PHYSIOLOGICAL EFFECTS OF NITRATE, LIGHT, AND INTERTIDAL POSITION ON THE RED SEAWEEDS *MAZZAELLA FLACCIDA* AND *MAZZAELLA SPLENDENS*

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PHYSIOLOGICAL EFFECTS OF NITRATE, LIGHT, AND INTERTIDAL POSITION ON THE RED SEAWEEDS *MAZZAELLA FLACCIDA* AND *MAZZAELLA SPLENDENS*

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ABSTRACT

Physiological Effects of Nitrate, Light, and Intertidal Position on
the Red Seaweeds *Mazzaella flaccida* and *Mazzaella splendens*
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California's intertidal seaweeds *Mazzaella flaccida* and *Mazzaella splendens* reside in different intertidal zones. The yellow-green *M. flaccida* is found in the high- and mid-intertidal, while the brown-purple *M. splendens* is found in the mid- and low-intertidal. These differences in intertidal position and blade color, in addition to minute differences in morphology, are typically used to differentiate these species in the field. However, a reciprocal transplant study by Foster (1982) found that, not only can *M. flaccida* and *M. splendens* reside in each other's zone, but the color of *M. splendens* can change to the yellow-green of *M. flaccida*. Thus, Foster (1982) suggested that these two species may be conspecifics. Presently, genetic evidence supports the separation of both species, however, little progress has been made towards determining the cause, mechanism, and impact of this chromatic plasticity on thallus physiology. The present study serves to further our understanding of this chromatic plasticity in *Mazzaella* through a series of field and laboratory experiments. In the field experiment, 360 individuals (180 of each species, 90 controls and 90 experimental) were reciprocally transplanted within the intertidal zones of 3 central California sites. Thereafter, transplants were monitored monthly from June – October for blade size and presence. In October, all transplants were removed for pigment analysis. In the laboratory experiments pigment concentrations of both species were quantified from seaweeds cultured in reduced or replete irradiances and nitrate concentrations. Differences in blade size, pigment composition, and survival between site, intertidal zone, species, and culture treatment were investigated with 2-way ANOVAs and non-parametric tests. In these experiments: 1) greening was documented only for seaweeds in the culture experiments, 2) survival was greatest in the low intertidal zone, 3) high intertidal seaweeds contained greater photoprotective pigment content, 4) *M. flaccida* exhibited increased capacity to regulate photoprotective pigments, and 5) *M. splendens* exhibited increased capacity of phycobilin pigments. The results of this study illustrate how these intertidal seaweeds can survive adverse conditions such as nutrient limitation or increased light stress/desiccation by cannibalizing phycobiliproteins and increasing photoprotective pigments. The differing extent of each species to regulate photoprotective and phycobilin pigments supports their current classification as separate species.

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INTRODUCTION

Darwin's (1859) theory of evolution is predicated on three key concepts: that populations have natural variation in phenotypes; that phenotypes are heritable; and that cohabitating organisms must compete for limited resources to survive. Pursuant to his theory, the natural environment selects for organisms whose phenotypes improve their survival, reproductive output, and competitiveness (Darwin 1859). However, phenotypes that an environment once selected for can rapidly become maladaptive when environmental parameters change, such as temperature and resource availability (Majerus 2009). Since such environmental changes can occur within an organism's lifetime (Bradshaw 1965), organisms may benefit from the ability to alter their phenotype in response to their current environment (Richards et al. 2006). Such phenotypic plasticity would grant these organisms an advantage over phenotypically static individuals and, over time, phenotypic plasticity may be selected for by natural selection (Bradshaw 1965, Schlichting 1986).

The study of phenotypic plasticity and coinage of the term began in the early 1900's (see reviews by Stearns 1989, DeWitt and Scheiner 2004). While observing successive generations of *Daphnia* clones in German lakes, Woltereck (1909) noticed that their head size increased as a function of nutrient availability and varied between *Daphnia* strains. This observation caused Woltereck (1909) to coin the term "reaction norm," which refers to all potential phenotypes an organism can exhibit as a function of its environment. Phenotypic plasticity has a similar definition, but more generally, it encompasses all types of phenotypic change induced by environmental variations (Bradshaw 1965, Stearns 1989, West-Eberhard 1989, DeWitt and Scheiner 2004); Bradshaw's (1965) paper on phenotypic plasticity in plants is often credited as

formalizing the modern study of phenotypic plasticity. In his paper, Bradshaw (1965) hypothesized that plasticity was heritable and subjected to natural selection.

Since these early publications, great strides have been made in understanding the causes and consequences of phenotypic plasticity. Bradshaw's (1965) hypothesis that plasticity was heritable and selected for has been experimentally demonstrated by numerous studies (Van Buskirk and Relyea 1998, Scheiner 2002, Nussey et al. 2005, Hairston et al. 2011). Genes underlying plastic change have been identified (Kelly et al. 2012) and the field is currently very active, spanning multiple scientific disciplines (Via and Lande 1985, Schlichting 1986, Langerhans and DeWitt 2002, Scheiner 2002, Price et al. 2003, DeWitt and Scheiner 2004, Kelly et al. 2012). Additionally, phenotypic plasticity in natural populations has been documented worldwide and is now considered common, where phenotypically static individuals are the exception (reviewed by Kelly et al. 2012).

The plethora of phenotypically-plastic organisms (Padilla and Savedo 2013), including humans (Fluck 2006), suggest that the ability to alter one's phenotype is of evolutionary significance and, therefore, worthy of scientific inquiry. Of particular importance is understanding the molecular mechanisms responsible for plasticity, its evolutionary significance, and how plasticity will influence ecosystems in coming years (Scheiner 2002, Miner et al. 2005, Kelly et al. 2012). Research on phenotypic plasticity can provide us with clues regarding how an organism may respond to gradual changes in its environment, such as those resulting from climate change (Gienapp et al. 2008). Since climate change is predicted to increase the intensity of weather events and alter the predictability of environmental seasons (Sydeman et al. 2014), organisms exhibiting greater plasticity may then receive a competitive advantage over others, thus altering the species composition and function of their ecosystems (Richards et al. 2006).

In marine and lacustrine environments, algae are of particular interest in plasticity studies because their plastic responses are easily quantified through changes in their morphology and/or physiology (Bradshaw 1965, Morales et al. 2002). Macroscopic algae, like terrestrial plants, are mostly fixed at their site of settlement and are unable to escape adverse environmental changes (Bradshaw 1965, Hurd et al. 2014). Therefore, many algae acclimate to environmental changes through alterations of their morphology and physiology (Padilla and Savedo 2013). Morphological and physiological plasticity has been documented in many genera (Padilla and Savedo 2013), from planktonic lacustrine *Desmids* (Hessen and Van Donk 1993) to the giant kelp *Macrocystis* (Demes et al. 2009). Factors that have been studied and shown to generate plastic responses are often those essential to algal development/ecology, such as water flow, nutrient availability, herbivory, and light availability (Hurd et al. 2014).

Water flow can dislodge entire seaweeds and influence seaweed size, shape, and nutrient uptake (Svendsen and Kain 1971, Gerard and Mann 1979, Druehl and Kemp 1982, Fowler-Walker et al. 2006, but see Table 1 in Koehl et al. 2008). The intensity of water flow varies both spatially and temporally as a function of exposure to swell, tides, winds, and storms (Hiscock 1983, Denny 1988, Hurd 2000, Hurd et al. 2014). Habitats with low flow often favor the development of large, ruffle blade morphologies (Svendsen and Kain 1971, Gerard and Mann 1979, Druehl and Kemp 1982, Fowler-Walker et al. 2006, Koehl et al. 2008). This morphology serves to maximize photosynthesis and reduce the nutrient boundary layer by increasing turbulence over blades (Hurd 2000, Roberson and Coyer 2004, Koehl et al. 2008). Conversely, high flow habitats favor the development of robust, low drag morphologies that serve to reduce the likelihood of dislodgement (Stewart and Carpenter 2003, Roberson and Coyer 2004, Wing et al. 2007, Kregting et al. 2008). Transplants from low- to high-flow intertidal zones have been

documented to reduce blade size (Blanchette et al. 1993) and increase stipe thickness (Shaughnessy and DeWreede 2001); whereas, high- to low-flow transplants have resulted in the growth of pneumatocysts (Stewart 2006), wider blades (Stewart and Carpenter 2003), and ruffled contours in blade shape (Svendsen and Kain 1971, Gerard and Mann 1979, Druehl and Kemp 1982, Fowler-Walker et al. 2006, Koehl et al. 2008). All of the aforementioned morphological changes helped the seaweeds reduce dislodgement, while maintaining nutrient uptake rates required for growth and photosynthesis.

The nutrient concentration of a seaweed's surrounding water is also crucial to its form and function (Boyd and Hurd 2009). Nutrients of interest have included sources of nitrogen, phosphorous, sulfur, iron, and carbon, which are needed for the synthesis of organic compounds such as amino acids, ATP, cell walls, and proteins (Roleda and Hurd 2012, Hurd et al. 2014). These nutrients are known to vary both seasonally and spatially in response to upwelling, atmospheric dust deposition, and eutrophication events from storm and agricultural runoff (Phillips and Hurd 2003, 2004, Hurd et al. 2014). Phenotypically-plastic responses to variations in nutrient concentration have been observed to change hyaline hair production, storage and utilization of nitrogen reserves, and even the replacement of a limited nutrient with an amino acid (Mazel and Marlière 1989). Hyaline hair in seaweeds are small sterile outgrowths from the thallus that may aid in nutrient absorption (Deboer and Whoriskey 1983, Hurd et al. 1993). Their presence is a plastic response to nutrient concentrations such as NH_4^+ and phosphate (Deboer and Whoriskey 1983, Hurd et al. 1993). When these nutrients are limited in supply, seaweeds, such as *Ceramium rubrum* and *Fucus*, create hyaline hairs, however, once nutrients return to higher concentrations, hair production ceases (Deboer and Whoriskey 1983, Hurd et al. 1993). In *Mazzaella splendens* nitrate concentrations were shown to affect pigmentation; in a nitrate-

depleted environment, the algal tissue paled from its original red to a light green, but reverted to original coloring with the addition of nutrients (Waaland 1973). An increase in nitrate concentrations above natural levels resulted in further darkening of thalli beyond that typically observed, causing Waaland (1973) to propose that the red accessory pigments may serve as a nitrogen reserve. Another example of how nutrient limitation prompts plastic responses was observed with the cyanophyte, *Calothrix*. When grown in a sulfur-depleted environment, *Calothrix* created photopigments that utilized amino acids devoid of sulfur rather than the typical sulfur containing amino acids utilized in a sulfur replete environment (Mazel and Marlière 1989).

Physical damage from herbivory, or abrasion, is another important factor that elicits plastic responses (Paul 1992, Lürling 2003, Hurd et al. 2014). These responses include wound healing, morphological variation, and chemical defenses (Menzel 1988, Paul 1992, Arnold et al. 1995, Pavia and Brock 2000, Lüder and Clayton 2004, Svensson et al. 2007). Wound healing in siphonous algae is of particular importance as damage to the cell can be lethal (Menzel 1988). Damaging the tissue of such an alga results in a series of responses that rapidly seal the wound in 5-10 min (Menzel 1988). Increased grazing pressures on the tropical alga *Padina sanctae-crucis* (was *Padina jamaicaensis*) triggers the formation of an herbivore-resistant turf morphology (Lewis et al. 1987). However, in *Halimeda* spp. (Paul 1992), *Ascophyllum nodosum* (Pavia and Brock 2000), and other Phaeophyceae (Arnold et al. 1995, Lüder and Clayton 2004, Svensson et al. 2007), similar grazing pressure induces the production of chemical defenses. In *Halimeda*, physical abrasion triggers the production of a potent feeding deterrent, halimedatrial, while in *Ascophyllum nodosum* and other Phaeophyceae it induces the accumulation of phlorotannins (Paul 1992, Arnold et al. 1995, Pavia and Brock 2000, Lüder and Clayton 2004, Svensson et al. 2007). When these same algae are protected from herbivores, however, halimedatrial and

phlorotannins production decreases and *Padina sanctae-crucis* morphology reverts back to the more susceptible, foliose form (Lewis et al. 1987).

Of all of the factors influencing algal ecology/physiology, light is arguably one of the most important because of its importance for photosynthesis (Seitzinger 1956, Kirk 2011, Hurd et al. 2014). Its quality (the range of wavelengths transmitted), intensity (the amount of photons received per $m^2 s^{-1}$), and duration vary seasonally and spatially, as a function of depth and shading (Jerlov 1976, Smith and Baker 1981, Kirk 2011, Hurd et al. 2014). Seasonally, light intensity and duration are at a maximum in summer and minimum during the winter for non-tropical latitudes (Kain 1989). Spatially, intensity and quality are at a maximum at the surface and minimum at depth, varying further as a function of water clarity, which is influenced by weather and plankton growth (Kirk 2011). The change in light quality/intensity as a function of depth is caused by selective absorption of chlorophyll-a from phytoplankters in the upper water column, as well as, water's intrinsic ability to attenuate and absorb light (Jerlov 1976, Smith and Baker 1981, Kirk 2011, Hurd et al. 2014). Water's light absorption capabilities results in transmission maxima at 465 nm and 565 nm for ocean and coastal waters respectively (Jerlov 1976, Dring 1981, Saffo 1987), with transmission becoming increasingly impaired at either side of these maxima (Jerlov 1976, Smith and Baker 1981, Kirk 2011). Therefore, a higher proportion of green light in pigment-rich waters is transmitted to deeper depths, prompting the chromatic adaptation hypothesis (Engelmann 1882, Dring 1981, Saffo 1987).

The chromatic adaptation hypothesis stated that the distribution of the three seaweed groups, Rhodophyta, Phaeophyceae, and Chlorophyta was limited by their ability to absorb green light for photosynthesis. Those seaweeds best able to absorb green light would prevail at depth, while the other seaweeds would grow closer to the surface. Since red algae (Rhodophyta) are

best at absorbing green light, brown algae (Phaeophycea) second best, and green (Chlorophyta) the worst, chlorophytes should dominate at the surface, phaeophytes in the middle, and rhodophytes at depth (Engelmann 1883, reviewed in Saffo 1987). Although the hypothesis was believed by many, it was ultimately disproven by photo-acclimation strategies that algae have evolved to respond to varying light environments (Ramus and Meer 1983, Saffo 1987).

Variation in light quality, intensity, and duration have been shown to induce both physiological and morphological changes influencing growth, pigmentation, and other critical, non-photosynthetic responses, such as protein synthesis or egg formation (Haxo and Blinks 1950, Dring 1981, López-Figueroa and Niell 1989, Algarra et al. 1991, López-Figueroa 1991, Talarico and Cortese 1993, Grossman et al. 1994, Carmona et al. 1996, Talarico 1996, Talarico and Maranzana 2000, Kirk 2011). When grown in green light, red algae in particular significantly up-regulate the production of phycoerythrin, relative to phycocyannin, as a means of maximizing photon absorption in the green wavelengths (Sagert and Schubert 1995). Similarly, Ramus (1976) found that pigment concentrations and proportions increased in response to decreased light intensity/quality from transplants across a depth gradient. Since photosynthetic pigments influence an alga's coloration, changes in their relative concentrations result in the alteration of color. In rhodophytes, reduction of phycoerythrin:phycocyanin ratios, or the loss of phycoerythrin, resulted in the greening of algal thalli (Waaland 1973, Algarra et al. 1991, Costa and Plastino 2011)

Seaweed phenotypic plasticity has made the identification of new or old species through anatomical or morphological criteria precarious (Hind et al. 2015). In fact, recent genetic studies have resulted in the significant reorganization of many algal clades, such as the Laminariales, Corallinales, Florideophyceae, and Gigartinales (Svendsen and Kain 1971, Hughey et al. 2001,

Stewart 2006, Koehl et al. 2008, Monro and Poore 2009, Hughey and Hommersand 2010, Gabrielson et al. 2011, Hind et al. 2014, Saunders and Millar 2014). Of particular note is the reorganization of kelp families (Lane et al. 2006), the synonymization of four *Macrocystis* species (Demes et al. 2009), the synonymization of two *Corallinales* species from two different genera (Gabrielson et al. 2011), as well as the reorganization of the Pacific Gigartinaceae (Hughey et al. 2001). California's species of *Macrocystis* are prime examples of how morphological plasticity confounded early taxonomists. Within this genus, *M. angustifolia, M. integrifolia, M. laevis,* and *M. pyrifera* were determined to be conspecifics due to a variety of factors, including: morphological plasticity, their ability to interbreed, and lack of genetic separation (Graham et al. 2007, Demes et al. 2009, Macaya and Zuccarello 2010). A similar synonymization occurred with *Calliarthron yessoense* and *Cheilosporum latissimum*, such that they are both classified as *Alatocladia modesta* (Gabrielson et al. 2011). Pacific Gigartinaceae are known to be particularly troublesome for morphological identification, since they can appear similar to each other, as well as other genera (Hughey et al. 2001). In Hughey et al.'s (2001) study, it was discovered that five assigned type specimens were describing incorrect species; three species of *Gratelupia* were identical to *Chondracanthus squarrulosus.* Furthermore this was the first study to substantively refute the hypothesis that *Mazzaella flaccida* and *Mazzaella splendens* are conspecifics (Abbott 1971, Foster 1982).

THESIS RESEARCH: THE GENUS *MAZZAELLA*

The possibility that *Mazzaella flaccida* and *Mazzaella splendens* may be conspecifics was suggested by Abbott (1971) due to their similar morphology. These algae have isomorphic diplohaplontic life histories and are abundant in the rocky intertidal zone. On rocks, multiple iridescent blades emerge from a perennial-crusting base. In their juvenile stages (blade

size < 3 cm), these two species are indistinguishable from each other; both exhibit a dark, red-purple coloration (Abbott 1971, Foster 1982). However, as blades grow to sizes larger than 3 cm, their coloration changes. *Mazzaella flaccida* develops a bright green thallus (sometimes with a purple base), while *M. splendens* remains purple (Figure 1 A & B). In addition to differences in pigmentation, these species also dominate different sections of the intertidal zone, although they can settle and co-occur in cleared quadrats (Foster 1982). *Mazzaella flaccida* is generally found from the mid- to high-intertidal zones, growing saxicolously or epiphytically in exposed or protected sites (Abbott and Hollenberg 1976, Foster 1982, Hannach and Waaland 1986); conversely, *M. splendens* grows in the lower intertidal in relatively protected sites (Abbott and Hollenberg 1976, Hannach and Waaland 1986). Although coloration and intertidal positioning were once used to differentiate the species, these traits are unreliable (Abbott 1971, Abbott and Hollenberg 1976, Foster 1982). The present description of each species includes coloration variability from red to light brown to black for *M. splendens* and green to brown for *M. flaccida* (Hughey and Hommersand 2010). Furthermore, Foster (1982) demonstrated that both species can persist in each other's zones and that *M. splendens* can alter its coloration to light green (2 of 9 changed color) when transplanted to the high intertidal (Foster 1982). The cause and consequences of this plastic response, however, have yet to have been examined.

Figure 1. Typical coloration of *Mazzaella splendens* (left) and *Mazzaella flaccida* (right).

The change in coloration that Foster (1982) observed when he transplanted *M. splendens* may be caused by decreased nutrient availability, increased irradiance, and/or increased desiccation relative to low intertidal conditions. Algae in the high intertidal adapted to reside in this zone by maximizing nutrient uptake, reducing pigment concentrations, creating photo-protective pigments (e.g., lutein & zeaxanthin), and resisting desiccation (Thomas et al. 1987a, 1987b, Rowan 1989, Hurd et al. 2014). In the lower intertidal zone, however, desiccation occurs only when algae are exposed at low tide, nutrients are in constant supply, and harmful UV rays are absorbed by the water (Jerlov 1976, Smith and Baker 1981, Kirk 2011, Hurd et al. 2014). Therefore, seaweeds in the lower intertidal may be less nutrient-limited and have less photo-protective pigments in comparison to the higher intertidal algae. Additionally, the steady nutrient supply may allow *Mazzaella splendens* to store excess nutrients within accessory pigments such as phycobilins (Waaland 1973, Talarico 1996).

OBJECTIVES AND HYPOTHESIS

The purpose of this study was to use laboratory and field experiments to investigate 1) the cause and 2) mechanisms of blade color change and the consequences of transplantation and color change on *Mazzaella flaccida* and *Mazzaella splendens*.

Hypothesis 1: *Mazzaella* thalli transplanted from the low intertidal to the high intertidal will decrease pigment concentrations, decrease phycobiliprotein concentrations, and increase photoprotective pigment concentrations. Conversely, those transplanted from the high intertidal to the low will increase pigment concentrations, increase phycobiliprotein concentrations, and decrease photoprotective pigment concentrations.

Hypothesis 2: *Mazzaella* thalli transplanted out of their native intertidal zone will experience greater mortality than those transplanted back into their native intertidal zone.

Hypothesis 3: *Mazzaella* thalli grown in nutrient-poor water will have lower phycobiliprotein content than thalli grown in nutrient-rich water.

Hypothesis 4: *Mazzaella* thalli grown in low irradiance values will have significantly greater phycobilin &chlorophyll-a concentrations, and significantly lower zeaxanthin concentrations than those grown at high irradiance values.

METHODS

SITE DESCRIPTION

To encompass greater genotypic diversity and a range of intertidal zone conditions, three sites were chosen for field transplants: Pigeon Point (37°10'55.86"N 122°23'33.61"W), Fanshell Beach (36°34'57.71"N, 121°58'15.12"W), and Soberanes Point (36°27'13.5"N 121°55'49.0"W). These sites were similar in exposure to a range of swell direction from the Northwest to the South and in the intertidal range of *Mazzaella* (≈1.3m), although, they differed in geology. Pigeon Point and Fanshell Beach were comprised of marine terrace sandstone (Wagner et al. 2002, Dibblee and Minch 2007), while Soberanes Point was a granitic formation (Brabb et al. 2000). At each site, *M. flaccida* and *M. splendens* were identified and reciprocally transplanted between zones of 80% cover, and monitored monthly for morphological changes from June – October 2016.

SPECIES & PLOIDY IDENTIFICATION

Blade color, blade shape, and medullary cell diameter of reproductive tissue, were utilized to ensure accurate identification of *M. flaccida* and *M. splendens* (Hughey and Hommersand 2010). 251 reproductive blades from both Fanshell Beach and Soberanes Point

were removed from the field, photographed, and cross-sectioned. These cross-sections included the carposporangium and were examined with a compound microscope for medullary cell width, according to the dichotomous key published in Hughey and Hommersand (2010).

At the lab, the ploidy of each individual utilized in the field transplant study was determined utilizing the resorcinol test, where haploid tissue turned the reagent red and diploid tissue failed to alter its color (Yaphe and Arsenault 1965, Garbary and Dewreede 1988, Shaughnessy and De Wreede 1991, Brown et al. 2004). Diploid tissue was not utilized in these experiments because medullary filament size is similar for each species (Hughey and Hommersand 2010) and because the extent of plasticity may differ between thalli of variable ploidy (Scheiner et al. 1999).

FIELD EXPERIMENT

At each site in June of 2016, 120 individuals in two treatments were reciprocally transplanted to determine the chromatic plasticity potential of *M. flaccida* and *M. splendens*. Seaweeds were removed from their intertidal zone with a masonry chisel and hammer, such that the substrate their holdfast was attached was not compromised. Thereafter, they were reduced to three blades <30cm, photographed, and epoxied (Z spar, Kopper's Company, Los Angeles, California, USA) into clean pre-chiseled holes, the epoxy was flush with its surrounding. In the experimental treatment, *Mazzaella flaccida* was transplanted into the low intertidal, while *Mazzaella splendens* was transplanted into the high intertidal; in the control treatment, *Mazzaella flaccida* was transplanted back into the high intertidal and *Mazzaella splendens* was transplanted into the low intertidal. Upon transplantation, individuals were labeled by engraving an ID into the epoxy and the location was marked for monthly monitoring.

MONITORING

Monthly monitoring consisted of revisiting each site during the lowest low tide, finding the seaweeds, documenting their status, size of their crust, size of their three largest blades, and (when possible) seaweeds were photographed. Individual status was noted as: alive, dead, or missing due to epoxy failure or overgrowth by other algae. Seaweeds were considered dead when their thalli whitened and failed to recover, or when blades and base were no longer present, but epoxy and the rock to which they were attached was still present. Size of the crust consisted of length by width measurements to nearest mm, where length was the longest measurable dimension and width was the longest measurable distance perpendicular to the axis of length. For blade dimensions, blade length consisted of the distance from the base of the blade's stipe to the edge of its tip, while blade width was the longest measurable distance perpendicular to the axis of blade length. Crust and blade area were approximated using the ellipse equation: $Area\ (mm^2) = \pi * \frac{W}{2} * \frac{L}{2}$, where W and L are the width and length respectively in mm. In addition to these measurements, blade color of the experimental treatment was haphazardly documented with a camera. In October of 2016, all seaweeds were removed from the field, frozen, photographed, and ground for pigment analysis using acetone and phosphate buffer extractions.

PHOTOSYNTHETIC PIGMENT EXTRACTION METHOD

Pigment extractions were accomplished using two different techniques and instruments. Chlorophyll-a, lutein, zeaxanthin, beta carotene and alpha carotene were extracted in 90% acetone and quantified with a High Performance Liquid Chromatograph (HPLC; Zapata et al. 2000), whereas phycobilins (phycoerythrin and phycocyanin) were extracted in 0.1M phosphate

buffer and quantified optically with a dual beam spectrophotometer (HP 8452A Diode Array) (Sampath-Wiley and Neefus 2007).

TISSUE PREPARATION

To prepare algal tissue for extraction, thalli were washed in seawater, lightly scrubbed to remove epiphytes, dabbed dry of excess water, and sliced into thin strips (≈1 cm wide). These strips were placed in a mortar, bathed in liquid nitrogen, and ground into a fine powder with a pestle. This powdered tissue was then scooped into pre-weighed microcentrifuge vials and weighed to determine the wet-mass of tissue added. This mass was then manipulated such that vials intended for acetone extractions contained 20-60 mg (wet weight) of tissue, while vials for phosphate buffer extractions received 60-120 mg (wet weight) of tissue.

PIGMENT EXTRACTION

For each extraction, the amount of extractant added was manipulated such that tissue mass comprised 3% and 5% of the final mass for acetone and phosphate buffer extractions respectively (Sampath-Wiley and Neefus 2007). Extractant mass was determined by reweighing the vials after each step. Once extractant was weighed, the mixture was vortexed and stored at 4 °C for phosphate buffer extractions or -20 °C for acetone extractions.

HPLC METHOD FOR CHLOROPHYLLS, CAROTENOIDS, AND XANTHOPHYLLS

Acetone extracts were analyzed within 2 months of extraction on an HPLC with a 15cm C-8 column (Waters Symmetry, 4.6 mm I.D., 3.5 µm bead size) using the HPLC System 1 gradient profile described in Zapata et al. (2000). Prior to analysis, samples were centrifuged at 17,000 g for 30 mins (Hermle Z 252 MK). The cleared solvent supernatant was used for HPLC analysis. Thereafter, the samples were injected by refrigerated autosampler (200 µL) which was

programmed to dilute each sample with lab grade water just before sample injection (1 part water: 2 parts acetone extract; vol : vol). Peaks from the chromatogram were identified from absorbance spectra (400-750 nm) collected on a Thermo UV 6000 diode array HPLC detector. Corresponding absorbance peaks were analyzed and compared against those reported in both Zapata et al. (2000) and Roy (2011) to determine the pigment type. The following major pigments (and elution times) were identified: Fucoxanthin (18.2 min), Zeaxanthin (27.8 min), Lutein (27.9 min), Chlorophyll a allomer (33.6), Chlorophyll a (34.1 min), alpha carotene (36.6 min), and beta carotene (36.8 min). For each sample, program peak areas at 440 nm were determined with Thermo ChromQuest chromatography software. These area units are representative of pigment concentrations in the extract and were used to calculate pigment area proportions. Pigment area proportion was calculated as $\frac{\text{pigment x area}}{\text{total pigment area}}$ for any given sample. This metric and that of pigment concentration was used to compare pigmentation among samples.

SPECTROPHOTOMETRIC METHOD FOR PHYCOBILIN CONTENT

Phycobilin concentrations were measured one day after extraction on a dual beam spectrophotometer (Jasco Model V-530) according to the methodology of Sampath-Wiley and Neefus (2007). Samples were centrifuged for 30 minutes at 17,000 g, ~500 μL of the supernatant was placed in a 1cm path length narrow bore cuvette, and the absorbance at 564 nm, 618 nm, and 730 nm were measured against a 0.1M phosphate buffer blank. These absorbance values were utilized to calculate the phycoerythrin and phycocyanin concentrations (mg/mL) utilizing the equations derived in Sampath-Wiley and Neefus (2007): R-Phycoerythrin = $0.1246((A_{564}-A_{730})-0.4583(A_{618}-A_{730}))$ and R-Phycocyanin = $0.154(A_{618}-A_{730})$. Where A_{564}, A_{618}, A_{730} correspond to absorption values at 564nm, 618nm, and 730nm respectively. This extract concentration, when

multiplied by extract volume and normalized to tissue wet weight, yielded the final pigment concentration per unit fresh weight (mg pigment/g FW).

LAB EXPERIMENTS

Four culture experiments were utilized to determine nitrate uptake rates and the influence of nutrient/light limitation on pigment composition. These will, hereafter, be referred to as the 1) nitrate uptake experiment, 2) culture experiment 1: *Mazzaella splendens*, 3) culture experiment 2:*Mazzaella flaccida* and *Mazzaella splendens*, and 4) culture experiment 3: constant flow. In these experiments, *Mazzaella splendens* and/or *Mazzaella flaccida* were grown in 19 L containers where nutrient concentrations were adjusted in nutrient-enriched artificial seawater media and incident light levels in outdoor conditions were adjusted with neutral density screening.

CULTIVATION VESSELS

Three laboratory experiments were conducted to determine if nitrogen limitation or shading can stimulate chromatic plasticity in *M. splendens* and/or *M. flaccida.* In these experiments, algae were grown in von Stosch (Grund) nutrient-enriched artificial seawater (VS media; Guiry and Cunningham 1984) in tumble-culture sufficiently agitated by bubbling to mimic turbulence typical of their intertidal environments (Waaland 1976). Thirty-two cultivation vessels were created from 19 L polyethylene buckets. Three holes were bored into each of these buckets. The first hole, located centrally at the bottom of the container, housed a ¼” air hose for circulation, while the other two holes allowed a ¼” cooling hose to wind around the inner base of the bucket and back out again. This cooling hose allowed Monterey Bay seawater to conductively cool the tanks without introducing weedy algae that had plagued preliminary attempts at culturing these seaweeds. Overall, this cooling system was effective in maintaining a

temperature within 2 °C of ambient Monterey bay water (≈14.6 °C) and kept temperatures from reaching 20 °C, as was recorded in a similar bucket without the cooling system.

EXPERIMENTAL DESIGN

Seaweed preparation

Seaweeds for all experiments were gathered from Fanshell Beach and transported to the laboratory in a chilled insulated cooler. At the lab, seaweeds were scrubbed in seawater to remove epiphytes and rinsed in filtered seawater. To ensure homogeneity amongst the samples, seaweeds were trimmed to remove reproductive blades and reduce their masses to <5 g before being placed in the culture vessels.

Nitrate uptake experiment

The purpose of the nitrate uptake experiment was to determine the uptake rate of each species as a function of initial nitrate concentrations. This information was needed to determine a nitrate addition schedule that would allow maintenance of nitrate concentrations within 10 uM of desired concentration in future experiments without the need to replace expensive artificial seawater.

In this experiment, nine individuals of each *Mazzaella* species (n=3) were subjected to three nitrate treatment levels: high nitrate (40 uM), medium nitrate (10 uM), and low nitrate (5 uM). These concentrations were obtained by modifying the nitrate portion of the von Stosch artificial seawater formula (Table 1). All VS media was created separately in large 190 L containers, thoroughly mixed, and then transferred to 18 cultivation vessels. For each treatment, one 30mL water sample was filtered and stored at -20 C for nitrate analysis. Subsequent nitrate samples were taken from each vessel at 10, 22.5, and 70.5 hrs. With these measurements, an

average uptake rate of 0.12 uM/hr was calculated. Thus, to remain within 10 uM of treatment nitrate concentrations, the equivalent of 8.64 uM nitrate (18 mg of nitrate) was added to each vessel every three days.

Table 1. von Stosch enriched artificial seawater solutions for nitrate treatments utilized in all culture experiments.

	Nitrate Treatments		
Compound	***High***	***Medium***	***Low***
Na_2β-glycerophosphate	2.48 uM	2.48 uM	2.48 uM
$NaNO_3$	40 uM	10 uM	5 uM
$FeSO_4 \cdot 7H_2O$	0.1 uM	0.1 uM	0.1 uM
$MnCl_2 \cdot 4H_2O$	1 uM	1 uM	1 uM
$Na_2EDTA \cdot 2H_2O$	1 uM	1 uM	1 uM

Culture Experiment 1: *Mazzaella splendens*

To determine the effect of nitrate concentrations and light levels on pigment concentrations for *Mazzaella splendens*, 36 individuals were cultured in nine treatments of 4 replicates. These treatments were full factorials of high (40 uM & 100%), medium (10um & 60%), and low (0uM & 10%) nitrate and light concentrations, respectively (Table 2). Light reductions were accomplished by layering neutral density screens over the top of the vessels until light levels were sufficiently reduced for each treatment, as measured with a PAR sensor.

Seaweeds were cultivated in the vessels for 14 days. On the 7th day, water samples were taken from each vessel and VS media was completely replaced; tissue samples of each alga were taken on day 0 and day 14, placed in labeled bags, and frozen until they could be processed in the lab for pigment extraction.

Table 2. Treatment design for culture experiment 1.

		Light		
		100%	60%	10%
Nitrate	40uM	N=4	N=4	N=4
	10uM	N=4	N=4	N=4
	0uM	N=4	N=4	N=4

Culture Experiment 2: *Mazzaella splendens* and *Mazzaella flaccida*

To determine the effect of nitrate concentrations and light levels on pigment concentrations for *Mazzaella splendens* and *Mazzaella flaccida*, 16 individuals of each species were cultivated in a total of 4 treatments with 4 replicates in each treatment. These treatments were, again, full factorials of high (50 uM & 100%) and low (0uM & 10%) nitrate and light concentrations. Like the previous experiment, tissue samples were taken on day 0 and day 14, whereas nutrient concentrations were sustained by adding 18 mg of nitrate every 3 days to the high nutrient treatments to yield a final concentration of 40 uM nitrate.

Culture Experiment 3: Constant Flow

During the analysis of previous experiments, it was apparent that the high nitrate treatments were unable to maintain concentrations of 40 uM. Thus, an experimental set-up was constructed to culture the algae indoors, in a continuous flow environment, where nitrates were resupplied continuously. This design consisted of 16 1L cylinders outfitted with a bubbler for mixing and placed in a water bath containing cold Monterey Bay water for cooling. Each cylinder was outfitted with a drip hose, set at a constant drip rate of 1 drop/5 seconds, to supply the cylinders with either 5 (high N) or 0.1 uM (low N) nitrate per hour. In this experiment, artificial light was supplied at 900 μmol m^{-2} s^{-1} with an 8:12 Light-Dark Cycle. Light levels

were consistent for all cylinders and only nitrate concentrations were modified. These two treatments received 4 replicates of each species and blade tissue samples were taken at day 1 and day 7 for pigment extractions.

To determine the ability of nitrate depleted seaweeds to recover pigment content, four of the 16 seaweeds were placed in a circular acclimation tank. This tank lacked a bubbler but received a high flow of sand-filtered Monterey Bay water jetted in from the sides. This jet action resulted in the cyclonic movement of water that concentrated seaweeds at the middle of the tank and jettisoned less dense material. This proved to be an effective culture technique that prevented overgrowth by pest algae. 39 days later these seaweeds were removed, and pigments were quantified.

STATISTICAL ANALYSIS

In the field experiment two-way ANOVAs were utilized to compare survival, pigment concentration/proportion, and morphometrics between site, treatment, species, and time. While in the laboratory experiments two-way ANOVAs were utilized to compare pigment concentration/proportion between treatment, species, and sample day. In each statistical test ANOVA assumptions of normality and homoscedasticity were tested for and transformed until assumptions were met. For data in which assumptions could not be met, non-parametric tests were utilized.

RESULTS

FIELD EXPERIMENT

Monitoring Status:

In October of 2016, 43% and 33% of the initial 180 transplants of *M. flaccida* and *M. splendens* survived respectively, while 12% and 29% died, 28% and 20% experienced epoxy failure, and 18% went missing (Table 3). These values were utilized to calculate survival, where $\text{survival} = \frac{\text{n survivng}}{\text{n transplanted} - (\text{n missing} + \text{n epoxy failure})}$. Survival from June to October decreased gradually and was greater in the low intertidal zone for both species (Figure 2).

Table 3. Survey status of *M. flaccida* and *M. splendens* for all sites combined during month of October.

	Status			
Species	**Alive**	**Dead**	**Epoxy Failure**	**Missing**
M. flaccida	43%	12%	28%	18%
M. splendens	33%	29%	20%	18%

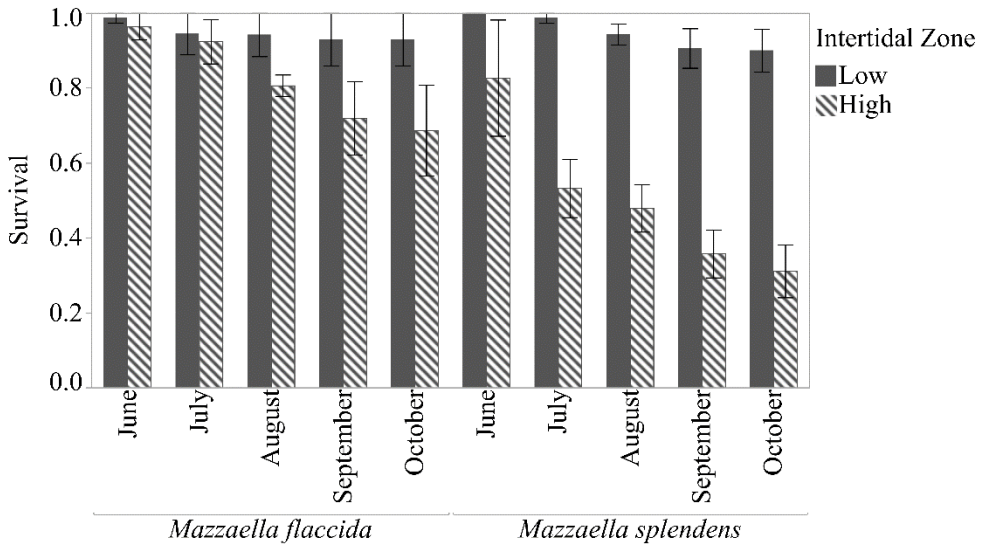

Figure 2. Mean survival (±SE) of *M. splendens* and *M. flaccida* between high and low intertidal zones from June – October.

During the month of October, there was no effect of species on the ANOVA (ANOVA p=0.0461; Species p=0.31; Intertidal Zone p=0.04; Interaction p=0.10; See Appendix Table A1-A2). Instead, significant differences in survival were found between intertidal zones, with the lower intertidal having greater survival than the high intertidal (Figure 3).

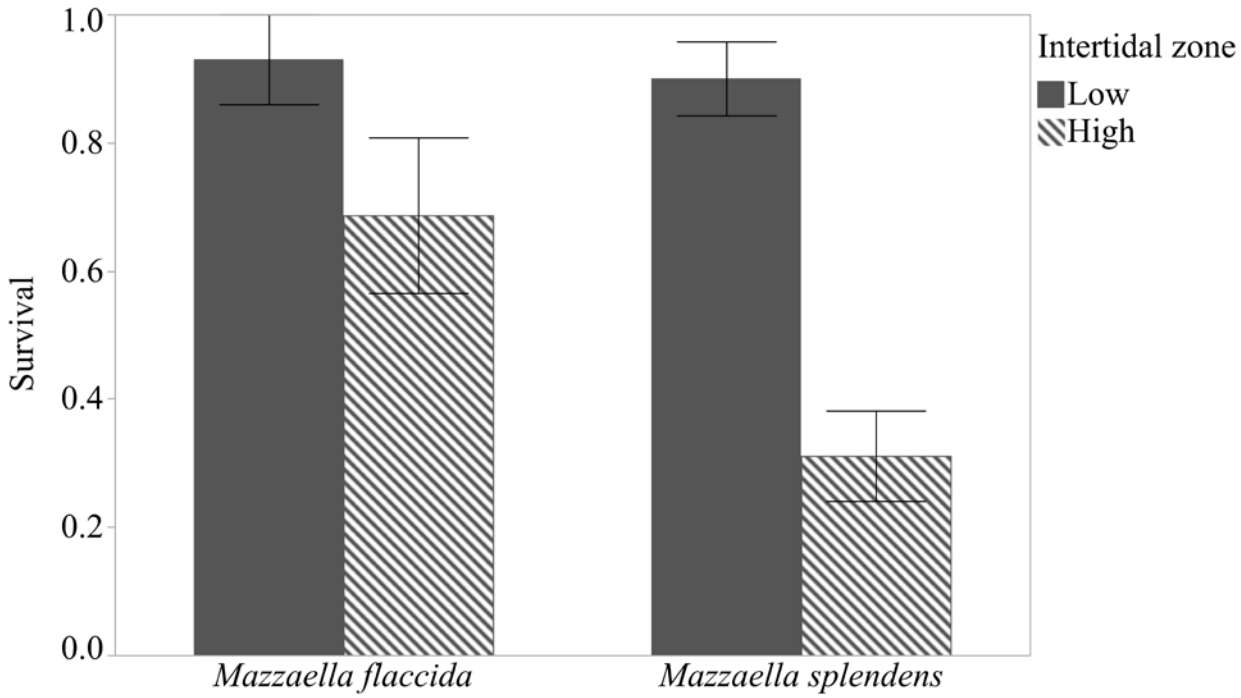

Figure 3. Mean survival (±SE) of *M. splendens* and *M. flaccida* between high and low intertidal zones of all sites for the month of October. * indicates significant difference as determined by t-test.

Mass and Blade Area:

Log-transformed blade area showed a significant effect of species, intertidal zone, and the interaction between species and intertidal zone, for the month of August and a significant effect of site for the month of June (Figure 4). However, no significant differences in total blade area

(total area of the 3 largest blades) were observed during any other month. Similarly, no significant differences were detected between blade mass by site, intertidal zone, or species (ANOVA p=0.65; Site p=0.92; Intertidal Zone p=0.70; Species p=0.89; Intertidal Zone*Species p=0.09; Site*Intertidal Zone*Species p=0.18; See Appendix Table A3-A8).

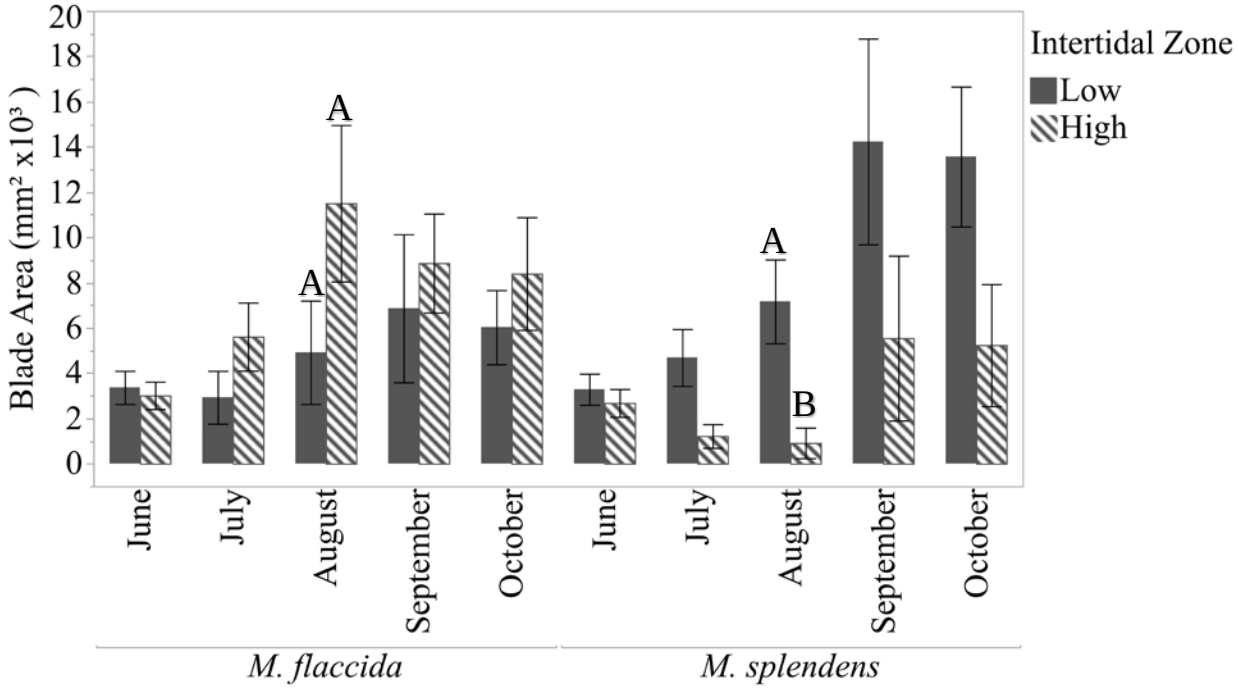

Figure 4. Mean total blade area (mm^2 x 10^3 ± SE) of the three largest blades for all sites by species, intertidal zone, and month. Means sharing a letter are not significantly different as determined by Tukey HSD.

Color changes:

None of the 90 *M. splendens* that were transplanted to the high intertidal transitioned to green coloration. The only color change observed was a browning of blades prior to bleaching and, ultimately, death (Figure 5). Surviving *M. splendens* in the high intertidal did so without altering their color (Figure 5).

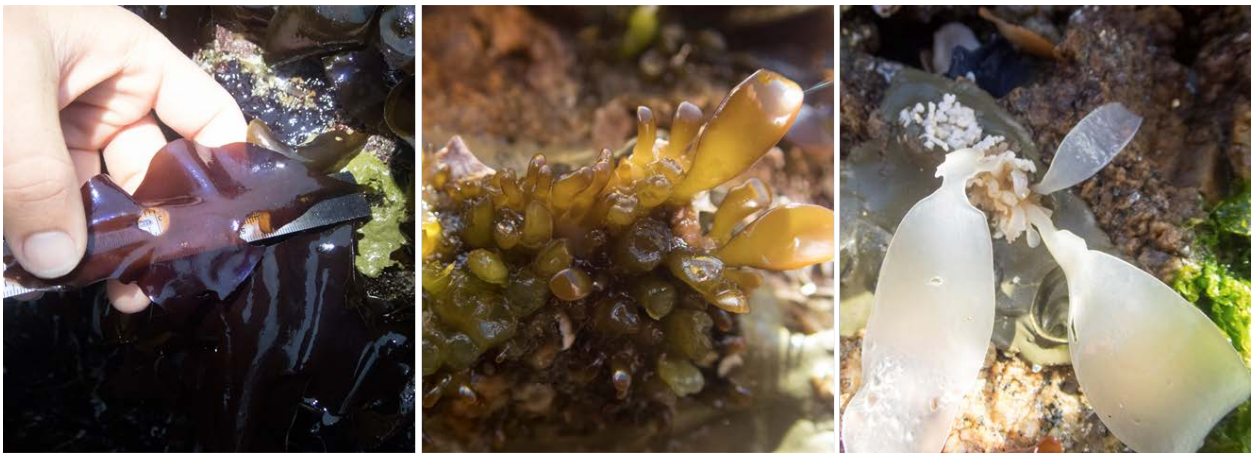

Figure 5. Coloration exhibited by *M. splendens* transplants in the high intertidal zone. Left: healthy transplant after 2 months. Middle: dying transplant after one week. Right: photobleached transplant after one week.

Pigments:

Phycobilins - The lack of green color change in the high intertidal was corroborated by phycoerythrin and phycocyanin content (mg/gFW) being approximately equal within species, between intertidal zones, and between sites (Figure 6; See Appendix Table A9-A10). Between species only phycoerythrin differed significantly, with *M. splendens* attaining greater content

(Figure 7).

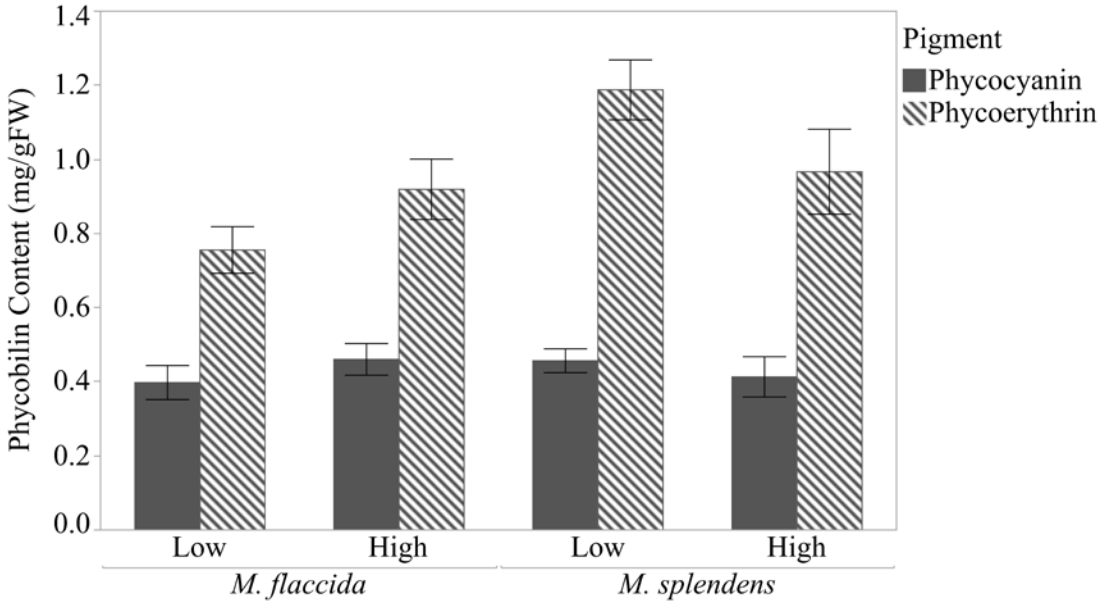

Figure 6. Mean phycobilin content (mg/gFW ± SE) by intertidal zone and species.

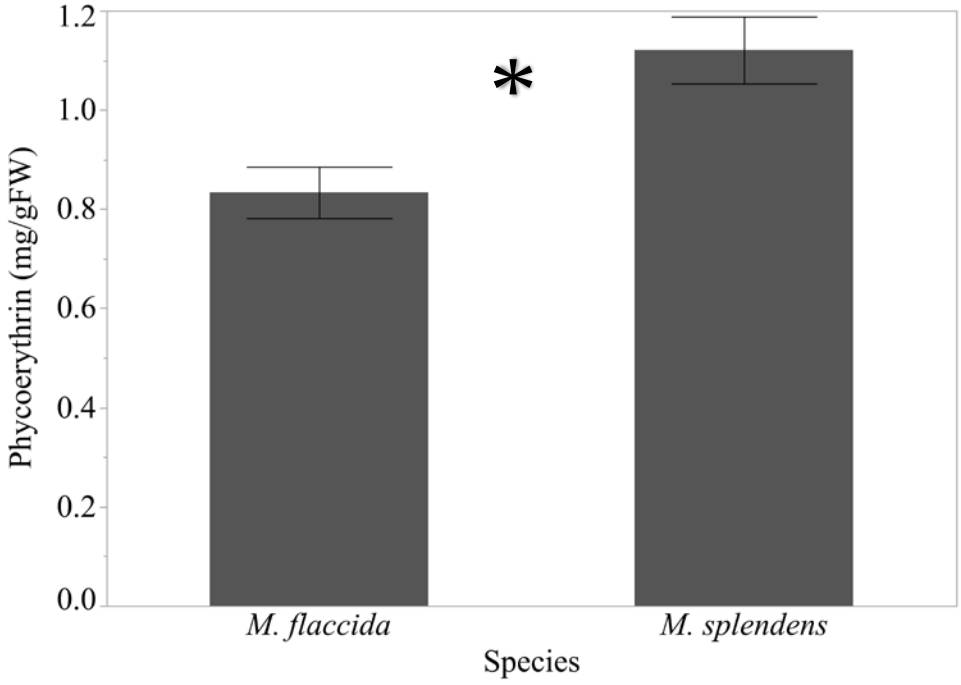

Figure 7. Mean phycoerythrin content (mg/gFW ± SE) between species. * indicates significant difference as determined by t-test.

Chlorophylls – Logit transformed Chlorophyll a (mg/gFW) similarly did not differ in proportion between intertidal zones, species, or site (Figure 8; See Appendix Table A9-A10). Chlorophyll a allomer proportion of total HPLC area, however, significantly differed among sites, species, and intertidal zone (Figure 9; See Appendix Table A9-A10). HPLC proportions were significantly greater in the low intertidal zone (Fig 9; See Appendix Table A9-A10).

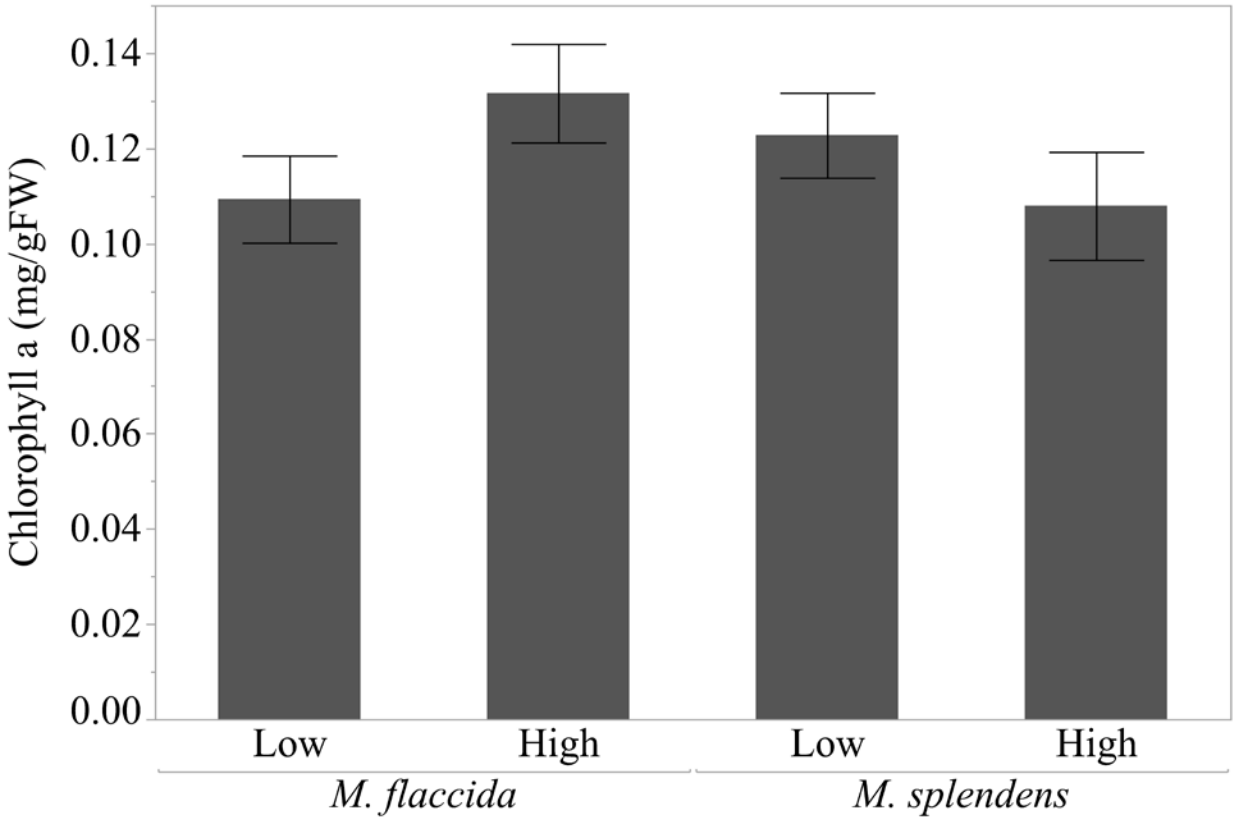

Figure 8. Mean chlorophyll a content (mg/gFW ± SE) by species and intertidal zone.

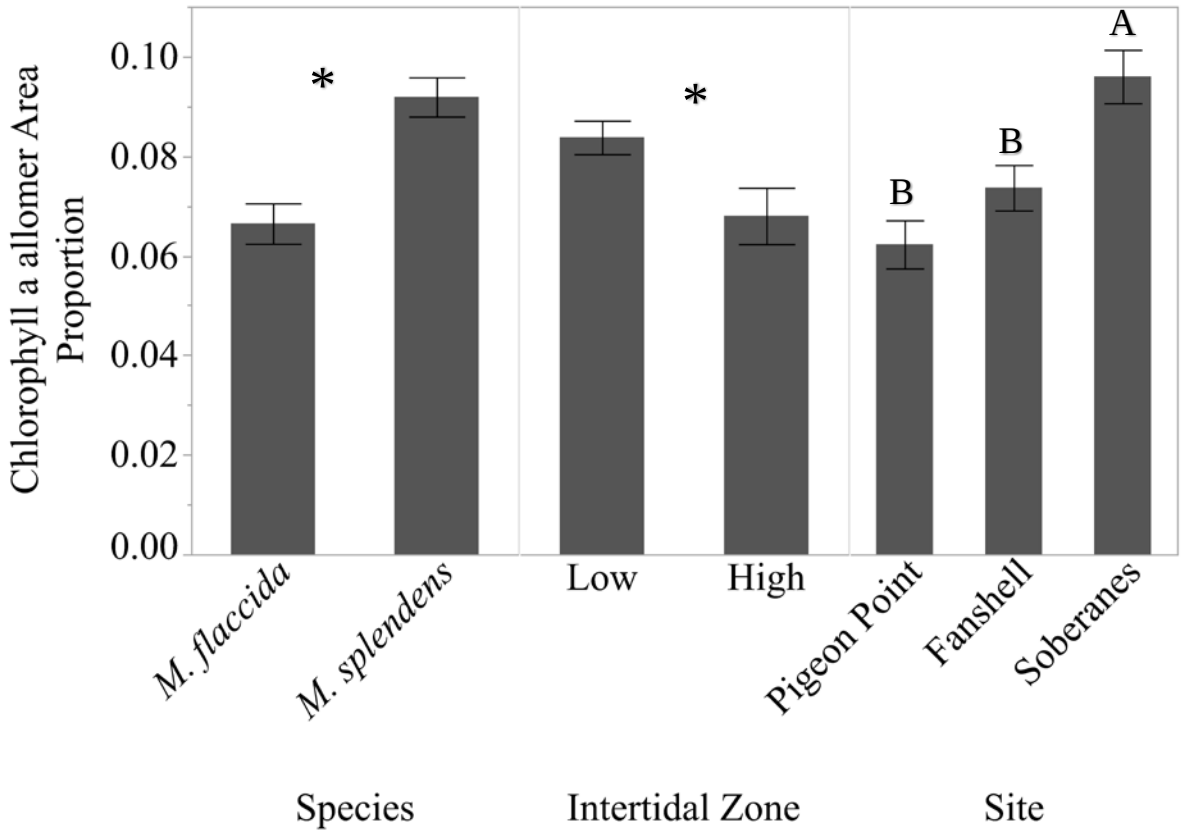

Figure 9. Mean chlorophyll a allomer area proportion (± SE) by species, intertidal zone, and site. *indicates significant difference as determined by t-test.

Xanthophylls – Fucoxanthin, a pigment typical of brown algae (derived from epiphyte or endophytes), differed by intertidal zone and species (ANOVA p<0.0001; Intertidal Zone p=0.005; Species*Intertidal Zone p=0.018; Site p=<0.0001; See Appendix Table A9-A10). Low intertidal *M. flaccida* contained a greater proportion of fucoxanthin than low intertidal *M. splendens* or high intertidal *M. flaccida* (Fig 10). This may be indicative of greater epiphyte and/or endophyte loads. Zeaxanthin on the other hand did not differ significantly between species, but was significantly different between intertidal zones (ANOVA p<0.0001; Site p=0.02; Species p=0.023; Intertidal Zone p=<0.0001; Site*Intertidal Zone p=0.0405; See Appendix Table A9-A10). Seaweeds grown in the high intertidal zone had greater proportions

and concentrations of zeaxanthin than those grown in the low intertidal zone (Fig 11). Lutein was significantly different between intertidal zone and species; *M. flaccida* in the high intertidal had a greater proportion of lutein comprising their photosystems than *M. flaccida* in the low intertidal and more lutein per unit wet-weight than *M. splendens* had in either zone (Fig 12).

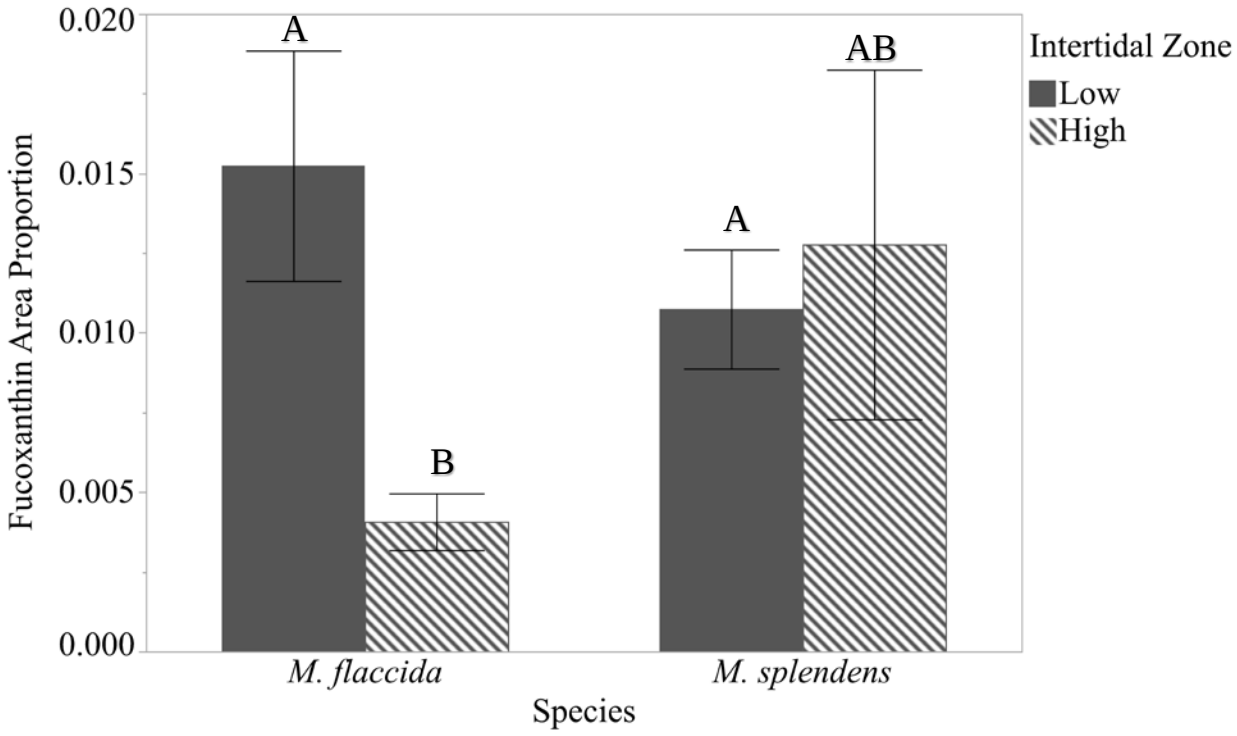

Figure 10. Fucoxanthin area proportion (±SE) by intertidal zone and species. Means not sharing letter are significantly different as determined by Tukey HSD.

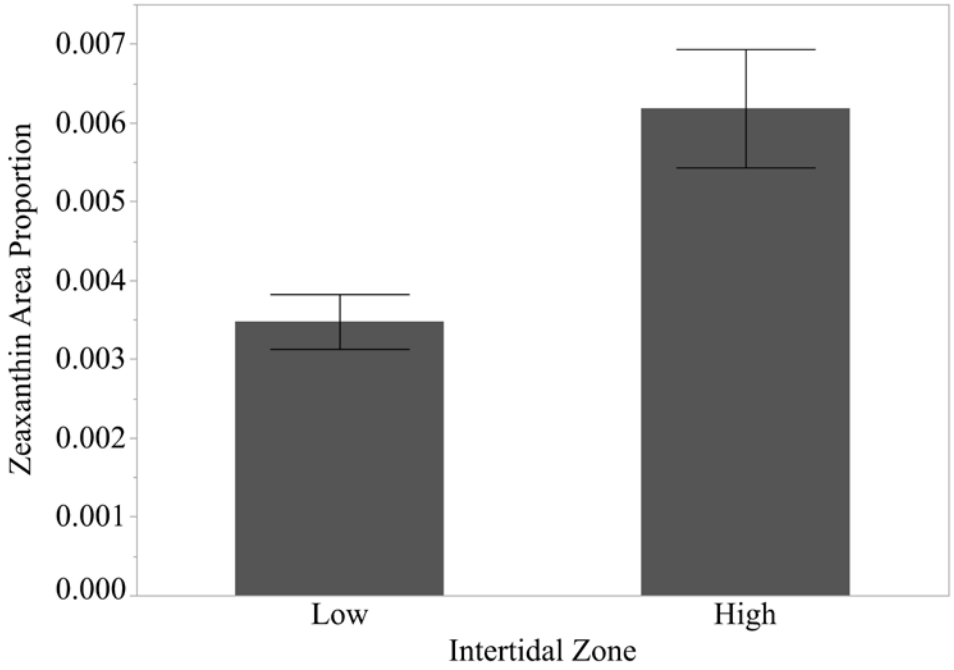

Figure 11. Mean zeaxanthin area proportion (±SE) by intertidal zone. * indicates significant difference as determined by t-test.

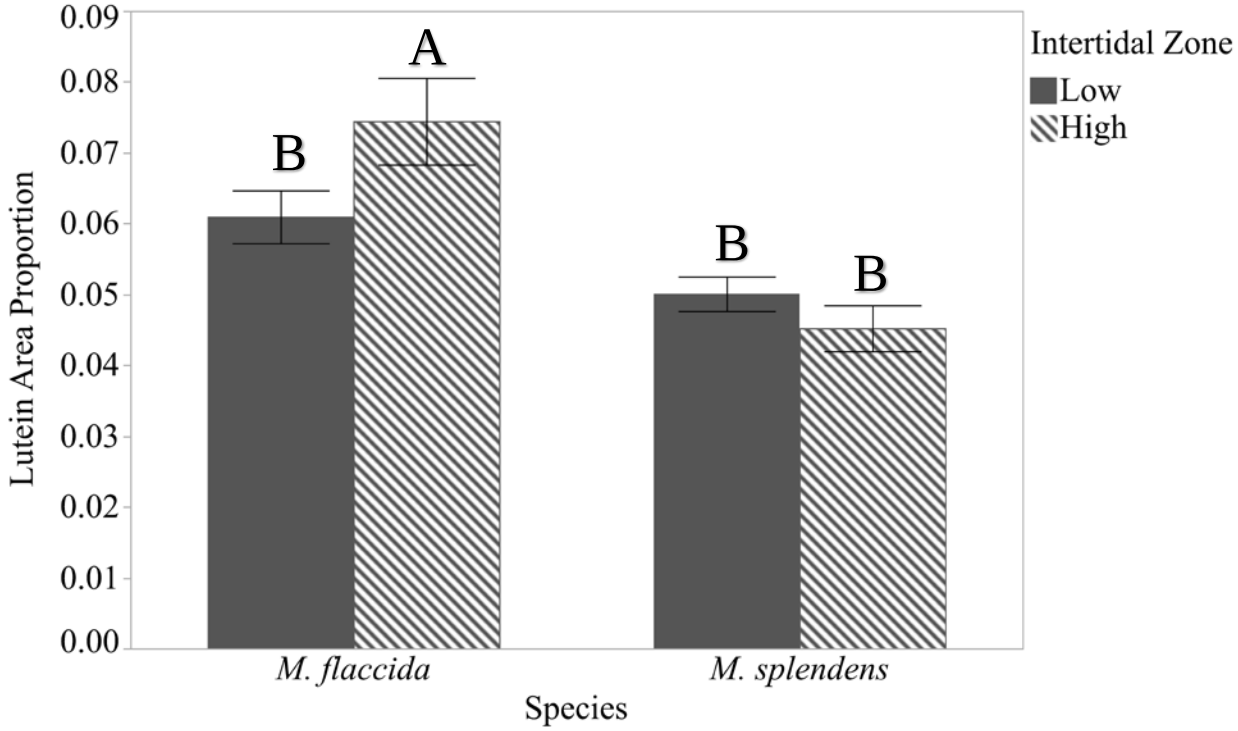

Figure 12. Mean lutein area proportion (±SE) by intertidal zone and species. Means not sharing letter are significantly different as determined by Tukey HSD.

Carotenoids – Alpha carotene proportions were significantly different between site and intertidal zone but not between species (Fig 13; See Appendix Table A9-A10). For Fanshell Beach and Pigeon Point, alpha carotenes were proportionally greater in the high intertidal zone and did not differ between these two sites (Fig 13). However, at Soberanes alpha carotene proportions didn't differ between intertidal zone. Beta carotene, on the other hand, differed only between intertidal site, such that those in the high intertidal had a greater proportion (Fig 14). The ratio of beta carotene to alpha carotene showed an interesting relationship that remained consistent between species and intertidal zones. The low intertidal seaweeds had values close to 1, while those in the high intertidal had values greater than 2 (Fig 15).

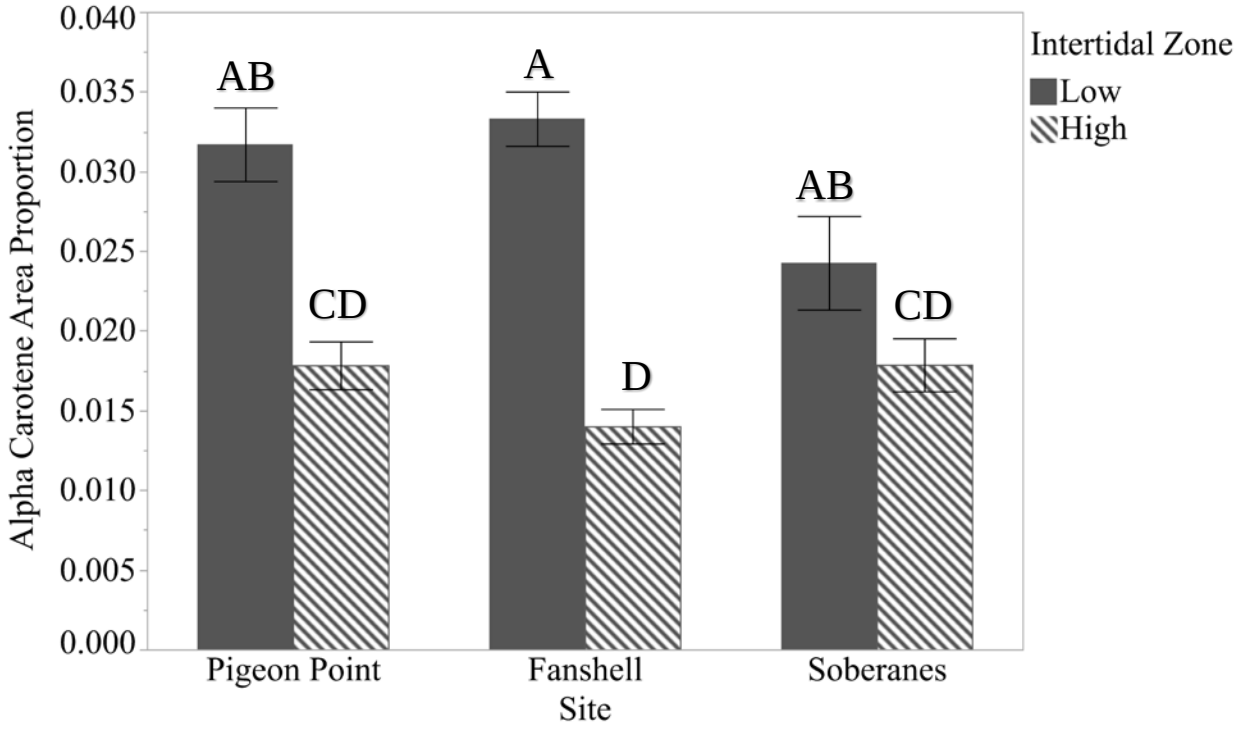

Figure 13. Mean alpha carotene area proportion (±SE) by site and intertidal zone. Means not sharing letter are significantly different by Tukey HSD.

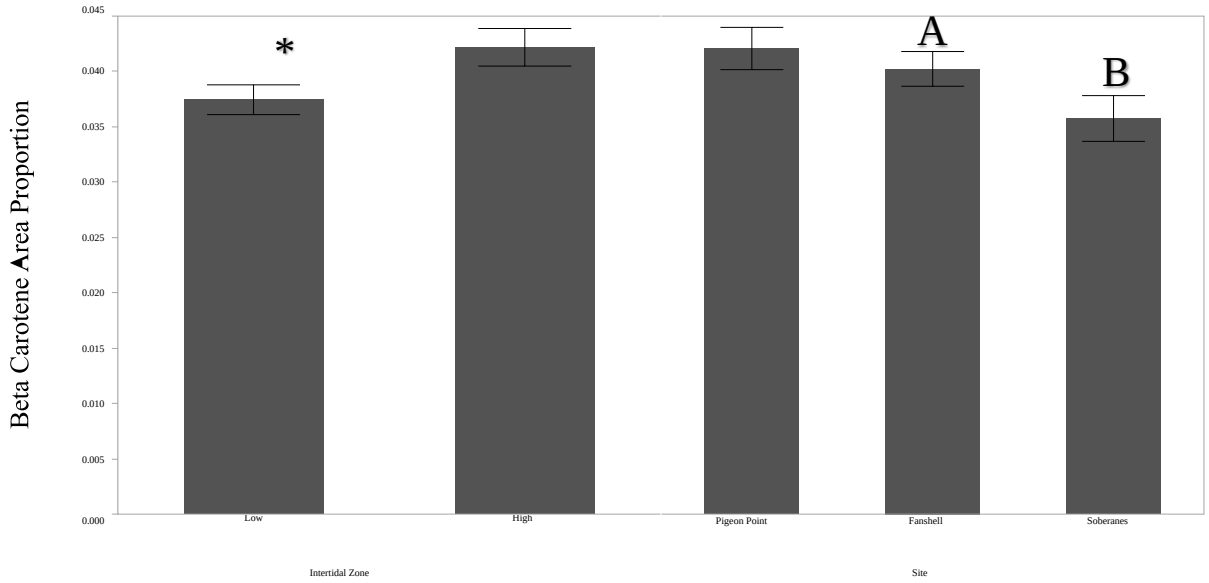

Figure 14. Mean beta carotene area proportion (±SE) by intertidal zone and site. * indicates significant difference as determined by t-test. Means not sharing a letter are significantly different as determined by Tukey HSD.

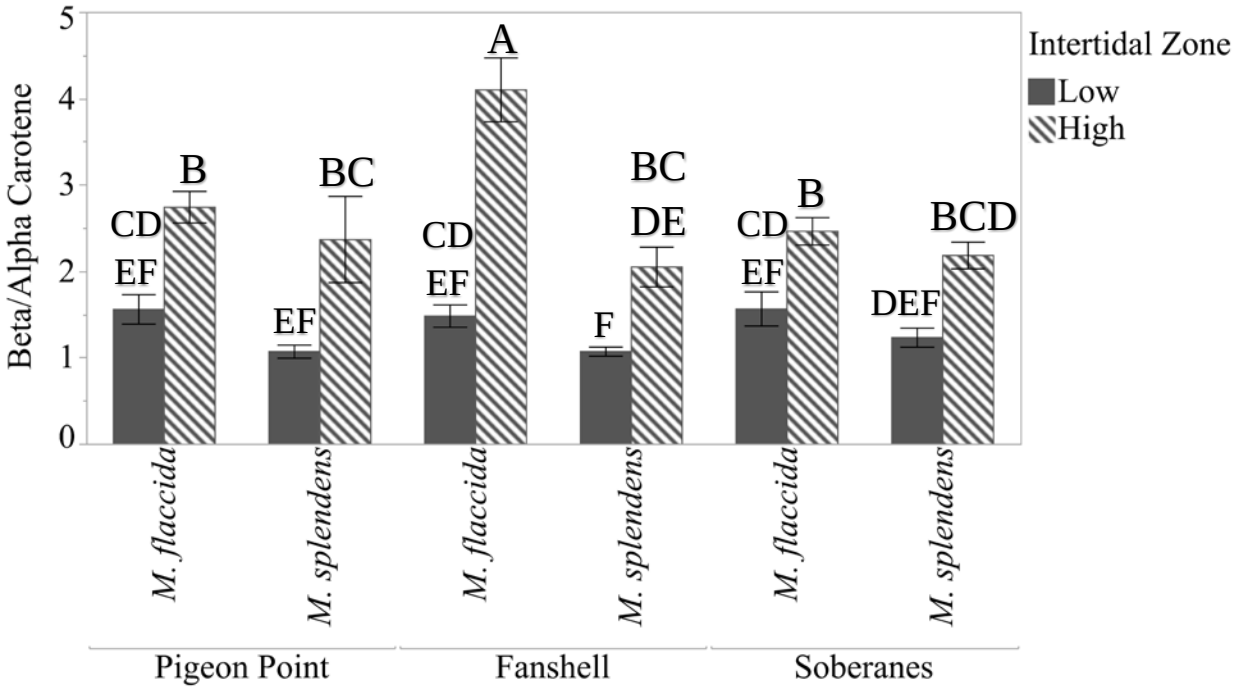

Figure 15. Mean beta to alpha carotene ratio (±SE). Means not sharing letter are significantly different as determined by Tukey HSD.

LABORATORY EXPERIMENTS

Culture experiment 1: *M. splendens*

Color – In this experiment, it was found that after 14 days all *M. splendens* blades began losing red pigmentation revealing a bright green blade (Fig 16).

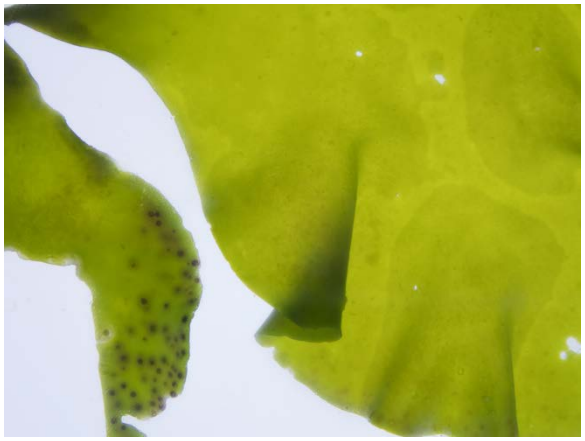

Figure 16. A bright green *M. splendens* blade after being grown for 2 weeks in a nitrate limited vessel.

Pigments – Between days 0 and 14, zeaxanthin, lutein, alpha carotene, beta carotene, phycoerythrin, and phycocyanin decreased significantly in proportion or content, while chlorophyll a allomer increased and chlorophyll a proportion remained unchanged (Figures 17-19; See Appendix Table A11-A12). Although chlorophyll a proportion remained unchanged, its content (mg/gFW) significantly decreased (Figure 20).

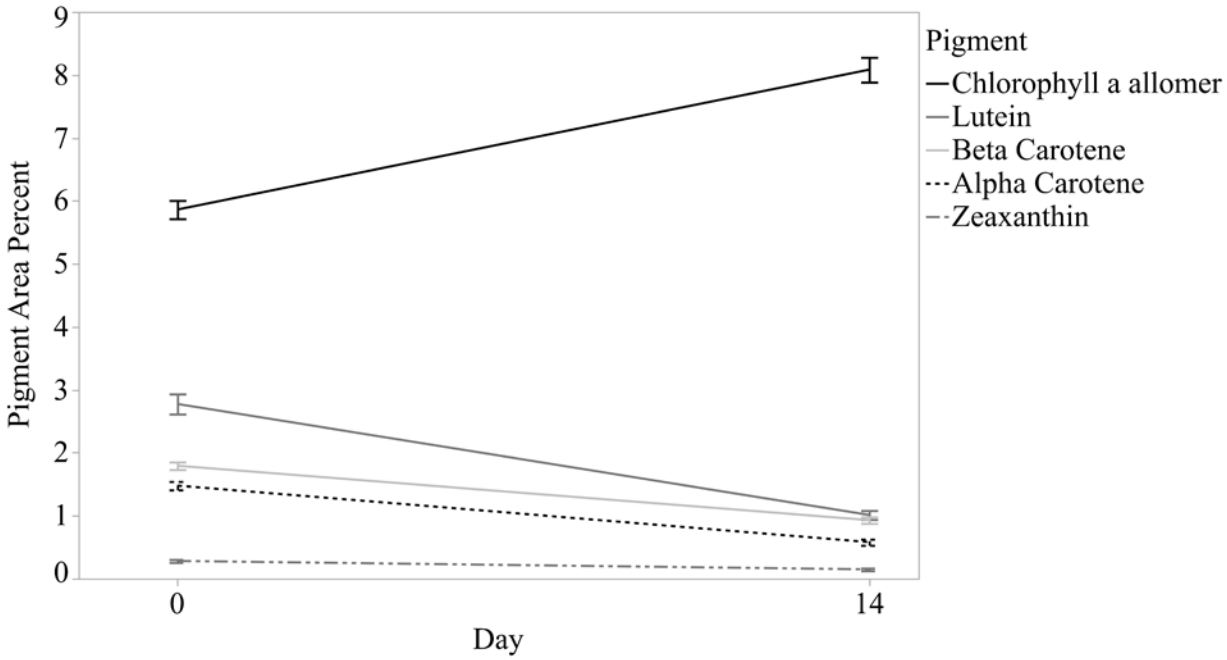

Figure 17. Mean pigment area percent (±SE) by pigment and sample day for *M. splendens* only.

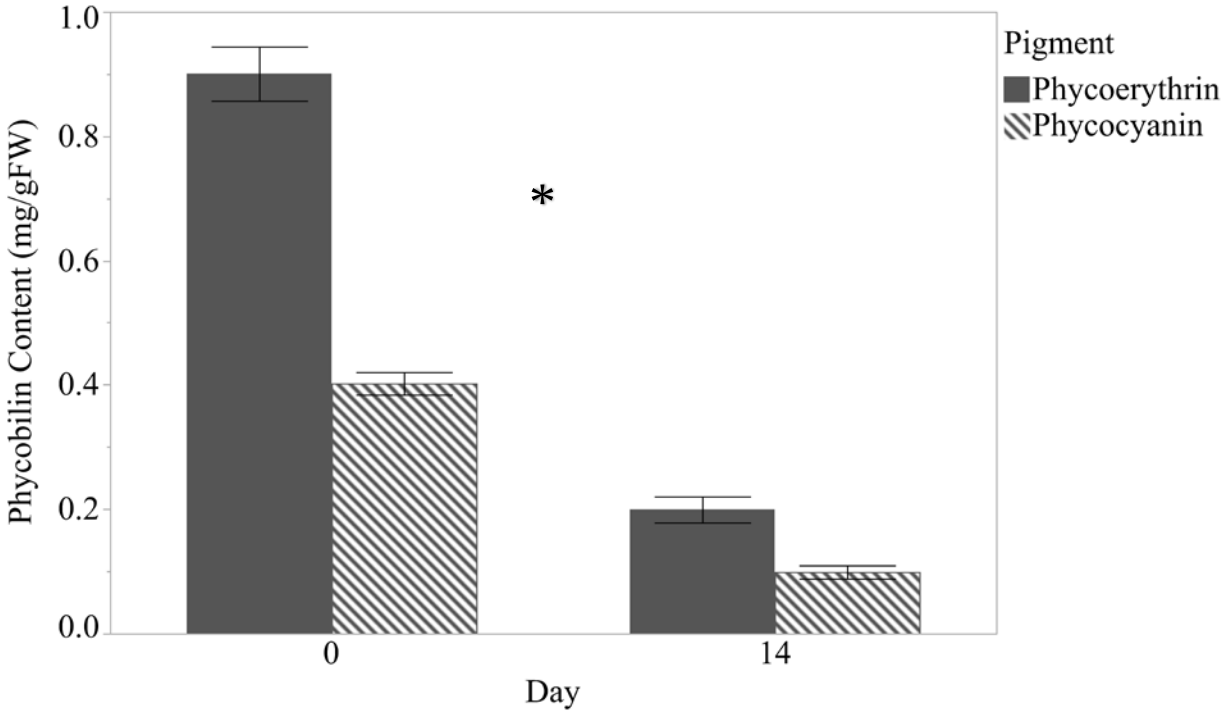

Figure 18. Mean phycobilin content (mg/gFW ± SE) by pigment and day for *M. splendens* only.
* indicates significant difference within pigments between sample day as determined by t-test.

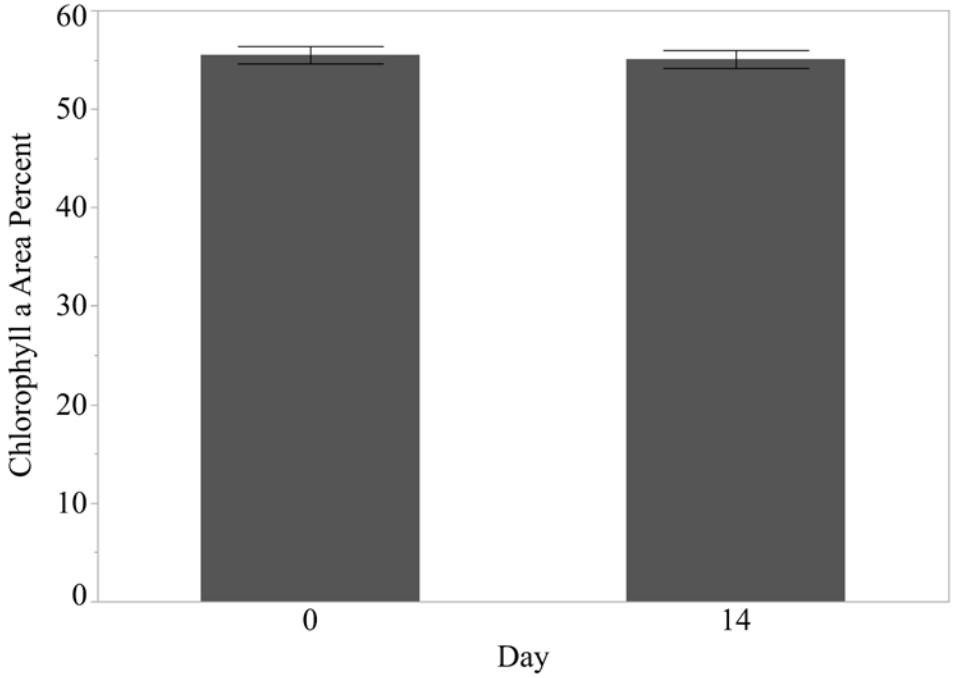

Figure 19. Mean chlorophyll a area percent (±SE) between sample day for *M. splendens*.

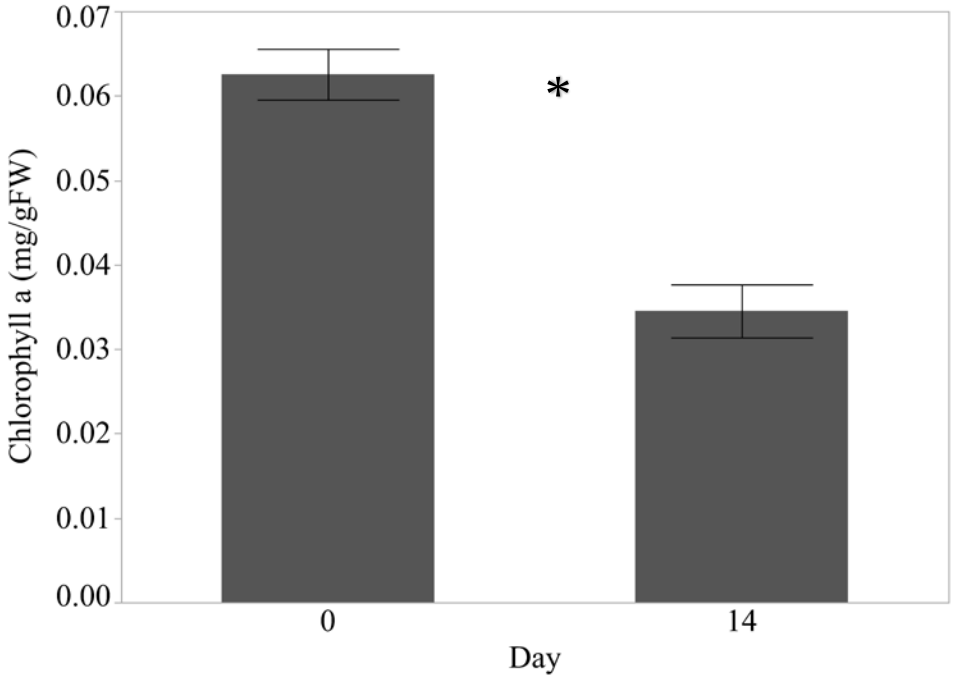

Figure 20. Mean chlorophyll a content (mg/gFW ±SE) by day for *M. splendens* only. * indicates significant difference as determined by t-test.

Culture experiment 2: *M. flaccida* & *M. splendens*

Pigments – Throughout the experiment, *M. flaccida* had higher zeaxanthin and lutein proportions than *M. splendens* (Figure 21; See Appendix Table A13-A14). Chlorophyll a allomer, chlorophyll a, and beta carotene increased over the 14 days for both species (Figure 22). Alpha carotene did not change over the period, but was significantly greater in *M. splendens* than *M. flaccida*. Phycoerythrin and phycocyanin content decreased over the 14 days for both species (Figure 23). Finally, the beta/alpha carotene ratio differed significantly between species and sample day (Fig 24).

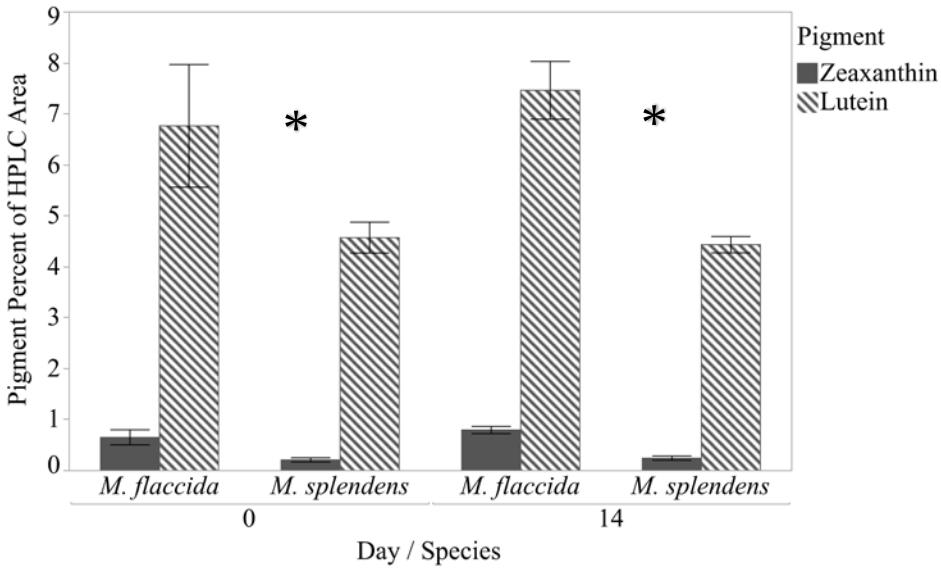

Figure 21. Mean zeaxanthin and lutein proportion (±SE) by species and day. * Indicates significant difference as determined by t-test.

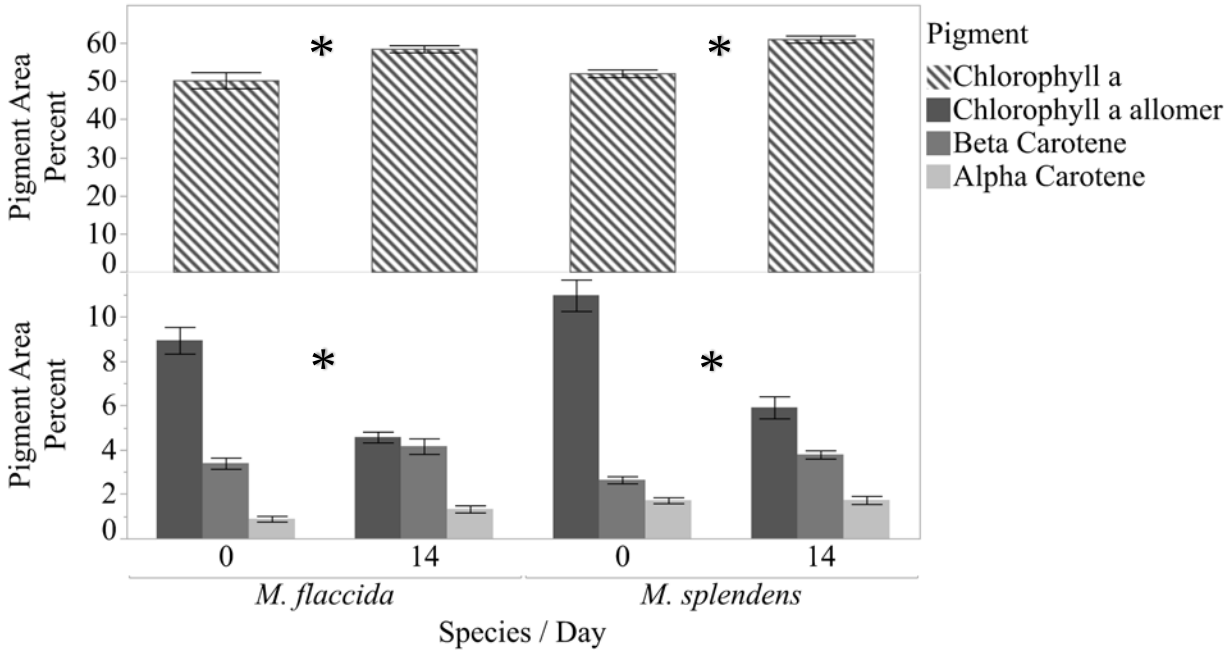

Figure 22. Mean pigment area percent (±SE) by species and day sampled. * indicates significant difference as determined by t-test.

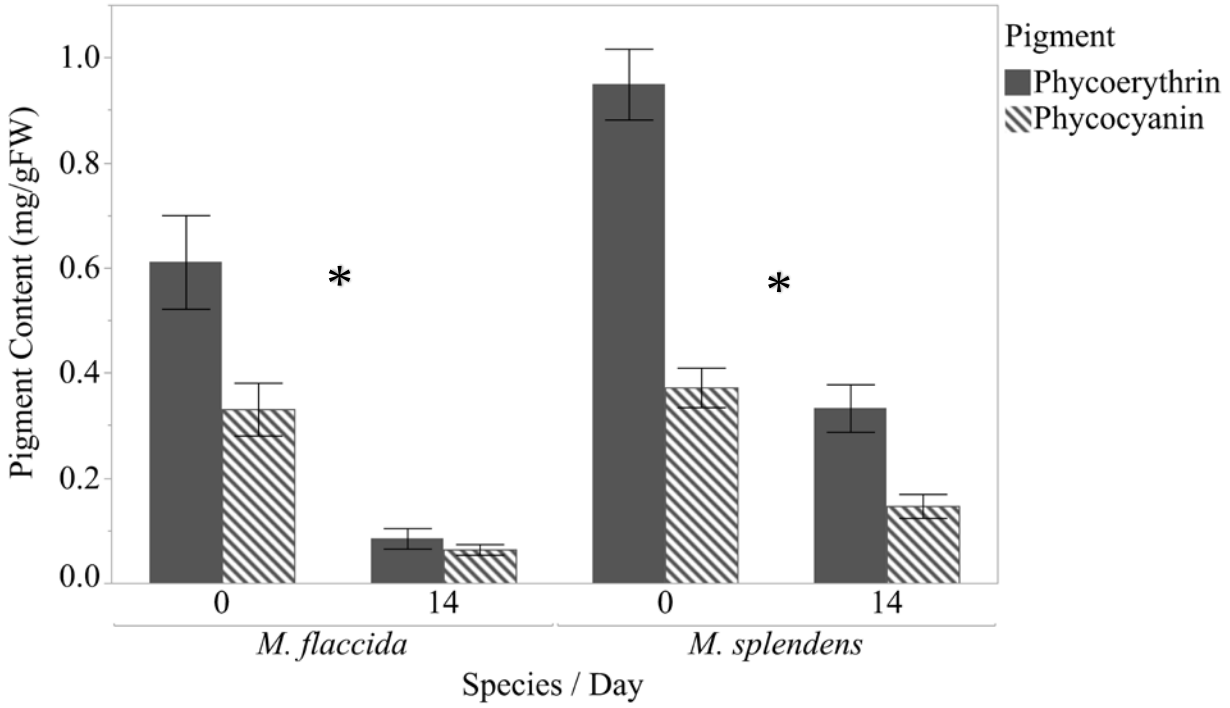

Figure 23. Mean phycobilin content (mg/gFW ±SE) by species, pigment, and sample day. * indicates significant difference as determined by t-test.

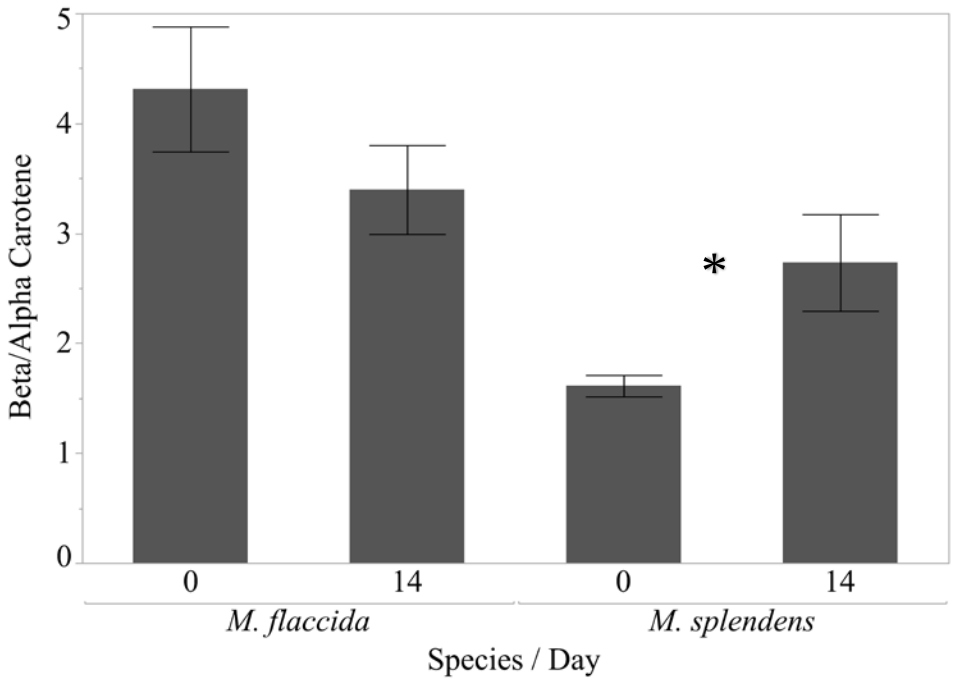

Figure 24. Mean beta/alpha carotene ratio (±SE) by species and day. * indicates significant difference as determined by t-test.

Culture experiment 3: Constant Flow

In the constant flow experiment difficulties arose with the "constant flow" it was not constant. Therefore, this experiment similarly lacked a truly non-limiting nitrate treatment. Nevertheless, over 7 days of culturing similar results to the other laboratory studies were seen. Firstly, phycoerythrin and phycocyanin significantly decreased for both species (Figure 25). Secondly, chlorophyll a proportions remained unchanged for *M. flaccida*, however, they significantly decreased for *M. splendens* (Table 4). Beta carotene and lutein significantly decreased in *M. flaccida* between sample day (Table 4). Finally, zeaxanthin significantly increased for *M. splendens* (Table 4). Transformed logit pigment proportion of alpha carotene and zeaxanthin for *M. flaccida* as well as lutein, alpha carotene, and beta carotene for *M. splendens* failed to meet the ANOVA assumption of equal variance. Therefore, a Wilcoxon test was utilized to compare these pigments between sample day. These tests indicated no significant

difference for zeaxanthin (p=0.43) and significant decrease for alpha carotene (p=0.0009) in *M. flaccida* by sample day. In *M. splendens* significant increase of lutein (p=0.02), significant decrease of alpha carotene (p=0.0009), and significant decrease of beta carotene (p=0.0007) was calculated for sample day.

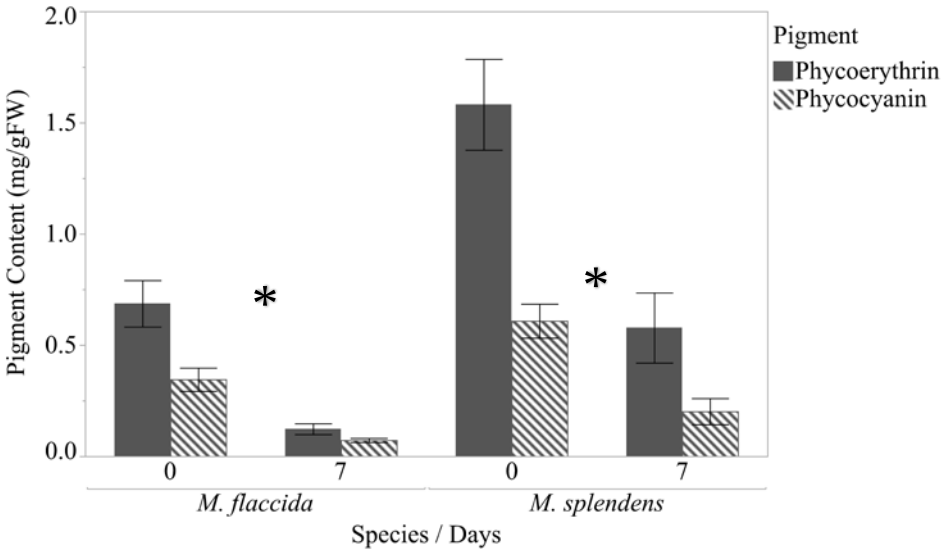

Figure 25. Mean phycobilin content (mg/gFW ±SE) by species and sample day. *indicates significant difference as determined by t-test.

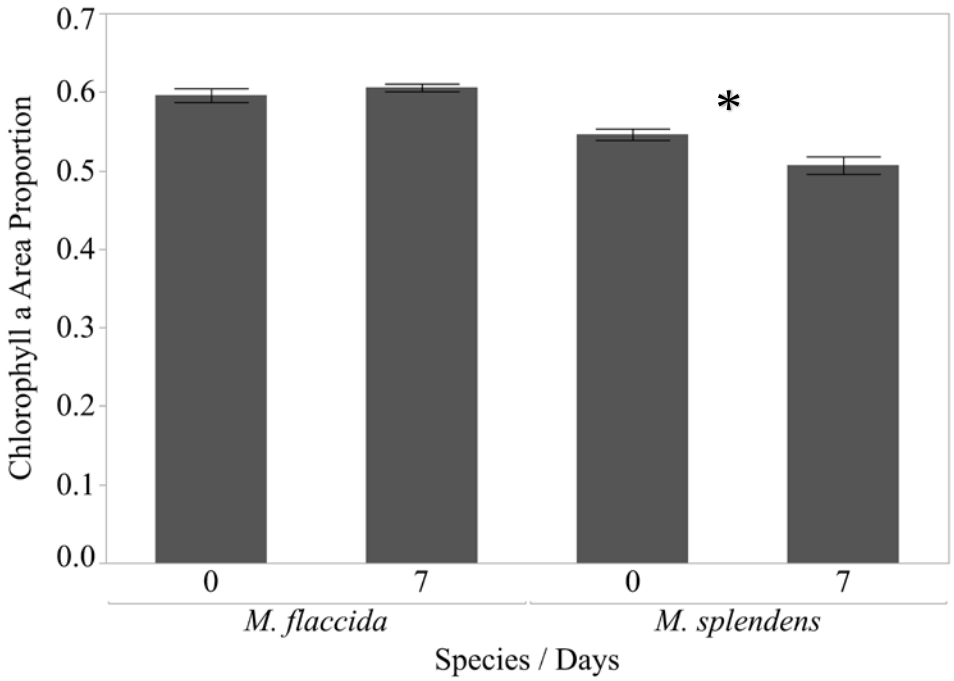

Figure 26. Mean chlorophyll a area proportions (±SE) by species and sample day. * indicates significant difference as determined by t-test.

Table 4. Pigment area percent between sample day and species. Cells not sharing letters are significantly different as determined by t-test or Wilcoxon test.

Species	Day	Zeaxanthin	Lutein	Chlorophyll a	α-carotene	β-carotene
M. flaccida	**0**	0.98 ± 0.23^{a}	9.00 ± 0.80^{a}	59.57 ± 0.88^{a}	1.46 ± 0.05^{a}	5.91 ± 0.24^{a}
M. flaccida	**7**	0.64 ± 0.06^{a}	6.80 ± 0.46^{b}	60.56 ± 0.49^{a}	0.75 ± 0.12^{b}	3.46 ± 0.22^{b}
M. splendens	**0**	0.2 ± 0.03^{a}	6.41 ± 0.07^{a}	54.59 ± 0.73^{a}	3.57 ± 0.19^{a}	4.66 ± 0.11^{a}
M. splendens	**7**	0.44 ± 0.07^{b}	7.93 ± 0.52^{b}	50.66 ± 1.11^{b}	1.28 ± 0.25^{b}	3.3 ± 0.17^{b}

HYPOTHESIS TESTING

The results of these studies have led to the partial rejection of hypothesis 1 and 3 and rejection of hypotheses 2 and 4.

Hypothesis 1 postulated that *Mazzaella* thalli transplanted from the low intertidal to the high intertidal will decrease pigment concentrations, decrease phycobiliprotein concentrations, and increase photoprotective pigment concentrations. After the field transplantation experiment, however, only photoprotective pigments increased in the high intertidal zone. No significant difference in phycobilins or chlorophyll pigment concentration was detected. Thus, hypothesis 1 is partially rejected and it is hypothesized that intertidal zone alone does not dictate phycobilin or chlorophyll a concentrations.

Hypothesis 2 postulates that *Mazzaella* thalli transplanted out of their native intertidal zone will experience greater mortality than those transplanted back into their native intertidal zone. However, mortality was significantly greater for the high intertidal zone regardless of species. Thus, hypothesis 2 is rejected.

Hypothesis 3 postulates that *Mazzaella* thalli grown in nutrient-poor water will have less phycobilins than thalli grown in nutrient-rich water. Although phycobilin concentrations in laboratory nitrate limitation experiments did decrease over time, this decrease occurred in both high and low nitrate treatments. Thus, hypothesis 2 is partially rejected since the decrease in the high nitrate treatment may be due to inadequate nitrate additions.

Finally, *hypothesis 4* postulates that *Mazzaella* thalli grown in low irradiance values will have significantly greater phycobilin & chlorophyll-a concentrations, and significantly lower zeaxanthin concentrations than those grown at high irradiance values. However, no significant effect of light treatment was detected. Thus, hypothesis 4 is rejected.

DISCUSSION

Overview

This study investigated the phenotypic response of *M. splendens* (a low intertidal seaweed) and *M. flaccida* (a high intertidal seaweed) to transplantation in the intertidal zone and cultivation at light and nutrient limiting conditions within the laboratory. Results indicate that: (1) field transplants could survive in both the high and low intertidal zones, (2) photoprotective pigment concentration differed between zones, indicating an ability to alter pigment content, and (3) intertidal zone alone does not dictate coloration of *Mazzaella* thalli. Lab experiments further elaborate on these results. From these experiments, it was determined that: (4) nitrate limitation induces reduction of phycobilin content and greening of both *M. splendens* and *M. flaccida* thalli (as seen in previous studies but not observed in our field experiment), (5) light availability (or shading) had no effect on pigment proportions, and that (6) phycobilin loss is reversible when allowed to acclimate in flowing seawater. These combined results illustrate that: seaweeds in the field experiment were likely not nitrate limited, phycobilins may act as nitrogen reserves, *M. splendens* and *M. flaccida* have differing ability to acclimate/tolerate intertidal stressors, and that photoprotective and phycobilin pigment regulation may be independently triggered by intertidal position and nutrient limitation respectively. Furthermore, the extent of plasticity of each alga may explain their broad intertidal range and ability to survive through varying environmental conditions, such as nutrient limitation events, high irradiance, seasonal storms, etc.

Intertidal zone alone does not dictate color change in Mazzaella spp.

Results from the present study indicate that intertidal position alone does not dictate coloration of either algae. In our field experiment, none of the 90 transplants to the high intertidal zone experienced greening of their blades. This result was not expected since approximately 29% of the *M. splendens* individuals transplanted in Foster's (1982) study (two of seven) changed coloration from purple to green. This greening described by Foster (1982) is similar to that seen in our laboratory experiments and those of Waaland (1976). In these experiments, nutrient limitation (instigated by decreased tank cycling rate or nitrate concentrations), caused greening of thalli via the selective loss of phycobilin pigments. Thus, the discrepancy seen between Foster's (1982) and the present study's results may be due to differences in environmental parameters that impact *M. splendens's* or *M. flaccida's* ability to absorb nutrients, such as wave exposure, nutrient content, and flow.

Total nutrient uptake in the high intertidal zone is positively correlated to the amount of time seaweeds absorb nutrients through wetted blades and the rate of this nutrient uptake (Reviewed in: Hurd et al. 2014). Therefore, if seaweeds remain dry for extended periods or if their rate of nutrient uptake is reduced they may become nutrient limited. This limitation is affected by environmental factors, such as intertidal height, wave splash, flow speeds, and seawater nutrient concentrations (Thomas et al. 1987a, Rico and Fernández 1996, Hurd et al. 2014, Flukes et al. 2015, Benes and Bracken 2016). These vary spatially and temporally, resulting in different seaweed nitrate content and uptake rates (Benes and Bracken 2016).

Total nutrient uptake can be increased in two ways. The first is by increasing the duration that algae are subjected to water/nutrients while the second is to increase the rate of nutrient uptake by increasing supply. Wave splash and spray increase the duration of nutrient uptake by wetting blades at low tide. This reduces desiccation stress and can lead to higher nutrient uptake

rates (Thomas et al. 1987b). Spray induced wetting is an effective method of nutrient supply and has been used in the cultivation of intertidal algae (Rheault and Ryther 1983, Pickering et al. 1995, Waaland 2004). Seaweeds cultivated in this method grew at a rate comparable to that of immersed cultures (4-5%/d) (Waaland 2004). Therefore, high intertidal seaweeds may receive sufficient nutrient supply from wave splash or spray during low tide. This nutrient supply, however, will vary with the amount of spray or wave energy reaching the intertidal zone. Thus, sheltered sites may receive less spray/splash than those exposed to waves and experience greater nutrient limitation.

The flux of nutrients into wetted seaweed thalli is affected by flow speed and total nutrient concentration (Reviewed in: Shibneva and Skriptsova 2015). Increased flow speeds reduce the thickness of the diffusive boundary layer (DBL) that forms on thallus surfaces (Wheeler 1980, Hurd et al. 1996, Koch and Gust 1999, Reviewed in: Hurd 2000). This DBL limits nutrient uptake rates to diffusive time scales (much slower than convective). Therefore, the rate of nutrient absorption can be increased by reducing the DBL thickness. This can be achieved by subjecting an alga to a high flow environment. This increases the rate of absorption and thus, reduces the amount of time required to meet nutrient requirement (Hurd and Pilditch 2011). In addition to flow speeds, events that increase nutrient concentrations, such as seasonal upwelling or land erosion, also allow for increased nutrient absorption of submerged seaweeds.

In summary, wave exposure (due to its effect on flow and splash) and upwelling regimes during monitoring may have affected nutrient availability at each site. Thus, differences in these parameters at this study's sites and the site of Foster (1982) may explain the greening seen in Foster's (1982) study and lack thereof in the present study. The geographical locations of this study's sites indicate exposure to a range of swells/waves originating from the northwest to

south. Foster's (1982) site of Hopkins, however, is protected from all but the NW swells by the Monterey peninsula and was found to experience 1/3 of the wave height of Fanshell Beach – just on the other side of the Monterey Peninsula (Site C: Graham et al. 1997). Exposure to swells from the open ocean increases flow and wave splash/spray. Therefore, it is reasonable to assume that Foster's (1982) protected site would receive less spray than the sites studied in the present study. Indeed, Foster (1982) reported that his high intertidal zone was often dry for more than 3 hrs. In the present study, however, spray and waves often wetted the high intertidal zone at all sites, regardless of tidal conditions. This wetting could have provided sufficient nutrients to maintain high phycobilin content. This observation is in contrast with Foster's (1982), which experienced a lack of spray and potentially lower flow in the high intertidal, which may have contributed to nitrogen limitation in the two *M. splendens* that greened. Unfortunately, flow and nutrient concentrations at each intertidal zone were not measured in the present study and these metrics are not mentioned in Foster's report (1982). Furthermore, the present study failed to include Hopkins Marine Station as a transplant site due to logistical issues presented by its status as a marine reserve. Future intertidal experiments may address the shortcomings of this study by including a flow sensor such as described by Bell and Denny (1994). They may also may be interested in constraining the spatial variability in nutrient exposure at different intertidal sites, since nutrient exposure can vary both temporally and spatially (Thomas et al. 1987a, Rico and Fernández 1996, Hurd et al. 2014, Flukes et al. 2015, Benes and Bracken 2016).

Demographics: Survival and Size

The present study agrees with size, and survival studies previously conducted on *Mazzaella flaccida* and *Mazzaella splendens* (Waaland 1976, Hansen 1977, Foster 1982). Generally, seaweeds attained their maximum size in summer and then senesce in fall/winter

(Waaland 1976, Hansen 1977, Foster 1982). As seaweeds grow and senesce, survival decreases to a minimum in the fall (October) (Shaughnessy and DeWreede 2001). The cause of this survival decrease was not extensively recorded in the present study. However, high intertidal death primarily stemmed from bleaching of tissue most likely induced by environmental stressors such as light and desiccation. Conversely, low intertidal mortality was seen in response to herbivory and entanglement with other seaweeds. Herbivores, such as limpets and chitons, seemed attracted to the smooth surfaces our epoxy created. These herbivores were sometimes pried off transplants to reveal a grazed stipe. However, it is not known if the blades were lost due to wave stress and then grazed, or if grazing induced blade loss. Other low intertidal seaweeds were ripped out, likely by entanglement with rough *Egregia menziesii* stipes. Towards the end of the monitoring, *E. menziesii* had grown so extensively that it needed to be relocated along large swaths of intertidal zone to reveal some transplants.

At the beginning of this experiment, it was hypothesized that survival would be greatest for each seaweed in their native intertidal zone. However, this was not the case; survival was significantly greater in the low vs. the high intertidal zone for both species. This result may indicate that physical and biological stressors in each zone act on different timescales. Indeed, the high intertidal zone saw greatest mortality for *M. splendens* in the first month of transplantation. In the first month, survival decreased by 0.3 for *M. splendens*, while survival for *M. flaccida* decreased by 0.04. Survival in the low intertidal lacked this high mortality in the first month for both species.

Physical and biological stressors in the high intertidal may act on shorter time scales than those in the low intertidal zone. While survival in the high intertidal zone is determined by the organism's ability to acclimate to its environmental conditions, the low intertidal's selective

pressures stem from herbivory and competition with other seaweeds (Schonbeck and Norton 1980, Foster 1982). Thus, survival in the low intertidal zone may decrease gradually, and over time would remove *M. flaccida* via competition or predation. Schonbeck and Norton (1980) observed this in their transplantation experiment. In their study, *Pelvetia canaliculata* transplanted below its zone grew faster and had higher survival those grown in the high intertidal (Schonbeck and Norton 1980). However, in December these low intertidal transplants experienced significant mortality (73%) and were overgrown in April. Therefore, the 5-month observation period in the present study may have been too short to observe significant changes in survival in the low intertidal zone.

Finally, the methodology implemented to determine survival between intertidal zones may not have been adequate. Studies have shown that survival is greater for species transplanted from extreme environments to calm environments than vice versa (Foster 1982, Blanchette et al. 1993). Future experiments may consider beginning their transplantation in winter months. During these months high intertidal stressors are at a minimum, since low tide occurs at night and light intensity/duration is decreased, while summer months experience low tide during daylight hours and are subjected to the greatest light intensity for a longer duration (Dethier and Williams 2009). Transplantation during the winter would allow transplanted seaweeds to gradually acclimate to the higher irradiances over months of time instead of being subjected to the maximum high intertidal stress within a few hours. Transplantation stress may also be reduced by raising transplanted seaweeds gradually (Hodgson 1980). Finally, future experiments may avoid this transplantation stress by transplanting spores or gametes into the high intertidal zone in addition to mature individuals. These gametes may be able to overcome ontogenetic

mechanisms that may have kept seaweeds in the present study from acclimating to the high intertidal zone.

Chromatic Plasticity

Chromatic plasticity was observed in both *Mazzaella flaccida* and *Mazzaella splendens* in response to intertidal position and nutrient limitation. *Mazzaella* spp. transplanted to the high intertidal contained greater proportions of photoprotective pigments than *Mazzaella* spp. transplanted to the low intertidal algae, while nutrient limited *Mazzaella* spp. rapidly cannibalized their phycobiliproteins. These two processes were determined to occur independently of each other, since high intertidal seaweeds did not alter their phycobilin composition and nutrient starved seaweeds did not alter their photoprotective pigments. Thus, there are likely two mechanisms that lead to photopigment regulation. One may be in response to photooxidative stress, while the other in response to nutrient limitation.

Although both species of *Mazzaella* could alter their pigment proportions, these responses differed by species. *M. flaccida* had a greater ability to increase its photoprotective pigments, while *M. splendens* could achieve higher total phycobilin concentrations. In the high intertidal zone, *M. flaccida* increased zeaxanthin, lutein, and β-carotene, while *M. splendens* increased only zeaxanthin and β-carotene. In the low intertidal zone however, *M. flaccida* was not able to obtain the elevated phycobilin concentrations that *M. splendens* exhibited. Therefore, the differences in plasticity extent between the two species may be indicative of the physical stressors inherent to their native intertidal zone and may further support their classification as distinct species.

Physical stressors in the intertidal zone differs by zone area. The high intertidal zone is characterized as having greater light and desiccation stress since seaweeds here are more regularly exposed to the air and full intensity of the sun (Demmig-Adams and Adams 2000, Choudhury and Behera 2001, Sampath-Wiley et al. 2008). In contrast, the low intertidal zone is characterized as having greater competitive stress since harmful UV rays are absorbed by the water itself and seaweeds are rarely exposed to the air (Talarico and Maranzana 2000, Hurd et al. 2014). Therefore, survival in the high intertidal zone is dictated by the alga's ability to withstand the physical elements. The differences between these two zones should correspond to differing importance of photopigments. For example, seaweeds in the high intertidal should rely more heavily on photoprotective pigments to buffer themselves from photooxidative damage. Thus, *M. flaccida*'s enhanced ability to increase both lutein and zeaxanthin concentrations may explain why it experienced lower mortality than *M. splendens* in the high intertidal zone.

In contrast to the high intertidal, where survival is dictated by resilience to photooxidative stress, survival in the low intertidal is dictated by an alga's ability to grow rapidly and withstand grazing pressure. Thus, in this zone photoprotective pigments are less critical. Instead, pigments that aid in the capture of sunlight and growth are important. Phycobilipigments can serve this purpose by extending the range of wavelengths captured by the photosystem. Primarily, these pigments allow the capture of green wavelengths that are rejected by competitors in the brown (Phaeophycea) and green (Chlorophyta) algal groups. This may explain why *M. splendens* have greater concentrations of phycobilin pigments than *M. flaccida*. *M. flaccida* does not experience competitive stress or wavelength restriction in the high intertidal zone. Thus, high concentrations of phycobilins are not necessary and may overly stress the photosystem. Therefore, it seems odd that every *M. flaccida* measured in this study contained phycobilipigments at all. I believe they

may have retained these pigments as a means of buffering themselves from nitrogen limitation events that occur on a seasonal basis in the Monterey Bay (Kudela and Dugdale 2000).

Phycobilins as Nutrient Reservoirs

Waaland's (1976) paper described the greening and reddening of *M. splendens* thalli in response to nutrient starvation and nutrient excess (fertilizer) respectively. He observed that thalli turned green when nutrient limited and dark red when fertilized. This result caused him to corroborate the hypothesis that the red phycobilin pigments, in addition to their function as accessory pigments, may act as nitrogen reservoirs. This role of phycobilins as nitrogen reservoirs is supported by the nutrient limitation and acclimation experiments of the present study. In these experiments, rapid phycobilin reductions occurred during nitrate limitation, while pigments obligatory for photosynthesis, such as chlorophyll a, remained near initial concentrations. These algae remained viable after the 14-day experiments and regained pigmentation when acclimated in flowing seawater (n=2, Figure 27).

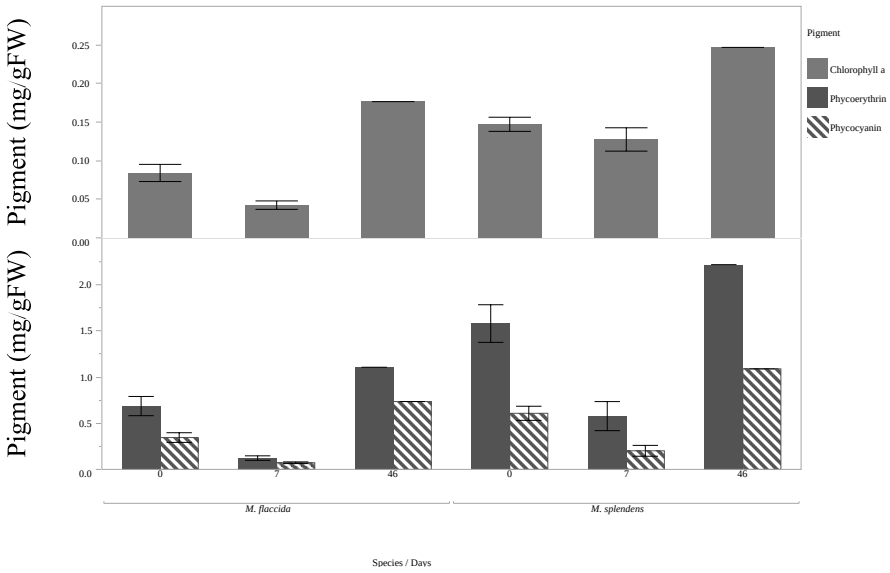

Figure 27. Mean pigment content (mg/gFW) by pigment, species, and day sampled.

Since Waaland's (1976) study, the role of phycobilins has been more extensively researched. Phycobilins were determined to comprise up to 73% of soluble proteins thus supporting their role as nitrogen reservoirs (Green and Neefus 2015). Their response to nutrient starvation has been extensively studied in the unialgal cultures of *Porphyridium purpureum* (Levy and Gantt 1990, Algarra and Rüdiger 1993). From these studies a few things became known. First, in *P. purpureum* nitrate limitation results in the immediate loss of phycobilins rendering the culture green (Levy and Gantt 1990). As phycobilins are lost up to 99%, chlorophyll a follows suit slowly decreasing, while photoprotective pigment concentrations remain unchanged. At the end of a two-week starvation period the entire phycobilisome (the structure that contains the phycobilipigments) disappears. While all of this is happening growth ceases. However, once nitrogen is added back to the system, phycobilisomes reappear, and phycobilipigments attach. Once these pigments are restored to initial concentrations, growth resumes (Levy and Gantt 1990).

Although it is known that phycobilins allow the utilization of green wavelengths, the functional benefit of phycobilins to intertidal algae is not entirely understood. Few studies have investigated how this pigment aids in survival. The fact that these phycobilins are the first to be cannibalized supports their status as non-obligatory pigments that may serve a role as nutrient reservoir. However, nutrients can be stored in differing formats such as amino acids, phospholipids, and proteins. Therefore, it is difficult to determine if these pigments give rhodophytes a competitive advantage over other non-phycobilin containing seaweeds, such as the Phaeophycea (brown algae) and Chlorophyta (green algae). Further clouding the importance of phycobilins to survival, one study with phycobilin diminished (not completely absent) mutants found that the green mutant grew at similar rates to those that contain higher concentrations of

phycobilins (Ursi et al. 2013). Furthermore, while conducting field sampling I have observed possible green *M. splendens* mutants living next to healthy purple *M. splendens* (Figure 28). Future studies implementing gene knockout trials targeting the phycobilin pigments, could offer great insight as to the importance of these pigments to red algae, specifically as they relate to survival in the low and high intertidal zones.

Figure 28. Possible *M. splendens* green mutant.

Paradoxical decrease of α-carotene

The analysis of photoprotective pigment composition between intertidal zone and species of *Mazzaella* led to the following curious result: while most photoprotective pigments were greater or unchanged in the high intertidal zone, α-carotene was consistently reduced. This pigment is a precursor to lutein. Since only *M. flaccida* upregulated its lutein content it is perplexing that α-carotene decreased in both species. This result may be explained by the underlying synthesis mechanism of these photoprotective pigments.

The photoprotective pigments lutein, zeaxanthin, α-carotene, and β-carotene are created by the modification of a singular lycopene molecule (Cunningham and Gantt 1998, Blatt et al. 2015). This process is fairly understood for land plants and green algae but requires more studies

for red algae (Takaichi 2011). In land plants and green algae, lycopene is modified by either lycopene ε-cyclase (LCYE) or lycopene β-cyclase (LCYB) (Cunningham and Gantt 1998, Blatt et al. 2015). LCYE adds a ε-ionine ring to one end of lycopene thereby creating δ-carotene, while LCYB adds a β-ionine ring to lycopene creating γ-carotene (Figure 29). Thereafter, the addition of another β-ionine ring by LCYB converts δ-carotene into α-carotene or converts γ-carotene into β-carotene. Other enzymes then convert α-carotene into lutein and β-carotene into zeaxanthin (Takaichi 2011). Although there is still much to learn about carotenoid synthesis in algae, it is reasonable to assume that *Mazzaella* spp. have similar processes of creating lutein and zeaxanthin from α- and β-carotene respectively (Takaichi et al. 2016).

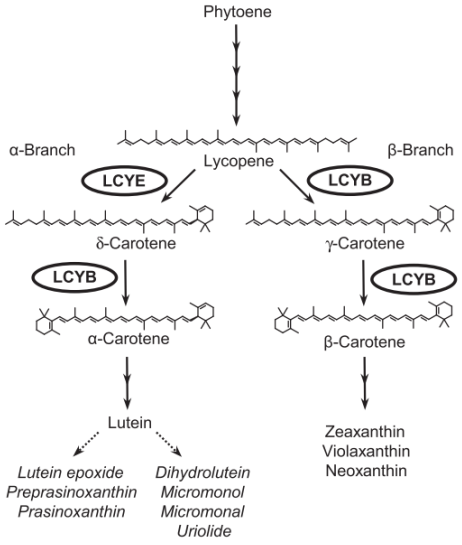

Figure 29. Lutein and zeaxanthin synthesis paths. From Blatt et al. 2015.

Knowing the approximate mechanism for carotenoid pigment creation we can hypothesize the process that led to the changes observed in this study's field transplant experiment. Upon transplantation into the high intertidal zone the photosystem of *M. splendens* and *M. flaccida* is excessively stimulated by light and begins the production of photoprotective pigments. Caches of α-carotene and β-carotene are converted into zeaxanthin and lutein by LYCB or LCYE homologs. This would lead to a decrease in alpha and beta carotene concentrations and increase in zeaxanthin and lutein concentrations. Thereafter, lycopene synthesis would create more β- and α-carotene until lutein and zeaxanthin concentrations

sufficiently buffer the seaweeds from light stress (Cunningham and Gantt 1998, Blatt et al. 2015).

In our experiment lutein, zeaxanthin, and β-carotene concentrations were increased in the high intertidal zone while alpha carotene concentrations were decreased. This decrease in alpha carotene may indicate a preference of beta carotene and lutein over alpha carotene. Unfortunately, little is known about the role alpha carotene plays in photoprotection. Zeaxanthin, beta carotene, and lutein, on the other hand, are known to play photoprotective roles in terrestrial plants (Takaichi 2011).

Although the paradoxical decrease of α-carotene is perplexing/exciting, this study does not allow the determination of causality for the reduction in α-carotene. It doesn't allow this because the information is based on a single time point from two different intertidal zones. Thus, it is not known how the seaweeds in this study responded as a function of time and more research needs to be conducted to determine whether the above mechanism is true. A study in which tissue samples are gathered from photo-acclimating seaweeds every hour could more accurately illustrate how these pigments are altered. Furthermore, future studies may wish to analyze enzyme activity of LYCB or LCYE in each sample. High concentrations of LYCB vs. LCYE could indicate favoring of α-carotene and lutein synthesis while the opposite would favor β-carotene and zeaxanthin.

Coloration of Mazzaella spp. may be indicative of pigment proportions

Mazzaella are reported to occur in a variety of colors (Abbott 1971, Waaland 1976, Foster 1982, Hughey and Hommersand 2010) and in this study were seen to be bright green, light brown, dark green, red, brown, and sometimes black. This coloration might correlate with

the pigment proportions and absolute abundances present in blade tissue since chlorophyll is green, carotenoids are yellow-orange, and phycobilins are blue-red. Thus, green blades may have high chlorophyll, low carotenoid, and low phycobilin content. Red blades may have moderate chlorophyll, low carotenoid, and high phycobilin content. Finally, yellow blades may have moderate chlorophyll, high carotenoid, and low phycobilin content. Since photoprotective pigments (carotenoids) are increased in response to high irradiances and phycobilins are reduced in response to nutrient limitation, color may be indicative of nutrient limitation or light stress. Therefore, future studies may seek to investigate how the natural color range reported of these seaweeds correlates to physical stressors afflicting these seaweeds at any given point.

CONCLUSION

M. flaccida and *M. splendens* are native to the high and low intertidal zone respectively (Abbott 1971, Foster 1982). Existence in these zones subjects them to significant environmental variability; nutrients, light, and desiccation vary by intertidal height, tide, wave exposure, and season. Generally, survival in the high intertidal zone is determined by the organism's ability to acclimate to light, nutrient, and desiccation stress, while survival in the low intertidal is determined by its ability to claim space, grow rapidly, and cope with grazing (Schonbeck and Norton 1980, Foster 1982, Hurd et al. 2014). From the present study it appears that *M. flaccida* and *M. splendens* respond to these environmental stressors through the modification of photoprotective and/or phycobilin pigment concentrations. Although both species can modify either pigment type, the modification capability differed by species. *M. flaccida*, for example, showed increased capacity to modify photoprotective pigments. This species increased both lutein, zeaxanthin, and beta carotene in response to transplantation into the high intertidal zone,

while *M. splendens* increased only zeaxanthin and beta carotene. Conversely, *M. splendens* shows greater ability to increase phycobilin concentrations. These differences in chromatic plasticity illustrate how each species is adapted to the stressors of its native intertidal zone but is capable of acclimating to a range of conditions. While it is difficult to ascertain the evolutionary significance of this plasticity, it appears to offer support to the current separation of these two species (Hughey and Hommersand 2010). From this study alone, it is unlikely that *M. splendens* and *M. flaccida* are ecotypes as Foster (1982) once hypothesized. However, more experiments are required that specifically investigate the claims made in the present study. These experiments should include: 1) laboratory experiments investigating response of either species to artificially increased irradiances 2) field experiments that transplant both mature thalli and spores/gametes 3) field/laboratory experiments that disrupt phycobilin synthesis to investigate the significance of this pigment to survival in the high or low intertidal zone. Finally, future studies may aim to optimize the culture method utilized in the present study.

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APPENDIX A

Table A1. ANOVA results of logit transformed survival proportions for the month of October.

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	9.810456	3.27015	5.6543
Error	5	2.891752	0.57835	**Prob > F**
C. Total	8	12.702207		0.0461*

Table A2. ANOVA effects test result of logit transformed survival data for all sites in month of October.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Intertidal zone	1	1	4.3484963	7.5188	0.0407*
Species	1	1	0.7259715	1.2552	0.3135
Intertidal zone*Species	1	1	2.2220339	3.8420	0.1073

Table A3. ANOVA results for mean total blade area for the month of June at all sites.

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	11	117.1962	10.6542	2.2445
Error	217	1030.0492	4.7468	**Prob > F**
C. Total	228	1147.2454		0.0133*

Table A4. ANOVA effects test for mean total blade area for all sites and species during month of June.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Site	2	2	65.314445	6.8799	0.0013*
Species	1	1	16.862032	3.5523	0.0608
Site*Species	2	2	9.970856	1.0503	0.3516
Intertidal Zone	1	1	4.150094	0.8743	0.3508
Site*Intertidal Zone	2	2	12.309630	1.2966	0.2756
Species*Intertidal Zone	1	1	4.478304	0.9434	0.3325
Site*Species*Intertidal Zone	2	2	12.675785	1.3352	0.2653

Table A5. ANOVA results for mean total blade area for the month of August for both species at all sites.

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	11	306.9803	27.9073	3.3157
Error	191	1607.5794	8.4166	**Prob > F**
C. Total	202	1914.5597		0.0003*

Table A6. ANOVA effects test for mean total blade area for the month of August for both species at all sites.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Site	2	2	50.09160	2.9757	0.0534
Species	1	1	44.63075	5.3027	0.0224*
Site*Species	2	2	37.45815	2.2252	0.1108
Intertidal Zone	1	1	37.95890	4.5100	0.0350*
Site*Intertidal Zone	2	2	4.57442	0.2717	0.7623
Species*Intertidal Zone	1	1	128.68382	15.2892	0.0001*
Site*Species*Intertidal Zone	2	2	12.84692	0.7632	0.4676

Table A7. Tukeys HSD test for the interaction of Species and intertidal zone during the month of August.

Level			Least Sq Mean
Mazzaella flaccida, High	A		2.6953002
Mazzaella splendens, Low	A		2.6197811
Mazzaella flaccida, Low	A		1.9419481
Mazzaella splendens, High		B	0.0753304

Table A8. ANOVA table for blade mass during the month of October.

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	11	46.14658	4.19514	0.7898
Error	115	610.84925	5.31173	**Prob > F**
C. Total	126	656.99583		0.6498

Table A9. ANOVA tables for all pigments quantified in the lab experiment during the month of October.

Pigment	Source	DF	Sum of Squares	Mean Square	F Ratio	Prob>F
Fucoxanthin	Model	11	35.84	3.26	6.18	<.0001
Fucoxanthin	Error	78	41.14	0.53		
Fucoxanthin	C. Total	89	76.97			
Zeaxanthin	Model	11	36.90	3.35	4.47	<.0001
Zeaxanthin	Error	89	66.85	0.75		
Zeaxanthin	C. Total	100	103.74			
Lutein	Model	11	39.51	3.59	4.90	<.0001
Lutein	Error	89	65.22	0.73		
Lutein	C. Total	100	104.74			
Chlorophyll a allomer	Model	11	48.45	4.40	7.19	<.0001
Chlorophyll a allomer	Error	89	54.50	0.61		
Chlorophyll a allomer	C. Total	100	102.95			
Chlorophyll a	Model	11	24.94	2.27	2.76	0.004
Chlorophyll a	Error	89	73.19	0.82		
Chlorophyll a	C. Total	100	98.13			
Alpha Carotene	Model	11	47.07	4.28	6.63	<.0001
Alpha Carotene	Error	89	57.44	0.65		
Alpha Carotene	C. Total	100	104.51			
Beta Carotene	Model	11	38.82	3.53	4.78	<.0001
Beta Carotene	Error	89	65.67	0.74		
Beta Carotene	C. Total	100	104.49			
Phycoerythrin	Model	11	24.83	2.26	2.93	0.0038
Phycoerythrin	Error	59	45.49	0.77		
Phycoerythrin	C. Total	70	70.32			
Phycocyanin	Model	11	17.60	1.60	2.02	0.0421
Phycocyanin	Error	59	46.72	0.79		
Phycocyanin	C. Total	70	64.32			
Beta/Alpha Carotene	Model	11	59.34	5.39	18.28	<.0001
Beta/Alpha Carotene	Error	89	26.26	0.30		
Beta/Alpha Carotene	C. Total	100	85.60			

Table A10. ANOVA effect test results by pigment type of significant sources such as site, intertidal zone, species, and interaction terms during the month of October.

Pigment	Source	Npar m	DF	Sum of Squares	F Ratio	Prob> F
Fucoxanthin	Site	2	2	14.93	14.15	<.0001

Fucoxanthin	Intertidal Zone	1	1	4.44	8.41	0.0048
Fucoxanthin	Species*Intertidal Zone	1	1	3.09	5.86	0.0179
Zeaxanthin	Site	2	2	6.14	4.09	0.02
Zeaxanthin	Species	1	1	4.00	5.32	0.0234
Zeaxanthin	Site*Species	2	2	4.99	3.33	0.0405
Zeaxanthin	Intertidal Zone	1	1	16.59	22.09	<.0001
Lutein	Site	2	2	8.43	5.75	0.0045
Lutein	Species	1	1	15.29	20.86	<.0001
Lutein	Species*Intertidal Zone	1	1	5.21	7.11	0.0091
Chlorophyll a allomer	Site	2	2	16.40	13.39	<.0001
Chlorophyll a allomer	Species	1	1	11.30	18.45	<.0001
Chlorophyll a allomer	Intertidal Zone	1	1	2.97	4.85	0.0302
Chlorophyll a	Site	2	2	15.49	9.42	0.0002
Alpha Carotene	Intertidal Zone	1	1	31.31	48.52	<.0001
Alpha Carotene	Site*Intertidal Zone	2	2	5.25	4.07	0.0204
Beta Carotene	Site	2	2	6.60	4.47	0.0141
Beta Carotene	Species	1	1	16.35	22.16	<.0001
Beta Carotene	Intertidal Zone	1	1	3.48	4.71	0.0326
Phycoerythrin	Species	1	1	7.08	9.19	0.0036
Phycoerythrin	Site*Species	2	2	9.46	6.13	0.0038
Phycocyanin	Site*Species	2	2	7.94	5.01	0.0097
Fucoxanthin	Species	1	1	1.19	2.26	0.136

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Fucoxanthin	Site*Species	2	2	2.90	2.75	0.0704
Fucoxanthin	Site*Intertidal Zone	2	2	0.11	0.10	0.9022
Fucoxanthin	Site*Species*Intertidal Zone	2	2	2.65	2.52	0.0873
Zeaxanthin	Site*Intertidal Zone	2	2	4.10	2.73	0.0706
Zeaxanthin	Species*Intertidal Zone	1	1	0.17	0.22	0.6389
Zeaxanthin	Site*Species*Intertidal Zone	2	2	1.30	0.86	0.4251
Lutein	Site*Species	2	2	3.75	2.56	0.0829
Lutein	Intertidal Zone	1	1	1.85	2.53	0.1153
Lutein	Site*Intertidal Zone	2	2	1.63	1.11	0.3339
Lutein	Site*Species*Intertidal Zone	2	2	3.89	2.65	0.0761
Chlorophyll a allomer	Site*Species	2	2	1.97	1.61	0.2058
Chlorophyll a allomer	Site*Intertidal Zone	2	2	1.49	1.21	0.3023
Chlorophyll a allomer	Species*Intertidal Zone	1	1	0.94	1.53	0.2189
Chlorophyll a allomer	Site*Species*Intertidal Zone	2	2	1.56	1.27	0.2857
Chlorophyll a	Species	1	1	0.44	0.53	0.467
Chlorophyll a	Site*Species	2	2	1.87	1.14	0.3259
Chlorophyll a	Intertidal Zone	1	1	1.55	1.89	0.1727
Chlorophyll a	Site*Intertidal Zone	2	2	0.33	0.20	0.8186
Chlorophyll a	Species*Intertidal Zone	1	1	0.10	0.13	0.7226
Chlorophyll a	Site*Species*Intertidal Zone	2	2	1.53	0.93	0.3984
Alpha Carotene	Site	2	2	2.84	2.20	0.1172

Alpha Carotene	Species	1	1	0.29	0.45	0.5044
Alpha Carotene	Site*Species	2	2	0.63	0.49	0.6162
Alpha Carotene	Species*Intertidal Zone	1	1	0.43	0.66	0.4184
Alpha Carotene	Site*Species*Intertidal Zone	2	2	2.34	1.81	0.1697
Beta Carotene	Site*Species	2	2	1.61	1.09	0.3404
Beta Carotene	Site*Intertidal Zone	2	2	2.15	1.45	0.239
Beta Carotene	Species*Intertidal Zone	1	1	1.04	1.41	0.2376
Beta Carotene	Site*Species*Intertidal Zone	2	2	1.04	0.71	0.496
Phycoerythrin	Site	2	2	1.91	1.24	0.2977
Phycoerythrin	Intertidal Zone	1	1	0.10	0.12	0.7268
Phycoerythrin	Site*Intertidal Zone	2	2	2.64	1.71	0.1889
Phycoerythrin	Species*Intertidal Zone	1	1	0.02	0.03	0.8582
Phycoerythrin	Site*Species*Intertidal Zone	2	2	1.22	0.79	0.459
Phycocyanin	Site	2	2	4.95	3.13	0.0512
Phycocyanin	Species	1	1	0.70	0.89	0.3505
Phycocyanin	Intertidal Zone	1	1	0.10	0.12	0.7278
Phycocyanin	Site*Intertidal Zone	2	2	1.57	0.99	0.3776
Phycocyanin	Species*Intertidal Zone	1	1	0.13	0.17	0.6855
Phycocyanin	Site*Species*Intertidal Zone	2	2	0.57	0.36	0.7002
Beta/Alpha Carotene	Site	2	2	1.88	3.19	0.0459
Beta/Alpha Carotene	Species	1	1	7.86	26.65	<.0001
Beta/Alpha	Site*Species	2	2	4.05	6.86	0.001

Carotene						7
Beta/Alpha Carotene	Intertidal Zone	1	1	34.03	115.34	<.0001
Beta/Alpha Carotene	Site*Intertidal Zone	2	2	3.26	5.52	0.0055
Beta/Alpha Carotene	Species*Intertidal Zone	1	1	0.80	2.72	0.1028
Beta/Alpha Carotene	Site*Species*Intertidal Zone	2	2	4.60	7.80	0.0008

Table A11. ANOVA table for culture experiment 1.

Pigment	Source	DF	Sum of Squares	Mean Square	F Ratio	Prob>F
Phycoerythrin	Model	17	18.30	1.08	7.19	<.0001
Phycoerythrin	Error	54	8.08	0.15		
Phycoerythrin	C. Total	71	26.38			
Phycocyanin	Model	17	20.15	1.19	7.10	<.0001
Phycocyanin	Error	54	9.01	0.17		
Phycocyanin	C. Total	71	29.16			
Zeaxanthin	Model	17	0.70	0.04	1.81	0.0515
Zeaxanthin	Error	52	1.19	0.02		
Zeaxanthin	C. Total	69	1.89			
Lutein	Model	17	64.41	3.79	5.55	<.0001
Lutein	Error	52	35.50	0.68		
Lutein	C. Total	69	99.91			
Chlorophyll a allomer	Model	17	101.18	5.95	4.73	<.0001
Chlorophyll a allomer	Error	54	67.94	1.26		
Chlorophyll a allomer	C. Total	71	169.13			
Alpha Carotene	Model	17	18.57	1.09	7.71	<.0001
Alpha Carotene	Error	54	7.65	0.14		
Alpha Carotene	C. Total	71	26.22			
Beta Carotene	Model	17	15.28	0.90	6.02	<.0001
Beta Carotene	Error	54	8.06	0.15		
Beta Carotene	C. Total	71	23.34			
Chlorophyll a	Model	17	327.15	19.24	0.50	0.9421
Chlorophyll a	Error	54	2079.93	38.52		
Chlorophyll a	C. Total	71	2407.08			

Table A12. ANOVA effects table for Cultivation Experiment 1

Pigment	Source	Npar	D	Sum of	F	Prob>

		m	F	Squares	Ratio	F
Chlorophyll a allomer	Day	1	1	86.29	68.58	<.0001
Phycocyanin	Day	1	1	17.24	103.28	<.0001
Phycoerythrin	Day	1	1	15.56	103.95	<.0001
Lutein	Day	1	1	58.79	86.13	<.0001
Zeaxanthin	Day	1	1	0.33	14.47	0.0004
Alpha Carotene	Day	1	1	16.48	116.33	<.0001
Beta Carotene	Day	1	1	14.09	94.41	<.0001
Chlorophyll a allomer	Day*Nutrients*Light	4	4	7.37	1.46	0.2261
Chlorophyll a allomer	Day*Nutrients	2	2	3.15	1.25	0.2941
Chlorophyll a allomer	Light	2	2	2.86	1.14	0.3279
Chlorophyll a allomer	Nutrients	2	2	0.33	0.13	0.8765
Chlorophyll a allomer	Day*Light	2	2	0.28	0.11	0.8958
Chlorophyll a allomer	Nutrients*Light	4	4	0.91	0.18	0.9476
Chlorophyll a Area Percent	Day*Nutrients	2	2	63.42	0.82	0.4444
Chlorophyll a Area Percent	Nutrients	2	2	59.77	0.78	0.4653
Chlorophyll a Area Percent	Day*Light	2	2	38.66	0.50	0.6082
Chlorophyll a Area Percent	Day	1	1	5.59	0.15	0.7048
Chlorophyll a Area Percent	Light	2	2	26.85	0.35	0.7072
Chlorophyll a Area Percent	Nutrients*Light	4	4	81.55	0.53	0.7147
Chlorophyll a Area Percent	Day*Nutrients*Light	4	4	51.30	0.33	0.8546
Phycocyanin	Nitrate	2	2	0.69	2.07	0.136

Phycocyanin	Day*Nitrate	2	2	0.54	1.63	0.2055
Phycocyanin	Day*Nitrate*Light	4	4	0.74	1.12	0.3589
Phycocyanin	Day*Light	2	2	0.30	0.90	0.4131
Phycocyanin	Nitrate*Light	4	4	0.47	0.71	0.5915
Phycocyanin	Light	2	2	0.16	0.48	0.6215
Phycoerythrin	Nitrate	2	2	0.50	1.66	0.1992
Phycoerythrin	Day*Nitrate*Light	4	4	0.88	1.48	0.2223
Phycoerythrin	Day*Nitrate	2	2	0.43	1.45	0.2441
Phycoerythrin	Day*Light	2	2	0.32	1.08	0.346
Phycoerythrin	Light	2	2	0.18	0.60	0.5515
Phycoerythrin	Nitrate*Light	4	4	0.42	0.70	0.5987
Lutein	Day*Light	2	2	1.02	0.74	0.48
Lutein	Light	2	2	0.91	0.67	0.5183
Lutein	Day*Nutrients*Light	4	4	1.96	0.72	0.5827
Lutein	Nutrients*Light	4	4	1.79	0.66	0.625
Lutein	Day*Nutrients	2	2	0.52	0.38	0.6871
Lutein	Nutrients	2	2	0.49	0.36	0.6987
Zeaxanthin	Light	2	2	0.11	2.32	0.1082
Zeaxanthin	Nutrients*Light	4	4	0.11	1.22	0.3152
Zeaxanthin	Day*Nutrients	2	2	0.05	1.14	0.328
Zeaxanthin	Day*Nutrients*Light	4	4	0.06	0.69	0.6016
Zeaxanthin	Nutrients	2	2	0.02	0.51	0.6034
Zeaxanthin	Day*Light	2	2	0.01	0.13	0.8781

Alpha Carotene	Nutrients	2	2	0.56	1.96	0.1508
Alpha Carotene	Light	2	2	0.51	1.79	0.1769
Alpha Carotene	Nutrients*Light	4	4	0.49	0.87	0.4884
Alpha Carotene	Day*Light	2	2	0.18	0.64	0.5296
Alpha Carotene	Day*Nutrients*Light	4	4	0.34	0.60	0.6624
Alpha Carotene	Day*Nutrients	2	2	0.00	0.02	0.9829
Beta Carotene	Nutrients	2	2	0.33	1.11	0.3379
Beta Carotene	Day*Nutrients*Light	4	4	0.38	0.64	0.6343
Beta Carotene	Light	2	2	0.11	0.38	0.6847
Beta Carotene	Nutrients*Light	4	4	0.28	0.46	0.7621
Beta Carotene	Day*Light	2	2	0.05	0.17	0.8466
Beta Carotene	Day*Nutrients	2	2	0.03	0.11	0.8919

Table A13. ANOVA table for Cultivation Experiment 2.

Pigment	Source	DF	Sum of Squares	Mean Square	F Ratio	Prob>F
Zeaxanthin	Model	15	23.68	1.58	2.49	0.0272
Zeaxanthin	Error	21	13.32	0.63		
Zeaxanthin	C. Total	36	37.00			
Lutein	Model	15	27.17	1.81	3.01	0.0057
Lutein	Error	28	16.83	0.60		
Lutein	C. Total	43	44.00			
Chlorophyll a allomer	Model	15	33.54	2.24	5.99	<.0001
Chlorophyll a allomer	Error	28	10.46	0.37		
Chlorophyll a allomer	C. Total	43	44.00			
Chlorophyll a	Model	15	30.86	2.06	4.38	0.0004
Chlorophyll a	Error	28	13.14	0.47		
Chlorophyll a	C. Total	43	44.00			
Alpha Carotene	Model	15	21.70	1.45	1.82	0.0837

Alpha Carotene	Error	28	22.30	0.80		
Alpha Carotene	C. Total	43	44.00			
Beta Carotene	Model	15	23.50	1.57	2.14	0.0398
Beta Carotene	Error	28	20.50	0.73		
Beta Carotene	C. Total	43	44.00			
Phycoerythrin	Model	15	35.74	2.38	8.86	<.0001
Phycoerythrin	Error	27	7.26	0.27		
Phycoerythrin	C. Total	42	43.00			
Phycocyanin	Model	15	30.63	2.04	4.67	0.0003
Phycocyanin	Error	26	11.37	0.44		
Phycocyanin	C. Total	41	42.00			

Table A14. ANOVA effects table for Cultivation Experiment 2.

Pigment	Source	Nparm	DF	Sum of Squares	F Ratio	Prob> F
Alpha Carotene	Species	1	1	9.10	11.43	0.0021
Beta Carotene	Day	1	1	7.99	10.91	0.0026
Chlorophyll a allomer	Species	1	1	2.90	7.77	0.0094
Phycoerythrin	Nutrients*Light*Species	1	1	1.62	6.03	0.0208
Phycocyanin	Species	1	1	2.58	5.90	0.0223
Lutein	Light*Species*Day	1	1	3.43	5.71	0.0239
Beta Carotene	Species	1	1	3.45	4.71	0.0387
Chlorophyll a	Species	1	1	2.11	4.50	0.0428
Phycocyanin	Nutrients*Light*Species	1	1	1.96	4.47	0.0442
Zeaxanthin	Species	1	1	19.41	30.60	<.0001
Lutein	Species	1	1	15.16	25.23	<.0001
Chlorophyll a allomer	Day	1	1	22.78	60.99	<.0001
Chlorophyll a	Day	1	1	21.74	46.33	<.0001
Phycoerythrin	Species	1	1	7.93	29.48	<.0001
Phycoerythrin	Day	1	1	19.11	71.08	<.0001
Phycocyanin	Day	1	1	15.90	36.34	<.0001
Lutein	Light*Species	1	1	2.37	3.95	0.0567
Lutein	Nutrients*Light	1	1	2.07	3.44	0.074
Chlorophyll a	Nutrients*Species	1	1	1.48	3.14	0.0871
Chlorophyll a	Nutrients*Light*Species	1	1	1.46	3.12	0.0884

Beta Carotene	Light	1	1	2.21	3.01	0.0936
Phycoerythrin	Light	1	1	0.76	2.83	0.1041
Phycocyanin	Nutrients	1	1	1.23	2.80	0.1062
Chlorophyll a allomer	Light*Species*Day	1	1	1.02	2.73	0.1097
Zeaxanthin	Nutrients*Light*Species	1	1	1.60	2.52	0.1272
Phycocyanin	Light	1	1	1.08	2.46	0.1286
Alpha Carotene	Nutrients*Day	1	1	1.91	2.40	0.1327
Beta Carotene	Light*Species*Day	1	1	1.53	2.09	0.1593
Chlorophyll a	Light	1	1	0.88	1.88	0.1809
Beta Carotene	Species*Day	1	1	1.33	1.82	0.188
Zeaxanthin	Nutrients*Light*Species*Day	1	1	1.14	1.81	0.1934
Chlorophyll a	Nutrients*Light	1	1	0.80	1.70	0.2025
Chlorophyll a allomer	Light	1	1	0.62	1.65	0.2091
Lutein	Nutrients	1	1	0.99	1.64	0.2108
Alpha Carotene	Light	1	1	1.26	1.58	0.2193
Phycocyanin	Nutrients*Light*Species*Day	1	1	0.65	1.48	0.2343
Chlorophyll a allomer	Nutrients*Species	1	1	0.53	1.43	0.2424
Alpha Carotene	Light*Day	1	1	1.12	1.41	0.2454
Phycoerythrin	Nutrients	1	1	0.38	1.40	0.2469
Phycoerythrin	Nutrients*Light*Species*Day	1	1	0.36	1.35	0.2562
Chlorophyll a	Light*Day	1	1	0.58	1.23	0.2761
Beta Carotene	Light*Day	1	1	0.87	1.19	0.2837
Alpha Carotene	Species*Day	1	1	0.94	1.18	0.287
Phycocyanin	Light*Species*Day	1	1	0.50	1.15	0.2937
Alpha Carotene	Nutrients	1	1	0.91	1.14	0.2954
Lutein	Light*Day	1	1	0.68	1.13	0.2971
Phycoerythrin	Nutrients*Species*Day	1	1	0.30	1.10	0.3041
Lutein	Nutrients*Light*Day	1	1	0.65	1.08	0.308
Beta Carotene	Nutrients*Species*Day	1	1	0.78	1.07	0.3101
Phycocyanin	Light*Day	1	1	0.46	1.05	0.3152
Alpha Carotene	Light*Species	1	1	0.74	0.93	0.3436
Chlorophyll a	Nutrients*Light*Species*Day	1	1	0.41	0.87	0.3579
Alpha Carotene	Day	1	1	0.69	0.87	0.3582
Chlorophyll a allomer	Light*Species	1	1	0.32	0.86	0.3611

Zeaxanthin	Day	1	1	0.51	0.80	0.3812
Phycoerythrin	Nutrients*Light	1	1	0.21	0.79	0.3824
Lutein	Nutrients*Species*Day	1	1	0.47	0.78	0.3862
Lutein	Nutrients*Species	1	1	0.46	0.76	0.39
Chlorophyll a allomer	Light*Day	1	1	0.27	0.73	0.4006
Chlorophyll a allomer	Nutrients*Light	1	1	0.26	0.71	0.4073
Phycoerythrin	Light*Species*Day	1	1	0.19	0.71	0.4076
Phycocyanin	Species*Day	1	1	0.24	0.54	0.4682
Phycocyanin	Light*Species	1	1	0.24	0.54	0.4687
Zeaxanthin	Nutrients*Light	1	1	0.32	0.50	0.4871
Phycoerythrin	Nutrients*Light*Day	1	1	0.12	0.45	0.5066
Phycocyanin	Nutrients*Light	1	1	0.20	0.45	0.5085
Lutein	Day	1	1	0.27	0.44	0.5116
Chlorophyll a	Nutrients*Species*Day	1	1	0.20	0.43	0.5168
Alpha Carotene	Nutrients*Species*Day	1	1	0.32	0.40	0.5309
Beta Carotene	Nutrients*Light*Day	1	1	0.26	0.35	0.5589
Lutein	Species*Day	1	1	0.20	0.34	0.5645
Alpha Carotene	Nutrients*Species	1	1	0.25	0.31	0.5803
Phycocyanin	Nutrients*Light*Day	1	1	0.13	0.30	0.5875
Lutein	Nutrients*Light*Species	1	1	0.17	0.29	0.5964
Beta Carotene	Nutrients*Day	1	1	0.21	0.29	0.5974
Alpha Carotene	Nutrients*Light*Species*Day	1	1	0.21	0.26	0.6113
Chlorophyll a allomer	Nutrients*Light*Species	1	1	0.09	0.25	0.6199
Alpha Carotene	Nutrients*Light	1	1	0.19	0.24	0.6312
Zeaxanthin	Light*Species*Day	1	1	0.14	0.21	0.6491
Chlorophyll a	Nutrients*Day	1	1	0.10	0.20	0.6558
Alpha Carotene	Nutrients*Light*Day	1	1	0.15	0.19	0.6704
Beta Carotene	Nutrients*Light*Species	1	1	0.13	0.18	0.6728
Beta Carotene	Nutrients*Light	1	1	0.13	0.18	0.6729
Beta Carotene	Nutrients*Light*Species*Day	1	1	0.13	0.18	0.6763
Phycoerythrin	Light*Day	1	1	0.05	0.17	0.6832
Chlorophyll a allomer	Nutrients*Day	1	1	0.06	0.17	0.6842
Phycocyanin	Nutrients*Species*Day	1	1	0.07	0.17	0.6866
Beta Carotene	Nutrients*Species	1	1	0.09	0.13	0.7217
Lutein	Nutrients*Light*Species*Day	1	1	0.08	0.13	0.7249

Zeaxanthin	Nutrients*Species	1	1	0.07	0.11	0.7402
Zeaxanthin	Light	1	1	0.06	0.10	0.7596
Alpha Carotene	Nutrients*Light*Species	1	1	0.07	0.09	0.7677
Zeaxanthin	Light*Day	1	1	0.05	0.08	0.7836
Zeaxanthin	Species*Day	1	1	0.04	0.07	0.7937
Phycoerythrin	Nutrients*Day	1	1	0.02	0.06	0.8078
Beta Carotene	Light*Species	1	1	0.04	0.06	0.8123
Phycoerythrin	Light*Species	1	1	0.01	0.04	0.8389
Lutein	Nutrients*Day	1	1	0.02	0.04	0.8415
Zeaxanthin	Light*Species	1	1	0.02	0.03	0.8601
Lutein	Light	1	1	0.02	0.03	0.867
Beta Carotene	Nutrients	1	1	0.02	0.02	0.8769
Chlorophyll a allomer	Species*Day	1	1	0.01	0.02	0.8864
Zeaxanthin	Nutrients*Day	1	1	0.01	0.02	0.8939
Chlorophyll a	Light*Species*Day	1	1	0.01	0.01	0.9112
Chlorophyll a	Species*Day	1	1	0.01	0.01	0.916
Phycocyanin	Nutrients*Species	1	1	0.00	0.01	0.9198
Zeaxanthin	Nutrients*Light*Day	1	1	0.01	0.01	0.9205
Chlorophyll a	Nutrients	1	1	0.00	0.01	0.9231
Phycocyanin	Nutrients*Day	1	1	0.00	0.01	0.9353
Chlorophyll a allomer	Nutrients*Light*Species*Day	1	1	0.00	0.01	0.9362
Zeaxanthin	Nutrients*Species*Day	1	1	0.00	0.00	0.9483
Chlorophyll a	Light*Species	1	1	0.00	0.00	0.9639
Chlorophyll a	Nutrients*Light*Day	1	1	0.00	0.00	0.9685
Chlorophyll a allomer	Nutrients	1	1	0.00	0.00	0.9694
Alpha Carotene	Light*Species*Day	1	1	0.00	0.00	0.9711
Phycoerythrin	Nutrients*Species	1	1	0.00	0.00	0.9715
Phycoerythrin	Species*Day	1	1	0.00	0.00	0.9718
Zeaxanthin	Nutrients	1	1	0.00	0.00	0.9738
Chlorophyll a allomer	Nutrients*Light*Day	1	1	0.00	0.00	0.9947
Chlorophyll a allomer	Nutrients*Species*Day	1	1	0.00	0.00	0.9976