 100 μE/m^2/s to reach saturation (Lüning 1981); mid to low intertidal species require 150-250 μE/m^2/s (Lüning 1981), similar to sun plants (Taiz and Zeiger 2002). The units of irradiance (μE/m^2/s and μmol/m^2/s) are approximately equal (Thimijan and Heins 1983).

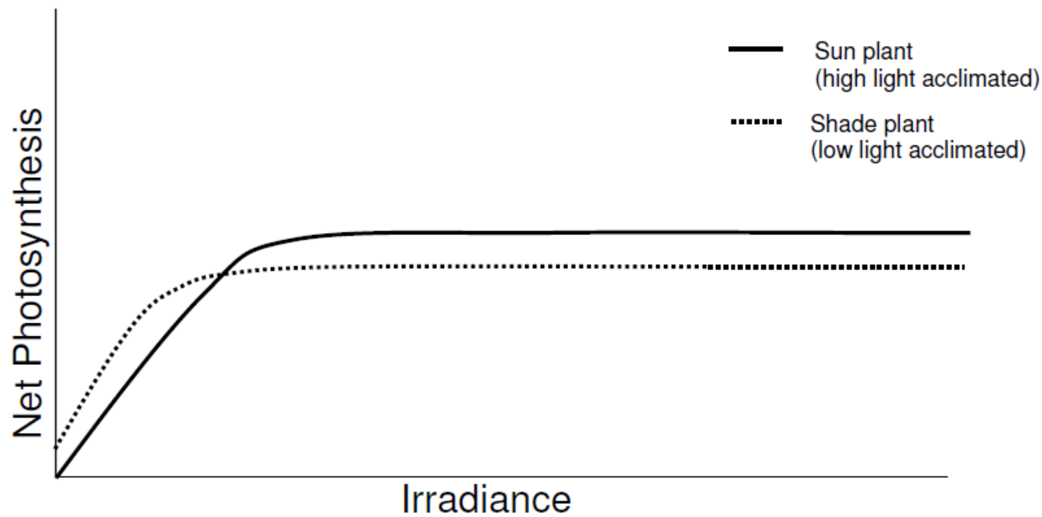


Figure 4: Theoretical light response curve for sun and shade adapted plants.

1.1.2 Pigmentation

Sun and shade plants exhibit differences in the organization and concentration of photosynthetic and photoprotective pigments (Larkum and Barett 1983). Differences in photosynthetic properties often correspond to differences in the photosynthetic apparatuses of sun and shade plants. Shade plants typically have fewer photosystem I (PSI) and photosystem II (PSII) reaction centres but larger antennae with more light harvesting pigments (Larkum and Barrett 1983), whereas sun plants tend to have more PSI and PSII reaction centres but smaller antennae. Therefore, shade plants are adapted to collect as much light as possible, whereas sun plants are adapted to efficiently convert light into energy and shuttle excess light toward photoprotective processes.

Generally, shade plants have larger chloroplasts, higher concentrations of chlorophyll *a*, more light harvesting pigments, and more chloroplasts per cell (Boardman 1977, Larkum and

Barrett 1983). Algae grown at low irradiances in the laboratory contained more light harvesting pigments than those grown at high irradiances (Waaland *et al.* 1974, Levy and Gantt 1988), and this trend is consistent with field data (Larkum and Barrett 1983, Rosenberg and Ramus 1983). Algae that are exposed to high irradiances, such as those algae living outside tidepools, have lower concentrations of chlorophyll *a* and accessory pigments than algae exposed to lower light irradiances, such as those living subtidally or deeply submerged in tidepools (Sagert 1997, Colombo-Pallota 2006). Additionally, decreases in phycobilisome number have been documented for algae grown in high irradiances (Staehelin *et al.* 1978).

Certain carotenoids, on the other hand, protect the photosynthetic apparatus of algae from photooxidative damage by quenching excited state chlorophyll molecules and reactive oxygen radicals (Larkum and Barrett 1983, Sierfermann-Harms 1987, Rowan 1989). Their levels tend to increase when algae are exposed to abiotic stressors such as high light (Sierfermann-Harms 1987) and desiccation stress (Sampath-Wiley *et al.* 2008). Since *Corallina* is likely adapted to a higher light environment and might experience more desiccation stress during emersion, I hypothesize that *Corallina* has more carotenoid pigment per unit biomass than *Calliarthron*.

1.1.3 Chapter Objectives

In order to explore the physiological response of marine macroalgae to environmental stress associated with low tide, it is necessary to document baseline physiological performance under non-stressful conditions. These baseline data provide a context in which to evaluate shifts in performance and allow for broad conclusions to be made about physiological performance.

This chapter explores the physiological differences between *Calliarthron* and *Corallina* under non-stressful submerged conditions. I generate light response curves to quantify differences in photosynthetic efficiencies (α), compensation points (I_c), saturation points (I_k) and maximum photosynthetic rates (P_{max}). I also extract light-harvesting and photoprotective pigments to compare pigment concentrations between the two species. Together, light response curves and pigment analyses allow for the exploration of adaptive differences between *Calliarthron* and *Corallina*. Since *Corallina* often is found growing outside tidepools during low tide, I hypothesize that it will perform physiologically similar to a sun plant with a low photosynthetic efficiency (α) and a high P_{max}. In relation to pigmentation, high-light adapted *Corallina* is expected to have a low concentration of light harvesting pigments (chlorophyll *a* and phycobilins) and a high concentration of photoprotective pigments (carotenoids). *Calliarthron* is exclusively found in areas that remain submerged throughout the tidal cycle; therefore, I hypothesize that it will perform physiologically like a shade plant, with a higher photosynthetic efficiency (α), and a lower $P_{max.}$ I also predict that *Calliarthron* will have a greater concentration of light harvesting pigments (chlorophyll *a* and phycobilins) and a lower concentration of photoprotective pigments (carotenoids).

1.2 Methods

1.2.1 Specimen Collection and Laboratory Conditions

Specimens were collected from Botanical Beach near Port Renfrew, British Columbia (48°31’03.18”N, 124°26’03.19”W) in July 2010 for light response curves, and from Sombrio Beach near Port Renfrew, British Columbia (48°30’04.19”N, 124°17’53.67”W) in August

2010 for pigment analyses. Individual fronds from different plants were collected haphazardly from tidepools in the low and mid intertidal zone. Fronds of *Calliarthron* were collected from tidepools, and fronds of *Corallina* were collected from outside the same tidepools. Specimens were transported to the lab at the University of British Columbia within 12 hours and were maintained in the dark, submerged in cooled seawater during transport.

Once in the lab, specimens were maintained at a naturally occurring photoperiod in a re-circulating seawater table at 12ºC, and dim light (5-10 µmol photons/m^2/s) at the University of British Columbia. Specimens were acclimated to lab conditions for approximately two days before beginning experiments. All experiments were completed within one week of collection.

1.2.2 Experimental Conditions

1.2.2.1 Light Response Curves

Photosynthetic rates were determined by oxygen concentration changes over a period of 5 minutes in natural seawater (12ºC). Figure 5 shows the experimental apparatus. Neofox oxygen and temperature probes (Ocean Optics) 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. Plumber's putty was used to seal the sample vial and ensure the system was air tight. Light was provided by a slide projector (Kodak Ektagraphic). This light source was chosen in order to obtain high light intensities such as would occur in the field, however, this light does not include the same wavelengths of light that occur in natural sunlight. Light levels were manipulated with a series of wire screens. A Li-Cor 250A light meter (LI-COR Biosciences, Lincoln, Nebraska)

with a Li-Cor 190 quantum sensor (LI-COR Biosciences, Lincoln, Nebraska) was used to take an average of five light measurements for each irradiance level. Water temperature was controlled with a re-circulating water chiller that was connected to tubing and a copper coil in a water bath. A stir plate was placed beneath the water bath to maintain water motion in the sample vials and ensure an even distribution of dissolved oxygen concentration via the stir bar.

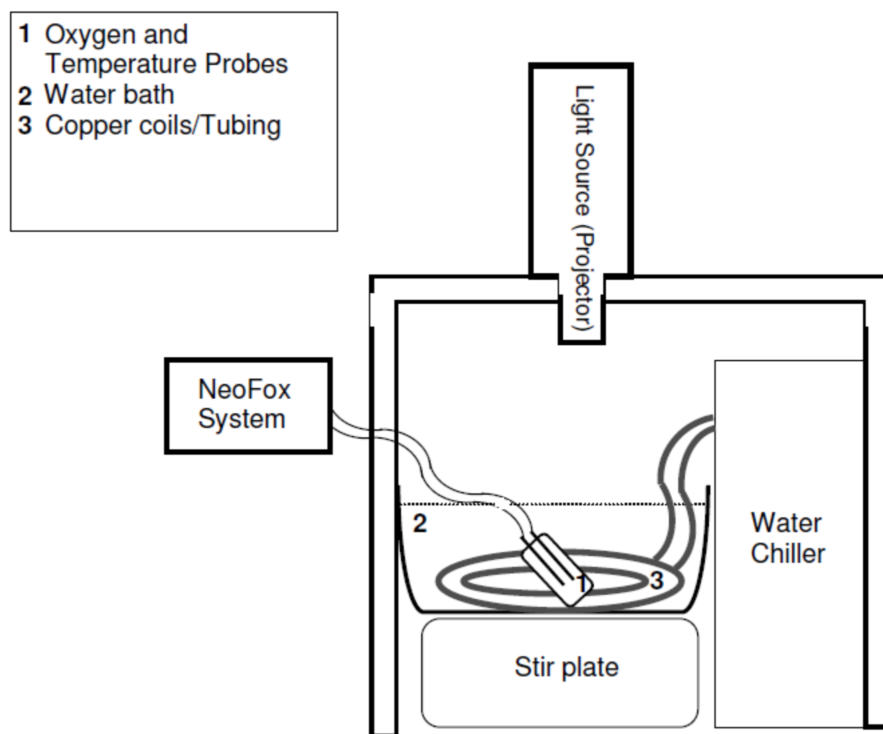


Figure 5: Experimental apparatus for measurement of photosynthesis.

Apical tissue of coralline fronds was used in all experiments (approximately 2 cm long, 100-300 mg). Specimens were cleaned with a soft brush to remove epiphytes and invertebrates before physiological measurements were taken. Coralline fronds were 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 in subsequent photosynthetic experiments (Chapters 2 and 3).

Oxygen evolution or consumption was measured at increasing irradiances (0, 12, 20, 31, 52, 89, 144, 245, 415 and 716 μmol photons/m^2/s) at 12ºC in submerged conditions. Before beginning light response curves, respiration was measured in darkness (0 μmol photons/m^2/s) by covering the entire water bath with a dark cloth. For each species, light response curves were constructed for five replicate fronds by measuring the rate of change in oxygen concentration over a five minute sampling period at each irradiance level. Photosynthetic rate was calculated in μmol O_2/g dry weight/minute. Dry weight of each sample was measured after drying the sample for 48 hours at 68ºC. The amount of calcified tissue was subtracted from weight measurements according to previously determined calcified to non-calcified dry weight ratios. The amount of calcium carbonate within species of coralline algae is highly conserved; *Calliarthron* fronds have 84 ± 0.3% $CaCO_3$ and *Corallina* fronds have 64 ± 0.7% $CaCO_3$ (K. Fisher and P. Martone, unpubl. data).

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

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

Where: P_{net} = Net Photosynthetic Rate (μmol O_2/gDW/min)
P_{max} = Maximum Photosynthetic Rate (μmol O_2/gDW/min)
P_0 = Respiration (μmol O_2/gDW/min)
α = Photosynthetic efficiency

I = Irradiance ($\mu mol/m^2/s$)

Mean α, mean respiration rate 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):

$$I_c = R_d/\alpha$$
$$I_k = (P_{max}+R_d)/\alpha$$

Where: R_d = dark respiration
α = photosynthetic efficiency

One-way ANOVAs were performed on the raw data (n=5) to test for differences in I_c, I_k, α and P_{max} among species.

1.2.2.2 Quantification of Light Harvesting and Photoprotective Pigments

Sub-samples for pigment analysis were taken before applying any treatment, frozen in liquid nitrogen and stored at -80°C until pigment analyses were performed. Phycobilin pigments were extracted first, followed by chlorophyll *a* and carotenoids. Samples were first ground with a mortar and pestle, and the sample was extracted in sodium phosphate buffer (0.1 M, 6.8 pH) at 4°C for 2 hours in darkness. The sample was then centrifuged (14000 rpm, 3 min) and the supernatent with the phycobilin pigment 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 re-suspended with 90% acetone overnight in darkness at 4°C to extract chlorophyll *a* and carotenoid pigments. The sample was centrifuged as above and pigment concentrations of the supernatent were again determined with a spectrophotometer. Repeated extractions according to the above protocol were then performed to remove all

chlorophyll *a* and carotenoid pigments from samples. Chlorophyll *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 pigment composition between species were tested using independent sample t-tests.

All pigment data was analyzed using SPSS 17.0 (SPSS Inc., Chicago, IL, USA), and a significance level of $p<0.05$ was considered to be significant. Normality for all statistical tests was confirmed with the Shapiro-Wilk test, and equal variance was confirmed with Levene's test.

1.3 Results

1.3.1 Light Response Curves

Light response curves are shown in Figure 6. The respiration rates between the two species were not significantly different ($p=0.36$, Table 1). *Calliarthron* had a respiration rate of -0.36 ± 0.05 µmol O_2/gDW/min (mean ± Std. error) and *Corallina* had a respiration rate of -0.27 ± 0.07. The maximum photosynthetic rates for *Calliarthron* and *Corallina* were significantly different ($p < 0.01$, Table 1). *Corallina* had a maximum photosynthetic rate of 2.14 ± 0.19 µmol O_2/gDW/min and *Calliarthron* had a maximum photosynthetic rate of 1.07 ± 0.19 µmol O_2/gDW/min (Table 1). *Corallina* had approximately twice the maximum photosynthetic rate as *Calliarthron*. The photosynthetic efficiencies (α) of *Calliarthron* and *Corallina* were not significantly different ($p = 0.41$, Table 1). *Corallina* had a photosynthetic efficiency of 0.01 ± 0.002 and *Calliarthron* had a photosynthetic efficiency of 0.02 ± 0.002 (Table 1). The compensation irradiances (I_c) of *Calliarthron* and *Corallina* were not significantly different ($p = 0.59$, Table 1). *Corallina* reached its compensation irradiance at 24.4 ± 7.2 µmol/m^2/s and *Calliarthron* reached its compensation point at 20.2 ± 1.7 µmol/m^2/s (Table 1). There was a significant difference in the saturation irradiance of

Calliarthron and *Corallina* ($p < 0.01$, Table 1). *Calliarthron* reached its saturation point at 83.9 ± 8.1 μmol/m^2/s, and *Corallina* reached its saturation point at 223.8 ± 41.6 μmol/m^2/s (Table 1).

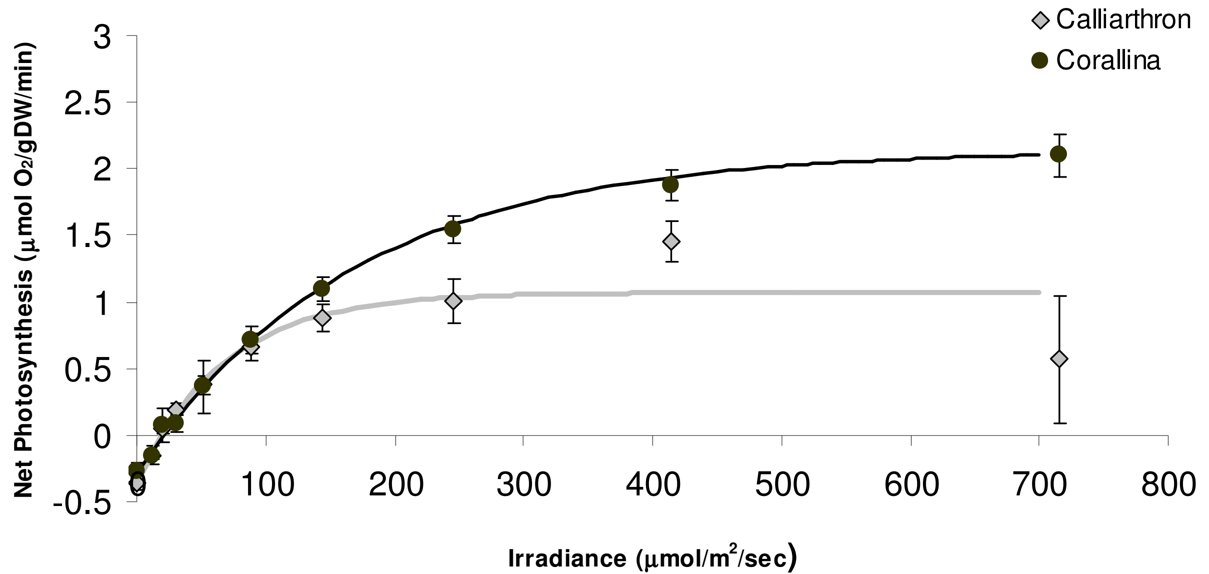


Figure 6: Net photosynthetic rates as measured by oxygen evolution by irradiance level. Smoothed lines are photosynthetic model (Webb *et al.* 1974), using estimated parameters P_{max} and α. Means ± SE, n=5.

Table 1: Photosynthetic Efficiency, Maximum Photosynthetic Rates, Compensation Irradiance, and Saturation Irradiance of *Calliarthron* and *Corallina*, 12°C, submerged. n=5. (Mean ± Std. Error)

	Calliarthron	*Corallina*	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***

1.3.2 Light Harvesting and Photoprotective Pigments

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 2). *Corallina* had 1.59 ± 0.13µg chlorophyll *a*/mg sample and *Calliarthron* had 1.49 ± 0.12 µg chlorophyll *a*/mg sample (p = 0.60, Table 2). *Corallina* had 0.99 ± 0.14 µg phycoerythrin/mg sample and *Calliarthron* had 1.43 ± 0.20 µg phycoerythrin/mg sample (p = 0.09, Table 2). *Corallina* had 0.35 ± 0.07 µg phycocyanin/mg sample and *Calliarthron* had 0.39 ± 0.08 µg phycocyanin/mg sample (p = 0.72, Table 2). *Corallina* had 0.45 ± 0.11 µg allophycocyanin/mg sample and *Calliarthron* had 0.61 ± 0.18 µg allophycocyanin/mg sample (p = 0.46, Table 2). *Corallina* had 0.57 ± 0.07 µg carotenoid/mg sample and *Calliarthron* had 0.40 ± 0.06 µg carotenoid/mg sample (p = 0.07, Table 2).

Table 2: Pigment concentrations of *Corallina* and *Calliarthron* under control conditions. Means ± Std. Error

Pigment (µg/mg sample)	*Corallina*	*Calliarthron*	p-value
Light Harvesting			
Chlorophyll *a*	1.59 ± 0.13 (n=13)	1.49 ± 0.12 (n=14)	0.60
Phycoerythrin	0.99 ± 0.14 (n=13)	1.43 ± 0.20 (n=14)	0.09
Phycocyanin	0.35 ± 0.07 (n=13)	0.39 ± 0.08 (n=14)	0.72
Allophycocyanin	0.45 ± 0.11 (n=13)	0.61 ± 0.18 (n=14)	0.46
Photoprotective			
Carotenoid	0.57 ± 0.07 (n=13)	0.40 ± 0.06 (n=14)	0.06

1.4 Discussion

1.4.1 Light Adaptation

Exploring the baseline physiological performance of *Calliarthron* and *Corallina* is the first step toward understanding the spatial separation of these two species in the intertidal zone. Intertidal *Calliarthron* fronds rarely live outside tidepools whereas intertidal *Corallina* fronds frequently live outside tidepools on bedrock. Given this distribution, I predicted that *Calliarthron* would perform like a shade adapted plant, whereas *Corallina* would perform like a sun adapted plant. The physiological data presented here does not fully support this hypothesis, since both species had similar values of light limited parameters (photosynthetic efficiency and compensation point). However, trends are suggestive of differences in light acclimation since species exhibited different values for light saturated parameters (maximum photosynthetic rate and saturation point).

Calliarthron and *Corallina* performed similarly in low light conditions. The two species had similar photosynthetic efficiencies, compensation points, and respiration rates when light was limiting. Performance in low light may be explained by the similar pigment profiles of the two species (Table 2). Photosynthetic efficiency is determined not only by the amount of incident light but also by the light harvesting ability of the alga, which is related to the amount of light harvesting pigments (Larkum and Barett 1983). Because *Calliarthron* and *Corallina* have similar light harvesting pigment concentrations (chlorophyll *a* and phycobilins), they likely have the same ability to harvest light. This data suggests that during high tide, when light levels are limiting, *Calliarthron* and *Corallina* photosynthesize at similar rates.

However, as light levels increased, the two species differed in their photobiology. *Corallina* exhibited significantly greater photosynthetic rates when light was saturating; the maximum photosynthetic rate of *Corallina* was approximately twice that of *Calliarthron.*

This suggests that *Corallina* performs like a sun adapted plant. Metabolic processes such as electron transport rate, RuBisCO activity, or the metabolism of triose phosphates may limit photosynthesis in *Calliarthron* in high light, but this remains to be determined. The differences in the light response curves may help to explain the habitat partitioning between *Calliarthron* and *Corallina*. Since *Corallina* has twice the maximum photosynthetic rate of *Calliarthron*, it performs best under high light conditions, explaining why it is commonly found outside tidepools or at the upper rim of mid-intertidal tidepools. *Calliarthron*, on the other hand, is likely to become photoinhibited at high irradiances, which may explain *Calliarthron*'s propensity to remain submerged where light levels are lower.

Calliarthron is not high light adapted, and is adapted to a lower light environment than *Corallina*. Although light limited photosynthetic parameters such as the compensation point and photosynthetic efficiency for *Calliarthron* were similar to *Corallina* and other sun plants, there were significant differences in light saturated parameters, such as saturation point and maximum photosynthetic rate. This data suggests that in the field, *Corallina* is twice as photosynthetically productive as *Calliarthron* when light levels are high, such as occurs during low tide.

Differences in the light-saturated parameters of the light response curve also suggest differences in the photosynthetic apparatuses of *Calliarthron* and *Corallina*, such as differing numbers of photosynthetic units (PSU's). Shade plants tend to have less PSU's but more pigments per PSU while sun plants tend have more PSU's and less pigment per PSU (Larkum and Barrett 1983, Lobban and Harrison 1997). This difference is reflected in the light response curves found for *Calliarthron* and *Corallina*. If *Corallina* has more PSU's,

then more light would be required to saturate all PSU's, resulting in a higher maximum photosynthetic rate, as was found in this study.

1.4.2 Pigmentation

Surprisingly, no significant differences were found in the light harvesting pigmentation of *Calliarthron* and *Corallina*. Due to habitat differences, I expected *Calliarthron* and *Corallina* to have different pigment profiles. Namely, I expected *Calliarthron* to have more light harvesting pigments than *Corallina* because it lives in a low light environment and therefore needs to collect light more efficiently than *Corallina*. However, both *Calliarthron* and *Corallina* performed similarly in the light limited region of the light response curve, which also explains their similarity in light harvesting pigment concentrations. In this region of the light response curve, photosynthetic rates are directly related to the ability of the alga to harvest light, which, in turn is correlated to the light harvesting pigmentation of the alga.

Previous studies have also documented a disparity between pigmentation and photosynthetic productivity. For example, Ramus *et al.* (1977) found that two species of rockweed, *Fucus vesiculosus* and *Ascophyllum nodosum*, had similar pigment profiles, but the maximum photosynthetic rate of *F. vesiculosus* was twice that of *A. nodoum*. Interestingly, in that study, *F. vesiculosus* (the more photosynthetically productive seaweed) was also the seaweed that occurred higher on the shore, similar to the trend found in this study for *Corallina*. This suggests that photosynthetic productivity and pigmentation are not necessarily directly correlated but that photosynthetic rates may be a determinant of algal zonation. Ramus (1977) also concluded that light quality and light quantity are not determinants of lower limits of macroalgae, rather, biotic factors such as competition and grazing dictate lower limits.

Because *Calliarthron* and *Corallina* live in different environments in the intertidal zone, it was hypothesized that they would have different photoprotective pigmentation. Living in higher light environments, *Corallina* would benefit from increased levels of photoprotective carotenoid pigmentation. However, despite differences in habitat, the two species had similar levels of carotenoid pigments. This is in contrast to the findings of other researchers. For example, Algarra and Niell (1990) found that carotenoid levels increased in sun morphotypes compared to shade morphotypes of a related coralline, *Corallina elongata*. The carotenoid values found in my study were slightly higher than those found by Algarra and Niell (1990); they are, however, within the standard deviation range as those found for *Corallina elongata*. In another high intertidal alga, *Porphyra umbilicalis*, carotenoid levels were in the range of 0.15 to 0.35 mg/g (Sampath-Wiley *et al.* 2008), slightly lower than the levels found in *Calliarthron* and *Corallina* in my study. However, carotenoid levels in *Porphyra* were significantly affected by emersion stress, but unaffected by sun versus shade habitats. This finding supports my observation that the photoprotective pigmentation between *Corallina* and *Calliarthron* did not differ due to sun or shade habitats, but does raise the possibility that desiccation stress might cause differences between the two species in pigmentation. This hypothesis will be further explored in the next chapter.

Similarities in pigment profiles among the two species may have resulted from pigment adjustments to conditions experienced in the lab. It has been well documented that algae can adjust pigmentation under varying light conditions. In my study, samples were collected in the field and maintained in the lab for several days before initiating pigment quantification experiments. Other researchers have observed short term pigment regulation in a related alga, *Corallina elongata*, where pigments shifted after just five hours of light treatment (Algarra

and Niell 1990). Figueroa *et al.* (1997) also found that photosynthetic pigments, chlorophyll *a* and phycobilins, changed throughout the day in *Porphyra leucosticta* depending on the light environment. This suggests that results for *Calliarthron* and *Corallina* may not be indicative of the pigmentation of the species found in the field, but suggests that the two species are able to adjust and regulate pigment levels equally at low light. If this same adjustment is occurring in the field, both species are likely to have the similar photosynthetic rates under low light conditions. However, the acclimation of pigment levels of *Calliarthron* and *Corallina* is in contrast to the findings for the differences in photosynthetic performance between the two species. Although both species may have acclimated pigmentation to lab conditions, the difference in photosynthetic performance can be explained by species-specific differences between *Calliarthron* and *Corallina* and suggests that, genetically, these two species are adapted to different light environments.

Chapter 2

A Physiological Comparison of *Calliarthron* and *Corallina* at Low Tide

2.1 Introduction

The upper boundary of macroalgal populations often is determined by tolerance to increased temperature, light and desiccation stress (Zaneveld 1969, Oates and Murray 1983), and intertidal algae are suddenly exposed to these stressors when the tide has receded. Excess light (Beer and Levy 1983, Häder *et al.* 2003, Sampath-Wiley *et al.* 2008), high temperature (Davison 1991, Bell 1993) and desiccation (Johnson 1974, Quadir *et al.* 1979, Bell 1993, Scrosati and Dewreede 1998) have been linked to zonation patterns along the shore.

When the tide is out, seaweeds experience high light levels that may cause photoinhibition (Davison and Pearson 1996). To adjust to varying light levels, many intertidal seaweeds enhance photosynthesis while submerged under non-stressful conditions and increase processes that dissipate excess energy when emerged (Davison and Pearson 1996). For example, *Porphyra* transfers energy away from PSII to protect itself from photodamage (Fork and Öquist 1981, Öquist and Fork 1982, Fork *et al.* 1986). Reductions in photosynthetic pigments have been linked closely to excess light exposure (Sampath-Wiley *et al.* 2008, Beach and Smith 1996). Sampath-Wiley *et al.* (2008) also found that reductions in photosynthetic pigments in *Porphyra umbilicalis* were affected more by light intensity than by emersion stress.

In general, algae can photosynthesize over a wide range of temperatures (Davison 1991); however, exposure to temperature extremes can cause irreversible damage to the photosynthetic apparatus (Berry and Björkman 1980). Plants that are grown in cool environments generally exhibit higher photosynthetic rates at low temperatures, and plants

grown in high temperatures have higher photosynthetic rates at warm temperatures (Berry and Björkman 1980). The high temperature tolerance of plants grown in high temperatures likely results from increased heat stability of the photosynthetic apparatus whereas low temperature tolerance in plants grown in low temperatures likely results from increased capacity of enzymatic steps of the photosynthetic process (Berry and Björkman 1980). Additionally, plants that are exposed to a wide range to temperature fluctuations naturally likely exhibit a greater potential for acclimation over a wide temperature range than do plants grown at relatively stable temperatures (Berry and Björkman 1980).

Desiccation stress impacts macroalgal physiology by reducing photosynthetic rates, carbon gain and growth (Dring and Brown 1982, Smith 1984, LaPointe *et al.* 1984, Matta and Chapman 1995). For example, photosynthetic processes shut down when exposed to severe desiccation due to an interruption in the electron transport between (PSII) and (PSI) (Bewley 1979). Desiccation can also cause the destruction of photosynthetic pigments (Sampath-Wiley *et al.* 2008, Martone *et al.* 2010). For example, in *Calliarthron tuberculosum*, a reduction of photosynthetic pigments, resulting in bleaching, has been documented to occur in response to desiccation stress alone (Martone *et al.* 2010).

In the intertidal zone, algae are exposed to abiotic factors, such as increased light, temperature and desiccation simultaneously. Therefore, understanding how these factors interact is important. For example, the effect of temperature on algal photosynthesis is dependent on whether light is saturating or sub-saturating (Davison 1991). Light saturated photosynthesis is much more sensitive to temperature than is light limited photosynthesis due to the sensitivity of enzymatic processes to temperature (Davison 1991). In *Fucus spiralis*, photosynthetic rates were greater in the water than in air at warm temperatures, but the

reverse was true at low temperatures (Madsen and Maberly 1990). Hunt and Denny (2008) demonstrated that desiccation can lead to thermotolerance in a high intertidal alga, *Endocladia muricata*, suggesting that desiccation can be beneficial to certain algae in tolerating other stressors. However, Martone *et al.* (2010) found that when combined, temperature, light and desiccation stress could induce significant pigment loss in *Calliarthron* fronds during emersion, more so than each factor individually.

Seaweeds resist environmental stress in several ways. For example, many algae are able to change levels of pigment concentration to acclimate to light and desiccation stress. For example, changes in carotenoid pigment concentration have been documented within the timeframe of a single tidal cycle (Sampath-Wiley *et al.* 2008) in response to increased light intensity and desiccation stress (Beach and Smith 1996, Sampath-Wiley *et al.* 2008). Conversely, to acclimate to reduced light levels, algae that are shaded or growing at depth often increase the concentration of light harvesting pigments (Ramus *et al.* 1977, Roberts 1996). These adjustments in pigment concentrations allow intertidal macroalgae to acclimate to the stressors associated with the low tide, and might underlie habitat differences between species in the intertidal zone.

Algae have evolved morphological strategies to delay or reduce desiccation. For example, some algae have sac-like morphologies that hold water for extended periods of time after being emersed (Oates 1986, Matta and Chapman 1995), others have highly branched or turf-like morphologies that can hold water like a paintbrush (Bell 1995, Hay 1981, Padilla 1984). Furthermore, dissected fronds desiccate at higher rates than bladed forms (Bell 1995).

Another way to limit some of the stresses associated with exposure during low tide is to live in tidepools (Metaxas and Scheibling 1993). In tidepools, organisms are exposed to less

temperature flux, reduced light and UV and no desiccation stress. Yet, tidepool organisms still may be negatively affected by increases in light and water temperature. For example, in Washington, USA, tidepool temperatures greater than 20°C have been recorded during the summer (Helmuth *et al.* 2002), compared to 9-12°C for ambient seawater. Furthermore, like algae, many animals find refuge in tidepools, avoiding desiccation and high temperatures during low tide (Dethier 1980). And so, macroalgae living in tidepools may be more susceptible to herbivory (Lubchenco 1978).

Surprisingly, some intertidal algae may actually benefit from low levels of desiccation. For example, some high intertidal seaweeds can photosynthesize in air, and emerged rates can exceed submersed photosynthetic rates (Johnson *et al.*, 1974, Quadir *et al.* 1979, Oates and Murray 1983, Bidwell and MacLachlan 1985, Oates 1985, 1986, Madsen and Maberly 1990). In such cases, emerged photosynthesis contributes significantly to the net carbon gain of the plant (Matta and Chapman 1995). Maximum photosynthetic rates in the air have a sharp peak with 10-20% desiccation (Quadir *et al.* 1979, Bell 1993, Madsen and Maberly 1990). As thalli dehydrate, the film of water coating the thallus surface, which inhibits gas exchange begins to evaporate. With a smaller boundary layer, gas exchange rates and hence photosynthetic rates increase. However, once the film has completely evaporated photosynthesis decreases as dehydration limits photosynthesis at the cellular level (Bell 1993). The reverse is true for species living in the low intertidal zone; species in this zone often photosynthesize better in the water than in the air (Kremer and Schmitz 1973, Johnson *et al.* 1974, Quadir *et al.* 1979). And so, the ratio of aerial photosynthesis to submerged photosynthesis correlates with the position of the alga on the shore.

Differential response to these environmental conditions contributes to differences in habitat usage along the shore (Wiltens *et al.* 1978, Hodgson 1981, Smith *et al.* 1982, Smith *et al.* 1986, Smith and Berry 1986).

2.1.1 Chapter Objectives

In this chapter, I compare the responses of *Calliarthron* and *Corallina* to the stresses associated with low tide. I explore differences in submerged photosynthesis, desiccation rates, aerial photosynthesis, and pigment degradation to determine whether habitat differences between the species can be explained by differences in stress tolerance.

First, I investigate the physiology of the two species in a submerged environment, simulating a tidepool increasing in temperature over the course of a low tide. Since *Corallina* often lives around the upper rim of tidepools, where submerged temperatures are highest, I predict that *Corallina* will not experience photosynthetic reductions with increasing temperature. On the other hand, I expect that since *Calliarthron* lives deeply submerged in tidepools, it will experience photosynthetic reductions with increasing seawater temperature.

Next, I investigate the ability of *Calliarthron* and *Corallina* to briefly photosynthesize in air. The morphology of *Corallina* undoubtedly provides desiccation resistance; however, it is unclear whether this is sufficient to allow it to survive outside tidepools. In this experiment, I explore physiological tolerance to emergence. I test the hypothesis that *Corallina* is able to photosynthesize in the air during emergence at low tide. This might permit *Corallina* to take advantage of aerial exposure. In tidepools, *Calliarthron* is rarely exposed to air, and therefore is unlikely to have the same ability.

Next, I explore pigment degradation with increasing desiccation stress, expanding upon the baseline pigment profiles of *Calliarthron* and *Corallina* described in Chapter 1. I predict

that desiccation will result in an increase of carotenoid pigment concentration along with the degradation of chlorophylls and phycobilins but that rates will differ between the two species. As an emergent species, *Corallina* might be more resistant to pigment degradation of chlorophylls and phycobilins, and might increase carotenoid pigments with increasing desiccation.

Finally, I quantify the ability of each species to delay desiccation through morphology. *Corallina* is finely branched sometimes forming turfs, and can hold water like a paint brush. Therefore, *Corallina* is able to resist desiccation through morphology, allowing it to live higher on the shore than other coralline algae. When *Corallina* fronds are thinned, thalli bleach and die within a few weeks (Padilla 1984) indicating that morphology is providing desiccation resistance and is critical to survival. Therefore, the fine branches of *Corallina* allow it to delay desiccation during low tide and live outside tidepools, but the extent of this ability has never been quantified.

2.2 Methods

2.2.1 Specimen Collection and Laboratory Conditions

Specimens were collected from Botanical Beach near Port Renfrew, British Columbia (48°31'03.18"N, 124°26'03.19"W) in July 2010 for submerged photosynthesis experiments. Specimens were collected from Sombrio Beach near Port Renfrew, British Columbia (48°30'04.19"N, 124°17'53.67"W) in August 2010 for emerged photosynthesis experiment. Specimens were collected from Prasiola Point near Bamfield, British Columbia (48°29'02"N, 125°10'06"W) in September 2010 for the desiccation tolerance experiment. All specimens were handled and transported to UBC as outlined in Chapter 2 except for the

desiccation tolerance experiment, which was completed at Bamfield Marine Sciences Centre (Bamfield, BC, Canada).

2.2.2 Experimental Set-up

2.2.2.1 Photosynthesis in Tidepools

To explore the effect of temperature and light on submerged photosynthesis, rates of oxygen production were measured as described previously (Chapter 1: Light Response Curve Section). This experiment was set up in a factorial design with three levels of temperature (12°C, 16°C, and 20°C) and two levels of light (sub-saturating: 50 μmol/m^2/s and saturating: 300 μmol/m^2/s). Sub-saturating and saturating light levels were selected by examining previously determined light response curves (Chapter 1, Fig. 1.5). Photosynthesis and respiration were measured in each treatment combination. Planned comparison t-tests were performed on net photosynthetic data from the high tide (12ºC, 50 μmol/m^2/s) treatment and the warm tidepool (20ºC, 300 μmol/m^2/s) treatment. All photosynthetic calculations were performed as described in Chapter 1.

To test the effect of light and temperature levels, a generalized linear model was performed on photosynthesis and respiration data for each species separately. The model was used because the data did not fit the ANOVA assumption of equal variances, and transformations were unsuccessful. In the generalized linear model, a normal distribution and an identity link function were used. The Shapiro-Wilk test confirmed that net photosynthetic data were normal for all treatment combinations. A two-way generalized linear model was performed for each species separately with temperature and light as fixed factors and net photosynthesis or respiration as the response variable.

2.2.2.2 Photosynthesis in Air

Emerged photosynthetic rates were tested for three different hydration levels. The treatments were: 'wet', 'blotted', and 'desiccated'. In the 'wet' hydration treatment, fronds were allowed to retain water within their branches. 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 55% ± 2% relative water content (RWC). To obtain comparable relative water contents for the two species, previously determined fresh weight to dry weight ratios were used to estimate approximate RWC. The desiccation level was determined by using this ratio to estimate the dry weight from the fresh weight of samples, which were weighed every five minutes. The dry weight of *Calliarthron* was found to be 74 ± 1.0% of the initial fresh weight (n=10); and the dry weight of *Corallina* was 63 ± 1.4% of the initial fresh weight (n=10). Saturating light levels (300 $\mu mol/m^2/s$) were provided during the desiccation period, and all samples were desiccated for less than 30 minutes.

An open system Infrared gas analyzer (IRGA) was used (Qubit systems, Inc., Kingston, Ontario, Canada) to measure CO_2 consumption or production in air. All photosynthetic measurements were taken at ambient CO_2 conditions (400-500 µmol 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 air-tight container. A hydrochron (iButtons, Maxim Integrated Products, Sunnyvale, CA USA) was placed in the sample chamber to measure temperature and relative humidity throughout photosynthetic measurements. Light was provided with a Kodak slide projector at a saturating irradiance (300 $\mu mol/m^2/s$) and measurements were taken at room temperature ($18^oC \pm 0.3^oC$). As with measurements in water, the rate of change in CO_2 concentration was

measured for five minutes to obtain photosynthetic rates. Dark respiration rate was taken by covering the entire sampling chamber with a dark cloth. 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.

To analyze emerged photosynthetic data, a two-way ANOVA was performed 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.

2.2.2.3 Pigment Analysis

From the above emerged photosynthesis experiment, sub-samples for pigment analyses were initially taken (baseline pigments reported in Chapter 1) and again after the photosynthetic measurements were performed. All specimens for pigment analysis were frozen in liquid nitrogen and stored at -80°C until pigment analyses were performed. A spectrophotometer (Ultrospec 2100, Biochrom Ltd., Cambridge, England) was used to quantify pigment concentrations on the same samples used in the emerged photosynthetic experiments. See Chapter 1 for pigment extraction protocol (Section 1.2.2.2).

To analyze pigment data, a two-way ANOVA was performed with treatment (3 levels: control ('wet', 'blotted', and 'desiccated') and species (2 levels: *Calliarthron* and *Corallina).* Initial pigment values were not included in statistical analysis (reported in Chapter 1 for baseline pigmentation), but were included in graphs for baseline comparison. Normality was confirmed with the Shapiro-Wilk test and equal variance was confirmed with Levene's test.

2.2.2.4 Desiccation Resistance

The amount of water lost through time was measured for *Calliarthron* and *Corallina* at two different relative humidity (RH) levels (56% RH and 74% RH) and two hydration levels ('wet', 'blotted'). In the 'wet' treatment, fronds were allowed to retain water within their branches. In the 'blotted' treatment, excess water was removed from fronds by gently blotting their branches with paper towels. A plastic bin was filled at the bottom with either water (for high RH) or with the desiccant, Drierite (for low RH). Each coralline apical frond was contained separately in a tin weighing dish (Figure 7).

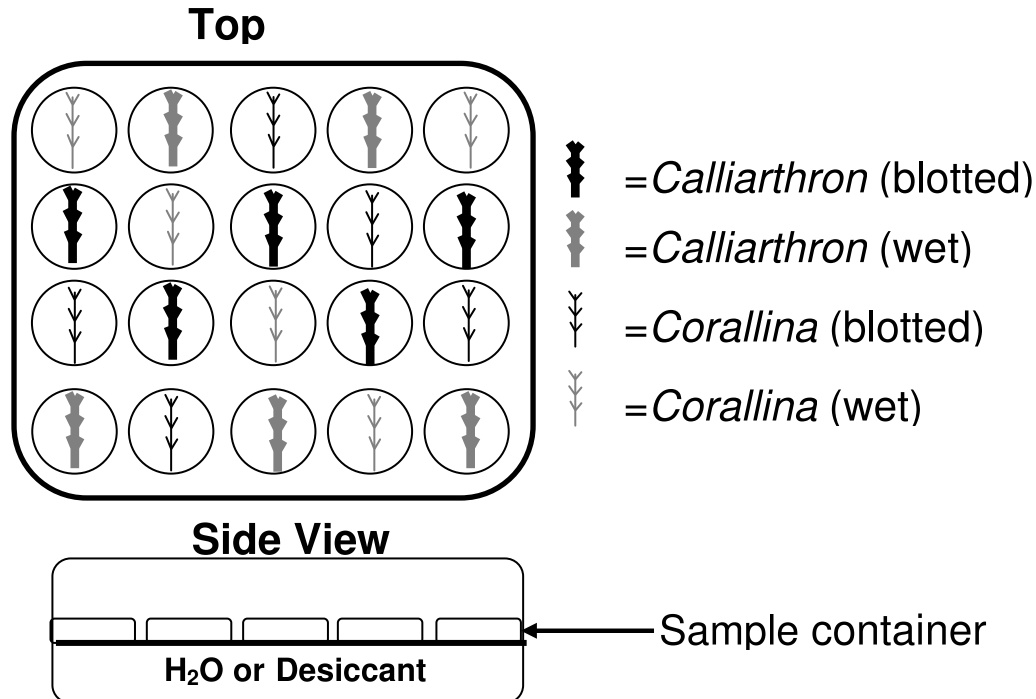


Figure 7: Experimental set up for desiccation trials. Two hydrochrons (iButtons, Maxim Integrated Products, Sunnyvale, CA USA) were placed at opposite ends of the bin to measure relative humidity and temperature throughout the sampling period. n=5 for all treatments. RH was manipulated by either drierite or water in the bottom of the bin. Experiments were performed at room temperature (18°C ± 3°C)

Desiccation experiments were performed at room temperature (18°C ± 3°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 minutes for 90 minutes to get a measure of relative water content of the thallus. After the 90 minute period, specimens were dried in a 60°C oven for 48 hours and oven dry weight measurements were taken. Relative water content of the fronds was calculated for each 15 minute time point according to the following formula (Slayter 1967):

$$\text{Relative Water Content (RWC)} = \frac{\text{Desiccated weight - Oven dry weight}}{\text{Initial fresh weight - Oven dry weight}}$$

To analyze the effect of relative humidity (2 levels: 56% and 74%) and species (2 levels: *Calliarthron* and *Corallina)* on relative water content loss through time, a repeated measures ANOVA was performed on the 'wet' and 'blotted' samples separately. Data from the treatments were not spherical, so degrees of freedom were corrected by using Greenhouse-Geisser. Normality was confirmed with Shapiro-Wilk for both treatments and equal variance was confirmed with Levene's Test. To analyze the effect of relative humidity and species on relative water content at the end of the 90 minute sampling period, a two-way ANOVA was also performed on the 90 minute sample time point.

2.3 Results

2.3.1 Photosynthesis in Tidepools

Increasing temperature had a negative effect on submerged photosynthesis of *Calliarthron* ($p < 0.05$, Table 3) whereas *Corallina* remained unaffected ($p = 0.07$, Table 3, Figure 8).

Under low light, *Corallina* photosynthesized in the lowest two temperature treatments (12ºC, 16ºC) but did not photosynthesize in the highest temperature treatment (20ºC) (Figure

8). In low light conditions, *Calliarthron* photosynthesized in the lowest temperature treatment (12ºC) but only respired as temperature increased ($p = 0.05$, Table 3, Figure 8).

Under high light, *Corallina* had high photosynthetic rates across all temperatures, even the highest temperature (Figure 8). Rates were significantly higher than those recorded in low light conditions ($p < 0.001$; Table 3, Figure 8). *Calliarthron* exhibited similar photosynthetic rates in both light treatments ($p=0.60$, Table 3). However, photosynthetic rates declined with increasing temperature ($p<0.05$, Table 3, Figure 8).

Respiration was always higher in fronds of *Calliarthron* than in fronds of *Corallina* ($p < 0.01$, Table 4, Figure 8), however, increases in temperature caused increased respiration rates for both *Calliarthron* and *Corallina* ($p<0.001$, Table 4, Figure 8).

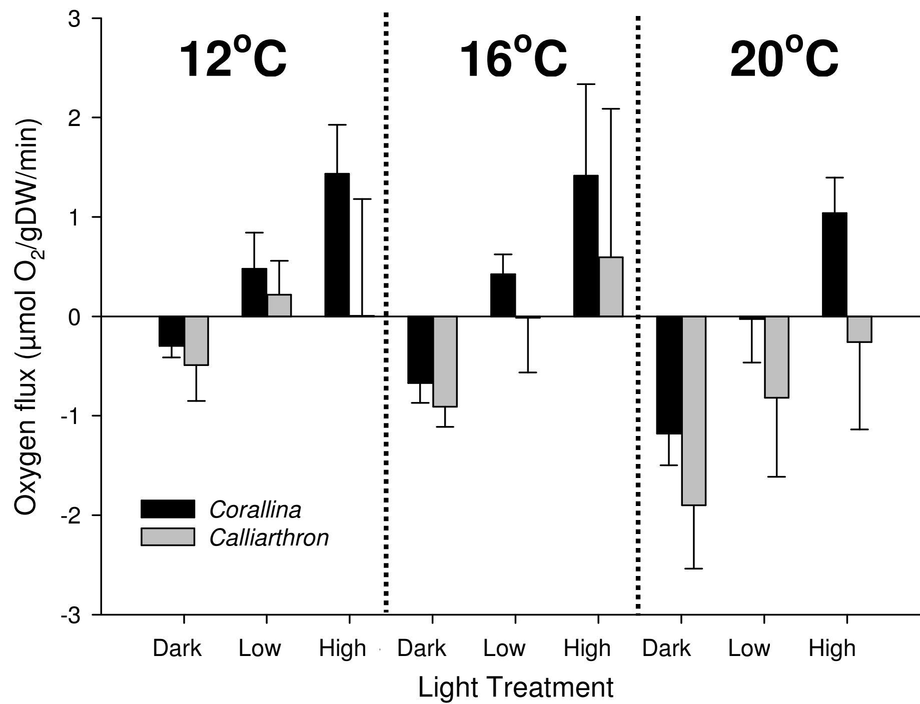


Figure 8: Net oxygen flux of *Calliarthron* and *Corallina* in submerged conditions by temperature. Dark = 0 µmol/m²/s (n=14); Low Light=50 µmol/m²/s (n=7); High Light=300 µmol/m²/s (n=7). Error bars are 95% confidence intervals.

Table 3: Results of generalized linear model for the effects of temperature and light on net photosynthesis of fronds of *Calliarthron* and *Corallina*

	Source	Wald Chi-Square	df	p-value
Calliarthron	Light	0.27	1	0.60
	Temperature	**5.85**	**2**	**0.05***
	Light * Temperature	1.90	2	0.39
Corallina	**Light**	**37.89**	**1**	**<0.001***
	Temperature	5.20	2	0.07
	Light * Temperature	0.58	2	0.75

Table 4: Results of generalized linear model for the effect of temperature and species on respiration rates of fronds of *Calliarthron* and *Corallina*.

Source	Wald Chi-Square	df	p-value
Temperature	**55.81**	**2**	**<0.001***
Species	**9.10**	**1**	**<0.01***
Temperature * Species	3.51	2	0.17

In the planned comparisons between simulated high and low tide conditions, *Calliarthron* and *Corallina* exhibited different photosynthetic rates under warm tidepool conditions ($p<0.01$, Table 5) but similar photosynthetic rates under high tide conditions ($p = 0.22$, Table 5). In the warm tidepool treatment, *Calliarthron* only respired and had a mean photosynthetic rate of -0.26 ± 0.36 μmol O_2/gDW/min, whereas *Corallina* had a mean photosynthetic rate of 1.04 ± 0.15 μmol O_2/gDW/min (Table 5). In the high tide treatment there was no difference in the photosynthetic rates; *Calliarthron* had a mean photosynthetic rate of 0.22 ± 0.14 μmol O_2/gDW/min and *Corallina* had a mean photosynthetic rate of 0.48 ± 0.15 μmol O_2/gDW/min (Table 5).

Table 5: Planned Comparison t-test of net photosynthesis between 12°C, 50 μmol/m^2/s (representing high tide) and 20°C, 300 μmol/m^2/s (representing low tide) treatments. n=7, Std. Error of mean.

	Calliarthron (μmol O_2/gDW/min)	***Corallina*** (μmol O_2/gDW/min)	p-value
High Tide (12ºC, 50 μmol/m^2/s	0.22 ± 0.14	0.48 ± 0.15	0.22
Warm Tidepool (20ºC, 300 μmol/m^2/s	**-0.26 ± 0.36**	**1.04 ± 0.15**	**0.01***

2.3.2 Photosynthesis Out of Tidepools

Independent of hydration level, both species exhibited very low photosynthetic and respiration rates in air (Figure 9). *Calliarthron* and *Corallina* photosynthesized at similar rates ($p = 0.19$, Table 6) at all hydration levels ($p = 0.70$, Table 6). *Calliarthron* had a mean photosynthetic rate of 0.02 ± 0.06 µmol O_2/gDW/min and *Corallina* had a mean photosynthetic rate of 0.07 ± 0.12 µmol O_2/gDW/min in the air (Figure 9). *Calliarthron* and *Corallina* ($p = 0.24$, Table 6) also demonstrated similar and very low respiration rates, regardless of hydration level ($p = 0.29$; Table 6; Figure 9). The mean respiration rate of *Calliarthron* was -0.01 ± 0.05 µmol CO_2/gDW/min, and the mean respiration rate of *Corallina* was -0.04 ± 0.08 µmol CO_2/gDW/min (Figure 9).

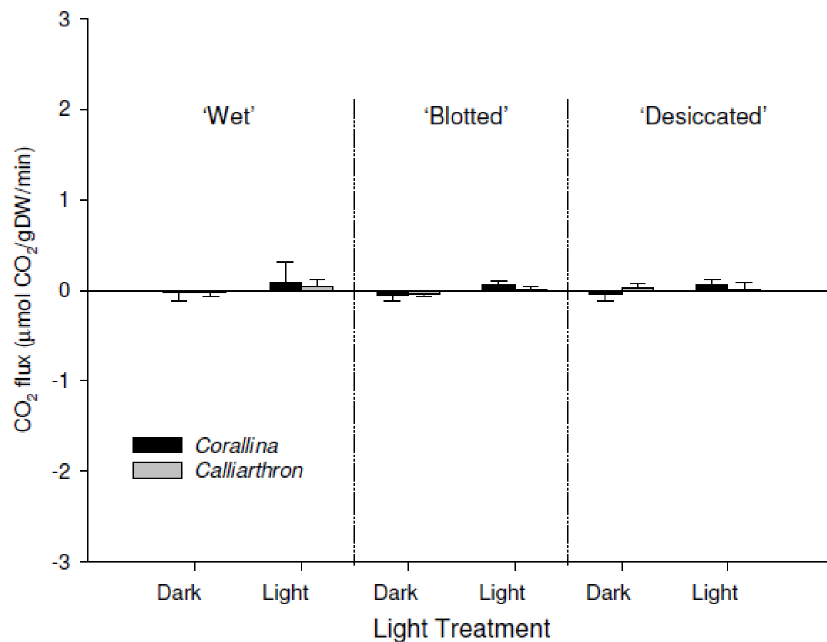


Figure 9: Respiration (Dark, 0 µmol/m²/s) and net photosynthesis (Light, 300µmol/m²/s) of *Calliarthron* and *Corallina* fronds in air. 'Desiccated' is 56% RWC, 'blotted' is with water removed from branches, and 'wet' is with water in branches. n=6. Error bars are 95% confidence intervals.

Table 6: Two-way ANOVA results of net photosynthesis and respiration of *Calliarthron* and *Corallina* in air.

	Source	Type III Sum of Squares	Df	Mean Square	F	p-value
Photosynthesis	Species	0.02	1	0.02	1.842	0.19
	Treatment	0.01	2	0.01	0.373	0.69
	Species * Treatment	0	2	9.80E-05	0.01	0.99
Respiration	Treatment	0.01	2	0.01	1.309	0.29
	Species	0.01	1	0.01	1.455	0.24
	Treatment * Species	0.01	2	0.00	0.502	0.61

2.3.3 The Effect of Desiccation on Pigments

Desiccation stress did not cause pigments to degrade in either *Calliarthron* or *Corallina*. Regardless of hydration level (p = 0.38, Table 7) *Calliarthron* and *Corallina* (p = 0.35, Table 7) had similar chlorophyll *a* concentrations: *Calliarthron* had a mean chlorophyll *a* concentration of 1.29 ± 0.50 µg /mg sample and *Corallina* had a mean chlorophyll *a* value of 1.46 ± 0.32 µg /mg sample (Figure 10).

Calliarthron and *Corallina* also had similar concentrations of phycoerythrin (p = 0.06), regardless of hydration level (p = 0.33, Table 7). *Calliarthron* had a mean value of 1.67 ± 0.81 µg phycoerythrin/mg sample and *Corallina* had a mean value of 1.14 ± 0.43 µg phycoerythrin/mg sample (Figure 10).

Calliarthron and *Corallina* (p = 0.47, Table 7) also had similar concentrations of phycocyanin pigment, regardless of hydration level (p = 0.37, Table 7). *Calliarthron* had a mean phycocyanin concentration of 0.54 ± 0.39 µg phycocyanin/mg sample and *Corallina* had a mean value of 0.44 ± 0.19 µg phycocyanin/mg sample (Figure 10).

Regardless of hydration level, *Calliarthron* and *Corallina* (p = 0.40, Table 7) also had similar concentrations of allophycocyanin pigment (p = 0.73, Table 7). *Calliarthron* had a mean value of 0.62 ± 0.43 µg allophycocyanin/mg sample and *Corallina* had a mean value of 0.48 ± 0.34 µg allophycocyanin/mg sample (Figure 10).

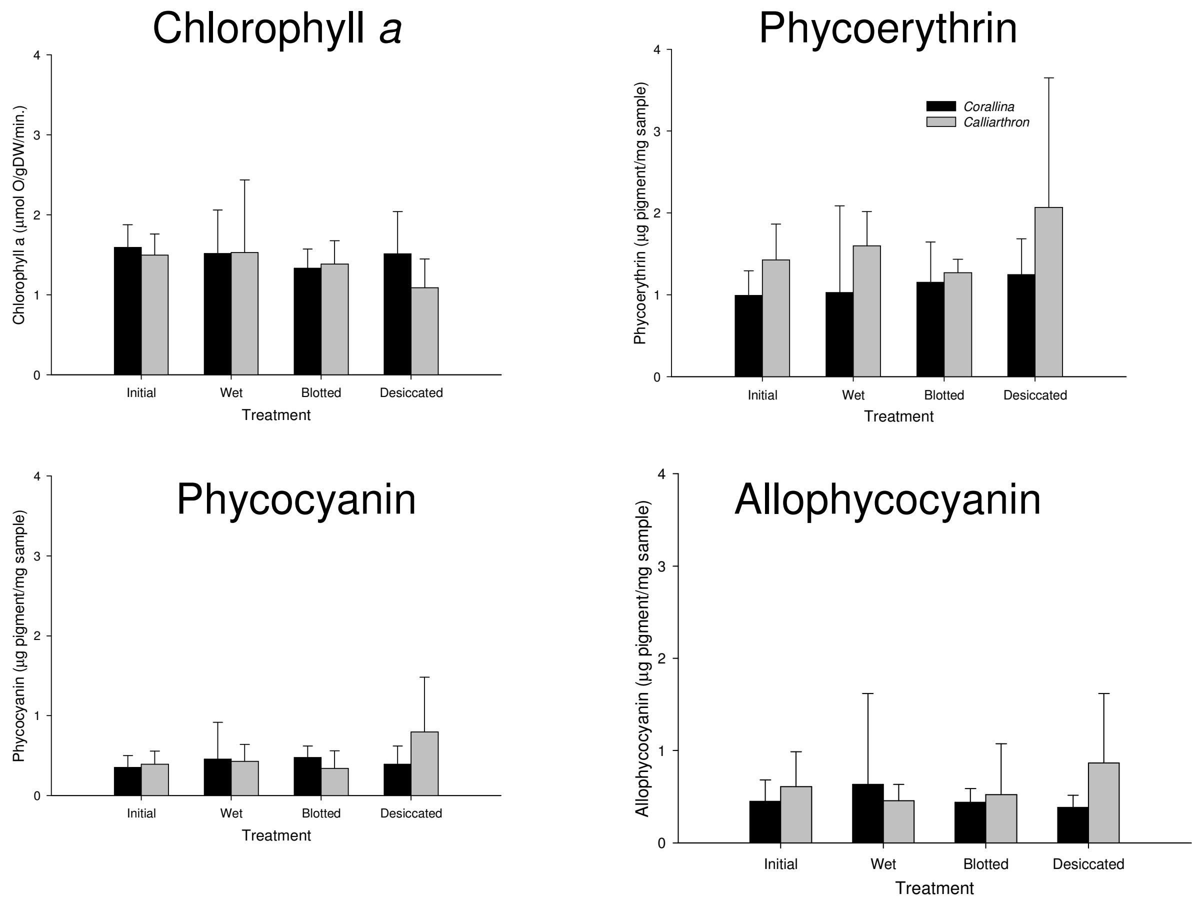


Figure 10: Pigment concentrations of *Calliarthron* and *Corallina* fronds with different hydration treatments. *Corallina*: Initial (n=13), Wet (n=4), Blotted (n=4), Desiccated (n=5). *Calliarthron*: Initial (n=14), Wet (n=5), Blotted (n=4), Desiccated (n=6). Error bars are 95% confidence intervals.

Table 7: Results of two-way ANOVA of light harvesting pigment concentrations in fronds of *Calliarthron* and *Corallina* in 'wet', 'blotted', and 'desiccated' treatments.

Pigment	Source	Type III Sum of Squares	df	Mean Square	F	p-value
Chlorophyll *a*	Species	0.16	1	0.16	0.91	0.35
	Hydration	0.35	2	0.18	1.01	0.38
	Species * Treatment	0.49	2	0.25	1.41	0.27
Phycoerythrin	Species	1.68	1	1.68	3.87	0.06
	Hydration	1.00	2	0.50	1.16	0.33
	Species * Treatment	0.56	2	0.28	0.64	0.54
Phycocyanin	Species	0.046	1	0.046	0.53	0.47
	Hydration	0.18	2	0.09	1.04	0.37
	Species * Treatment	0.38	2	0.19	2.21	0.14
Allophycocyanin	Species	0.11	1	0.11	0.73	0.40
	Hydration	0.09	2	0.05	0.32	0.73
	Species * Treatment	0.52	2	0.26	1.76	0.20

Calliarthron had less carotenoid pigment than *Corallina* (p = 0.001, Table 8); but desiccation did not affect carotenoid concentrations (p = 0.83, Table 8). This is in contrast to the baseline pigment analyses performed in Chapter 1, however, this disparity is most likely due to differences in sample sizes between the two experiments. *Calliarthron* had a mean value of 0.34 ± 0.10 μg carotenoid/mg sample and *Corallina* had a mean value of 0.53 ± 0.12 μg carotenoid/mg sample (Figure 11).

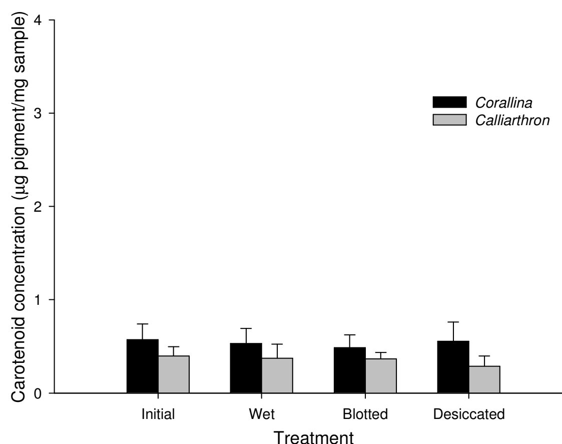


Figure 11: Carotenoid concentration of *Calliarthron* and *Corallina* fronds with different hydration treatments. *Corallina*: Control (n=13), Wet (n=4), Blot (n=4), Desiccated (n=5). *Calliarthron*: Control (n=14), Wet (n=5), Blot (n=4), Desiccated (n=6). Error bars are 95% confidence intervals.

Table 8: Results of two-way ANOVA of carotenoid concentrations in fronds of *Calliarthron* and *Corallina*.

Source	Type III Sum of Squares	df	Mean Square	F	p-value
Species	**0.22**	**1**	**0.22**	**16.01**	**<0.001***
Treatment	0.01	2	0.00	0.19	0.83
Species * Treatment	0.02	2	0.01	0.90	0.42

2.3.4 Desiccation Resistance

Fronds of both species desiccated faster in the lower RH treatment (p<0.001, Table 9, Figure 12A & B). At the lowest humidity (56% RH), 'wet' fronds of *Calliarthron* were able to delay desiccation by 15 minutes before starting to lose water from its tissues (Figure 12A) and 'wet' fronds of *Corallina* were able to delay desiccation by approximately 60 minutes before losing water from its tissues (Figure 12A). When water was 'blotted' from the branches, both *Calliarthron* and *Corallina* desiccated at similar rates (Figure 12A).

At high (74%) relative humidity, 'wet' *Calliarthron* fronds delayed desiccation for 30 minutes before starting to lose water from its tissues (Figure 12B). At this higher humidity,

desiccating *Calliarthron* fronds retained excess water for twice as long as fronds in the low (56%) humidity treatment. 'Wet' *Corallina* fronds never reached 100% RWC in the 90 minute sampling period and continued to retain water in their branches at the end of the experiment (Figure 12B).

The results from the repeated measures ANOVA for the 'wet' treatment showed two interactions (Table 9). Desiccation rate depended on relative humidity ($p < 0.001$, Table 9) and on the species identity ($p < 0.01$, Table 9). In the 'blotted' treatment, *Calliarthron* desiccated significantly faster than *Corallina*. Desiccation rate depended on all three factors, relative humidity, species identity and time ($p < 0.05$, Table 10).

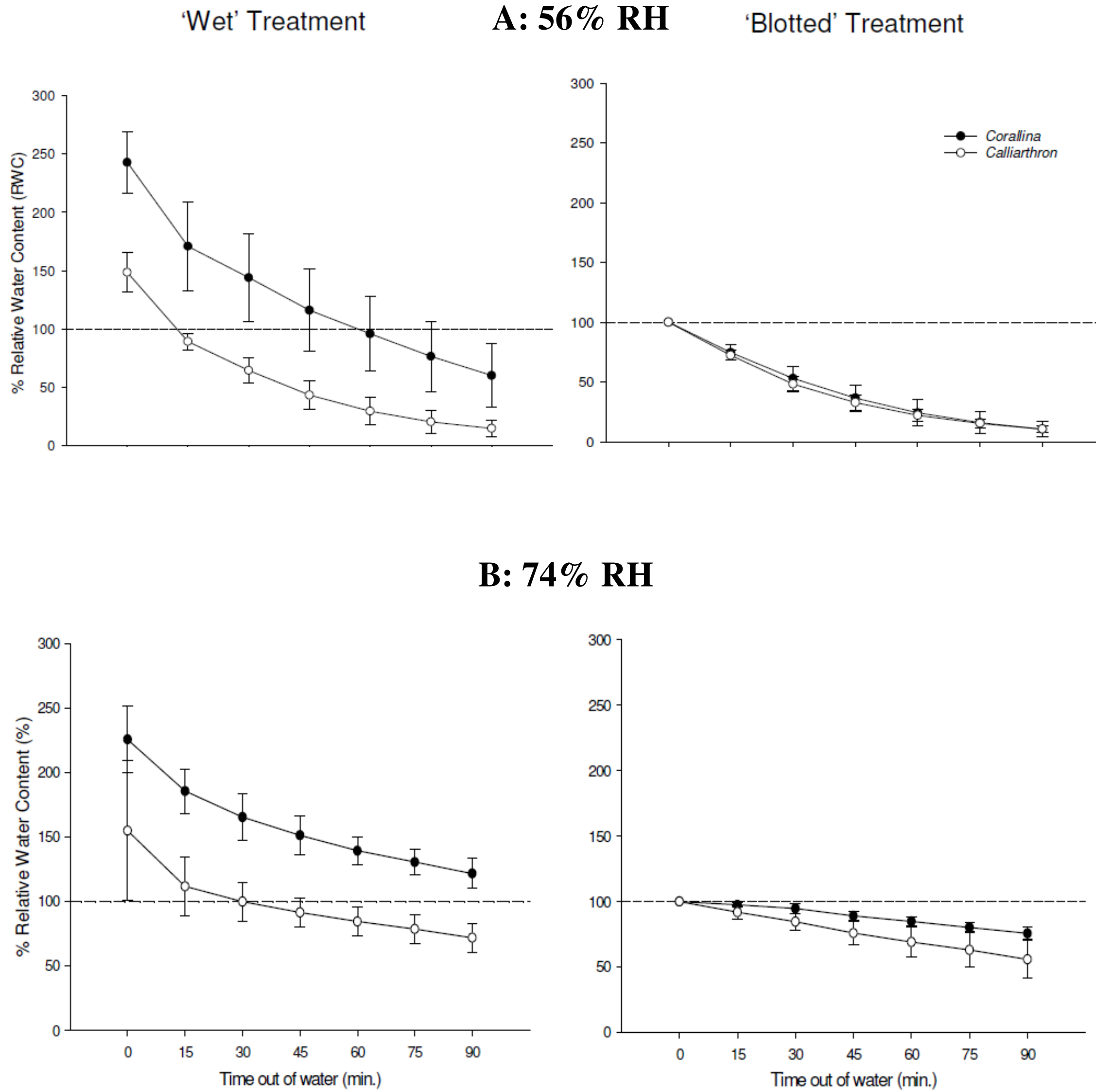


Figure 12: The change in relative water content of fronds of *Calliarthron* and *Corallina* with increasing time out of water. Fronds were allowed to retain water in branches in 'wet' treatment, and water was removed from fronds in 'blotted' treatment. Experiment performed at 56% (A) and 74% (B) relative humidity. n=5, error bars are 95% confidence intervals.

Table 9: Results of repeated measures ANOVA on desiccation level of 'wet' samples

Source	Type III Sum of Squares	df	Mean Square	F	p-value
Species	**151937.30**	**1**	**151937.30**	**84.11**	**<0.001***
Relative Humidity	**44575.38**	**1**	**44575.38**	**24.73**	**<0.001***
Species * Relative Humidity	854.82	1	854.82	0.47	0.50
Time	**227250.04**	**1.483**	**153223.01**	**417.07**	**<0.001***
Time * Species	**4861.40**	**1.483**	**3277.80**	**8.92**	**<0.01***
Time * Relative Humidity	**16365.52**	**1.483**	**11034.45**	**30.04**	**<0.001***
Time * Species * Relative Humidity	553.05	1.483	372.89	1.02	0.36

Table 10: Results of repeated measures ANOVA on desiccation level of 'blotted' samples

Source	Type III Sum of Squares	df	Mean Square	F	p-value
Species	**2089.70**	**1**	**2089.70**	**10.95**	**<0.05***
Relative Humidity	**55766.06**	**1**	**55766.06**	**292.19**	**<0.001***
Species * Relative Humidity	533.14	1	533.14	2.79	0.11
Time	**60597.97**	**2.263**	**26775.44**	**869.75**	**<0.001***
Time * Species	**447.78**	**2.263**	**197.86**	**6.43**	**<0.05***
Time * Relative Humidity	**13621.21**	**2.263**	**6018.59**	**195.50**	**<0.001***
Time * Species * Relative Humidity	**491.24**	**2.263**	**217.07**	**7.05**	**<0.05***

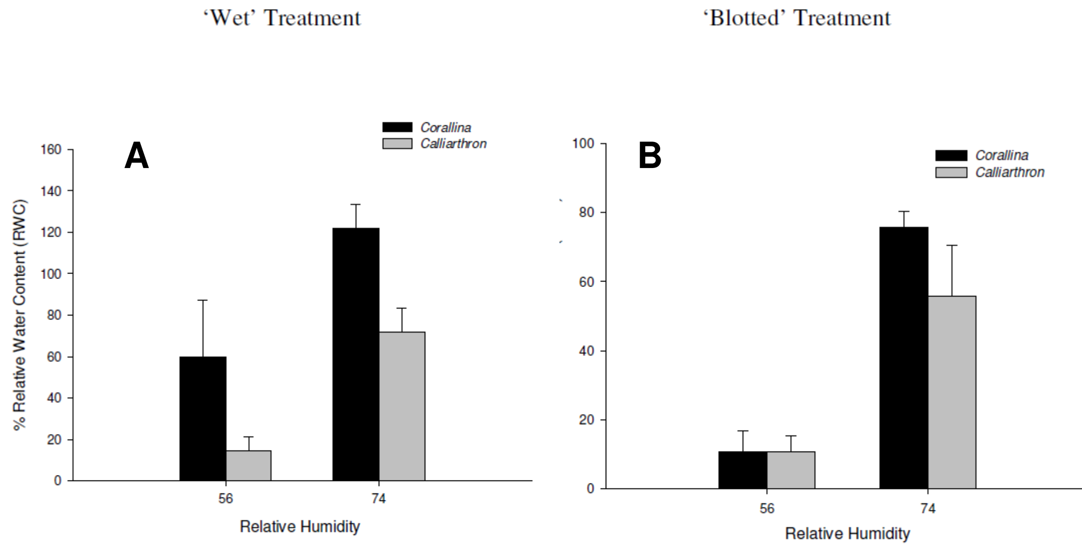


Figure 13: A: Percent relative water content of fronds of *Calliarthron* and *Corallina* in the 'wet' treatment after 90 minutes out of water at two different relative humidity levels. B: Percent relative water content of fronds of *Calliarthron* and *Corallina* in the 'blotted' treatment after 90 minutes out of water at two different relative humidity levels. n=5. Error bars are 95% confidence intervals.

Table 11: Results of ANOVA at 90 minute sampling point of wet and blotted fronds of *Calliarthron* and *Corallina*.

	Source	Type III Sum of Squares	df	Mean Square	F	p-value
'Wet'	**Species**	**11326.77**	**1**	**11326.77**	**66.29**	**< 0.001***
	Relative Humidity	**17939.11**	**1**	**17939.12**	**104.99**	**< 0.001***
	Species * Relative Humidity	24.79	1	24.79	0.15	0.71
'Blotted'	**Species**	**491.30**	**1**	**491.30**	**10.14**	**< 0.01***
	Relative Humidity	**15124.97**	**1**	**15124.97**	**312.09**	**< 0.001***
	Species * Relative Humidity	**498.24**	**1**	**498.24**	**10.28**	**< 0.01***

After 90 minutes in the 'wet' treatment, fronds of *Calliarthron* contained less water than *Corallina* (Figure 13A). *Calliarthron* and *Corallina* desiccated at different rates ($p < 0.001$, Table 11). Fronds desiccated faster at lower relative humidity ($p < 0.001$, Table 11, Figure 13A). At the end of the sampling period in low humidity (56% RH), 'wet' *Calliarthron*

fronds had 14.4 ± 5.5 % RWC, while 'wet' *Corallina* fronds still had 59.8 ± 22.1 % RWC (Figure 13A). In the higher humidity (74% RH), 'wet' fronds of *Calliarthron* had 72.1 ± 9.1% RWC and 'wet' fronds of *Corallina* never desiccated, maintaining 121.9 ± 9.2% RWC (Figure 13A).

After 90 minutes in the 'blotted' treatment, water content of *Calliarthron* and *Corallina* in the 'blotted' treatment depended on species identity and relative humidity (Fig. 2.7B, $p<0.01$, Table 11). In the 56% RH treatment, blotted *Corallina* fronds had 10.7 ± 5.0 % RWC and blotted *Calliarthron* fronds had 10.7 ± 3.7% RWC (Figure 13B). In the higher humidity (74% RH), 'blotted' fronds of *Calliarthron* had 55.7 ± 11.9% RWC and 'blotted' fronds of *Corallina* had 75.6 ± 3.8 % RWC (Figure 13B).

2.4 Discussion

The results from experiments in this chapter indicate that habitat partitioning of *Calliarthron* and *Corallina* may be driven by both physiological and morphological differences between the two species at low tide. Results presented in this study suggest that physiological and morphological mechanisms permit *Corallina* to survive emergence stress and confine *Calliarthron* to tidepools.

2.4.1 Physiology

In submerged conditions, *Corallina* is tolerant of high temperatures and high light making it well-adapted to shallow intertidal habitats and emergent conditions. However, *Corallina* is limited in low light conditions, its photosynthetic performance decreases significantly in low light compared to high light. Therefore, its photosynthetic performance is reduced during high tide as compared to low tide. *Calliarthron*, on the other hand, photosynthesizes significantly less as temperature increases during low tide, suggesting that

Calliarthron is ill suited to temperature stress and fares best in tidepools that do not increase in temperature at low tide. This explains why *Calliarthron* is often found deeply submerged in large, shaded tidepools.

Respiration is an important metabolic process occurring along with photosynthesis. During respiration, photosynthetic products are used to generate ATP, which can be used in the metabolism or maintenance of other cellular components (Taiz and Zeiger 2002). The respiration rate of *Calliarthron* was always greater than the rate for *Corallina*, and in both species respiration rates increased approximately 3-fold over the experimental temperatures. A similar trend has been documented for other intertidal macroalgae (Lapointe *et al.* 1984, Davison *et al.* 1991). The rate of respiratory increase often is related to the temperature the algae experienced during development (Davison *et al.* 1991). For example, as experimental temperatures were increased, respiration rates of *Laminaria saccharina* grown at 5ºC increased more than those grown at 15ºC.

Neither *Corallina* nor *Calliarthron* photosynthesize in the air, and apparently 'shut down' photosynthesis when the tide is out. Similarly, respiration also declines under emerged conditions. Thus, *Corallina*'s ability to live outside tidepools is not linked to an enhanced ability to photosynthesize in air, but is instead likely related to its unique ability to delay desiccation.

Field measurements of growth indicate that both *Calliarthron* and *Corallina* collected from Botanical Beach have similar growth rates (Fisher, unpubl. data). Both *Calliarthron* and *Corallina* go dormant when exposed to aerial conditions. Since both species are only productive in water, they must remain submerged to retain a positive carbon gain in their tissues. Therefore, *Corallina* fronds that are emerged during low tide are not productive

compared to fronds of *Corallina* submerged at the upper rims of tidepools. *Corallina* is often exposed to aerial conditions at low tide whereas *Calliarthron* is never exposed to aerial conditions. This means that in the field, *Corallina* alternates between high productivity while submerged and low productivity during emergence. This may account for the finding that they have similar growth rates despite their different photosynthetic rates.

Severe desiccation did not result in pigment degradations of *Calliarthron* or *Corallina*, even after desiccating to approximately 50% RWC. This suggests that pigments in both species are robust to dehydration, and that pigment degradation is not responsible for the decline in photosynthetic performance. However, pigments may have been damaged but not lost. Martone *et al.* (2010) found that fronds that were bleached during a stressful low tide needed to be re-submerged in seawater for a few days before pigment loss became evident. However, in the Martone *et al.* (2010) study, pigments were estimated using a basic color analysis. In the present study, pigments were determined with a spectrophotometer, and so immediate pigment degradations should have been detectable. Nevertheless, it is possible that pigments did not degrade immediately following desiccation stress, and that pigment degradations would have been measurable after a few days.

2.4.2 Morphology

Corallina can resist desiccation for over an hour when the tide is out by holding water in its branches. This enables *Corallina* to survive outside of tidepools during low tide as long the desiccation period is not too long. However, the experimental desiccation times in this study were much shorter than exposure times in their natural habitats. For a typical summer low tide, *Corallina* living out of tidepools is likely exposed for 3-4 hours. Unlike *Corallina*, *Calliarthron*, is very desiccation prone and dries out within 15-30 minutes depending on the

relative humidity. This supports the results of Martone *et al.* (2010), who found that desiccation was the primary stress that limited *Calliarthron* during low tide. Desiccation of *Calliarthron* tissues happens very quickly after emergence since the sparse branches of *Calliarthron* are unable to hold excess water. It is likely that *Calliarthron* is limited to tidepool habitats in order to avoid desiccation due to this morphological limitation. Interestingly, when water was blotted from their branches, *Calliarthron* and *Corallina* desiccated at a similar rate. This suggests that the calcified thalli are equally susceptible to desiccation, but that the arrangement of fine branches in *Corallina* provides a significant morphological advantage.

In conclusion, *Corallina* is well adapted to high light and high temperature and is able to resist desiccation through morphology. These results help explain why *Corallina* is the only articulated coralline species in the Northeast Pacific that is able to live outside tidepools or at the rims of tidepools, where tidepool temperatures can exceed 20ºC and light levels can reach 2000 $\mu mols/m^2/s$. On the other hand, *Calliarthron* is well adapted to low light and cooler water temperatures, and desiccates rapidly when emerged, helping to explain why *Calliarthron* is found only deeply submerged in tidepools. However, both species had similar concentrations of light harvesting pigments suggesting that other processes likely dictate differences in maximum photosynthetic rates. However, in regards to photoprotective pigments, *Corallina* had more carotenoids than *Calliarthron* which may explain why it can live emergently at low tide. Yet, this finding was in contrast to the findings for baseline pigments in Chapter 1. This difference is likely due to differences in sample sizes between the two pigment quantification experiments. Since neither species photosynthesized in the air

and both species had similar light harvesting pigmentation, data suggest that morphology rather than physiology is allowing *Corallina* to survive outside of tidepools during lowtide.

Chapter 3

Physiological Recovery of *Calliarthron* and *Corallina* After a Low Tide

3.1 Introduction

Many plants have the ability to survive extreme desiccation and recover photosynthetic activity after re-hydration. For example, resurrection plants (Rascio and Rocca 2005), bryophytes (Proctor 2001), and many algae (Bell 1993, Smith and Berry 1986, Gray *et al.* 2007) shut down their metabolism during stressful events (Hunt and Denny 2008, Rascio and Rocca 2005, Chapter 2 this study) only to quickly re-hydrate and regain photosynthetic processes when re-hydration occurs (Bell 1993, Lipkin *et al.* 1993).

There are several ways plants and algae resist or avoid damage due to desiccation during stressful events. According to Roscio and Rocca (2005), "desiccation survival of a plant or organ depends on its ability to successfully achieve the following: 1) limit cellular damage to a reparable level upon drying, 2) maintain structural and physiological integrity of cells in the dried state, and 3) repair cell damage rapidly and efficiently upon rehydration." For example, in the high intertidal alga, *Fucus spiralis*, desiccation resistance results from the ability to recover from a high degree of cellular dehydration (Dring and Brown 1982, Madsen and Maberly 1990), suggesting that this alga can limit cellular damage to a reparable level. To maintain structural integrity of cells while desiccating, land plants produce osmolytes such as sucrose, polyols, glycine-betaine, or proline (Geigenberger *et al.* 1997, Pelah *et al.* 1997, Clifford *et al.* 1998, Hare *et al.* 1998, Mundree *et al.* 2000) which serve to protect cell membranes and macromolecules while cells are drying (Hare *et al.* 1998). The accumulation of osmolytes in cells is accompanied by the influx of water into cells, or at the

very least, a reduction in the efflux of water from cells, thus providing a way to reduce cellular dehydration (Hare *et al.* 1998).

The degree of tolerance to low tide stress depends on the rate and extent of photosynthetic recovery after re-immersion (Smith and Berry 1986, Lüning 1990, Davison and Pearson 1996). Provided that algae can survive low tide stressors, their ability to recover when the tide returns is critical. Macroalgae that are not able to recover from the stresses of exposure during low tide, are physiologically compromised when the tide returns, and this inability to recover has obvious implications for growth and reproduction, which invariably affects the structure of the community (Lubchenco and Menge 1978).

Recovery from stress likely is one of the factors that determine the habitat distribution of species within the intertidal zone (Doty 1946, Johnson *et al.* 1974, Hodgson 1981, Smith and Berry 1986). Most likely, desiccation is the primary factor in determining the upper limit of intertidal algae, rather than temperature or light regimes (Hodgson 1981, Smith and Berry 1986). For example, Hodgson (1981) found that neither light levels nor temperature *in situ* were damaging to photosynthesis, but severe desiccation resulted in marked decreases in photosynthetic rates. Smith and Berry (1986) found that desiccation tolerance was correlated with tidal distribution of macroalgae. High intertidal algae were able to tolerate a higher degree of desiccation than low intertidal algae, and this pattern held even for conspecifics growing in different habitats (Smith and Berry 1986).

The relative importance of temperature on algal recovery is equivocal. Past studies found that limits of temperature tolerance did not correlate to tidal height for several intertidal macroalgae (Smith and Berry 1986). But Bell (1993) found that extreme temperature, rather than desiccation, affected the rates of photosynthetic recovery in the high intertidal alga,

Mastocarpus papillatus. However, desiccation protected high intertidal algae from temperature stress (Hunt and Denny 2008), suggesting that desiccation can assist algae in surviving in the high intertidal zone. This interaction makes it difficult to test the importance of either factor individually.

3.1.1 Chapter Objectives

Both *Calliarthron* and *Corallina* are negatively impacted by low tide stressors. Photosynthetic performance declines with increased temperature and desiccation stresses (see Chapter 2). In this chapter, I explore the ability of *Calliarthron* and *Corallina* to recover from submerged and emerged stressors associated with the low tide after the tide returns. The extent of photosynthetic recovery after a stressful low tide event was used as a metric to quantify the stress tolerance of *Calliarthron* and *Corallina*. First, I document their ability to recover from warm, submerged conditions, mimicking a warm tidepool. I predict that both *Corallina* and *Calliarthron* will recover rapidly and completely since both *Corallina* and *Calliarthron* naturally occur in warm tidepools. Then, I quantify their ability to recover from exposure to temperature and desiccation stresses, mimicking low tide emergence. Because *Corallina* often occurs outside tidepools, I predict that *Corallina* will recover after emergence stress, and that *Calliarthron*, which is highly susceptible to desiccation stress, will not recover at all.

3.2 Methods

3.2.1 Specimen Collection and Laboratory Conditions

Specimens were collected from Prasiola Point, near Bamfield, BC, Canada (48°50'N, 125°08'W). All collection and transport of specimens was performed as outlined in Chapter

1. Specimens were kept in running seawater at 12ºC, and low light conditions (5-10 μmol/m^2/s) at Bamfield Marine Sciences Centre (Bamfield, BC, Canada).

3.2.2 Experimental Set-up

3.2.2.1 Submerged Recovery

Initial photosynthetic rates (as described in Chapter 1: Light Response Curve Section) at 12°C were measured before any treatment was applied. After initial photosynthetic measurements, *Calliarthron* and *Corallina* fronds were placed into one of two submerged temperature treatments: 12ºC, representing continued high tide (control), or 20ºC, representing a warm tidepool during low tide. In the 12ºC treatment, fronds were placed in vials of pre-chilled seawater and were placed back into the circulating water bath under saturating irradiance (~300 μmol photons/m^2/s). In the 20ºC treatment, fronds were placed into vials of room temperature seawater, and saturating light was provided (~300 μmol photons/m^2/s). In both treatments, a temperature probe (Ocean Optics Neofox) was placed in the vial to monitor temperature for the duration of the treatment. In both treatments, temperature varied by < 1ºC. Coralline fronds were exposed to temperature treatments for 30 minutes. After the 30 minute treatment period, coralline fronds were placed into a new vial of pre-chilled seawater (12ºC). Photosynthesis and respiration were then recorded every five minutes for one hour. After recovery measurements were taken, the specimens were dried in a 60°C oven for 48 hours to obtain the dry weights. The amount of calcium carbonate was then accounted for in order to obtain the decalcified dry weight of the algae for photosynthetic calculations (see methods in Chapter 1).

3.2.2.2 Emerged Recovery

Initial photosynthetic rates were taken as described above and in Chapter 1. After initial photosynthetic measurements were taken, fronds were placed into temperature and desiccation treatments. Fully factorial manipulations of temperature (2 levels: 16ºC and 25ºC) and desiccation (2 levels: 75% and 50% RWC) were used. All fronds were desiccated under saturating light conditions, as explained in Chapter 2. Fronds were not allowed to desiccate for more than 30 minutes to avoid differential exposure to light. Fronds that took longer than 30 minutes to desiccate were discarded. Previously determined fresh to dry weight ratios were used to determine the relative water content of desiccating fronds, as described in Chapter 2 (Emerged Photosynthesis Section). Temperature and relative humidity in treatments were measured with a hydrochron (iButtons, Maxim Products). Since fronds of *Calliarthron* and *Corallina* desiccate at different rates, either 2 fronds of *Corallina* or 2 fronds of *Calliarthron* were treated and analyzed simultaneously. After the treatment period, fronds were placed into a new vial of fresh seawater at 12ºC and photosynthetic and respiration rates were measured starting at 20 minutes, measurements were recorded every five minutes for one hour. The specimen was then dried in a 68ºC oven for 48 hours to obtain a dry weight. The amount of calcium carbonate was subtracted from this dry weight to obtain the dry weight of only algal material as described in Chapter 1.

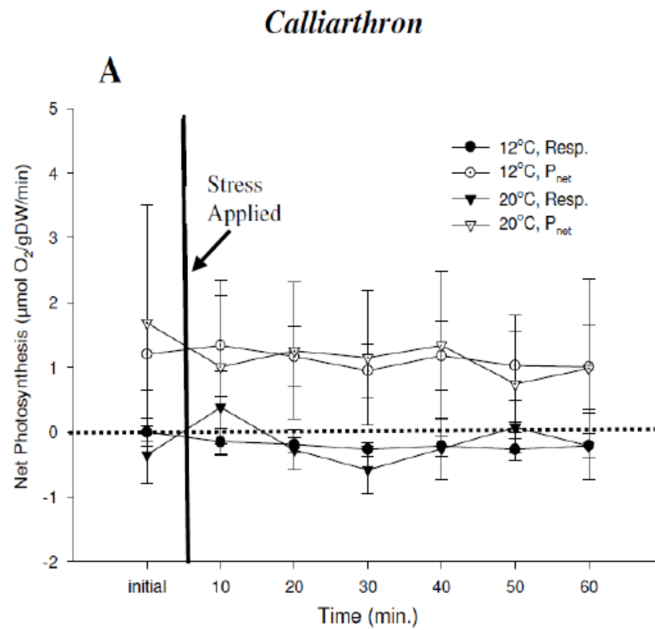
A repeated measures ANOVA design was used to analyze photosynthetic recovery data. First, only photosynthetic data in the recovery period was tested with an ANOVA and no significant effect of time was found. Because of this, any significant effect of time is due to a difference between initial photosynthetic measurements and recovery measurements. Homogeneity of variance was confirmed with Levene’s test. Sphericity was tested with

Mauchly's test. Data had equal variances but was not spherical. Mauchly's test indicated that the assumption of sphericity had been violated $\chi 2$ (F=51.019, $p<0.05$), and therefore degrees of freedom were corrected for using Greenhouse Geisser estimates of sphericity. A two way mixed repeated measures ANOVA was performed on the data, including the initial measurement and five recovery measurements.

3.3 Results

3.3.1 Submerged Recovery

Fronds were considered to recover if net photosynthetic rates were positive after stress treatments were applied. Both *Calliarthron* and *Corallina* recovered from warm tidepool conditions (Figure 14A & B). After 10 minutes, photosynthetic rates of both *Corallina* and *Calliarthron* were positive, and in addition, they were not significantly different from initial rates. However, the two species exhibited significantly different photosynthetic rates ($p<0.01$, Table 12). In the 12ºC treatment, the mean photosynthetic rate was 1.09 ± 0.20 µmol O_2/gDW/min for *Calliarthron* (Fig. 3.1A) and 1.80 ± 0.21 µmol O_2/gDW/min (Fig. 3.1B) for *Corallina*. At 20ºC, the mean photosynthetic rate for *Calliarthron* was 1.18 ± 0.23 µmol O_2/gDW/min (Fig. 3.1A) and 1.88 ± 0.20 µmol O_2/gDW/min (Fig. 3.1B) for *Corallina*.



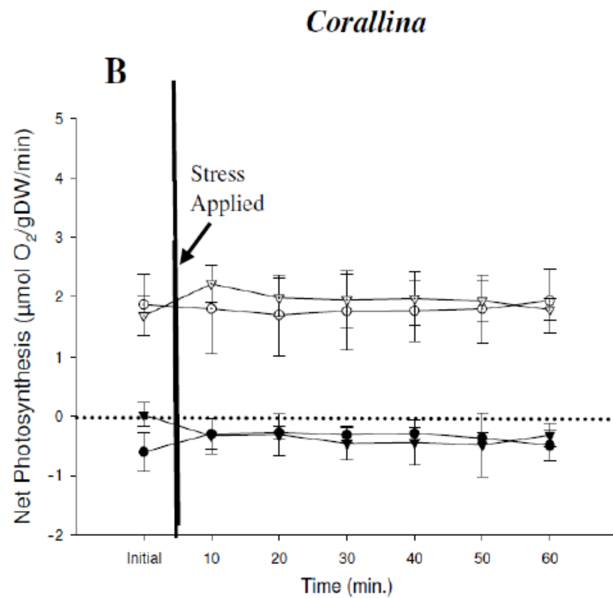


Figure 14: Net photosynthetic and respiration rates of *Calliarthron* (A) and *Corallina* (B) before and after exposure to submerged temperature treatments. Initial rates are shown to the left of the vertical solid line and recovery rates are to the right of this line. Horizontal dashed line represents 0 net photosynthesis. Open symbols indicate net photosynthesis and closed symbols represent respiration rates. Circles are the 12ºC treatment and triangles are the 20ºC treatment. *Calliarthron* (A): n=7 for 12ºC and n=5 for 20ºC; *Corallina* (B): n=6 for 12ºC and n=7 for 20ºC. Error bars are ± 95% confidence intervals.

Table 12: Repeated measures ANOVA results of recovery of net photosynthesis after temperature treatments (12ºC and 20ºC).

Source	Type III Sum of Squares	df	Mean Square	F	p-value
Temperature	0.27	1	0.27	0.17	0.69
Species	**18.51**	**1**	**18.51**	**11.63**	**< 0.01***
Time	0.82	2.34	0.35	1.08	0.38
Temperature*Species	0.00	1	0.00	0.00	0.98
Time*Temperature	0.46	2.34	0.20	0.61	0.70
Time*Species	1.51	2.34	0.65	1.98	0.09
Time*Temperature*Species	1.05	2.34	0.45	1.39	0.24

Table 13: Repeated measures ANOVA results of recovery of dark respiration rates after temperature treatments (12ºC and 20ºC).

Source	Type III Sum of Squares	df	Mean Square	F	p-value
Temperature	0.00	1	0.00	0.01	0.91
Species	0.93	1	0.93	3.65	0.07
Time	0.51	5	0.10	1.72	0.14
Temperature * Species	0.08	1	0.08	0.31	0.58
Time* Temperature	0.60	5	0.12	2.02	0.08
Time*Species	0.50	5	0.10	1.69	0.14
Time* Temperature *Species	**1.82**	**5**	**0.36**	**6.10**	**<0.001***

The interactive effect of time, temperature and species on respiration rates made it difficult to describe clear trends in respiration due to physical factors ($p<0.001$, Table 13). However, over the course of the experiment, temperature affected respiration rates differently in each species (Fig. 3.1A & B, $p<0.001$, Table 13). Respiration rates for *Calliarthron* were -0.21 ± 0.06 μmol O_2/gDW/min. (Fig. 3.1A). Respiration rates for *Corallina* were -0.37 ± 0.06 μmol O_2/gDW/min. (Fig. 3.1B).

3.3.2 Emerged Recovery

Calliarthron did not recover from the combination of temperature and desiccation stress (Figure 15A). 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* ($p<0.001$, Table 14). Photosynthetic rates of fronds of *Calliarthron* were negatively affected by all temperature and desiccation treatments (see Fig. 3.2A), 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 (Fig. 3.2A, $p<0.01$,Table 15) on respiration rates. However, in the recovery phase, photosynthetic productivity was the same whether or not light was applied (net photosynthesis v. respiration) after temperature and desiccation stress was applied. Fronds of *Calliarthron* did not photosynthesize after stress treatments, but continued to respire (Figure 15A).

Unlike *Calliarthron*, *Corallina* fronds recovered after temperature and desiccation stress (Figure 15B). Recovery measurements all exhibited a positive net photosynthetic rate after the temperature and desiccation stress treatments, which is in contrast to the trend seen in *Calliarthron* (Figures 15A & B). In fronds of *Corallina*, there was an interactive effect of temperature and desiccation on net photosynthesis, such that greater temperatures exacerbated the negative effect of desiccation ($p<0.05$, Table 16). Additionally, there was an

effect of time ($p<0.05$, Table 16), confirming that there was a difference between initial and recovery measurements ($p<0.001$, Table 16).

Respiration rates of *Corallina* fronds were unaffected by temperature and desiccation treatments (Figure 15B, Table 17). There was no significant change in respiration rates between initial and recovery measurements ($p = 0.07$, Table 17) nor after the application of temperature ($p = 0.58$, Table 17) or desiccation stresses ($p = 0.72$, Table 17).

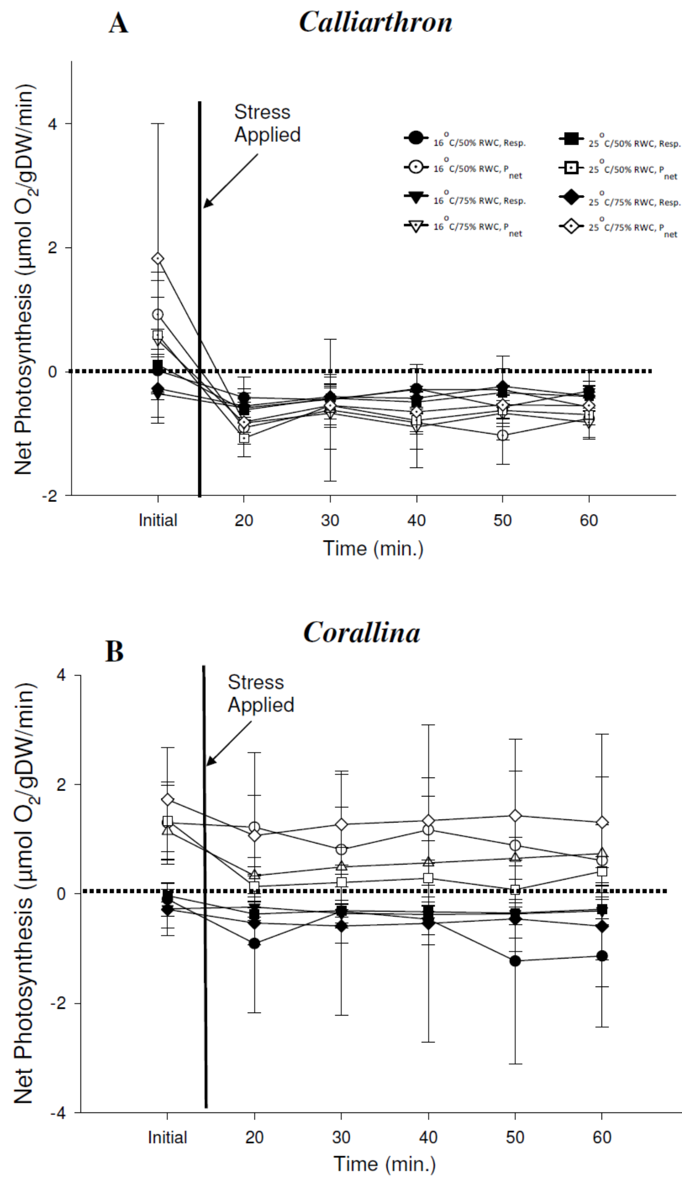


Figure 15: Net photosynthetic and respiration rates of *Calliarthron* (A) and *Corallina* (B) before and after emergent stress. Initial rates are shown to the left of the vertical solid line and recovery rates are to the right of this line. Horizontal dashed line represents zero net photosynthesis. Open symbols indicate photosynthetic rates and solid symbols represent respiration rates. Circles are the 16°C/50% RWC treatment, triangles are 16°C/75% RWC treatment, squares are 25°C/50% RWC, diamonds are 25 °C/75% RWC. *Calliarthron* (A): n=8 for 16°C/50% RWC, n=10 for 16°C/75% RWC, n=13 for 25 °C/50% RWC, and n=8 for 25 °C/75% RWC. *Corallina* (B): n=7 for 16°C/50% RWC, n=10 for 16°C/75% RWC, n=12 for 25 °C/50% RWC, and n=8 for 25 °C/75% RWC. Error bars are ± 95% confidence intervals.

Table 14: Repeated measures ANOVA results of recovery of net photosynthesis for *Calliarthron* after exposure to temperature and desiccation treatments.

Source	Type III Sum of Squares	df	Mean Square	F	p-value
Temperature	1.86	1	1.856	2.302	0.14
Desiccation	1.13	1	1.13	1.401	0.25
Temperature * Desiccation	1.63	1	1.632	2.024	0.16
Time	**92.53**	**1.96**	**47.144**	**29.203**	**<0.001***
Time*Temperature	1.69	1.96	0.86	0.532	0.59
Time*Desiccation	1.22	1.96	0.624	0.386	0.68
Time*Temperature*Desiccation	5.16	1.96	2.628	1.628	0.20

Table 15: Repeated measures ANOVA results of recovery of respiration for *Calliarthron* after exposure to temperature and desiccation treatments

Source	Type III Sum of Squares	df	Mean Square	F	p-value
Temperature	0.00	1	0.00	0.011	0.92
Desiccation	0.16	1	0.16	0.518	0.48
Temperature * Desiccation	0.06	1	0.06	0.185	0.67
Time	**3.61**	**3.01**	**1.20**	**4.060**	**< 0.01***
Time * Temperature	0.67	3.01	0.22	0.753	0.52
Time * Desiccation	1.71	3.01	0.57	1.926	0.13
Time * Temperature * Desiccation	0.32	3.01	0.11	0.363	0.78

Table 16: Repeated Measures ANOVA results for *Corallina* for recovery of net photosynthesis after exposure to temperature and desiccation treatments

Source	Type III Sum of Squares	df	Mean Square	F	p-value
Temperature	0.16	1	0.161	0.03	0.86
Desiccation	4.88	1	4.877	0.98	0.33
Temperature * Desiccation	**22.49**	**1**	**22.49**	**4.5**	**<0.05***
Time	**12.33**	**2.22**	**5.553**	**6.93**	**<0.001***
Time * Temperature	1.39	2.22	0.625	0.78	0.47
Time * Desiccation	2.11	2.22	0.951	1.19	0.31
Time * Temperature * Desiccation	3.01	2.22	1.353	1.69	0.19

Table 17: Repeated Measures ANOVA results for *Corallina* for recovery of respiration after exposure to temperature and desiccation treatments

Source	Type III Sum of Squares	df	Mean Square	F	p-value
Temperature	0.74	1	0.74	0.31	0.58
Desiccation	0.30	1	0.30	0.13	0.72
Temperature * Desiccation	4.74	1	4.74	1.98	0.17
Time	4.45	2.19	2.03	2.77	0.07
Time * Temperature	1.63	2.19	0.74	1.01	0.37
Time * Desiccation	2.80	2.19	1.28	1.74	0.18
Time * Temperature * Desiccation	2.20	2.19	1.00	1.37	0.26

3.4 Discussion

Both *Calliarthron* and *Corallina* are well adapted to living in tidepools, but data suggest that their survival strategies differ. Experiments from the previous chapter demonstrated that *Corallina* is tolerant of warm water conditions, and continues to function even as tidepools heat up. *Calliarthron*, on the other hand, experiences a significant reduction in photosynthesis with increasing water temperature. The results of this experiment

demonstrate that although *Calliarthron* is stressed by warm temperatures, it recovers rapidly when the tide returns and temperatures are returned to cool conditions. *Corallina*, on the other hand, was not stressed by warm temperatures under submerged conditions (see Chapter 2, Submerged Photosynthesis Section, Section 2.3.1), and so, likely retained photosynthetic productivity while exposed to warm temperatures in this experiment. *Corallina* can live at the upper rim of tidepools, because it is not stressed by high temperatures. *Calliarthron* can recover after exposure to warm tidepool conditions, but is stressed by these conditions (See Chapter 2). These results help to explain habitat partitioning within tidepools between the two corallines.

Calliarthron and *Corallina* showed significant differences in their capacity to recover from aerial exposure. *Corallina* regained positive net photosynthetic rates after emergence stress with the return of the tide. After application of stress treatments, *Corallina* recovered in that it maintained positive photosynthetic rates in the recovery period. Photosynthetic rates in recovery were not significantly different from each other but they were significantly different than initial photosynthetic rates. This indicates that *Corallina* is somewhat stressed by emergent conditions, but maintained positive net photosynthesis. The percent of recovery of *Corallina* was highly variable, and no concrete trends in relation to temperature and desiccation treatments could be established. *Calliarthron*, however, never recovered in any desiccation treatment. This finding supports the results of previous studies (Martone *et al.* 2010) and confirms that *Calliarthron* is extremely sensitive to emergent stresses (see Chapter 2), and cannot recover from temperature and desiccation stress.

In this study, *Corallina* recovered photosynthetic rates to positive values, but not to the extent demonstrated for other intertidal algae. In other intertidal species, such as

Mastocarpus papillatus (Bell 1993), *Endocladia muricata* (Britting 1992, Hunt and Denny 2008), and *Fucus spiralis* (Dring and Brown 1982, Madsen and Maberly 1990), desiccation tolerance has also been found to occur after extreme dehydration. In *Mastocarpus papillatus*, desiccation tolerance stems from the ability of the alga to rapidly re-hydrate and regain photosynthesis upon re-immersion in seawater (Bell 1993). Thalli of *Mastocarpus papillatus* are able to completely recover within 10 minutes of re-immersion, provided that temperature exposure was not extreme (Bell 1993). A similar response was found for *Fucus spiralis*, complete recovery of photosynthesis occurred after extreme desiccation after only 35 minutes due to this alga's ability to rehydrate upon re-immersion in seawater. In *Endocladia muricata*, there is a trade-off between remaining hydrated and hence continuing photosynthesis, and dehydrating and being more thermotolerant (Hunt and Denny 2008). It is likely that *Corallina*'s desiccation tolerance also stems from its ability to re-hydrate and regain photosynthesis up re-immersion, as long as desiccation is not too extreme, but this hypothesis was not tested in this study and requires further experimentation.

Past studies have demonstrated that repeated emergent stress may have a compounding effect. For example, Hodgson (1981) found that repeated desiccation events had different recovery outcomes than single desiccation events; as thalli were repeatedly desiccated, their ability to recover was greatly reduced. Because the present study only investigated the effect of a one-time desiccation event, repeated desiccation events typical of field conditions may have yielded different results. It is possible that if repeatedly desiccated, *Corallina* may not be able to recover photosynthesis. This hypothesis also requires further experimentation.

Desiccation rate affects the recovery capability of many desiccation tolerant plants (Roscio and Rocca 2005). For example, resurrection plants die if they dry out too rapidly

(Navari-Izzo and Rascio 1999, Bartels and Salamini 2001), since a certain amount of time is required to induce many of the mechanisms that assist in cellular protection (Oliver *et al.* 2000). In this study, desiccation rates were rapid but not unrealistic. All desiccation treatments were performed in 30 minutes or less. This may or may not have reflected desiccation rates in the field. It is possible that, given slower desiccation times, representative of field conditions at high relative humidities, *Calliarthron* and *Corallina* could utilize additional cellular processes to assist in recovery from desiccation.

Differences in photosynthetic rates may help explain the difference in recovery documented here. *Corallina* has a higher maximum photosynthetic rate than *Calliarthron* and may, therefore, produce more sugars or other osmolytes over the course of a day. Production of sugars may assist plants in recovering from desiccation, as sugars such as sucrose act as a cellular replacement of water (Ghasempour *et al.* 1998, Hoekstra *et al.* 2001). If more sucrose is stored in the cells of *Corallina* during non-stressful periods than in cells of *Calliarthron*, *Corallina* could use this sucrose to protect its cells from damage during dehydration. However, further experimentation is needed to determine if sugar storage assists *Corallina*.

In conclusion, the ability of *Calliarthron* and *Corallina* to recover from emergent conditions during low tide is a critical factor in determining the habitat partitioning between the two species. Both species are able to recover from increasing water temperatures, representing tidepools heating up during low tide. This finding supports the observation that both species occur in tidepools in the intertidal zone. However, only *Corallina* is able to recover from desiccation, explaining why this coralline can resist emergent stresses along the

margins of tidepools and out of tidepools while *Calliarthron* is relegated to tidepools in the intertidal zone.

Conclusion

This study demonstrated physiological and morphological differences underlying the spatial distribution of two intertidal macroalgae along the shore. Differences in morphology and physiological performance documented throughout the course of the tide both contribute to the pattern of habitat partitioning between the two species.

At high tide under saturating light levels, *Corallina* has a greater maximum photosynthetic rate than *Calliarthron*. This is not dependent upon pigments, which were not different between the two species, but likely reflects a difference in metabolism and enzymatic processes between the two species. When submerged under sub-saturating light levels at high tide, the two species performed similarly, reflecting the similarity in pigment profiles.

At low tide, the two species are affected by temperature stress differently when growing in tidepools. *Corallina* is less affected by increased temperatures and increased light, helping to explain how it is able to persist near the margins of pools or survive emergence at low tide. *Calliarthron*, on the other hand, is negatively affected by temperature, helping to explain why it rarely grows near the surface of tidepools, but generally grows deeper in tidepools where temperatures are buffered. When exposed to air, *Corallina*'s morphology allows it to retain water for more than 1 hour, helping this species resist desiccation. *Calliarthron*'s morphology does not hold water for more than 30 minutes when exposed to air, making it more susceptible to water loss. Once exposed to air, both species shut down physiologically, halting both respiration and photosynthesis, so an ability to delay desiccation is tantamount.

When the tide returns, both species are able to recover from temperature stress, if they are submerged in tidepools. However, only *Corallina* is able to recover from stresses associated with emergence. Once desiccated, *Calliarthron* never recovers, explaining why it is rarely found living outside tidepools.

Data presented here suggest several mechanisms preventing *Calliarthron* from surviving outside tidepools in the *Corallina* zone. However, it remains unclear why we do not see *Corallina* living deep in tidepools in the *Calliarthron* zone, since *Corallina* photosynthesizes at the same or higher rate than *Calliarthron* at all temperatures and light levels. What defines the lower limit of *Corallina* in a tidepool?

Herbivory may help explain this pattern. The mean number of molluscan herbivores was found to increase deeper in tidepools and *Corallina* is susceptible to herbivores (Padilla 1984). For example, *Corallina* was consumed by *Katharina* in experimental tidepools (Padilla 1984). *Calliarthron*, on the other hand, is resistant to common molluscan herbivores (*Acmaea, Notoacmaea, Katharina* and *Tonicella*) (Padilla 1984). This resistance is not chemical, but rather structural in nature – the large calcified segments and sparse branches are difficult for herbivores to eat (Padilla 1984). Since many herbivores also find refuge in tidepools during the low tide, *Corallina* may live outside of tidepools to avoid herbivory while *Calliarthron* persists.

Spore survival may also limit the downward extension of *Corallina* in tidepools. For example, preliminary experiments suggest that spores of *Corallina* may need to be slightly desiccated in order to germinate (Kieswetter and McConnell, unpubl. data, Bamfield Fall Program 2010). Further research on this topic is warranted.

During the summer months, low tides coincide with the hottest parts of the day but during the winter low tides occur at night, when temperatures are the lowest. This study was the first to document physiological responses over the course of a tidal cycle, however, it only explored physiological responses to daytime low tides. It did not explore physiological responses to winter low tides, where low temperatures likely play a role in the habitat partitioning between the two species. Further research to explore physiological responses of *Calliarthron* and *Corallina* to low temperatures such as would occur during winter low tides are also warranted.

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