# COMPARATIVE TIME COURSE OF PHOTOACCLIMATION IN HAWAIIAN ENDEMIC AND INVASIVE SPECIES OF *GRACILARIA* (RHODOPHYTA)

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# Traits that Characterize Invasive Seaweeds

## *Introduction*

The spread of organisms to new environments is one of the major drivers of speciation, since it exposes them to novel suites of environmental pressures (Mayr 1942). Recently, however, human activity has greatly accelerated the spread of organisms to new environments and altered ecosystem conditions in ways that often benefit these newcomers at the expense of pre-existing biota (Palumbi 2001). Although most research has focused on terrestrial species introductions, a recent meta-analysis determined that marine species introductions currently outpace them tenfold (Sorte et al. 2010).

Numbers of introduced marine species have increased exponentially in the last 200 years, and rates of introductions continue to increase due to trade, climate change and various other anthropogenic disturbances (Ruiz et al. 2000, Raitsos et al. 2010). It is harder to quantify the number or rate of marine algal introductions, as they are often overlooked or even encouraged (Inderjit et al. 2006). Despite representing a minority of reported marine species introductions, alien macroalgae can have profound economic and ecological impacts on existing systems (Schaffelke et al. 2006, Schaffelke & Hewitt 2007). Prevention of these impacts necessarily involves identification not only of algal species likely to become invaders but also of attributes that contribute to the invasiveness of potential nuisance algae (Anderson 2007). It is worth noting that many

successful introduced algae have not been observed to negatively impact their new environments and so are not considered invasive (Boudouresque & Verlaque 2002); these cases are not pertinent to this review.

Several attributes have been identified or proposed that may play roles in an alga's invasiveness (e.g. Chapman 1999, Wright & Davis 2006); however, many of these characteristics are shared by native algae living alongside these invaders (Schaffelke et al. 2006), making attributes definitive of invasibility elusive. Additionally, conditions or habitats that may help to enable the success of invasive alien seaweeds can also promote aggressive behavior in native algae (Smith et al. 2004, Dailer et al. 2010). Many investigators draw a distinction between these latter, "bloom-forming" natives with overly-successful exotic species, but in fact there seems to be little fundamental difference aside from ascribed origin. Changes in ambient conditions affecting otherwise non-aggressive native algae can cause behavior and impacts identical to those associated with alien invaders.

The discipline of invasive species risk assessment (the evaluation of the likelihood and severity of potential invasions based on known characteristics of potential invaders, recipient systems and vectors) has been around for less than two decades, so that even recent endeavors to assess seaweed invasions can be referred to as "early attempts". One such important early attempt was made by Boudouresque and Verlaque (2002), who analyzed data from 22 studies of species introductions in the Mediterranean, comparing potentially diagnostic life history and physiological traits of species known to

be invasive with those not considered so. Only traits that were thought to enable invaders to succeed once established were considered. Although no trait was shared by all nine of the invasive species considered and no species possessed all traits of invasiveness proposed, several traits less common to the non-invasive algae were made apparent: large size, perennial life history, lack of a resting stage, prolific vegetative reproduction, possession of toxic metabolites and lack of, or, escape from grazers.

Nyberg and Wallentinus (2005) provided a more comprehensive weed risk assessment for invasive algae. They conducted a meta-analysis of 113 introduced European macrophytes and 113 natives, comparing three categories of traits potentially diagnostic of invasiveness: 1) those that may enable dispersal, 2) those that help invaders become established and 3) those that may have disproportionate ecological effects once established. In all, 13 traits were assigned to a category and arranged in gradients, some qualitative, others quantitative depending on the nature of the trait. The largest differences in risk between native and introduced species were: probability of being transported (an index of size and attachment strategy), habitat effect size (a combination of depth affinity and area covered), tolerance to pollution (including nutrient enrichment) and to how well-distributed the plant already was. Some characteristics not explicitly influential because of the study design, but still considered important by the authors were prolific vegetative reproduction, fragmentation ability and tentatively, survival time in darkness.

Both of these studies, while offering procedures that the authors hoped would be useful to other investigations, were concerned with European algae and it is conceivable that their conclusions would be different if the study were conducted elsewhere; weed risk assessment for tropical and cold water systems will need to be conducted to achieve a more holistic and therefore comprehensive evaluation of potentially invasive traits.

Several traits that have been identified or proposed, including those from the above studies along with a select few others deemed commonly influential are discussed below.

*Association with humans*

An association with humans is the one characteristic that by definition, all introduced macroalgae, including invasives, share (Williams and Smith 2007, Sorte et al. 2010), having been distributed with the aid of humans both intentionally and accidentally. Many species are introduced for the purposes of, or in association with aquaculture (Williams and Smith 2007), and many of the traits listed in this review are intentionally selected for in algaculture species (Naylor et al. 2001). Another other major vector is shipping, where many macroalgae are transported on the hulls of ships or in ballast. Invasive seaweeds are frequently found in close proximity to areas of human population density, in part due to human dispersal mechanisms and the requirements of aquaculture, but also because of human-induced nutrient loading and other types of marine pollution that encourage the growth of opportunistic algae (Klein & Verlaque 2007, Anderson 2007).

Since Banner (1974) determined that algal blooms and simultaneous reef degradation in Oahu's Kaneohe Bay were strongly linked to terrestrial discharge into the Bay, a good deal of progress has been made in linking nutrient pollution to the success of invasive algae. More recently, several studies have used stable isotope analyses to trace $\delta^{15}N$ present in invasive algal tissue to sewage effluent sources (Lin 2007, Dailer et al. 2010). Massive seasonal blooms of *Ulva prolifera* along China's east coast have been connected to nori aquaculture (Hu et al. 2010). Anthropogenic eutrophication can transform formerly inconspicuous native seaweeds and otherwise merely introduced algae into highly destructive ecosystem engineers (Stimson & Larned 2000, Wallentinus & Nyberg 2005).

*Resistance to herbivory*

The enemy release hypothesis (ERH), which says that an invader's success or impact is related to the scarcity of predators in the recipient system as compared to the invader's native system, is one of the most cited explanations for invasive success (Sorte et al. 2010), but support for this concept as an explanation for invasiveness is inconsistent (Colautti et al. 2004, Hill 2006). One shortcoming of the ERH is that it takes little account of any defenses the invader might have developed to discourage predation. Macroalgae have developed various defenses to herbivory but those probably most relevant to invasibility are chemical and morphological defenses, both of which affect how palatable algae are to herbivores (Duffy & Hay 1990).

Morphological defenses include structural characteristics of a plant that make it difficult or unpleasant to eat, e.g. tissue density, texture or composition. Some plants may be too tough or calcareous for a given herbivore, while other grazers prefer those qualities (Steneck & Watling 1982). Chemical defenses include algal compounds that are toxic or unpleasant enough to an herbivore so as to be repellent. There are many well-known chemical defenses that inhibit herbivory in invasive seaweeds, including caulerpynene, a terpene present in *Caulerpa* species (Hay & Fenical 1988), dimethylsulfoniopropionate (DMSP) in *Codium fragile* ssp. *tomentosoides* (van Goor) P.C.Silva (Lyons et al. 2007), the phenol avrainvilleol, present in some species of *Avrainvillea* (Hay et al. 1990) although not yet confirmed in *A. amadelpha* (Montagne) A.Gepp & E.S.Gepp, and various halogenated metabolites, primarily bromoform, in *Asparagopsis armata* Harvey (Paul et al. 2006, Vérges et al. 2008). If a given defense type does not work on a specific herbivore, the plant may have others at its disposal. For instance, the sea hare *Dolabella auriculara* is undeterred by many secondary metabolites that are known to deter other herbivores, but is deterred by textural qualities such as plant toughness and calcification (Pennings and Paul 1992).

*Vegetative reproduction*

In their survey of 113 Mediterranean algal invasions, Nyberg & Wallentinus (2005) reported that invasive red algae reproduced more often asexually than sexually, brown algae the reverse and in green algae asexually about as often as sexually. Though many native red and green algae have the ability to reproduce in either mode, the ability

to reproduce both sexually and asexually is one of the traits most commonly attributed to invasive seaweeds (Sakai et al. 2001, Smith et al. 2002, Inderjit et al. 2006), as this flexibility in reproduction may greatly increase reproductive output. Asexual reproduction may be of especial value to invasive species as environmental cues necessary for sexual reproduction may not be present in the new environment. Vegetative propagation may occur in invasive marine plants by way of parthenogenesis, fragmentation or clonal development. All three are known to occur in *Codium fragile* ssp. *tomentosoides* including both budding and rhizomatous clonal growth (Watanabe et al. 2009).

Reproduction by vegetative fragmentation is the simple breaking or dissociation of the plant and subsequent regrowth of fragments into plants in their own right. In Hawai'i, both *Gracilaria salicornia* (C.Agardh) E.Y.Dawson and *Euchuema denticulatum (N.L. Burman) F.S. Collins & Hervey* are mat-forming invaders thought to propagate mainly by this method, though both are capable of sexual reproduction (Smith et al. 2001, Dailer 2006). In these species, mechanical damage or axial disintegration results in fragmentation, with fragments drifting about the benthos until eventual attachment to a substrate is accomplished. Fragmentation alone does not assure reproductive success, as many fragments will not settle in locations suitable for continued growth or be of sufficient size to survive. Both *G. salicornia* and *E. denticulatum* are negatively buoyant and relatively massive macroalgae and are highly efficient local colonizers but with unlikely long-distance colonizers due to limited depth range to sinkage (Smith et al. 2002, 2004). Fragment success is dependent on physiological requirements and

constraints that are species-specific. Smith & Walters (1999) tested fragment viability of three species of *Caulerpa*. Larger fragments had higher survivorship, which is to be expected for coenocytes with a costly wound response. Smith et al. (2002) examined fragment viability of four invasive algae in Hawai'i by cutting fragments into various size classes and growing them in small cages in the field and in culture. Results showed that fragmentation potential may be constrained by minimum size requirements in some species, but not all.

Stoloniferous clonal development allows for great lateral expansion of propagules and followed by fragmentation can aid in forming dense stands of plants (Davis & Wright 2006). Outside the Bryopsidales, few invasive algae possess this type of growth, *Asparagopsis taxiformis* being one exception (Ni Chualain et al. 2004).
For plants that use more than one of these methods to propagate asexually, there may be differential advantages to them based on how well established an invader is. For instance, *Caulerpa taxifolia* colonization is a multi-stage process, wherein first fragments recruit before stolons are present, then vegetative growth via stolons increases abundance, and finally positive feedback system is established as vegetative growth and fragmentation alternate increase stand density (Davis & Wright 2006).

*Dispersal potential*

Invasive algae may disperse via several different vectors, mostly famously object fouling, but also by rafting, hitchhiking on faunal aquaculture species, ship ballast or

fishing gear, and by intentional introduction as food or materials for human use (Williams and Smith 2007). Most of these vectors are incidental to the transport of seaweeds, i.e. no particular trait of the alga contributes to the transport, with the exceptions of buoyancy and fouling ability.

Dislodged plants or fragments can be carried by currents away from their original site. However, if the plant is not sufficiently buoyant it may not get far, as in the case of *G. salicornia* (see above). The phenomenon of rafting, the transport by sea of organisms on or attached to floating miscellany, has been well documented and has been observed to be a source of marine invasions since at least the 1940s (Thiel & Gutow 2005). Mat-forming buoyant marine macrophytes are often the vector for rafting (ibid.), or may themselves float as single plants until some attachment opportunity presumably presents itself, and reattachment to the substratum is accomplished. The invasive fucoid *Turbinaria ornata* (Turner) J. Agardh can form mats up to 2,500 $m^2$ that float offshore in the wake of storms (Stewart 2008); thalli from these mats may remain fertile for up to three months, suggesting dislodgement *en masse* as a dispersal opportunity. The success of another invasive fucoid, *Sargassum muticum* (Yendo) Fensholt, has been in part explained by the presence of pneumatocysts and post-dislodgment similar to the previous example (Harries et al. 2007). The highly invasive coenocyte *Codium fragile* ssp. *tomentosoides* is a buoyant alga that does not contain gas vesicles, but it does contain numerous gas bubbles trapped in its medulla that reportedly contribute to its dispersal potential (Dromgoole 1982). It is an open question as to whether this feature is unique to this species of *Codium*.

Very few studies have been conducted testing traits or properties of marine organisms that confer fouling potential. In an effort to identify adaptations that may provide competitive advantages allowing organisms to remain attached to boat hulls long enough to be introduced to new environments, Murray et al. (2012) calculated two parameters: 1) attachment strength, using a spring scale and pulling on the organism until breakage occurred, and 2) drag coefficient (the resistance of an object in a fluid environment), by determining at what vessel velocity an organism becomes dislodged. In all, three native and five introduced hull fouling animals of various taxa were tested. Results were mixed, but indicated that invasives tended to have higher attachment strengths and lower drag coefficients, qualities predicted to be higher in invasives. Although several invasive seaweeds are documented as having been introduced or transported in this way, including *Undaria pinnatifida* (Harvey) Suringar and various *Ulva* species, to date no study has focused on macroalgal fouling ability.

Qualities that contribute to the intentional dispersal of macroalgae by humans are based more on human requirements than on traits that directly enable invasiveness. That being said, any agarophyte or carageenophyte might be said to have moderate dispersal potential, as these algae possess economically valuable compounds and are likely to be exchanged around the globe for the foreseeable future. This may also be said of species palatable or aesthetically compelling to humans, although this quality is inconsistent and often ephemeral.

*Tolerance to Abiotic Stresses*

A broad tolerance to environmental variation enables invaders to colonize areas beyond the range of less plastic algae. Tolerance helps alien algae to survive shorter-term transport-associated stresses as well as longer-term challenges posed by environments different from those of their native habitats.

Several invasive species display euryhaline tendencies believed to contribute to their invasiveness including *Codium fragile* ssp. *tomentosoides* (Hanisak 1979), *Gracilaria salicornia* (Smith et al. 2004) and *Grataloupia turuturu* (Simon et al. 2001).
The bloom-forming *Ulva perusa* maintains positive growth rates at salinities between 5 and 40 ‰ (e.g. Choi et al. 2009). Steen (2004) demonstrated that *Sargassum muticum* germlings rapidly acquire tolerance to salinity as they age.

Tolerance to desiccation has also been observed to enable increased survival in invaders. Smith et al. (2004) reported that in Hawai'i, a tropical environment with a relatively low intertidal species richness, *Gracilaria salicornia* survived up to three hours of low humidity emersion without inhibiting growth rate and up to six hours of emersion with only minor decreases in growth rate. In an effort to simulate net entanglement as a plausible vector for transport, Schaffelke and Dean (2005) found that *Codium fragile* ssp. *tomentosoides* was capable of recovering from nearly three months of high humidity emersion.

The success of several invasions has been attributed to thermal tolerance. While many algae thrive in either arctic and tropical temperatures, few can tolerate both. One notable exception is *Gracilaria tikvahiae*, an opportunistic bloom forming rhodophyte, has a current Eastern Atlantic range from Nova Scotia to the Caribbean, and maintains positive growth rates between 15 and 30ºC (Lapointe et al. 1984); invasive populations of this species have been located in Hawai'i (Peyton 2009). *Grataloupia turuturu*, with populations ranging from as far south as Ivory Coast and north as far as Russia develops in waters ranging from 4 to 28 ºC (Archino et al. 2007).

Determinants of invasive success are to some degree a product of climatic characteristics of the recipient location as well as climatic tolerances of the invader (Sorte et al. 2010). Global climate change may promote phase shifts as the environment of native plants changes in ways they are not equipped to handle and are outcompeted by those more tolerant species, native and alien (Hughes et al. 2007).

*Survival in Darkness*

Dark survival, the extent to which an alga can tolerate or rebound from long periods of darkness, is a commonly investigated trait attributed to phytoplankton to help explain the survival in the absence of light during pelagic mixing of unattached microalgae in the water column. Little work has been published on dark survival in macroalgae, despite its presumed importance to high-latitude seaweeds, survival in ballast and burial in soft-sediments; dark survival may also have implications for tropical species, contributing to

the success of self-shading, mound-forming morphologies. Nyberg and Wallentinus (2005) initially intended to include this factor in their meta-analysis of invasive macroalgal traits, but declined to due to lack of published material on the subject. Prior to 2005, most studies on seaweed dark survival were concerned largely with wintering. However, interest is slowly shifting in the direction of dark survival as a trait diagnostic of invasiveness.

Dark survival for some algae may be associated with low temperatures that inhibit respiration (Kirst & Wiencke 1995). Investigating the apparent rarity of sexual reproduction or settled germlings of bloom forming *Ulva* species of Veerse Meer lagoon (Netherlands) it was discovered that overwintering *Ulva* sp. plants survive burial at temperatures below 5 ºC for up to two weeks (Kamermans et al. 1998). Glasby et al. (2005) found that *Caulerpa taxifolia* can also survive surprising periods of darkness at temperatures well above freezing. Fragments extracted from Botany Bay, New South Wales in were buried partially or completely in 5 cm of sediment at 20 ºC for periods up to 17 days. By the end of the experiment, 35% of the totally buried fragments survived and slowly recovered mass lost during burial.

Nyberg & Wallentinus (2009) found that *Gracilaria vermiculophylla* can survive up to five months of darkness at low temperatures and bereft of a liquid medium (and associated nutrients). Plants were shaken dry and stored in plastics bags at 8 ºC during which time they grew slightly. After reintroduction to light, growth resumed normal growth rates. After two to four weeks plants dissociated into fragments, which continued to grow.

The first study focusing on macroalgal dark survival in ballast water was conducted by Flagella et al. (2007). Ballast water from 12 ships was collected, and although no macroscopic macrophytes were found, 15 species of seaweeds, including 10 species of *Ulva*, surviving in microscopic uni- or multicellular stages were cultured from the collected water and raised to macroscopic sizes. The time in complete darkness spent by the algae varied from 4-34 days.

*Morphological plasticity*

Phenotypic plasticity is the ability of a genotype to exhibit alternative phenotypic characteristics in response to environmental conditions (Fordyce 2006). Although often cited as a trait common to invasive plants in general (see Agrawal 2001), studies that seek to demonstrate specific advantages conferred by phenotypic plasticity are rare and there is little in the literature that explicitly attributes the invasiveness of macroalgae to this morphological variability.

Stiger and Payri (1999) attributed the persistence of an invasive *Turbinaria ornata* on Tahitian fringing reefs to dwarfism in plants exposed to high wave action. These dwarfs were observed to be considerably more fertile than the more sheltered, taller plants. A rhizomatous growth form was discovered for *Codium fragile* ssp. *tomentosoides*, a species previously believed only to attach to hard substrates via basal discs.

Investigators attributed this form as a response to growth on soft sediment substrates (Garbary et al. 2004).

Morphological variation is frequently mentioned in passing as a trait possessed of or possibly contributing the invasiveness of several genera rich in invasive species. Examples of this include *Kappaphycus* spp. (Smith et al. 2002, Conklin et al. 2009), *Caulerpa* spp. (Anderson 2007, Cebrian & Ballesteros, 2009) and *Gracilaria* spp. (Dawes 1994, Abbott 1999). Research specifically oriented at testing hypothesis concerning phenotypic plasticity is sorely needed.

*Photosynthetic plasticity*

In terrestrial macrophytes, the term “photosynthetic plasticity” generally refers to “the range of phenotypic expression of leaves emerged and expanded under a constant light condition” (Yamashita 2000). In the study of marine plants, the phrase is used more broadly, and here is intended to expand upon Fordyce’s definition of phenotypic plasticity (see above), i.e. it is the ability of a genotype to exhibit alternative, reversible phenotypic characteristics in response to environmental irradiance. These characteristics may include changes in photosynthetic performance or requirements, changes in light capture ability, habit, pigment composition and other photophysiological variables.

As a potential trait of invasive plants in general, photosynthetic plasticity has only recently been considered. Studies comparing invasive terrestrial embryophytes with noninvasive species have revealed that among several traits considered, including resource capture and retention, photosynthetic efficiency and rapid leaf development, the trait that appears to be common to invasives is the ability to acclimate to a wide range of irradiances, often called 'photoacclimation potential' (Yamashita et al. 2000, Yamashita 2002, Feng, et al. 2007 a & b). Very few studies concerning photosynthetic plasticity in invasive macroalgae have been published as of yet. The success of invasive *Caulerpa racemosa* in the Mediterranean has been attributed to a combination of morphological and photosynthetic plasticity, referring specifically to that species' high photoacclimation potential, both in shallow microhabitats and across a range of depths (Raniello et al. 2004, Raniello et al. 2006). Dailer (2006) determined the velocity of photoacclimation to be a factor in the success of *E. denticulatum* in Hawai'i's Kaneohe Bay.

Photoacclimation is the common theme in these studies investigating photosynthetic plasticity in invasives and will be the subject of the remainder of this review.

## Photoacclimation

Differences in photosynthetic characteristics within a given species, and often within a single plant, are largely the result of relative exposure to solar irradiance.

Marine algae inhabit highly variable light environments, which results in significant stresses being applied to the photosynthetic apparatus of these organisms. Changes within the cell combat these stresses at a variety of scales to maintain homeostasis and generative capacity. The modulation of light harvesting dynamics and protection and repair of photosystems are key processes governing the success of photosynthetic species.

Photoacclimation is conventionally described as the ability of the photosynthetic apparatus to change in composition and function in response to changes in irradiance (Raven & Geider 2003), although it may be more accurate to say it is a response to changes in absorbed light, which is itself a function of differential incident irradiance and an organism's optical properties (Kana et al. 1997). Macroalgal acclimation to low irradiances typically involves an increase in chlorophyll and other light harvesting pigments and decreased photosynthetic output, while acclimation to high light involves increasing photosynthetic output and the synthesis of photoprotective pigments. Irradiance-induced adjustment of the photosynthetic apparatus has been divided into three categories based of the timescale of changes in the photosynthetic unit (PSU). Photoadaptation, a term formerly used interchangeably with photoacclimation, is now reserved for those changes that occur in the genome as a result of natural selection over the course of many generations. Photoregulation refers to rapid changes in the PSU that occur in the order of minutes or less: Rubisco activity, state transitions and the xanthophyll cycle (Raven & Geider 2003). Intermediate on this timescale, photoacclimation is concerned with changes that occur in the span of hours to days (or

months), including changes in pigment content and complement, in the relative size and abundance of photosystems (PS) I and II, and to changes in the persistence of Calvin-Benson cycle enzymes, particularly that of Rubisco.

The extent to which an alga can adjust its photosynthetic machinery may determine its distribution and tolerance to variation in light intensity that helps define its niche (Beach 2000). The broad range of vertical depths inhabited by populations of phytoplankton and corals (Moore et al. 1998, Hoogenboom et al. 2009), regional distribution in kelps (Wing et al. 2007) and general macroalgal community composition (Toohey et al. 2004) are all strongly influenced by the potential for photoacclimation inherent in a species. Gap formation in both terrestrial and kelp forests can benefit species with rapid photoacclimatory responses (Yamashita et al. 2000, Watanabe et al. 1994), a trait which may also play a role in the success of invasive plants (Yamashita et al. 2000, Dailer 2006).

Algae or algal tissues acclimated to low light (often called “shade acclimated”) conditions are characterized by a larger PSU size and/or increased number of PSUs (usually calculated by dividing the concentration of chl *a* by the number of PSI reaction centers) (Talarico et al. 2000), as a means of absorbing low levels of light. As a result of increased pigmentation, low light acclimated plant tissue often appears darker or brighter than higher light acclimated tissue, which is often pale. However, ambient nutrients can also increase pigment content depending on availability, so pigmentation alone cannot be used as an index of photoacclimation (Talarico 1996). Shade

acclimated plants generally have lower growth rates, lower Rubisco concentrations and photosynthetic output than high light acclimated ones, tradeoffs for the reduced light requirements characteristic of low light acclimation (Cunningham et al. 1992). When an alga acclimates to higher light (i.e. “sun acclimated”) conditions, these features are reversed and until they maximize at the point of light saturation. Carotenoid pigments may play a role in photoprotection or light harvesting, depending on the taxon-specific carotenoid composition and characteristics of the pigments themselves.

*Photoacclimation in Red Algae*

Red algae have been common subjects of study in photoacclimation research; their differences from other algae in pigment content and complement have led many to expect them to be especially adapted to specific depths or irradiances, e.g. the theory of chromatic adaptation (see Saffo 1987 for a review and gentle rebuttal of the theory). However, any differences inherent to red algal photobiology are not so obvious, although they do have this distinction: taken as a group, red macrophytes are able to survive at the broadest range of irradiances of all macroalgae, from deep-dwelling crustose corallines growing at ca. 0.008 μmol quanta $m^{-2}$ $s^{-1}$ photosynthetically active radiation (PAR) to tropical intertidal turf species at irradiances in excess of 2400 μmol quanta $m^{-2}$ $s^{-1}$ PAR (Littler et al. 1985, Beach et al. 2000), a difference of some five degrees of magnitude. Thus, rather than being limited to the depths, they are a ubiquitous and species-rich algal phylum.

Photoacclimation is a complex process, and in red algae it takes on an added dimension given the uniqueness of their pigment complement and the resulting complexity of their photophysiology. Chromoproteins called phycobilins are the primary light harvesting pigments in red algae (Haxo & Blinks 1950), and not chlorophyll *a* as in the other algae. Of the autotrophs containing phycobilin accessory pigments, cryptophytes, glaucophytes, cyanobacteria and rhodophytes, only in the latter three are they organized into phycobilisomes (PBS). Moreover, rhodophyte light harvesting complex arrangements are unique among algae in that the PSI reaction center is surrounded by a carotenoid/chlorophyll complex, while the reaction center of PSII instead has a phycobilisome, loosely bound to the thylakoid membrane (Gantt & Cunningham 2001). The pigment groups named above all play major roles in rhodophyte photoacclimation: chlorophyll, phycobiliproteins and carotenoid.

All oxygenic photosynthetic organisms contain chlorophyll *a* (Chl *a*) as it is essential to the activity of photosynthetic reaction centers. Chl *a* is the only chlorophyll associated with photosynthesis in red algae, and the great majority of it is associated with PSI (Levy & Gantt 1988). During acclimation to changing irradiances, cellular concentrations of Chl *a* tend to increase in low light and decrease in high light (Stevens 1992), although the change is often slight compared to other pigments (Levy & Gantt 1998) and are sometimes absent altogether (Ramlov et al. 2011). Chl *d* has generally been discounted as present in rhodophytes, although recent studies on the cyanobacterium *Acaryochloris marina* have revealed that it not only has a role in photosynthesis, but it

may replace Chl *a* has the principle light harvesting pigment in this organism (Larkum & Kühl 2005).

Phycobiliproteins, the major light harvesting antennae of red algae, absorb a large range of visible light (ca. 495-650 nm) that Chl *a* alone is unable to capture. Low light acclimation is accompanied by increases in Chl *a*, as in other algae, but in red algae is accompanied by even greater increases in phycobilin content and phycobilisome proliferation (Talarico & Maranzana 2000). Similar to changes in photosystem reactions to light intensity, PBS size and number may also change in response to irradiance. Changes in size are effected by increasing or decreasing rod lengths and numbers, with the vast majority of additions and subtractions attributed to PE, hinting at the existence of pool of free, nonfunctional PE (Lüder et al. 2002).

When functional, phycobiliproteins are organized into phycobilisomes, usually hemidiscoidal or hemispherical structures functionally attached to PSII reaction centers. The stable PBS core is composed of allophycocyanin (APC) cylinder complexes, from which projects rods composed of disks composed of phycocyanin (PC) on the inside and phycoerythrin, usually outnumbering the other phycobilins, on the periphery (Glazer & Clark 1986). Light energy is transferred from PE to PC to APC before being passed to PSII.

A large concentration of phycobilins may be indicative of a low number of PBS. As mentioned above, rhodophyte phycobilins are not always organized into

phycobilisomes, especially when more are present than can be utilized (Lüder et al. 2002). “Free” phycoerythrin appears to play a role in nitrogen storage (Lüder et al. 2002, Ramlov et al. 2011) and protection from photoinhibition. Detachment of PE from the PBS may result from exposure to excessive UV A & B radiation (Poppe et al. 2003) or high PAR levels (Talarico 1996), inhibiting photosynthesis while protecting the apparatus. PSII lacks photoprotective carotenoids, though the PBS provides some measure of protection by the decoupling of PE from the PBS at high irradiances thereby preventing absorption of excess light (Liu et al. 2009).

Carotenoids, associated exclusively with PSI in Rhodophyta, are carbon chains bookended by six-carbon rings embedded in the reaction center, serving to both stabilize reaction center structure and protect PSI from excessive light energy (Schubert et al. 2011). There is little evidence to suggest that carotenoids play an important light harvesting role in rhodophytes, although some energy transfer is evident (Goedheer 1968). The carotenoid profile of red algae can vary greatly between orders, with either lutein or zeaxanthin and antheraxanthin being the major pigments or, in the case of the Gracilariales, xanthophyll cycle (XC) pigments (Schubert et al. 2006a), although the XC itself has not been demonstrated to occur in any red algae (Schubert et al. 2011). The carotenoid pigment complement may be indicative of an alga’s photoprotective strategy, as different pigments and combinations of pigments appear to have different energy quenching capacities (Schubert et al. 2006b). The photochemical reactions governing carotenoid photoprotection happen at short-time scales and are therefore properly

regarded as aspects of photoregulation, but the synthesis and degradation of the pigments themselves are photoacclimatory processes.

*Measurement tools: Photosynthesis, Growth and Pigments*

The most common metric used to evaluate acclimation to changes in irradiance has been the comparison of photosynthetic and cellular respiration rates with the use of the oxygen electrode (Clark 1956), a device that measures the amount of dissolved oxygen (DO) present in a solution. Photoautotrophic tissue or cells are placed in the electrode's chamber with some fixed irradiance directed at the sample; measurements of oxygenic uptake and production are recorded, from which the parameters that constitute the photosynthesis irradiance curve (PI) can be inferred, notably $P_{max}$ (the light-saturated rate of photosynthesis), $I_c$ (compensation irradiance), $I_k$ (saturating irradiance, sometimes called the Talling Constant) and $\alpha$ (the initial slope of the photosynthesis-irradiance curve) and respiration (R).

The development of the Clark electrode in 1956 made measuring photosynthesis and associated processes as they occurred possible for the first time and replaced, to a large extent, previous wet chemistry techniques such as the Winkler method.

In a similar way, but to a lesser degree, the development of 'active' fluorescence methods (Kolber & Falkowski 1993), particularly pulse amplitude modulation (PAM) fluorometry (Schreiber et al. 1986), has been replacing the oxygen electrode as the

primary tool in studies of photosynthesis, especially those focusing on photoacclimation. PAM measures chlorophyll fluorescence by first obtaining the minimal fluorescence from a sample before a saturating pulse closes all open PSII reaction centers, followed by a series of actinic light pulses of increasing intensity (Maxwell and Johnson 2000). Each fluorescence response received by the fluorometer returns information about the speed and extent of PSII recovery, culminating in what is termed a rapid light curve (RLC) and resembles, but is not equivalent to, a PI curve. The parameters that constitute the RLC, $ETR_{max}$ (maximum electron transport rate, called *J* by some authors), $E_k$ (light saturation coefficient) and α (the slope of initial of the rapid light curve, sometimes called the maximum light use coefficient), by convention also correspond to, but are not equivalent to DO parameters (Beer & Axelsson 2004, Perkins et al. 2006). Notably absent from PAM parameters is an analog of $I_c$: since PAM does not measure cellular respiration, a compensation point cannot be calculated, ensuring that the DO method will remain in use for the foreseeable future. PAM-derived parameters are often used as proxies for DO-derived parameters due to the rapidity and ease of use of the former compared to the latter.

Acclimation to high photosynthetic photon flux density (PPFD) is associated with increased photosynthetic capacity, indicated by elevations in $P_{max}$ or $ETR_{max}$ (as more photosynthesis is occurring), increased $I_k$ or $E_k$, (as more light is required to saturate photosynthesis), and a lower $\alpha$ (as light harvesting efficiency increases) (Pan et al. 1996, Perkins et al. 2006).

Measurements of growth and growth rate can also be important indices of photoacclimation. These may be accomplished by cell counting in planktonic species or by dry or wet weight for multicellular algae. Dry weight is the most accurate measure but usually results in the death of the plant; the accuracy of wet weight measurements can be compromised by evaporation and desiccation so consistency of sample preparations becomes more important. In general, acclimation to higher PPFD is associated with higher growth rates, though excessive irradiance can result in photooxidation or cell death (Gant 1990). Likewise, excessively low PPFD may be insufficient for the reproduction and growth energy needs of a plant (Lobban & Harrison 1997).

Chloroplast pigment quantification and characterization are common methods of assessing photoacclimation in algae. These may be studied with a variety of different techniques including fluorometry, chromatography, spectrophotometry, microscopy and most recently, laser micromanipulation (see review by Bayoudh et al. 2001 on optical tweezers applications for chloroplast study). Chloroplast arrangement, size and quantity are plastic characteristics that are relatively easy to observe with microscopy and fluorometry. Pigment analysis is typically more invasive due to the requirement of most methods to chemically extract the pigments before analysis via HPLC or spectrophotometry. Analyses that require extraction make studying time-course acclimation problematic in that they require destructive sampling. *In vivo* absorbance provides us with a tool that obtains spectra while leaving the plant intact and alive, allowing pigment changes in the same plant or tissue to be measured over time (Smith & Alberte 1994, Beach et al. 2000).

Chlorophyll *a* content, because of its universality in photosynthetic tissue, is the most oft-reported pigment in red macroalgal photoacclimation studies. The presence of phycobilins in red algae gives the study of photoacclimation in this group added complexity. These pigment concentrations, along with ratios of pigment to pigment and pigment to nutrient (e.g. Chl *a*: C) are also frequently evaluated as photoacclimation parameters as they may be subject to change with changes in light intensity.

Measurement of photosynthetic acclimation almost always involves the comparison of one or more parameters obtained from the aforementioned methods from organisms exposed to more than one irradiance level for some length of time.

*Measuring the time-course of photoacclimation*

Photoacclimation is usually described as taking days or weeks to occur (Gantt 1990, Raven & Geider 2003) yet the time required for completion is rarely recorded, and when it is, it is often an incidental notation. Rarer still are studies that compare photoacclimation time between organisms, and without that comparison to give the measurement some context, there is little meaning to the result. The rate of photoacclimation, that is, how fast an organism responds to changes in light intensity, may be indicative of how successful it may be in situations that can alter that environment (Dailer 2006). Of the studies that do measure the length of time it takes for a plant to acclimate from one state to another, most use different methods, making

comparison of photoacclimation rates reported in past studies difficult. Other studies may report acclimation rates of some constituents of the photoacclimatory process (e.g. $P_{max}$ or chlorophyll concentration) or report data that do not explicitly mention acclimation time (yet allows the reader to infer it). Additionally, much of the early (pre-1990s) literature equates the terms photoadaptation and photoacclimation, further confounding searches for data specific to the latter process. Also, statistical methods are used inconsistently in many of these studies; the achievement of a steady state sometime after the organism's introduction to a new light environment is often deemed evidence of acclimation. For the determination of photoacclimation speed, the end-point of the process requires delineation.

Early experiments on photoacclimation involved maintaining algae in different light regimes for some (often arbitrary) length of time deemed appropriate for acclimation to have occurred, often several months, before analyses were conducted (Yocum & Blinks 1957, Waaland et al. 1974). Most studies that examined time course adjustments in photoacclimation were concerned mainly with stepwise changes in photosynthetic rate in response to different light levels (Steemann et al. 1962, Sheridan 1972). By the 1990s, as photoacclimation became a commonly researched topic, more attention was focused on the acclimation of other components of the photosynthetic apparatus, especially chlorophyll *a*.

There is no pattern that indicates that one taxonomic group acclimates faster or slower than another, although the most rapid acclimators appear to be single-celled. To date,

most studies on the time course of acclimation to irradiance have concerned planktonic species and symbiotic dinoflagellates. By measuring chlorophyll content and oxygen evolution, Fisher et al. (1996) used DO and Chl *a* pigment analyses to find that the eustigmatophyte *Nannochloropsis* sp. adjusted to low light conditions in 87 hours (ca. 3.5 days). Acclimation of pigmentation to low light conditions in the dinoflagellate *Gonyaulax polyedra* occurs within four days (Prézelin and Matlick 1983). A recent study by Nymark et al. (2009) combined physiological (PAM fluorometry) and molecular (transcriptional profiling) methods to assess high light acclimation in the diatom *Phaeodactylum tricornutum*, which takes a maximum of 48 hours. The rapid rate of acclimation ascribed to this last study may be due to the fact that pigment adjustments were not considered. Acclimation of photosynthesis often occurs well before that of pigmentation (Prézelin & Matlick 1983, Beach 2000). Indeed, the above time course studies that account for pigment adjustments only consider chlorophyll *a*, which may synthesize or degrade at different rates than accessory pigments (Prézelin & Matlick 1980). Studies on cyanobacterial photoacclimation indicate that chlorophyll *a* concentrations can reach stable states before other parameters (including photosynthetic ones) when introduced to new light environments. For example, chlorophyll *a* content in shade-acclimating *Anacystis nidulans* stabilized in around 30 hours, but another 50 hours was required before phycocyanin levels acclimated (Lönneborg et al. 1985); likewise, *Anabaena variabilis* chlorophyll *a* and carotenoid concentrations finished adjusting to low light in only one day, while $I_k$ took an additional three days to reach a decreased steady state (Collins & Boylen 1982).

Several studies on photoacclimation have revealed photoacclimation rates in dinoflagellate zooxanthellae, which are slower than those reported for phytoplankton. Anthony & Hoegh-Guldberg (2003) measured acclimation in both increases and decreases in irradiance in the Pacific coral *Turbinaria mesenterina* (Lamarck) using oxygen respirometry. Until this point, studies had focused on one irradiance shift or the other. Fragments of coral were maintained in flowing seawater tanks and pre-acclimated to one of five different artificial light regimes (ranging in PAR from 20-400 μmol quanta $m^{-2}$ $s^{-1}$ PAR) for one month. Fragments were then reciprocally transplanted to one of three alternate light regimes (ranging in PAR from 40-400 μmol quanta $m^{-2}$ $s^{-1}$ PAR). Using only $I_k$ as an index, *T. mesenteria* were adjudged to have acclimated to high light in eight days and to low light in 10 days.

Photoacclimation in corals has added complexity because not only does pigment concentration change in response to changing irradiance, but so does zooxanthellae density in coral tissue. A study by Titlyanov et al. (2001) sought to elucidate the dynamics of low-light acclimation in *Stylophora pistillata* using only algal cell and chlorophyll quantification. Acclimation to low irradiance was achieved by increases in zooxanthellae chlorophyll concentration as well as increases in zooxanthellae density in coral branch tissue. The change in chlorophyll concentration was completed within four days, while the increase in algal density took an additional five weeks. Harland and Davies (1994) examined zooxanthellae density, pigment content and DO-derived photosynthetic parameters in the anemone *Anemonia viridis*, concluding that all parameters had reached stable, acclimated states in 28 days.

Macrophytes have been underrepresented as study organisms in measurements of photoacclimation time course. Terrestrial embryophytes have been the focus of several of these examinations, often using unconventional (to phycologists at least) diagnostic parameters. Yamashita et al. (2000) measured acclimation to increased irradiance in five species of subtropical forest plants by tracking chlorophyll concentrations and PAM-derived parameters $F_o$ (initial fluorescence of PSII) and $F_v/F_m$ (quantum efficiency of PSII) over time. Other than for chlorophyll *a*, they did not discuss photoacclimation times explicitly, so photosynthetic acclimation time can only be inferred from their data using parameters that are typically used to assess photosynthetic "health" rather than photosynthetic activity (Maxwell & Johnson 2000). Chlorophyll degradation took seven days to reach a steady state for four out of five of the species, while $F_v/F_m$ stabilized to new steady states around 20 days for most species. Sims & Pearcy (1990) had more definitive results assessing sun and shade acclimation using reciprocal transplants on the tropical aroid *Alocasia macrorrhiza*, tracking rates of change in $A_{max}$ (a measure of carbon fixation), R and leaf nitrogen content. Notably, the investigators established sun and shade control treatments as benchmarks for change in the experimental treatments. *A. macrorrhiza* acclimated to higher and lower light levels in 10 days according to photosynthetic parameters, but leaf N levels changed only a little, and not in meaningful ways.

Marine macroalgae inhabit highly dynamic light environments at a variety of scales, leading to a broad recognition that photoacclimation potential is an important quality for

seaweeds (Beach et al. 1997, Figueroa & Gomez 2001, Payri et al. 2001, Raniello et al. 2004). Presumably, timeframes of photoacclimation processes and completion are important as well, however, little attention has been devoted to this subject. Unfortunately, no studies to date have recorded acclimation rates for phaeophyte macroalgae, although Watanabe et al. (1992) noted that pigment concentrations in *Pterygophora californica* were unaffected by *Macrocystis pyrifera* canopy removal even after 28 days, indicating the potential for very slow photoacclimation.

The chlorophyte*, Ulva rotundata*, when reciprocally transplanted in outdoor culture at three different irradiances over a five day period, responded to decreasing light with a sudden drop in R but no decrease in $P_{max}$ and increasing Chl *a* content for four days before reaching values comparable to low light controls. High light acclimation saw R and $P_{max}$ increase over the span of five days to reach control values, with Chl *a* content decreasing for four to five days before reaching high light control levels (Henley and Ramus 1989). The controls in this experiment were starting values of control treatments, which varied greatly over time. It seems likely that had the experiment extended beyond five days, $P_{max}$ adjustment would have eventually decreased in response to decreasing light, revealing a more complete estimate for photoacclimation rate in this species.

The rates of photoacclimation in red macroalgae are the slowest recorded of the marine algae, excluding zooxanthellae symbionts, and may be comparable to land plants in the length of time required to complete the process. These low rates are likely a result of

metabolic changes in phycobilin and carotenoid content, the combination of which is unique to rhodophytes. Pigment extractions from cultures of *Gracilaria tenuistipitata* over the course of 10 days revealed differing relative rates of change in pigment concentrations (Carnicas et al. 1999). Increasing irradiance was accompanied by a rapid decrease in Chl *a*, *β*-carotene and zeaxanthin and a slower decrease in phycobilin content. After a decrease in irradiance, increasing Chl *a* and *β*-carotene amounts stabilized much faster than phycobilin content, which by the study's end were still increasing. While showing that phycobilin synthesis may be a comparatively slow photoacclimatory process, the study did not seek to quantify rates or completion times for pigment changes in this species, which it would have been unable to do anyway, given the abbreviated nature of the study.

The time required for completion of the process in rhodophytes is comparable to that for embryophytes. Stevens (1992) reciprocally transplanted *Gracilaria chilensis* C.J.Bird, McLachlan & E.C.Oliveira in outdoor tanks with sun and shade treatments. Analyses including pigment extractions (Chl *a*, PE and PC) and DO-derived photosynthetic parameters, revealed that complete adjustment from high to low light levels occurred in all parameters in seven days, except for PE which took 10 days. Pigment acclimation to high light occurred in seven days while photosynthetic parameters stabilized at day nine.

In a combination of reciprocal transplant studies and field manipulations, *Ahnfeltiopsis concinna*, a Hawaiian intertidal turf alga adjusted to changes in solar irradiance very

slowly (Beach et al. 2000). Measurements of photosynthetic parameters and pigments were compared with controls based on initial measurements of canopy (shade) and understory (sun) treatments. Analyses were based on DO analysis and pigment extraction and *in vivo* based spectrophotometry. Regression analysis indicated that absorbance at 440 nm, a carotenoid-associated maxima and the slowest of the selected diagnostic absorbance peaks to change, reached a steady state acclimation level at around 29 days.

In a similar study, Dailer (2006) conducted an *in situ* investigation of the overall speed of photoacclimation in the mound-forming invasive *Euchuema denticulatum*. This was perhaps the first study to focus specifically on photoacclimation rates. Cores of algal tissue were taken from the field in canopy and understory regions of algal mounds, inverted and plugged back into the slots cut out of the mound. Every other day for nine days in summer and again in winter, a portion of the cores were retrieved from the field and brought into the lab for pigment and fluorometric analyses. A total of eight days was required for complete acclimation of the plugs to their new orientation, with phycobilins in the summer acclimated in four days, ahead of the other parameters.

Table 1 lists all study species for which an estimate of photoacclimation time could be obtained from the literature. Comparing these estimates may not be appropriate, due to the variety of study design and methodology, where the study was conducted (rates obtained in the laboratory may not be comparable with those from field studies) and the lack of an agreed-upon delineation of the end-point of photoacclimation, i.e. at what

point can we say that the process is complete? Without direct comparisons of rate, it is difficult to judge how important accurate measurements of the process are. Comparative studies of photoacclimation in microalgae have suggested that species-specific acclimation strategies may play a role in the differential presence of bloom forming algae (Van Leeuwe et al. 2005, Suggett et al. 2007). Comparatively rapid photoacclimatory responses to gap formation in terrestrial forests contribute to the success of invasive trees (Yamashita 2000). Similar studies comparing macroalgal species or taxa may broaden our understanding of the importance of time-course photoacclimation.

## SUMMARY

The introduction of exotic marine macroalgae is a problem of increasing international import, and has the potential to have major global impact, especially given the ecosystem engineering and structural potential of many seaweeds. To address problems posed by invasive algae, it is important to not only halt and reverse invasions already in progress but to prevent future introductions. To this end, many potential characteristics of algal invaders have been identified or proposed, with the presumption that identifying these characteristics enable us to arrest the spread of species that possess such traits. Of these, the ability to acclimate to a wide range of irradiances is an understudied aspect of invasive algae research.

Photoacclimation is a complex process that can be assessed in a variety of manners and has long held the interest of biologists, although the importance of the process has been inadequately evaluated. The importance of photoacclimation potential in red algae (due mainly to its unique pigment content), in particular, has long been the subject of speculation. It is also one of the few groups of plants that have been the subject of photoacclimation rate studies. Time-course acclimation to irradiance has been a topic of interest among phycologists since at least the late 1970s, but very little work has been done on macrophytes. The variety of experimental approaches, inconsistency in measurement practice and methodology and the lack of consensus on what constitutes 'complete' photoacclimation make comparing published studies of limited value.

Although several studies have examined photoacclimation velocity in rhodophytes (Stevens 1992, Carnicas 1999, Beach 2000, Dailer 2006), none have measured the responses of more than one species, and each has used a different method in determining change. The relative importance of photoacclimation both in general, and as applied to invasive species research, can be best assessed by direct comparison of more than one species to another.

## Hypothesis:

Does a rapid photosynthetic response to changes in light intensity give invasive rhodophytes a competitive advantage over non-invasive rhodophytes?

$H_A$: Invasive red algae photoacclimate faster than non-invasive red algae.

## REFERENCES


Abbott, IA (1999) Marine red algae of the Hawaiian Islands. Bishop Museum Press, Honolulu, HI, 477 pp

Agrawal AA (2001) Phenotypic plasticity in the interactions and evolution of species. Science 294: 321-326

Anderson LWJ (2007). Control of invasive seaweeds. Botanica Marina 50: 418–437

Anthony KRN & O Hoegh-Guldberg (2003) Kinetics of photoacclimation in corals. Oecologia 134: 23-31

Banner AH (1974). Kaneohe Bay, Hawai'i: Urban pollution and a coral reef ecosystem. Proceedings of the 2nd International Coral Reef Symposium. 2, 685–702.

Bayoudh S, M Mehta, H Rubinsztein-Dunlop, NR Heckenberg & C Critchley (2001) Micromanipulation of chloroplasts using optical tweezers. Journal of Microscopy 203: 214–222

Beach, K, C Smith & R Okano (2000). Experimental analysis of rhodophyte photoacclimation to PAR and UV -Radiation using *in vivo* absorbance spectroscopy. Botanica Marina. 43: 525-536.

Beach, KS, HB Borgeas, NJ Nishimura & CM Smith (1997) *In vivo* absorbance spectra and UV-absorbing compounds in tropical reef macroalgae. Coral Reefs 16: 21-28

Beer, B & L Axelsson (2004). Limitations in the use of PAM fluorometry for measuring photosynthetic rates of macroalgae at high irradiances. European Journal of Phycology Volume 39: 1-7

Boudouresque CF & M Verlaque (2002) Biological pollution in the Mediterranean Sea: invasive versus introduced macrophytes. Marine Pollution Bulletin 44: 32–38

Callaway RM, SC Pennings & CL Richards (2003) Phenotypic plasticity and interactions among plants. Ecology 84: 1115–1128

Carnicas E, C Jiménez & FX Niell (1999) Effects of changes of irradiance on the pigment composition of *Gracilaria tenuistipitata var. liui* Zhang et Xia. Journal of Photochemistry and Photobiology B: Biology 50: 149-158

Chapman AS (1999) From introduced species to invader: What determines variation in the success of *Codium fragile* ssp. *tomentosoides* (Chlorophyta) in the North Atlantic Ocean? Helgoland Marine Research 52: 277-289

Cebrian E & E Balleseteros (2009) Temporal and spatial variability in shallow- and deep-water populations of the invasive *Caulerpa racemosa var. cylindracea* in the Western Mediterranean. Estuarine, Coastal and Shelf Science 83: 469-474

Collins CD & CW Boylen (1982) Ecological consequences of long-term exposure of *Anabaena variabilis* (Cyanophyceae) to shifts in environmental factors. Applied and Environmental Microbiology 44: 141-148

Clark L (1956) Monitor and control of blood and tissue oxygen tensions. Transactions – American Society for Artificial Internal Organs 2: 41-48

Conklin KY, A Kurihara & AR Sherwood (2009) A molecular method for identification of the morphologically plastic invasive algal genera *Eucheuma* and *Kappaphycus* (Rhodophyta, Gigartinales) in Hawaii. Journal of Applied Phycology 21:691–699

Cunningham FX Jr, A Vonshak, & E Gantt (1992) Photoacclimation in the red alga *Porphyridium cruentum*. Plant Physiology 100: 1142- 1149

Dailer ML 2006. Photoecological strategies influencing the invasive success of marine macrophytes *Euchuema denticulatum* on Hawaiian coral reefs. Master's Thesis. Botany Department, University of Hawai'i, Honolulu.154 pp

Dailer ML, RS Knox, JE Smith, M Napier & CM Smith (2010) Using $\delta^{15}N$ values in algal tissue to map locations and potential sources of anthropogenic nutrient inputs on the island of Maui, Hawai'i, USA. Marine Pollution Bulletin 60: 655–671.

Dromgoole FI (1982) The buoyant properties of *Codium*. Botanica Marina 25: 391–397.

Duffy JE & ME Hay (1990) Seaweed Adaptations to Herbivory. BioScience, 40: 368-375

Figueroa FL & I Gomez (2001) Photosynthetic acclimation to solar UV radiation of marine red algae from the warm-temperate coast of southern Spain: A review. Journal of Applied Phycology 13: 235–248

Fine M, L Steindler & Y Loya (2004) Endolithic algae photoacclimate to increased irradiance during coral bleaching. Marine and Freshwater Research 55: 115-121

Fisher T, J Minnaard & Z Dubinsky (1996) Photoacclimation in the marine alga *Nannochloropsis sp*. (Eustigmatophyte): a kinetic study. Journal of Plankton Research 18: 1797-1818

Flagella MM, M Verlaque, Alessio Soria & MC Buia (2007) Macroalgal survival in ballast water tanks. Marine Pollution Bulletin 54: 1395–1401

Fordyce J A (2006) The evolutionary consequences of ecological interactions mediated through phenotypic plasticity. Journal of Experimental Biology 209: 2377–2383.

Gantt E (1990) Pigmentation and photoacclimation. *In* Cole, K.M. & Sheath R.G. [Eds.] *Biology of the Red Algae*, Cambridge University Press, Cambridge, U.K., pp. 203-220.

Gantt E & FX Cunningham (1992) Algal Pigments. Encyclopedia of Life Sciences, a web-based encyclopedia at www.els.net. John Wiley & Sons, Ltd.

Glasby TM, PT Gibson & S Kay (2005) Tolerance of the invasive marine alga *Caulerpa taxifolia* to burial by sediment. Aquatic Botany 82: 71–81

Glazer AN & JH Clark (1986) Phycobilisomes: Macromolecular structure and energy flow dynamics. Biophysics Journal 49: 115-116

Hanisak MD (1979) Growth patterns of *Codium fragile ssp. tomentosoides* in response to temperature, irradiance, salinity and nitrogen source. Marine Biology 50:319–332

Harland AD & PS Davies (1994) Time-course of photoadaptation in the symbiotic sea anemone *Anemonia viridis*. Marine Biology 119: 45-51

Harries DB, E Cook, DW Donnan, JM Mair, S Harrow & JR Wilson (2007) The establishment of the invasive alga *Sargassum muticum* on the west coast of Scotland: Rapid northwards spread and identification of potential new areas for colonization. Aquatic Invasions 2: 367-377

Haxo FT & LR Blinks (1950) Photosynthetic action spectra of marine algae. Journal of General Physiology 33: 389-422.

Henley WJ & J Ramus (1989) Time course of physiological response of *Ulva rotundata* to growth irradiance transitions. Marine Ecology Progress Series 54: 171-177

Hoogenboom MO, SR Connolly & KRN Anthony (2009) Effects of photoacclimation on the light niche of corals: a process-based approach. Marine Biology 156: 2493-2503

Hughes TP, MJ Rodrigues, DR Bellwood, D Ceccarelli, O Hoegh-Guldberg, L McCook, N Moltschaniwskyj, MS Pratchett, RS Steneck & B Willis (2007) Phase shifts, herbivory, and the resilience of coral reefs to climate change. Current Biology 17: 360-365

Hu, C, D Li, C Chen, J Ge, FE Muller-Karger, J Liu, F Yu, & M-X He,. (2010) On the recurrent *Ulva prolifera* blooms in the Yellow Sea and East China Sea. Marine Science Faculty Publications. Paper 58.

Inderjit, D Chapman, M Ranaletti, & S Kaushik (2006) Invasive marine algae: An ecological perspective. The Botanical Review 72: 153-178

Kamermans P, E Malta, JM Verschuure, L F Lentz & L Schrijvers (1998) Role of cold resistance and burial for winter survival and spring initiation of an *Ulva spp.* (Chlorophyta) bloom in a eutrophic lagoon (Veerse Meer lagoon, The Netherlands) Marine Biology 131: 45-51

Kana TM, Geider RJ & C Critchley (1997) Regulation in photosynthetic pigments in micro-algae by multiple environmental factors: a dynamic balance hypothesis. New Phytologist. 137: 629-638

Kirst GO &C Winecke (1995) Ecophysiology of polar algae. Journal of Phycology 31: 181-199

Kolber Z & PG Falkowski (1993) Use of active fluorescence to estimate phytoplankton photosynthesis in situ. Limnology & Oceanography 38: 1646-1665

Lapointe, BE, KR Tenore & CJ Dawes (1984) Interactions between light and temperature on the physiological ecology of *Gracilaria tikvahiae* (Gigartinales: Rhodophyta). Marine Biology Volume 80: 171-178

Lapointe BE., PJ Barile, MM Littler & D S Littler (2005) Macroalgal blooms on southeast Florida coral reefs: II. Cross-shelf discrimination of nitrogen sources indicates widespread assimilation of sewage nitrogen. Harmful Algae 4: 1106-1122

Larkum AWD & M Kühl (2005) Chlorophyll d: the puzzle resolved. Trends in Plant Science 10: 355–357

Levy I & E Gantt (1988) Light acclimation in *Porphyridium purpureum* (Rhodophyta): growth, photosynthesis and phycobilisomes. Journal of Phycology 24: 452-458

Lin, HJ, CY Wu, SJ Kao & PJ Meng (2007) Mapping anthropogenic nitrogen through point sources in coral reefs using $\delta$15N in macroalgae. Marine Ecology Progress Series 335: 95–109.

Littler MM, DS Littler, SM Blair & JN Norris (1985) Deepest known plant life discovered on an uncharted seamount. Science 227: 58-59

Liu, LN., TJ Aartsma, JC Thomas, BC Zhou, & YZ Zhang (2009) FRAP analysis on red alga reveals the fluorescence recovery is ascribed to intrinsic photoprocesses of phycobilisomes than large-scale diffusion. PloS One 4: 5295–5305

Lobban CS & PJ Harrison (1997). Seaweed ecology and physiology. Cambridge University Press, New York. 366 pp

Lönneborg A, LK Lind, SR Kalla, P Gustafsson, & G Öquist (1985) Acclimation

Processes in the light-harvesting system of the cyanobacterium *Anacystis nidulans* following a light shift from white to red light. Plant Physiology 78: 110-114

Lüder UH, C Wiencke & J Knoetzel (2002) Acclimation of photosynthesis and pigments during and after six months of darkness in *Palmeria decipiens* (Rhodophyta): a study to simulate Antarctic winter sea ice cover. Journal of Phycology 38: 904–913

Maggs, CA, & H Stegenga 1999. Red algal exotics on North Sea Coasts. Helgoland Marine Research 52: 243–258.

Mathieson AC, J Pederson & CJ Dawes (2008) Rapid assessment surveys of fouling and introduced aeaweeds in the Northwest Atlantic. Rhodora 110:406-478.

Maxwell K & GN Johnson (2000) Chlorophyll fluorescence—a practical guide. Journal of Experimental Botany 51: 659-668

Mayr E (1942) Systematics and the origin of species from the viewpoint of a zoologist. New York: Columbia University Press. 334 pp

Moore LR, G Rocap & SW Chisholm (1998) Physiology and molecular phylogeny of coexisting *Prochlorococcus* ecotypes. Nature 393: 464-467

Naylor RL, SL Williams, & DR Strong 2001. Aquaculture - A gateway for exotic species. Science 294:1655–56

Nyberg, CD & I Wallentinus (2005). Can species traits be used to predict marine macroalgal introductions? Biological Invasions 7: 265-279

Nyberg CD & I Wallentinus (2009). Long-term survival of an introduced red alga in

adverse conditions. Marine Biology Research 5: 304-308

Palumbi SR (2001) Humans as the world's greatest evolutionary force. Science 293: 1786-1790

Pan Y, DVS Rao & KH Mann (1996) Acclimation to low light intensity in photosynthesis and growth of *Pseudo-nitzschia multiseries* Hasle, a neurotoxigenic diatom. Journal of Plankton Research 18: 1427-1438

Payri CE, S Maritorena, C Bizeau & M Rodière(2001) Photoacclimation in the tropical coralline alga *Hydrolithon onkodes* (Rhodophyta, Corallinaceae) from a French Polynesian reef. Journal of Phycology 37: 223–234

Perkins RG, JL Mouget, S Lefebvre & J Lavaud (2006) Light response curve methodology and possible implications in the application of chlorophyll fluorescence to benthic diatoms. Marine Biology 149: 703–712

Peyton KA (2009) Aquatic invasive species impacts in Hawaiian soft sediment habitats. PhD Dissertation. University of Hawai'i, Honolulu. 150 pp

Poppe F, RAM Schmidt, D Hanelt & C Wiencke (2003) Effects of UV radiation on the ultrastructure of several red algae. Phycological Research 51: 11–19

Prézelin BB & IA Matlick (1980). Time-course of photoadaptation in the photosynthesis-irradiance relationship of a dinoflagellate exhibiting photosynthetic periodicity. Marine Biology 58: 85-96

Prézelin BB & IA Matlick (1983). Nutrient-dependent low-light adaptation in the dinoflagellate *Gonyaulax polyedra*. Marine Biology 74: 141-150

Raitsos DE, G Beaugrand, D Georgopoulos, A Zenetos, AM Pancucci-Papadopoulou, A

Theocharis, & E Papathanassiou (2010) Global climate change amplifies the entry of tropical species into the Eastern Mediterranean Sea. Limnology & Oceanography 55: 1478-1484

Ramlov F, JMC Souza, AF Faria, M Maraschin, PA Horta, & NS Yokoya (2011) Growth and accumulation of carotenoids and nitrogen compounds in *Gracilaria domingensis* (Kütz.) Sonder ex Dickie (Gracilariales, Rhodophyta) cultured under different irradiance and nutrient levels. Brazilian Journal of Pharmacognosy 21: 255-261

Raniello R, M Lorenti, C Brunet & MC Buia (2006) Photoacclimation of the invasive alga *Caulerpa racemosa* var. *cylindracea* to depth and daylight patterns and a putative new role for siphonaxanthin. Marine Ecology 27: 20–30

Raniello R, M Lorenti, C Brunet & MC Buia (2004) Photosynthetic plasticity of an invasive variety of *Caulerpa racemosa* in a coastal Mediterranean area: light harvesting capacity and seasonal acclimation. Marine Ecology Progress Series 271: 113–120

Raven JA & RJ Geider (2003) Adaptation, acclimation and regulation in algal photosynthesis *in* Larkum, AWD, SE Douglas, and JA Raven, *eds*, Photosynthesis in algae. Kluwer Academic Publishers, Netherlands, pp. 385-412.

Ruiz GM, PW Fofonoff, JT Carlton, MJ Wonham & AN Hines (2000) Invasion of coastal marine communities in North America: Apparent patterns, processes, and biases. Annual Review of Ecology, Evolution & Systematics 31:481–531

Saffo MB (1987) New light on seaweeds. BioScience 37: 654-664

Sakai AK, FW Allendorf,, JS Holt,, DM Lodge,, J Molofsky, KA With,, S Baughman, RJ Cabin, JE Cohen,, NC Ellstrand,, DE McCauley,, P O'Neil,, IM Parker,, JN Thompson &

SG Weller (2001) The population biology of invasive species. Annual Review of Ecology and Systematics 32: 305–332

Schaffelke B & D Deane (2005) Desiccation tolerance of the introduced marine green alga *Codium fragile ssp. tomentosoides* – clues for likely transport vectors? Biological Invasions 7: 577-587

Schaffelke B, JE Smith & CL Hewitt (2006) Introduced macroalgae – A growing concern. Journal of Applied Phycology 18: 529–541

Schaffelke B & CL Hewitt (2007) Impacts of introduced seaweeds. Botanica Marina 50: 397–417

Schreiber U, U Schliwa & W Bilger (1986) Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. Photosynthesis Research 10: 51–62.

Schubert H, M Andersson & P Snoeijs (2006a) Relationship between photosynthesis and non-photochemical quenching of chlorophyll fluorescence in two red algae with different carotenoid compositions. Marine Biology 149: 1003–1013

Schubert N, E Garcia-Mendoza & I Pacheco-Ruiz (2006b) Carotenoid composition of marine red algae. Journal of Phycology 42: 1208–1216

Schubert N, E Garcia-Mendoza, & S Enriquez (2011) Is the photo-acclimatory response of Rhodophyta conditioned by the species carotenoid profile? Limnology & Oceanography 56: 2347–2361

Sheridan, RP (1972). Kinetics of chlorophyll *a* and platoquinone *a* changes in response to light intensity. Journal of Phycology 8: 166-169

Sims DA & RW Pearcy (1990) Photosynthesis and respiration in *Alocasia macrorrhiza* following transfers to high and low light. Oecologia 86: 447-453

Smith, CM & RS Alberte (1994) Characterization of *in vivo* absorption features of chlorophyte, phaeophyte and rhodophyte algal species. Marine Biology 118: 511-521.

Smith JE, CM Smith & CL Hunter (2001) An experimental analysis of the effects of herbivory and nutrient enrichment on benthic community dynamics on a Hawaiian reef. Coral Reefs 19: 332-342

Sorte JB, SL Williams & JT Carlton (2010) Marine range shifts and species introductions: comparative spread rates and community impacts. Global Ecology and Biogeography 19: 303–316

Steernann NE, VK Hansen & EG Jorgensen (1962). The adaptation to different light intensities in *Chlorella vulgans* and the time dependence upon transfer to a new light intensity. Physiologia PIantarum 15: 505-517

Stevens MLG (1992) Aspects of photoadaptation in the intertidal red alga *Gracilaria chilensis*. PhD Dissertation. University of Auckland, New Zealand pp. 205

Suggett DJ, E Le Floc'H, GN Harris, N Leonardos, & RJ Geider (2007) Different strategies of photoacclimation by two strains of *Emiliana huxleyi* (Haptophyta). Journal of Phycology 43: 1209–1222

Stewart HL (2008) The role of spatial and ontogenetic morphological variation in the expansion of the geographic range of the tropical brown alga, *Turbinaria ornata.* Integrative and Comparative Biology 48: 713-719.

Stiger V & CE Payri (1999) Spatial and seasonal variations in the biological

characteristics of two invasive brown algae, *Turbinaria ornata* (Turner) J. Agardh and *Sargassum mangarevense* (Grunow) Setchell (Sargassaceae, Fucales) spreading on the reefs of Tahiti (French Polynesia). Botanica Marina 42: 295-306

Steneck RS & L Watling (1982) Feeding capabilities and limitation of herbivorous molluscs: A functional group approach. Marine Biology 68: 299-319

Talarico L (1996) Phycobiliproteins and phycobilisomes in red algae: adaptive responses to light. Scientia Marina 60 (Suppl. 1): 205-222

Talarico L & G Maranzana (2000) Light and adaptive responses in red macroalgae: an overview. Journal of Photochemistry and Photobiology B: Biology 56: 1–11

Titlyanov EA, TV Titlyanova , K Yamazato & R van Woesik (2001). Photo-acclimation dynamics of the coral *Stylophora pistillata* to low and extremely low light. Journal of Experimental Marine Biology and Ecology 263: 211–225

Toohey B, GA Kendrick, T Wernberg, JC Phillips, S Malkin & J Prince (2004) The effects of light and thallus scour from *Ecklonia radiata* canopy on an associated foliose algal assemblage: the importance of photoacclimation. Marine Biology 144: 1019–1027.

Van Leeuwe MA, B van Sikkelerus, WWC Gieskes & J Stefels (2005) Taxon-specific differences in photoacclimation to fluctuating irradiance in an Antarctic diatom and a green flagellate. Marine Ecology Progress Series 288: 9–19

Watanabe JM, RE. Phillips, NH Allen & WA Anderson (1992) Physiological response of the stipitate understory kelp, *Pterygophora californica* Ruprecht, to shading by the giant kelp, *Macrocystis pyrifera* C. Agardh. Journal of Experimental Marine Biology and Ecology Volume 159: 237–252

Watanabe S, A Metaxes & RE Schiebling (2009) Dispersal potential of the invasive green alga *Codium fragile ssp. fragile.* Journal of Experimental Marine Biology and Ecology 381: 114–125

Whitman, DW & AA Agrawal (2009). What is phenotypic plasticity and why is it important? *in* Phenotypic plasticity of insects: Mechanisms and consequences (ed. D. W. Whitman and T. N. Ananthakrishnan). Enfield: Scientific Publishers. pp. 1-63.

Williams SL & JE Smith (2007) A Global review of the distribution, taxonomy, and impacts of introduced seaweeds. Annual Review of Ecology, Evolution, and Systematics 38:327–359

Wright JT & AR Davis (2006) Demographic feedback between clonal growth and fragmentation in an invasive seaweed. Ecology 87: 1744-1754

Yamashita N, A Ishida, H Kushima & N Tanaka (2000) Acclimation to sudden increase in light favoring an invasive over native trees in subtropical islands, Japan. Oecologia 125: 412–419

Yamashita N, N Koike & A Ishida (2002) Leaf ontogenetic dependence of light acclimation in invasive and native subtropical trees of different successional status. Plant, Cell and Environment 25: 1341–1356

Table 1: Species-specific timeframes for completion of photoacclimation processes.

| Taxon | Species | Timeframe | Reference |
|---|---|---|---|
| Ochrophyta | *Phaeodactylum sp.* | 2 days | Nymark et al 2009 |
| | *Nannochloropsis sp.* | 3.6 days | Fisher et al 1996 |
| Cyanophyta | *Anacystis nidulans* | 3 days | Lönneborg et al 1985 |
| | *Anabaena variabilis* | 4 days | Collins & Boylen 1982 |
| Dinophyta | *Gonyaulax polyedra* | 4 days | Prézelin & Matlick 1983 |
| | *Anemonia viridis* | 28 Days | Harland & Davies 1994 |
| | *Turbinaria mesenterina* | 8-10 Days | Anthony & Hoegh-Guldberg 2003 |
| | *Stylophora pistillata* | 34 days | Titlyanov et al 2001 |
| Chlorophyta | *Ulva rotundata* | 5 days | Henley & Ramus 1989 |
| Embryophyta | *Alocasia macrorrhiza* | 10 days | Sims & Pearcy 1990 |
| | *various (5 species)* | 20+ days | Yamashita et al 2000 |
| Rhodophyta | *Eucheuma denticulatum* | 8 days | Dailer 2006 |
| | *Gracilaria chilensis* | 9-10 days | Stevens 1992 |
| | *Ahnfeltiopsis concinna* | 29 days | Beach et al 2000 |

Chapter 2

# COMPARATIVE RATES OF PHOTOACCLIMATION IN HAWAIIAN ENDEMIC AND INVASIVE SPECIES OF *GRACILARIA* (RHODOPHYTA)

## INTRODUCTION

Invasive marine algae are a major threat to coral reef ecosystems in Hawai‘i (Smith et al. 2002). Invasive algae can negatively impact nearshore marine environments in many ways, including the reduction of species diversity by outcompeting corals, native macroalgae, and other benthic organisms, altering environmental parameters such as water quality and substrate conditions to unfavorable states for pre-existing organisms, and reducing faunal species diversity by interfering with established food webs (Schaffelke & Hewitt 2007, Olenin et al. 2007, Smith et al. 2001). Identifying characteristics of algae that may play a role in their invasiveness can contribute to preventing future introductions as well as aid in the early identification of potential invaders.

One characteristic that may be instrumental in invasiveness is photoacclimation, the adjustment of the photosynthetic apparatus in response to changes in irradiance. There has been growing interest in examining photoacclimatory capacity to diagnose invasive potential in both terrestrial (Yamashita 2002, Feng et al. 2007) and marine (Raniello et al. 2006, Dailer 2006) plants. Evidence suggests that plants that can acclimate to a broader range of light conditions may exercise competitive advantage over other plants (Raniello et al. 2004, Feng 2007a). High shade tolerance and a large relative increase in photosynthetic rate after high light exposure are also photoacclimatory attributes that appear to favor invasive plants (Yamashita 2000, Feng 2007b). Such attributes are

important to identify to aid management and prevention efforts, particularly in areas at high risk of biological invasions like Hawaiian coastal waters.

There is some evidence that invasive algae acclimate to changing irradiance faster than non-invasive Hawaiian algae (Beach 1996, Dailer 2006), giving invasives an advantage in rebounding from disturbances that scour marine substrates or displace existing algae; the former can generate intense competition for the newly exposed substrate while the latter may introduce a potential invader to a novel environment. Rapid photoacclimation may also imbue an invasive with the ability to adjust to several light regimes that might otherwise be occupied by less phenotypically flexible algae (algae that have evolved to fit less expansive niches). Photoacclimation response time may be a factor in determining future threats in the form of both native and alien algae and if so, will help us make decisions regarding what to look for in terms of invasive characters.

Photoacclimation allows a plant to both avoid the potentially damaging effects of high irradiances as well as allow for increased light harvesting at lower irradiances. Photoacclimation may refer to one or more reversible changes to the size and/or number of photosynthetic units (PSU) in a cell, changes in the relative pigment content within a cell, or changes in photochemistry (Falkowski & LaRoche 1991, Cunningham et al. 1992) as a result of the plant's exposure to higher ("sun" state) or lower ("shade" state) photon flux densities. For rhodophytes, acclimation to changing irradiance includes reciprocal changes in the concentration of chlorophyll *a* and phycobilins, and

corresponding changes in carotenoid concentration and photosynthetic output (Waaland et al. 1974, Levy & Gantt 1988, Beach 2000, Payri 2001).

Photoacclimation is often described as requiring several days to weeks to occur (Gantt 1990), but acclimation time has in fact rarely measured and the point of acclimation occurrence remains poorly delineated. Phytoplankters have been reported to fully acclimate in a matter of hours (Prézelin & Matlick 1980) to days (Fisher et al. 1996). Measurements of corals acclimate somewhere between a few days to several weeks (Titlyanov et al. 2001). The few measurements made of macrophyte photoacclimation time range from eight days for the invasive *Euchuema denticulatum* (Dailer 2006) to about one month for the Hawaiian non-invasive? *Ahnfeltiopsis concinna* (Beach et al. 1997). However, among the various estimates there has been little congruence as to photoacclimation determination methodology.

*Gracilaria salicornia* is an invasive alien alga that is currently found along much of O‘ahu’s south and windward shores; it most often is found as freestanding plants that can occasionally reach lengths of 30 cm. Along several south shore beaches that experience particularly high levels of human disturbance *G. salicornia* forms extensive mats that can be over 10 cm thick and 2 or more meters across (Beach et al.. 1997, Larned 1998). Within a mat tissues range in color from yellow in the canopy regions where they may be exposed at low tide to a dark purple, a result of self-shading (Beach 1997). These mats have profound influence over the nearshore environment allowing this species to outcompete other benthic organisms for light, space and nutrients (Smith

et al. 2004). *Gracilaria salicornia* mats experience wave action and human mechanical disturbance that often leads to breakage of the mat and the formation of tumbleweed-like fragments. Many of these find purchase in crevices or otherwise fuse to hard substrates (Smith et al. 2004), often in a vertical orientation inverted from its previous state (pers. obs.). It can be assumed that a newly attached plant would require some rapid level of photoacclimatory readjustment as the plant rights itself physiologically to accommodate its new light environment. A more rapid shift in photoacclimatory recovery would facilitate first survival and then a return to higher growth rates in plants newly exposed to direct sunlight or the ability to continue harvest light despite sudden shading. Though this potential would be of advantage to any benthic alga, this is the type of phenotypic plasticity that has been suggested as a predictive trait for invasive species (Raniello et al. 2004, Nyberg & Wallentinus 2009).

Few past studies have tracked both photosynthetic and pigment acclimation in response to changes in irradiance over time in tropical rhodophytes (Beach et al. 2000, Dailer 2006), with each examining a single species. Here we compare two phylogenetically related species with similar local distribution, habitat and morphology. The objective of this study was to elucidate the extent to which the photosynthetic response time of invasive algae is indicative of their invasiveness. This is a novel approach to the study of photoacclimation, as researchers have generally evaluated the presence, importance or magnitude of photoacclimation but have rarely considered the speed at which it occurs to be a physiological response worthy of note.

## METHODS

To determine the rate of change from one photoacclimation state to another, reciprocal transplants were performed in culture, comparing the transition of *G. salicornia* to that of *Gracilaria coronopifolia* J. Agardh, a comparatively small (usually > 8 cm long and 1-2 mm in diameter) shallow-subtidal alga that is endemic to the Hawaiian Islands. This species was selected due to several factors:

- Both species are restricted to waters above a 4 m depth, and though only *G. salicornia* is truly intertidal, both are euryhaline, tolerating wide ranges of nutrients and salinity (Amato 2011), and occur often in the same locations, sometimes less than a meter apart. Though both exhibit a tendency to local dominance (Smith 2004, pers. obs.), there is no evidence as yet of any interaction between the two species.
- Both species exhibit analogous, if visually dissimilar, ranges of pigmentation resulting from differential exposure to incident solar irradiance, with *G. coronopifolia* a pale yellow to white corresponding to the orange-yellow of *G. salicornia* tissue in areas with high exposure to sunlight and pink to red corresponding to the latter's purple to brown where tissues are shaded.
- Both species are closely related, being members of the same genus, and are morphologically and structurally similar.

### *Collections*

Tissues of *G. salicornia* and *G. coronopifolia* were collected at the seaward end of Paiko Lagoon, on Oahu's south shore and at Kualoa Beach on Oahu's windward shore (Fig. 1). Additional samples of *G. salicornia* were collected from Kaimana Beach on the south shore, and of *G. coronopifolia* from the windward Malaekahana Beach. Species determinations for each population were made in the field using morphological characters, and in the laboratory using primers and methods after Sherwood and Presting (2007) for the universal plastid amplicon (UPA) marker, part of the plastid 23S rDNA. Samples were selected for sun- or shade-acclimated pigmentation, i.e. paler or darker, respectively.

*Culture*

Cultures were maintained in aerated, filtered seawater-filled 5L aquaria set in water baths on the 6th floor balcony of the St. John building on the University of Hawai'i at Mānoa campus. Seawater was collected from the environs of Makai Pier, near the easternmost tip of O'ahu, and enriched with 10 μM $NaNO_3$ and 2 μM $PO_4$. Culture media was replaced every two days. Water temperature of the cultures was maintained between 25 and 30 ºC, measured with HOBO data loggers. Salinity was monitored periodically with a Reichert temperature compensated refractometer and adjusted to 34 ‰ with additions of distilled water. Salinity varied between 34 and 38 ‰ during the duration of the experiment. Water baths were shielded from rain and UV radiation with polymethylmethacrylate sheet roofs.

Samples of both species were initially acclimated to either full sunlight, with daily maxima ranging from ca. 1500 to 3300 μmol $m^{-2}$ $s^{-1}$ PAR (average = 2450 μmol $m^{-2}$ $s^{-1}$ PAR), or shade, full sunlight, but covered in three layers of medium grade shade-cloth, resulting in daily maxima ranging from ca. 70 to 180 μ mol $m^{-2}$ $s^{-1}$ PAR (average = 115 μmol $m^{-2}$ $s^{-1}$ PAR). This acclimation period lasted for 9-14 days or until relative uniformity and stability of plant color was achieved among samples. PAR was continuously monitored during each of five experimental trials between June 21, 2012 and November 13, 2012 with LI-COR LI-193 underwater spherical quantum sensors. Mean irradiance was sampled at 15-min intervals and stored in LI-COR LI-1000 data loggers.

After the initial acclimation period, samples from each irradiance treatment were placed in their correspondingly alternate irradiance regimes, i.e. HL acclimated samples were placed in LL water baths and vice versa.

*PAM fluorometry*

PAM fluorometer parameters were measured using a Waltz JUNIOR-PAM fluorometer every other day starting from the day that the samples were switched. Photosynthetic parameters were assessed by exposing the second or third node from the tip (ca. 3 - 6 cm from the tip for *G. salicornia* samples and 2 - 4 cm for *G. coronopifolia*) on each specimen to the fiber optic cable of the PAM. Rapid light curves (RLC) were generated by exposing tissue to a series of saturation pulses applied after 10 s exposures to actinic irradiance of increasing exposure ranging from 25 to 420 μmol $m^{-2}$ $s^{-1}$ PAR in

low-light (LL) acclimated samples and from 66 to 820 µmol $m^{-2}$ $s^{-1}$ PAR in high-light (HL) acclimated samples. Different PAR ranges were required for HL and LL acclimated samples as lower level PAR exposure in HL samples resulted in an incomplete RLC, while PAR pulses higher than 420 resulted in a depressed effective quantum ((Fm′-F)/Fm′) yield below a reliable threshold (following Beer and Axelsson 2004) in LL samples. The photosynthetic parameters $rETR_{max}$ (relative maximum electron transport rate), and $E_k$ (light saturation index) were obtained from RLC data with Wincontrol-3 software (Heinz Walz GmbH).

In vivo *absorbance analyses*

An *in vivo* absorbance spectrum (400 to 750 nm) was acquired from each sample every two days starting from the day that the samples were switched. *In vivo* absorbance spectra were obtained using a Shimadzu UV 2101 scanning spectrophotometer with a 150 mm Shimadzu integrating sphere attachment. Spectra were obtained by placing a non-overlapping layer of plant between two white cards with a mask window cut of 1.0 cm by 0.7 cm. The spectrum sampling interval was set at 0.2 nm. Spectra were normalized to 1.0 at 678 nm maxima after spectra acquisition.

*In vivo* absorbance spectra were normalized to chlorophyll *a* maxima at ~678nm after Smith and Alberte (1994). Specific absorbance maxima of accessory pigments at 650 nm (allophycocyanin, APC), 625 nm (phycocyanin, PC), 568 nm (phycoerythrin, PE), 540 nm (PE), 495 nm (a combination of PE and carotenoids) and 440 nm (a combination of Chl *a* and carotenoids) were selected for analysis, with pigment

identities based on published absorbance maxima (Haxo & Blinks 1950, Smith & Alberte 1994, Beach et al. 1997, Beach et al. 2000).

*Growth*

Cumulative change in mass was measured by obtaining wet weight immediately after each spectrum was obtained. Several *G. coronopifolia* plants dissolved completely in shaded culture, while others maintained their vigor; *G. salicornia* plants responded more favorably to culture, although fragmentation often occurred.

*Data analyses*

The effects of the change in irradiance regime over time on photosynthetic parameters, pigment to chlorophyll ratios and growth rates were investigated using repeated measures analysis of variance (ANOVA) using SPSS version 19 (IBM), with the main factor of irradiance regime change (hereafter termed “light”) and time as the repeated measure. Greenhouse–Geisser corrections were applied in those instances where the assumption of sphericity was violated according to Mauchly’s test. For ANOVA models that indicated significant interaction effects, post-hoc t-tests were conducted to determine how many days photosynthetic parameters and pigment to chlorophyll ratios took to reach corresponding control values.

## RESULTS

Each trial consisted of two replicates for each of four treatments (sun to shade with its corresponding shade control and shade to sun with its corresponding sun control) of each of two species. Between June and November of 2011, five trials were conducted with ten replicate plants per treatment for a total of 80 individuals for the entire experiment. *Gracilaria coronopifolia* did not perform as well as *G. salicornia* in culture, so measuring days did not extend beyond 14 days for the former, in contrast to the latter alga which was measured for 18 - 20 days. Several samples, mostly of *G. coronopifolia,* degenerated to the point of dissolution near the end of some trials. *Gracilaria salicornia* shade-acclimated controls lost no mass even after 20 days in the shade, while all other shade treatments declined. The irradiance experienced by experimental samples in this study closely approximated the range of irradiance (ca. 117- 2389 μmol $m^{-2}$ $s^{-1}$ PAR) reported for *G. salicornia* field conditions in Beach et al. (1997); *G. coronopifolia* is unlikely to experience these extremes *in situ*.

Parameters diagnostic of acclimation included in this study are: PAM fluorometer-derived $rETR_{max}$ and $E_k$ and *in vivo* spectrophotometer-derived pigment to chlorophyll *a* ratios 650:678 nm, 625:678 nm, 568:678 nm, 540:678 nm, 495:678 nm and 440:678 nm. Acclimation was considered to have been achieved when treatment values of each parameter first ceased to be statistically different from corresponding control values over the course of the experiment (e.g. Fig. 2). The number of days required for this condition to be met varied by parameter, by treatment and by species. Many treatment

values that achieved acclimation did not remain static, but deviated from control values after first acclimating to the new light environment. Similarly, parameter values for most control groups did not remain static, particularly for those samples exposed to prolonged periods of intense sunlight.

Sun-acclimated samples exhibited high absorbance values at the carotenoid-associated absorbance maxima 440:678 nm and 495:678 nm (Fig. 3). Sun-acclimated samples also exhibited reduced absorbance values at all phycobilin-specific maxima relative to shade-acclimated plants of both species. Similarly, phycobilin absorbance values increased and the carotenoid-associated absorbance maxima decreased for shade acclimated plants. The PE to Chl a ratio 568:678 nm absorbance did not change in *G. salicornia* regardless of light environment. Likewise, no change at the PE to Chl a ratio 568:678 nm absorbance was detected in *G. salicornia* transferred from shade to sun even after 20 days, though that maximum did increase when plants were transferred from sun to shade. Curiously, plants of both species became more flexible as they acclimated to high light and more turgid as they acclimated to low light.

*Transition from Sun to Shade acclimation*

There were significant time effects for all parameters with the exception of the ratio 495:678 nm absorbance comparison for *G. coronopifolia.* The effects of changing light environment from sun to shade, as well as the interaction of the change in light environment and time, were significant for all parameters with the exception of the ratio

540:678 nm absorbance for *G. salicornia* (Table 2.1). Post-hoc t-tests (Table 2.2) indicated that *G. coronopifolia* transitioned from sun-acclimated to shade-acclimated states faster than *G. salicornia* for every parameter measured (Table 2.3), with the exception of the 540:678 nm absorbance ratio, which was not comparable between species. Values of the 540:678 nm absorbance ratio for *G. salicornia* were not statistically different over the course of the experiment.

There was no significant difference in the growth rate between controls and treatments for either species.

$rETR_{max}$ values from samples of *G. coronopifolia* declined faster than average values of *G. salicornia,* averaging losses of 30.9% and 13.8% $d^{-1}$ respectively until control-level values were reached (Table 2.5). Values of $E_k$ also declined faster in *G. coronopifolia* (41.7 % $d^{-1}$ to 19.2 % $d^{-1}$ for *G. salicornia*). Pigment adjustments were usually characterized by an initial rapid change followed by a longer period of decreased changes before acclimation was reached. The phycocyanin to Chl *a* ratio of 625:678 nm absorbance varied equally between highs and lows throughout the experiment, but the rate of change was faster in tissues of *G. coronopifolia* due to its lower absorbance value. Not only did absorbance ratios specific for PE adjust at much slower rates in *G. salicornia* but they also changed less in value overall than did *G. coronopifolia* (Fig. 5d-5e), particularly for the 540 peak, which did not change in any meaningful way from its starting value in; in contrast to this, PC absorbance in *G. coronopifolia* increased 33.7% before acclimation was reached.

Absorbance at carotenoid-associated peaks technically adjusted much faster in *G. coronopifolia*, with maxima at 440 nm reaching control-level values three times faster and at 495 nm eight times faster. However, the initial drop in the 440 nm maximum was actually higher in *G. salicornia*, 11.8% to *G. coronopifolia's* 8.0% decrease (Fig. 5g), but that rate soon decreased leaving another eight days before equivalence to shade state control-values were reached. In *G. coronopifolia,* the 495 nm maximum took only one two-day, between-measurements period to reach control values, but this was partly due to the sun-acclimated treatment 495 nm absorbance ratio being only 3.3% higher than that of the shade-acclimated control during this period. The *G. salicornia* 495 nm maximum absorbance declined 11.3% in the first two days, indicating that *G. salicornia* may have had more carotenoids to lose, eliminating them at a faster rate (70.1%) than *G. coronopifolia* in that time period. In both carotenoid-associated maxima, an initial rapid decrease in absorbance was followed by a continued decrease at a slower rate until control-level values were reached (Figs. 5f), with the mentioned exception of the *G. coronopifolia* 495 nm maximum, for which a finer timescale is needed to sort out actual acclimation rates.

*Transition from Shade to Sun acclimation*

The effect of time was significant for all parameters with the exception of *G. salicornia* at the 440:678 nm, 540:678 nm and 568:678 nm absorbance maxima and *G. coronopifolia* at the 540:678 nm maximum (Table 2). The effect of light was significant for all

parameters except for *G. salicornia* at the 540nm absorbance maximum, while the interaction of light and time was significant for all parameters with the exception of the *G. salicornia* at the 540:678 nm and 568:678 nm absorbance maxima and for both species at the 440:678 nm maximum.

Post-hoc t-tests (Table 2) indicated that *G. coronopifolia* transitioned from shade-acclimated to sun-acclimated states faster than *G. salicornia* as measured by the PAM fluorometer-derived parameters $ETR_{max}$ and $E_k$ (Table 2.4). However, PC-specific maxima adjusted more slowly in *G. coronopifolia*. As neither 540:678 nm nor 568:678 nm absorbance maxima in *G. salicornia* treatments differed from controls, acclimation times for these parameters could not be compared across species. Absorbance maxima at 440:678 nm and 495:678 nm could not be compared across species as *G. coronopifolia* for both maxima and *G. salicornia* at 440:678 nm ceased to be significantly different than controls in shade to sun treatments throughout the course of the experiment.

There was a significant interaction effect of light regime shift and time on the growth of *G. salicornia* ($F_{2.046, 36.831}$=4.583, p=0.016), where mean mass increased nearly 58% more in treatments compared to controls (Fig. 4). The change in light environment had no effect on the growth of *G. coronopifolia*.

$rETR_{max}$ for samples of *G. coronopifolia* increased by an average of around twice that of *G. salicornia*, while $E_k$ increased at about the same rate (Table 2.6). Absorbance at 650

nm and at 625 nm did not begin to decline until after four days in *G. coronopifolia,* whereas a steadier decline originating at day 0 was observed in *G. salicornia.*

Shade to sun treatment plants of *G. salicornia* did not have the visual appearance of high light acclimated control samples by the experiment's end, retaining an intermediate light brown pigmentation, with the exception of branch tips, which were yellow. All other treatments in this study acquired the visual appearance of their corresponding controls with regards to pigmentation.

## DISCUSSION

This study compared side-by-side rates of photoacclimation of two species of marine macrophytes to as a first step in determining whether a faster response to changes in light environment was a trait specific to invasive algae, as suggested by Dailer (2006). This hypothesis did not hold true for these two *Gracilaria* species, though these results suggest that invasive algae possess other advantageous photoacclimatory characteristics not shared by noninvasive species. Feng et al. (2007a) compared several species of invasive and non-invasive embryophytes, and determined that invasive plants could acclimate to a broader range of irradiances. Yamashita et al. (2000) found that an invasive tree recovered from a sudden increase in light faster than non-invasives. Both of these observations were supported by the results of this study.

Photoacclimation is a process composed of several components, not all of which are necessarily codependent. Light-harvesting and photoprotection in red algae are two processes requiring different suites of pigments that require different resources to

operate, primarily nitrogen for phycobilins and carbon for carotenoids (Beach 1996). These processes occur physically separated in the rhodophyte chloroplast with carotenoids associated exclusively with photosystem I and phycobilins exclusively with photosystem II (Gantt & Cunningham 2001), and although adjustment of either pigment type is a response to ambient irradiance, there is no reason for them to occur in synchrony.

*Sun to Shade*

During acclimation to shade environments, red algal chloroplasts decrease photosynthetic output and carotenoid content while simultaneously synthesizing phycobilins as a means of absorbing additional light (Beach 1996). Complete conversion of carotenoid-rich, sun-acclimated tissue to a phycobilin-saturated, shade acclimated state occurred in *G. coronopifolia* 100% faster than in *G. salicornia*. Photosynthetic acclimation was particularly rapid in *G. coronopifolia*, occurring 50% faster than in the invasive species. Carotenoid concentrations decreased markedly slower in the invasive alga, a result consistent with both its higher carotenoid: Chl *a* ratio compared to *G. coronopifolia*, as well as with the photoprotective requirements of a truly intertidal tropical marine alga (Beach & Smith 1996). Rapid carotenoid degradation would be disadvantageous in an intertidal alga that experiences frequent periods of burial by sand and wrack and sudden re-exposure to direct sunlight. Though *G. coronopifolia* is most often found at depths of less than 1m at low tide, in waters so turbid that collections for this study were often made difficult, it rarely experiences

emersion for more than an instant at a time (pers. obs.). The slow degradation of carotenoids, 16 days in *G. salicornia* and 29 in *Ahnfeltiopsis concinna*, may be peculiar to the needs intertidal algae (Beach et al. 2000). Phycobilin increases in *G. coronopifolia* were slower than decreases in photosynthetic and carotenoid parameters, consistent with observations that anabolic processes are resolved faster than catabolic (Beach 1996), and this holds true to a less obvious extent in *G. salicornia*. The slow degradation of APC and PC in *G. salicornia* contrasts with the relatively rapid adjustment of the 568 nm PE maximum, but as the treatment was indistinguishable from the control at 540 nm, it is difficult to make the claim that PE degraded either faster or slower or to speak of phycobilin acclimation in general terms for this species. It should be noted that as PE at 568 nm acclimates faster in both *G. coronopifolia* treatments, is it is possible that in *G. salicornia* sun to shade acclimation, the change at 540 nm is simply very slow. This lack of change in the *G. salicornia* 540 nm maximum coupled with a definite though comparatively small increase at 568 nm suggest that phycoerythrin concentration remains at or near shade-acclimated levels even in high photosynthetic photon flux density (PPFD) environments. It is possible that samples for this treatment were not completely light-acclimated, but that seems unlikely given the appearance and location of collected samples and the fact that all other parameters were characteristic of a rhodophyte acclimated to high PPFD.

Although growth was unaffected by light treatment, *G. salicornia* shade plants (i.e. those plants selected for a shade acclimated appearance in the field and where used for shade control samples) were the only block in the sun to shade category that did not

lose mass over time. Indeed, the shade control samples were the healthiest in appearance of all treatments in this experiment.

*Shade to sun*

Acclimation from low to high PPFD environments in rhodophytes is characterized by increased photosynthetic capacity, increased concentration of photoprotective carotenoids and decreased light-harvesting phycobiliprotein content (Gantt 1990, Ramlov et al. 2011). As with the sun to shade treatment, photosynthetic acclimation occurred faster in the native *G. coronopifolia* than in the invasive species as they transitioned from shade to sun states.

Carotenoid acclimation comparisons could not be made due to a combination of difficulties. *Gracilaria salicornia* carotenoid concentration increases leveled out before control values were reached, while *G. coronopifolia* control values increased in tandem with, and indeed more than, treatment values. Control samples of sun acclimated plants had a great deal longer to acquire carotenoid pigments as they were selected by appearance and so were predisposed to contain elevated carotenoid concentrations. In addition, an unforeseen tendency in both species to continue to accumulate photoprotective pigments made obtaining a saturation level for either absorbance maximum elusive. A full factorial experimental design such as the one this study employed appears to be inappropriate for obtaining shade to sun acclimation times for carotenoid concentrations.

Though APC and PC degradation occurred somewhat faster in *G. salicornia* than in *G. coronopifolia*, PE concentration did not decrease in the former species when exposed to full sunlight, but remained at levels consistent with a shade acclimated state, whereas all *G. coronopifolia* phycobilin concentrations declined at similar rates. This discrepancy in phycobilin contemporaneousness is unusual, as phycobilisome components are commonly reported to increase or decrease together (Beach & Smith 1996a, Dailer 2006, Ramlov et al. 2011), although Beach and Smith (1996a) reported that *Laurencia mcdermidae* I.A.Abbott sustained levels of APC and PC, while PE degraded with exposure to high PPFD. Rhodophyte phycobilisomes are composed of APC cores surrounded first by PCs and then by PEs on the periphery of the assembly (Gantt 1975). Energy transfer of these antenna pigments follows PE to PC and then to the APC core before being passed to chlorophyll a (Yamazaki et al. 1984). In *G. salicornia*, it would appear that APC and PC degrade much faster than PE, the persistence of which still lends the plant some appearance of its former shade acclimated state, albeit of a lighter hue than truly shade acclimated plants.

Shade acclimated rhodophytes exposed to prolonged, excessive irradiance experience high light stress reactions, including photoinhibition and eventual photooxidation. Recent studies have revealed phycobilisome decoupling from the PSII reaction centers in response to high light stress to be a photoprotective mechanism (Liu et al. 2008, 2009). Phycobilisome decoupling (specifically, the energetic decoupling of PE within the phycobilisome rod) as a result of high light stress is generally followed by disassociation of the phycobilisome (Liu et al. 2008, Tamary et al. 2012), but not necessarily

phycobiliprotein degradation. These “free” biliproteins, now present in the stroma and unattached to the thylakoid membrane, play no role in light harvesting, but their continued ability to absorb solar radiation dissipates excess light energy, preventing photoinhibition and photooxidative damage to some degree (Liu et al. 2009). These free phycobilisomes are also believed to play a role in nitrogen storage (Talarico and Maranzana 2000). Phycobilin-specific absorbance values in this study suggest that free PE persists in *G. salicornia* tissue long after PC and APC phycobilisome components have degraded, and that some mechanism of selective degradation is responsible for this.

The significant increase in growth *G. salicornia* exhibited in response to the shift to high PPFD was comparable to that reported by Laing et al. (1989) for *G. chilensis.* That increase was attributed to the plant’s underutilization of stored nutrients due to light limitation, but when the plant was exposed to high PPFD, those nutrients were available for growth. The vitality exhibited by plants selected for shade acclimation in this study suggests that *G. salicornia* can weather long periods of light limitation, provided nutrients are not also limiting, before rapidly exploiting a sudden exposure to high PPFD.

*Conclusions*

Nutrient availability is a limiting factor in the spread of invasive plants (Inderjit 2006). Nutrient runoff not only positively influences the growth rate of algae in general, but it

may also lead to the competitive success of invasive algae able to capitalize on the excess nutrient (Nyburg & Wallentinus 2005, Vermeij et al. 2009). Hawaiian coastal waters, which typically contain very low levels of dissolved nutrients, experience algal blooms resulting from anthropogenic eutrophication (Stimson& Larned 2000, Dailer et al. 2010). The island of O‘ahu is particularly affected, being by far the most populous island and also because a number of nonindigenous algae have been introduced intentionally there, among them *G. salicornia* (Smith et al. 2002, Lapointe & Bedford 2011). The invasive potential of this species has been attributed to its ability to sequester nutrients, to asexual reproduction via fragmentation and to a tolerance to a wide range of irradiance regimes (Beach et al. 1997, Larned 1998, Smith et al. 2004). However, this species' response to changes in light environment does not appear to be more rapid than that of its less dominant congener, *G. coronopifolia*.

All plants in this study were collected in waters close to streams or other sources of frequent terrestrial discharge in addition to experiencing seasonal brown water events. In these nutrient replete conditions, *G. salicornia* may have the luxury of storing large amounts of both pigment classes, despite exposure to irradiance extremes, as both degradation of phycobiliproteins in high PPFD and degradation of carotenoids in low PPFD are much slower than they are in the native species. This ability to retain pigment longer may in part be a function of the plant's morphology: *G. salicornia* possesses larger pigment-containing cortical cells, more cortical layers and greater branch and axial circumference than *G. coronopifolia* (Abbott 1999). Retention of accessory

pigments may be a strategy that allows *G. salicornia* to be better situated in the event of a change in a plant's light environment.

This study reinforces the notion that *G. salicornia* possesses broad tolerance to solar irradiance, thriving both in direct tropical sunlight and to long periods of deep shade, and seems poised to succeed when these extremes alternate. In environments where neither carbon nor nitrogen are limiting, retaining luxury levels of both carotenoids and phycobiliproteins by forestalling pigment degradation is a strategy that may be less costly than synthesis and may allow a plant to better weather light-altering disturbance events.

In addition to *G. salicornia* in Hawai'i, several other *Gracilaria* species have been identified as invasive or bloom-forming in various locales including G. *vermiculophylla, G. tikvahiae* and an as yet unidentified *Gracilaria* species in New Zealand (Thomsen et al. 2006, Teichberg 2008, Wilcox et al. 2007). Other *Gracilaria* species have been reported to display characteristics associated with invasiveness (Thompsen and Wernberg 2009, Israel et al. 1999, Schaffelke et al. (2006). Like *G. salicornia*, *G. coronopifolia* displays several weedy traits, including a tendency to grow in dense stands, a broad tolerance to salinity and nutrients (Smith et al. 2004, Amato 2009) and as this study shows, irradiance (albeit to a lesser extent than the former alga). Based on the results of this study, a rapid photoacclimatory response, as proposed by Dailer (2006), does not appear to be a diagnostic for invasiveness. Similar experiments using non-*Gracilaria* species should be conducted before ruling out this hypothesis.

Comparisons of photoacclimation time should be performed involving multiple species, from a range of depths and lineages, to further investigate the role of photosynthetic plasticity in the invasiveness of macroalgae.

## REFERENCES


Abbott IA (1999) Marine red algae of the Hawaiian Islands. Bishop Museum Press, Honolulu. 477 pp.

Amato D (2009) Physiological effects of simulated submarine groundwater discharge on the Hawaiian endemic edible alga *Gracilaria coronopifolia*. Master's Thesis. Department of Botany, University of Hawai'i, Honolulu. 106 pp

Beach KS (1996) Photoadaptive strategies of Hawaiian macroalgae. PhD Dissertation. Department of Botany, University of Hawai'i, Honolulu. 325 pp.

Beach KS & CM Smith (1996 a) Ecophysiology of tropical rhodophytes I: microscale acclimation in pigmentation. Journal of Phycology 32: 701-710.

Beach KS, HB Borgeas, NJ Nishimura & CM Smith (1997) *In vivo* absorbance spectra and UV-absorbing compounds in tropical reef macroalgae. Coral Reefs 16: 21-28

Beach K, C Smith & R Okano (2000) Experimental analysis of rhodophyte photoacclimation to PAR and UV -Radiation using in vivo absorbance spectroscopy. Botanica Marina 43: 525-536.

Beer B & L Axelsson (2004) Limitations in the use of PAM fluorometry for measuring photosynthetic rates of macroalgae at high irradiances. European Journal of Phycology 39: 1-7

Cunningham FX Jr, A Vonshak, & E Gantt (1992) Photoacclimation in the red alga *Porphyridium cruentum*. Plant Physiology 100: 1142- 1149

Dailer M (2006) Photoecological strategies influencing the invasive success of marine macrophytes *Euchuema denticulatum* on Hawaiian coral reefs. Master's Thesis. Department of Botany, University of Hawai'i, Honolulu. 154 pp.

Falkowski PG & J LaRoche (1991) Acclimation to spectral irradiance in algae. Journal of Phycology 27: 8-14

Feng YL, JF Wang & WG Sang (2007a) Irradiance acclimation, capture ability, and efficiency in invasive and non-invasive alien plant species. Photosynthetica 45: 245-253

Feng YL, JF Wang & WG Sang (2007b) Biomass allocation, morphology and photosynthesis of invasive and noninvasive exotic species grown at four irradiance levels. Oecologia 31: 40-47

Gantt E (1975). Phycobilisomes: Light harvesting complexes. BioScience 25: 781-788

Gantt, E (1990) Pigmentation and photoacclimation. *In* Cole, K.M. & Sheath R.G. [Eds.] *Biology of the Red Algae*, Cambridge University Press, Cambridge, U.K., pp. 203-220.

Haxo FT & LR Blinks (1950) Photosynthetic action spectra of marine algae. Journal of General Physiology 33: 389-422.

Inderjit, D Chapman, M Ranaletti, & S Kaushik (2006) Invasive marine algae: An ecological perspective. The Botanical Review 72: 153–178

Laing WA, JT Christelier & BE Terzaghi (1989) The effect of temperature, photon flux density and nitrogen on growth of *Gracilaria sordida* Nelson (Rhodophyta). Botanica Marina 32: 439-445.

Lapointe BE & BJ Bedford (2011) Stormwater nutrient inputs favor growth of non-native macroalgae (Rhodophyta) on O‘ahu, Hawaiian Islands. Harmful Algae 10: 310–318

Larned ST (1998). Nitrogen versus phosphorus limited growth and sources of nutrients for coral reef macroalgae. Marine Biology 132: 409-421

Levy I & E Gantt (1988) Light acclimation in *Porphyridium purpureum* (Rhodophyta): growth, photosynthesis and phycobilisomes. Journal of Phycology 24: 452-458

Nyberg CD & I Wallentinus (2005). Can species traits be used to predict marine macroalgal introductions? Biological Invasions 7: 265-279

Nyberg CD & I Wallentinus (2009). Long-term survival of an introduced red alga in adverse conditions. Marine Biology Research 5: 304-308

Olenin S, D Minchin & D Daunys (2007) Assessment of biopollution in aquatic ecosystems. Marine Pollution Bulletin 55: 379–394

Payri CE, S Maritorena, C Bizeau & M Rodière (2001) Photoacclimation in the tropical coralline alga *Hydrolithon onkodes* (Rhodophyta, Corallinaceae) from a French Polynesian reef. Journal of Phycology 37: 223–234

Prézelin BB & IA Matlick (1980). Time-course of photoadaptation in the photosynthesis-irradiance relationship of a dinoflagellate exhibiting photosynthetic periodicity. Marine Biology 58: 85-96

Ramlov F, JMC Souza, AF Faria, M Maraschin, PA Horta, & NS Yokoya (2011) Growth and accumulation of carotenoids and nitrogen compounds in *Gracilaria domingensis* (Kütz.) Sonder ex Dickie (Gracilariales, Rhodophyta) cultured under

different irradiance and nutrient levels. Brazilian Journal of Pharmacognosy 21: 255-261

Raniello R, M Lorenti, C Brunet & MC Buia (2006) Photoacclimation of the invasive alga *Caulerpa racemosa* var. *cylindracea* to depth and daylight patterns and a putative new role for siphonaxanthin. Marine Ecology 27: 20–30

Raniello R, M Lorenti, C Brunet & MC Buia (2004) Photosynthetic plasticity of an invasive variety of *Caulerpa racemosa* in a coastal Mediterranean area: light harvesting capacity and seasonal acclimation. Marine Ecology Progress Series 271: 113–120

Schaffelke B & CL Hewitt (2007) Impacts of introduced seaweeds. Botanica Marina 50: 397–417

Sherwood AR & GG Presting (2007) Universal primers amplify a 23S rDNA plastid marker in eukaryotic algae and cyanobacteria. Journal of Phycology 43:605-608.

Smith, CM & RS Alberte (1994) Characterization of *in vivo* absorption features of chlorophyte, phaeophyte and rhodophyte algal species. Marine Biology 118: 511-521.

Smith JE, CM Smith & CL Hunter (2001) An experimental analysis of the effects of herbivory and nutrient enrichment on benthic community dynamics on a Hawaiian reef. Coral Reefs 19: 332-342

Smith JE CL Hunter & CM Smith (2002) Distribution and reproductive characteristics of nonindigenous and invasive marine algae in the Hawaiian Islands. Pacific Science 56: 299-315

Smith JE, CL Hunter, EJ Conklin, R Most, T Sauvage, C Squair, & CM Smith (2004)

Ecology of the invasive red alga *Gracilaria salicornia* (Rhodophyta) on O‘ahu, Hawai‘i. Pacific Science 58: 325–343

Stimson J & ST Larned (2000) Nitrogen efflux from the sediments of a subtropical bay and the potential contribution to macroalgal nutrient requirements. Journal of Experimental Marine Biology and Ecology 252: 159–180

Teichberg M, SE Fox, C Aguila, YS Olsen, & I Valiela (2008) Macroalgal responses to experimental nutrient enrichment in shallow coastal waters: growth, internal nutrient pools, and isotopic signatures. Marine Ecology Progress Series 368:117-126

Thomsen MS, C Frederico, D Gurgel, S Fredericq, & KJ McGlathery (2005) *Gracilaria vermiculophylla* (Rhodophyta, Gracilariales) in Hog Island Bay, Virginia: a cryptic alien and invasive macroalga and taxonomic correction. Journal of Phycology 42: 139–141

Thomsen MS & T Wernberg (2009) Drift algae, invasive snails and seagrass health in the Swan River: patterns, impacts and linkages. Report prepared for the Swan River Trust. Report no. 2009-2 from the Centre for Marine Ecosystems Research, Edith Cowan University, Perth. Pp. 82.

Titlyanov EA, TV Titlyanova , K Yamazato & R van Woesik (2001). Photo-acclimation dynamics of the coral *Stylophora pistillata* to low and extremely low light. Journal of Experimental Marine Biology and Ecology 263: 211–225

Vermeij MJA, TB Smith, ML Dailer & CM Smith (2009). Release from native herbivores facilitates the persistence of invasive marine algae: a biogeographical comparison of the relative contribution of nutrients and herbivory to invasion success. Biological Invasions 11:1463–1474

Waaland, JR, SD Waaland & G Bates (1974). Chloroplast structure and pigment composition in the red alga *Griffithsia pacifica*: regulation by light intensity. Journal of Phycology 10: 193-199.

Wilcox SJ, N Barr, J Broom, RH Furneaux & WA Nelson (2007). Using gigartinine to track the distribution of an alien species of *Gracilaria* in New Zealand. Journal of Applied Phycology 19:313–323

Yamashita N, N, A Ishida, H Kushima & N Tanaka (2000) Acclimation to sudden increase in light favoring an invasive over native trees in subtropical islands, Japan. Oecologia 125:412–419

Yamazaki I, M Mimuro, T Murao, T Yamazaki, K Yoshihara, & Y Fujita (1984) Excitation energy transfer in the light harvesting antenna system of the red alga *Porphyridium cruentum* and the blue-green alga *Anacystis nidulans*: analysis of time resolved fluorescence spectra. Photochemistry and Photobiology 39:233-240.

Figure 1: Map of collection locations for *G. coronopifolia* (shaded circles) and *G. salicornia* (open circles).

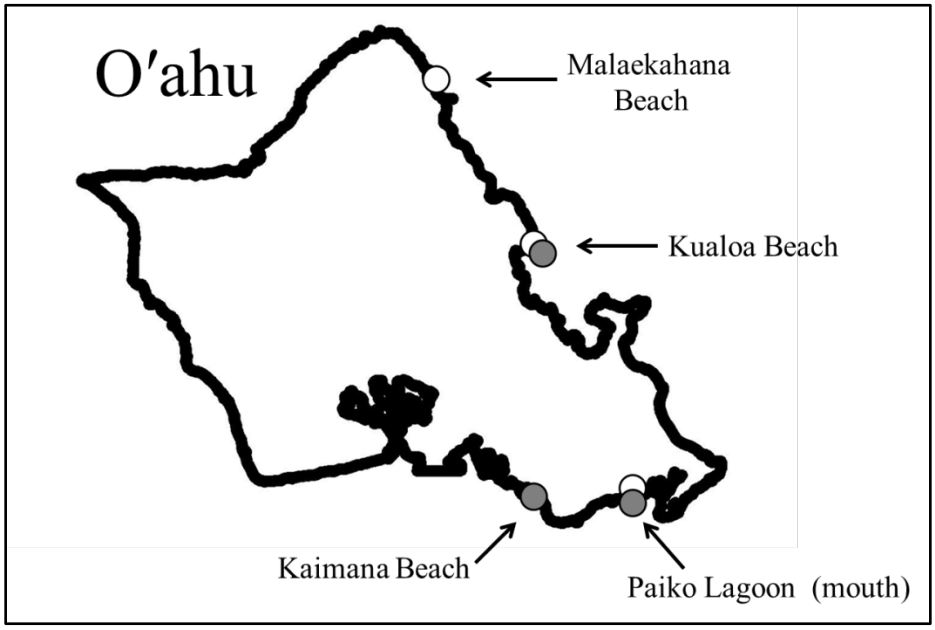


Figure 2: Change in the ratio of 568:680nm absorbance thru time for replicates of *Gracilaria coronopifolia* samples transferred from sun to shade environment (solid line). Samples were considered acclimated when parameter values ceased to be significantly different than control values (dashed line). Mean ± SE, n=10.

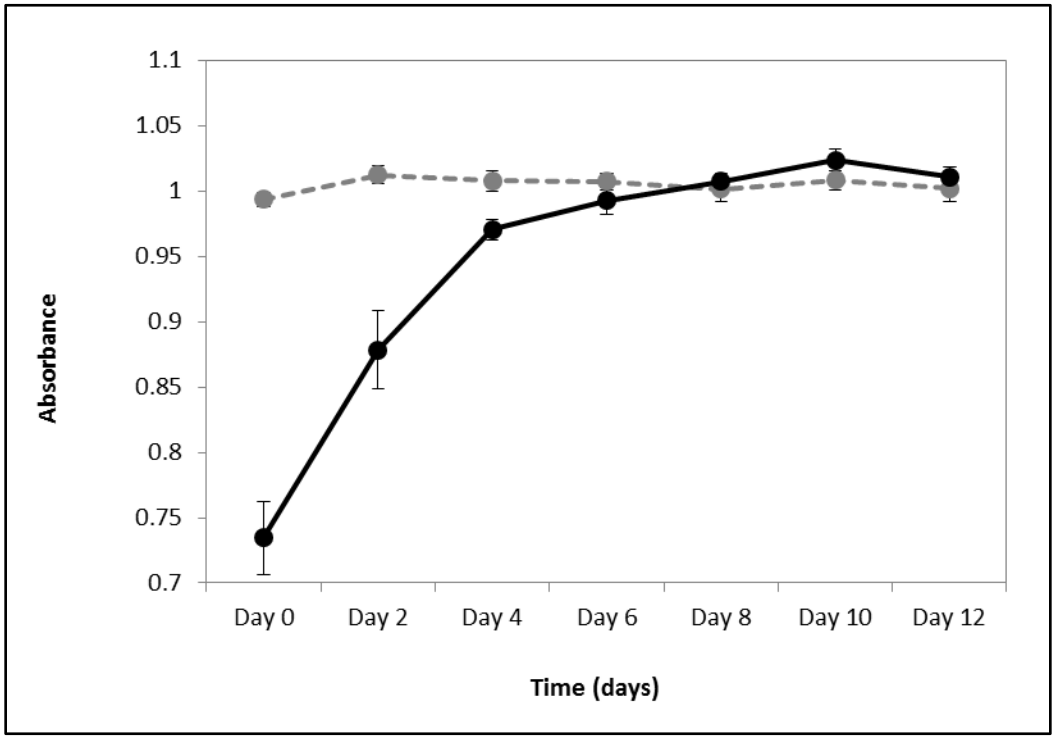


Figure 3: *In vivo* absorbance spectra of sun-acclimated (grey lines) and shade acclimated (black lines) *G. salicornia* (triangles) and *G. coronopifolia* (circles). All spectra normalized to chlorophyll *a* absorbance peak at 678 nm.

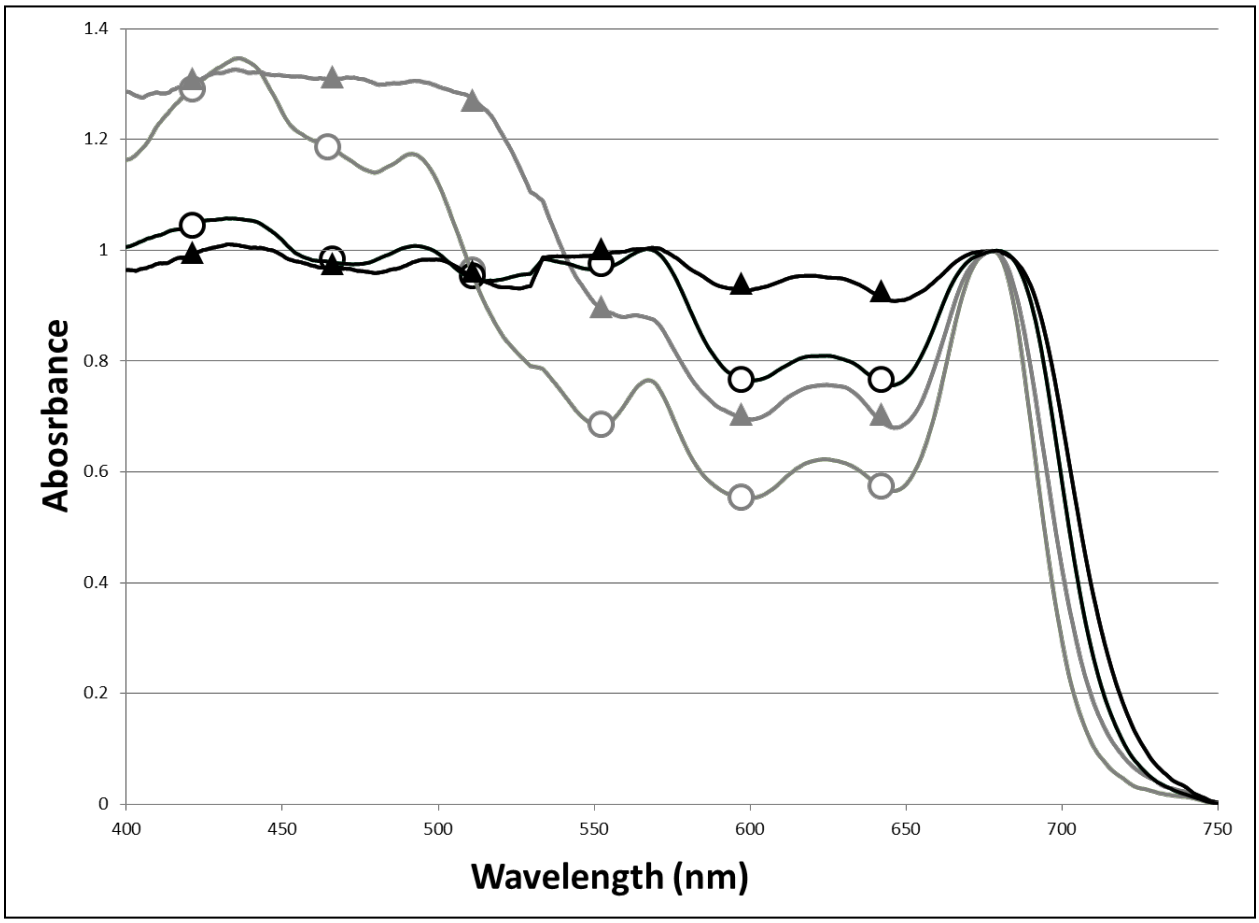


Figure 4: Cumulative growth of *G. salicornia* (triangles) and *G. coronopifolia* (circles) shade to sun treatments (black solid lines) and sun controls (grey shaded lines). Mean ± SE, n=10.

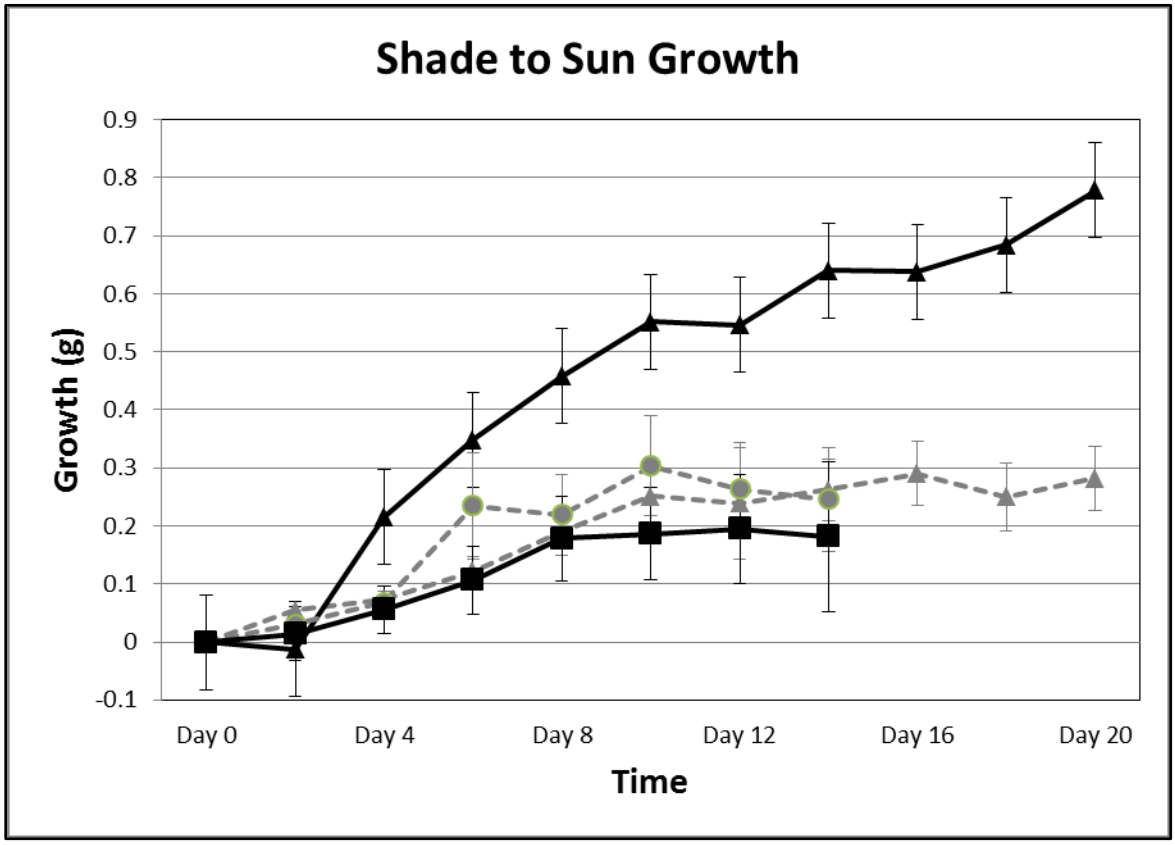


Figure 5a: Change in $rETR_{max}$ thru time in treatments (solid lines) and controls (dashed lines) for replicates of *G. salicornia* (triangles) and *G. coronopifolia* (circles). Mean + SE, n=10.

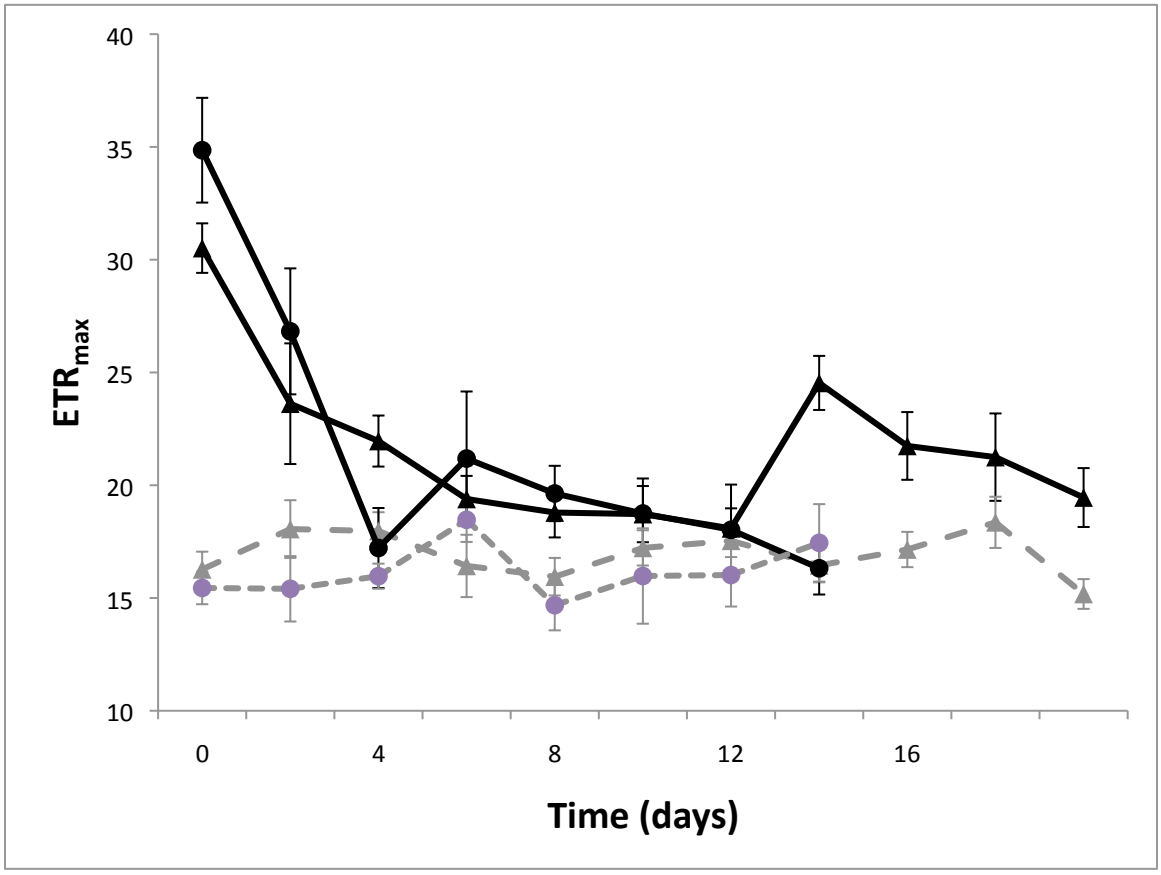


Figure 5b: Change in $E_k$ thru time in treatments (solid lines) and controls (dashed lines) for replicates of *G. salicornia* (triangles) and *G. coronopifolia* (circles). Mean + SE, n=10.

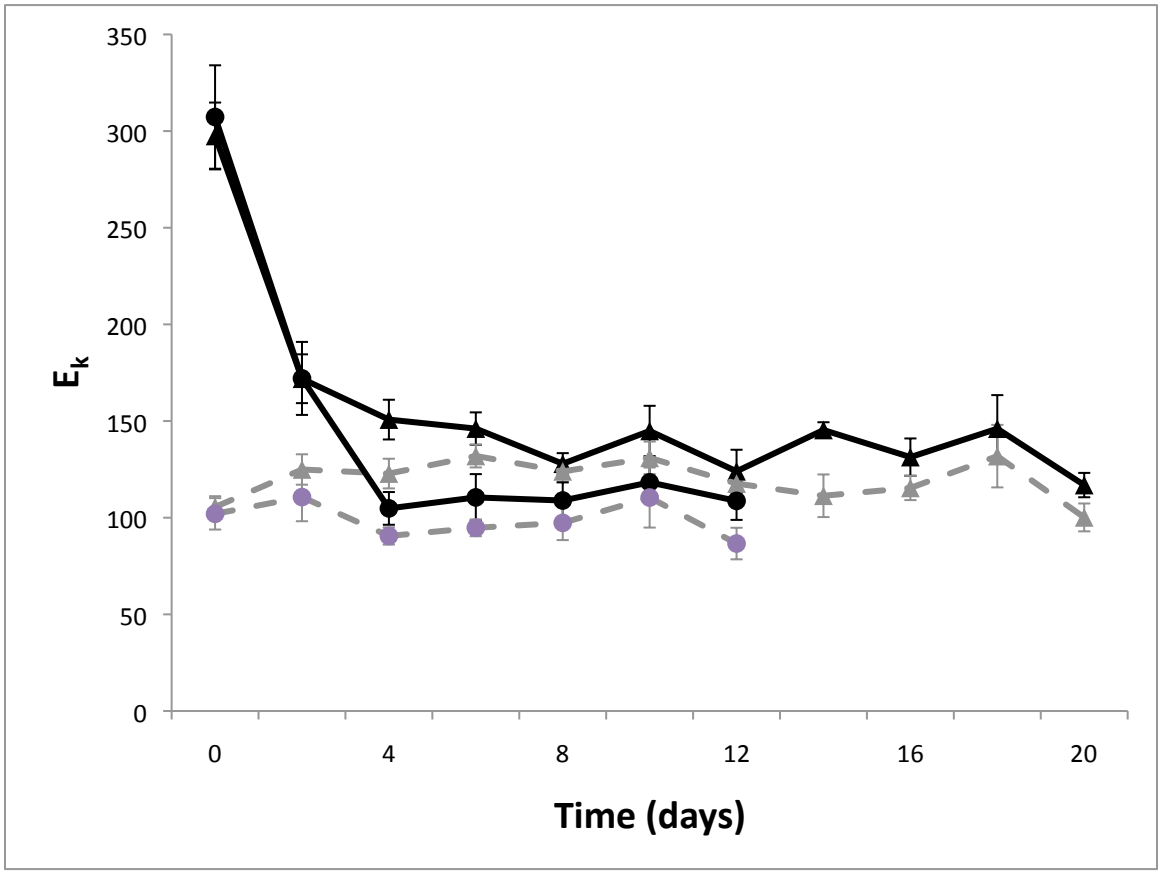


Figure 5c: Change in the ratio of 650:678 nm absorbance thru time in treatments (solid lines) and controls (dashed lines) for replicates of *G. salicornia* (triangles) and *G. coronopifolia* (circles). Mean + SE, n=10.

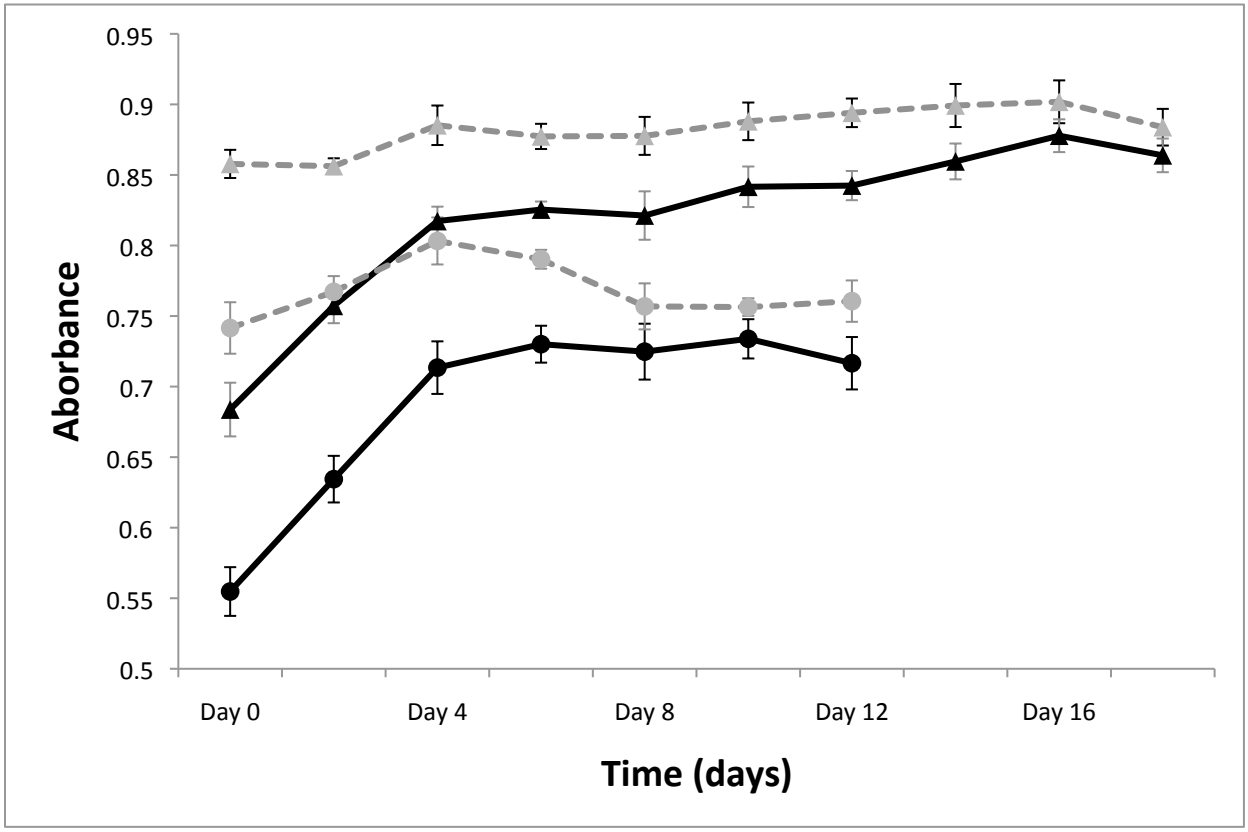


Figure 5d: Change in the ratio of 625:678 nm absorbance thru time in treatments (solid lines) and controls (dashed lines) for replicates of *G. salicornia* (triangles) and *G. coronopifolia* (circles). Mean + SE, n=10.

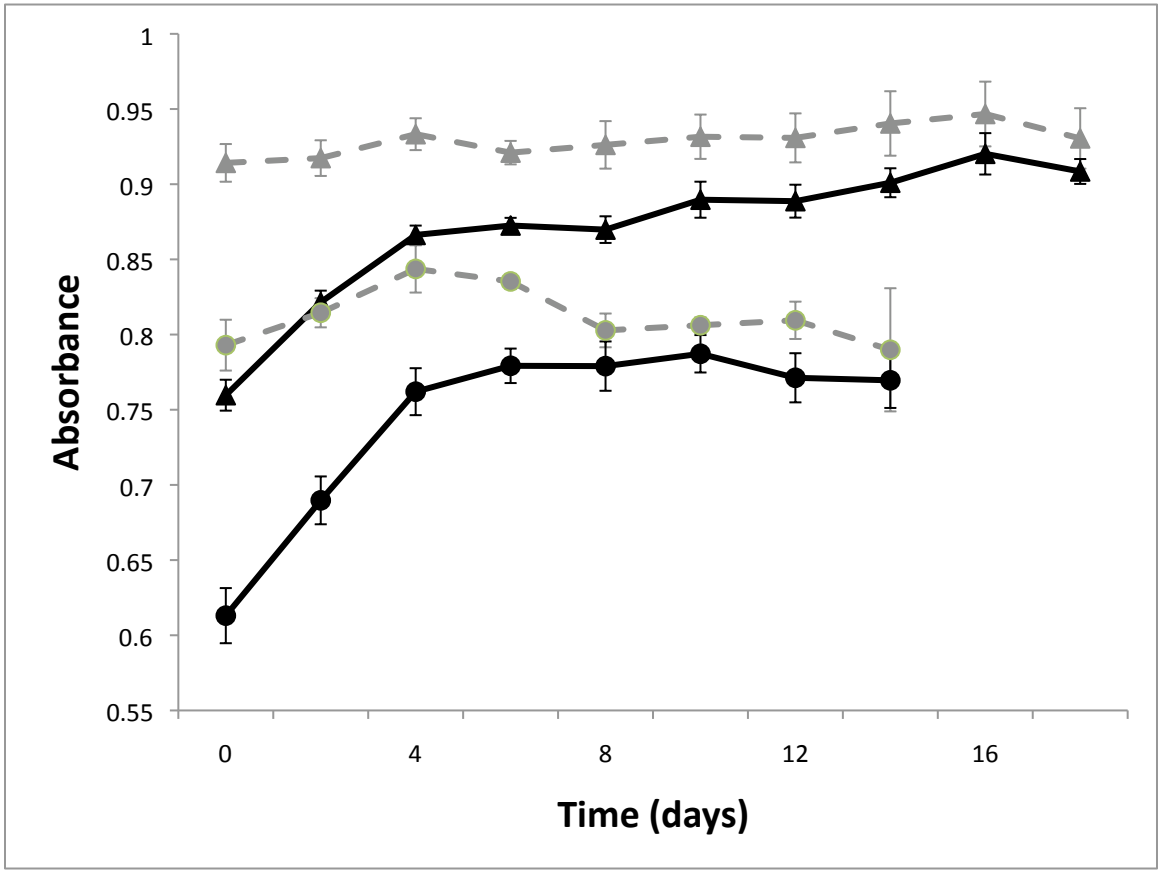


Figure 5e: Change in the ratio of 568:678 nm absorbance thru time in treatments (solid lines) and controls (dashed lines) for replicates of *G. salicornia* (triangles) and *G. coronopifolia* (circles). Mean + SE, n=10.

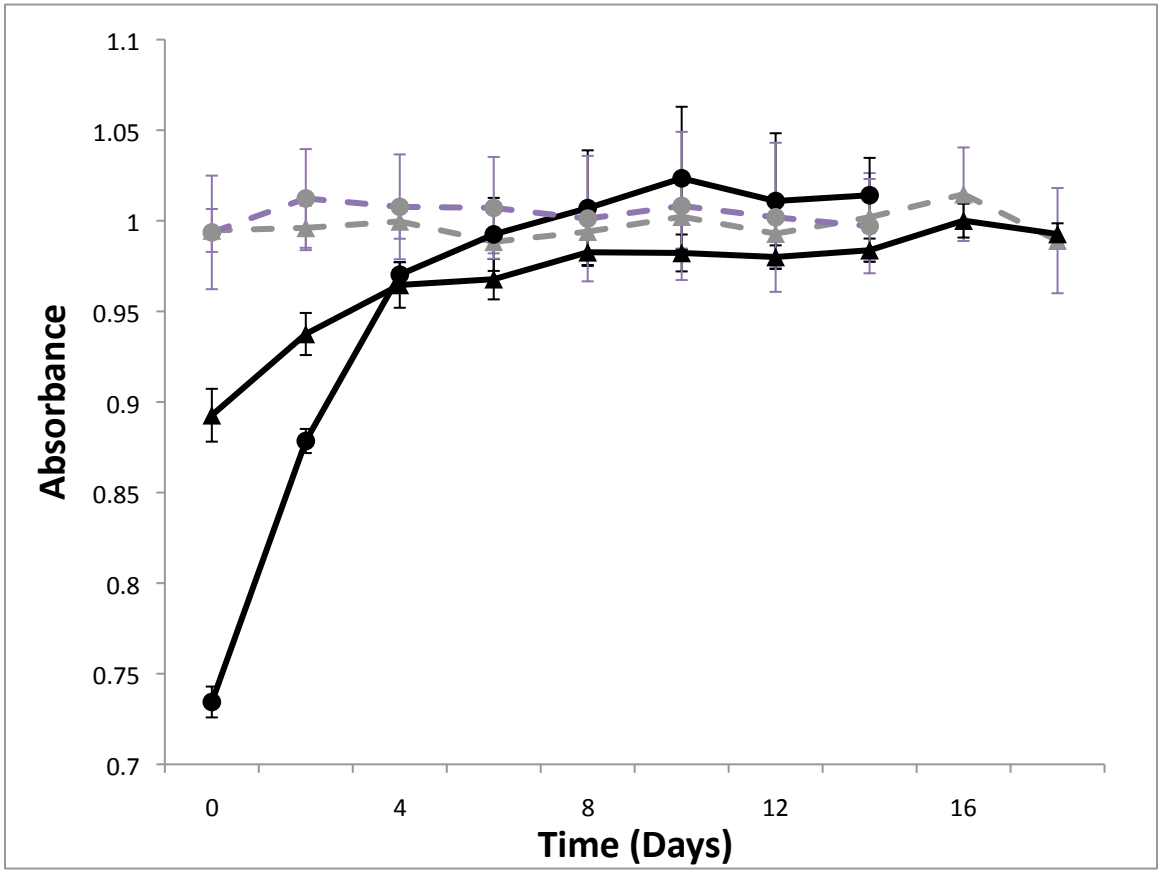


Figure 5f: Change in the ratio of 540:678 nm absorbance thru time in treatments (solid lines) and controls (dashed lines) for replicates of *G. salicornia* (triangles) and *G. coronopifolia* (circles). Mean + SE, n=10.

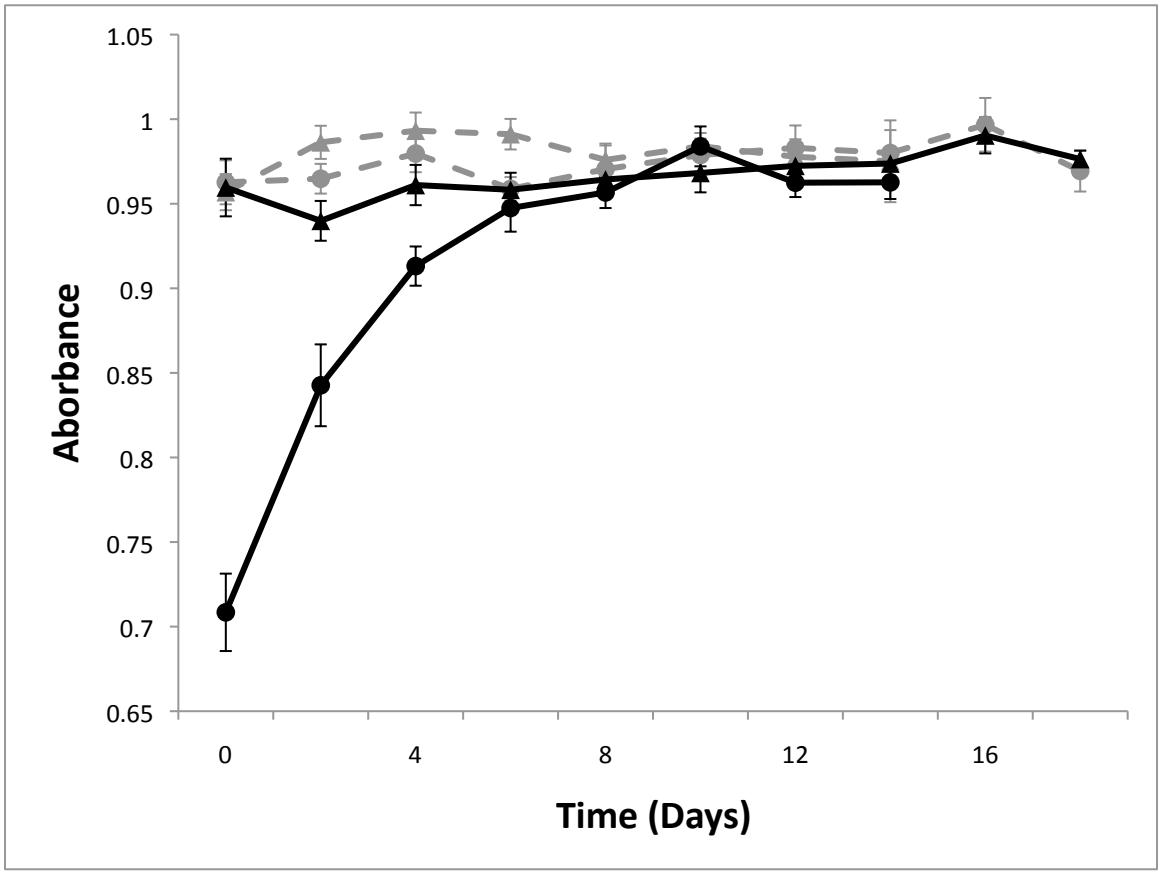


Figure 5g: Change in the ratio of 495:678 nm absorbance thru time in treatments (solid lines) and controls (dashed lines) for replicates of *G. salicornia* (triangles) and *G. coronopifolia* (circles). Mean + SE, n=10.

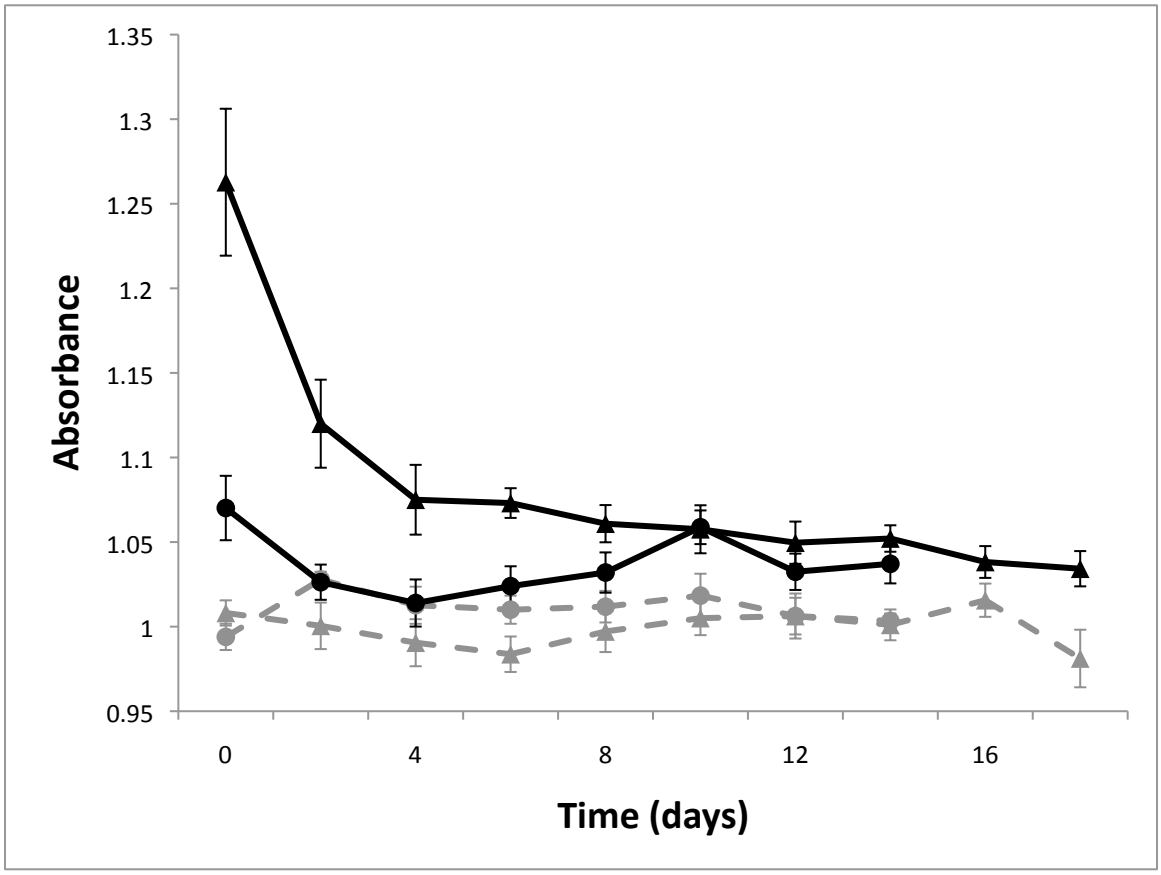


Figure 5h: Change in the ratio of 440:678 nm absorbance thru time in treatments (solid lines) and controls (dashed lines) for replicates of *G. salicornia* (triangles) and *G. coronopifolia* (circles). Mean + SE, n=10.

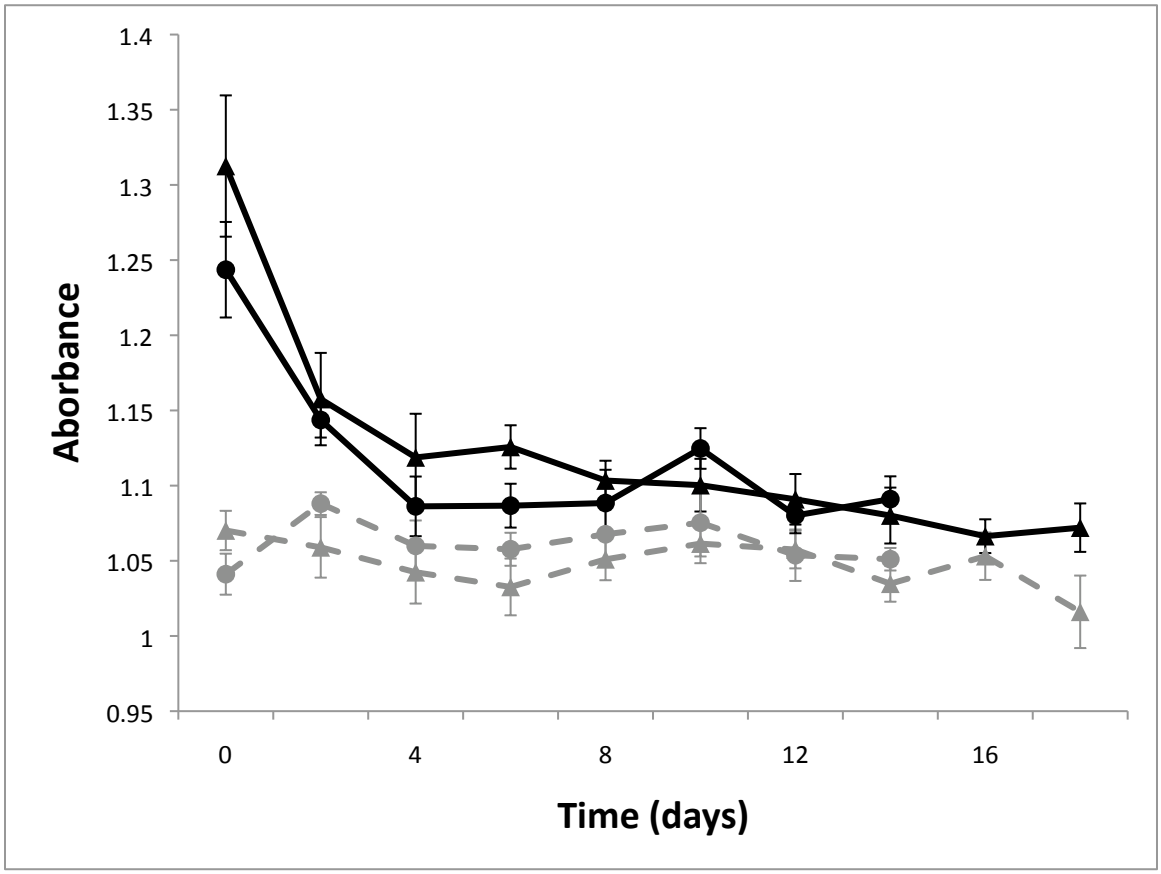


Figure 6a: Change in $rETR_{max}$ thru time in treatments (solid lines) and controls (dashed lines) for replicates of *G. salicornia* (triangles) and *G. coronopifolia* (circles). Mean + SE, n=10.

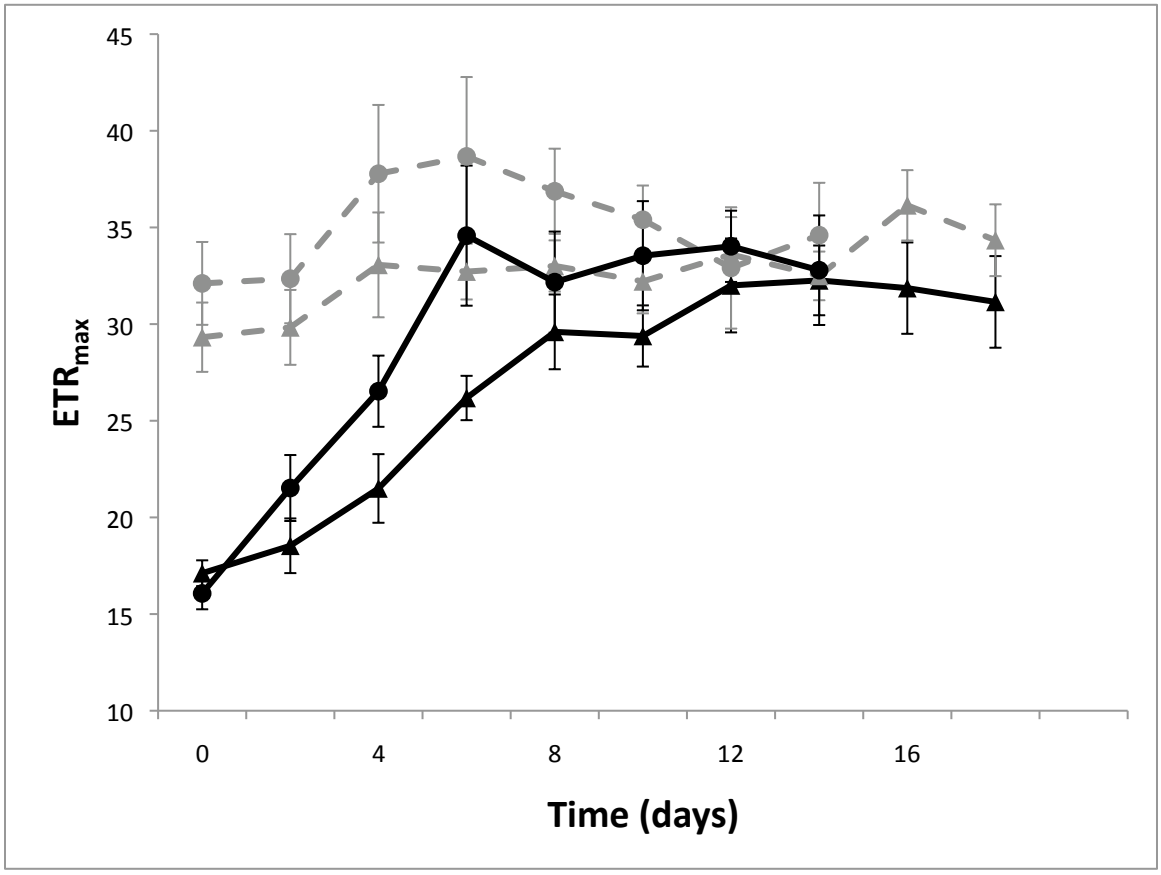


Figure 6b: Change in $E_k$ thru time in treatments (solid lines) and controls (dashed lines) for replicates of *G. salicornia* (triangles) and *G. coronopifolia* (circles). Mean + SE, n=10.

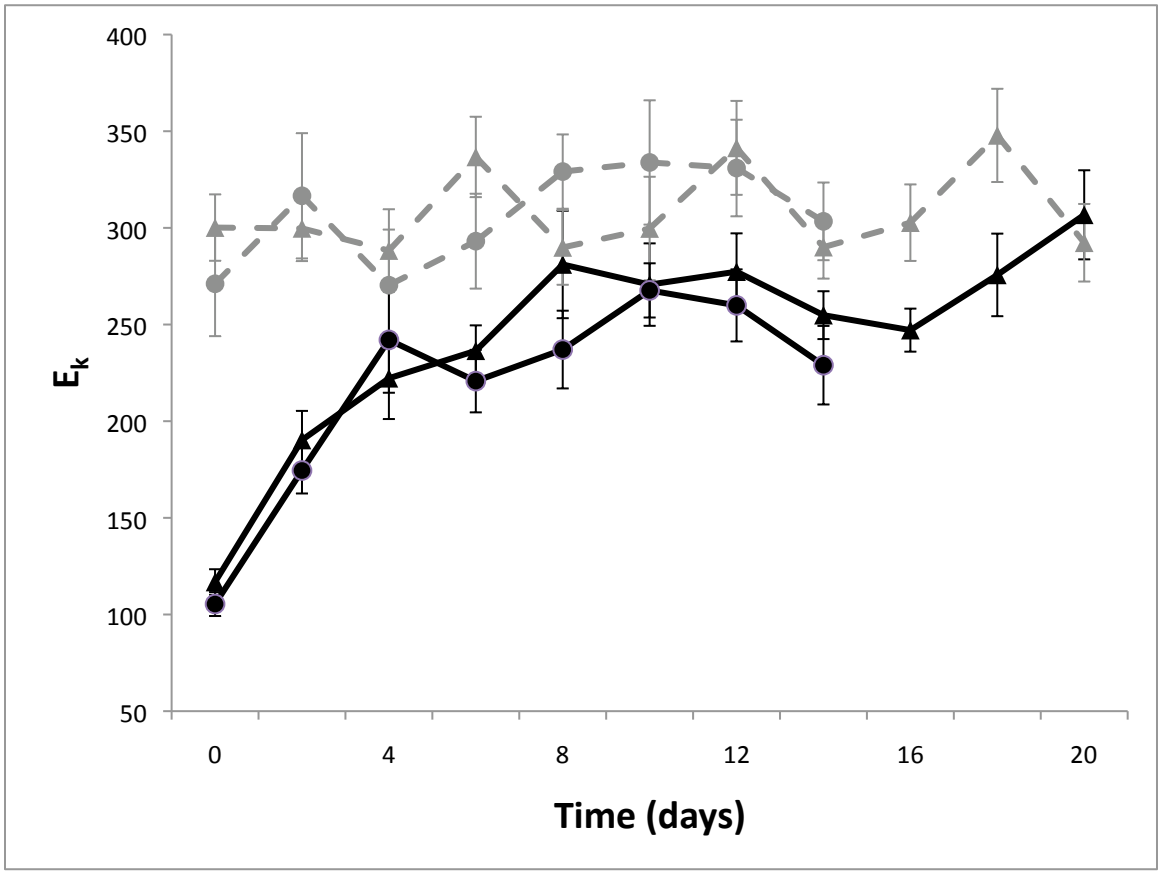


Figure 6c: Change in the ratio of 650:678 nm absorbance thru time in treatments (solid lines) and controls (dashed lines) for replicates of *G. salicornia* (triangles) and *G. coronopifolia* (circles). Mean + SE, n=10.

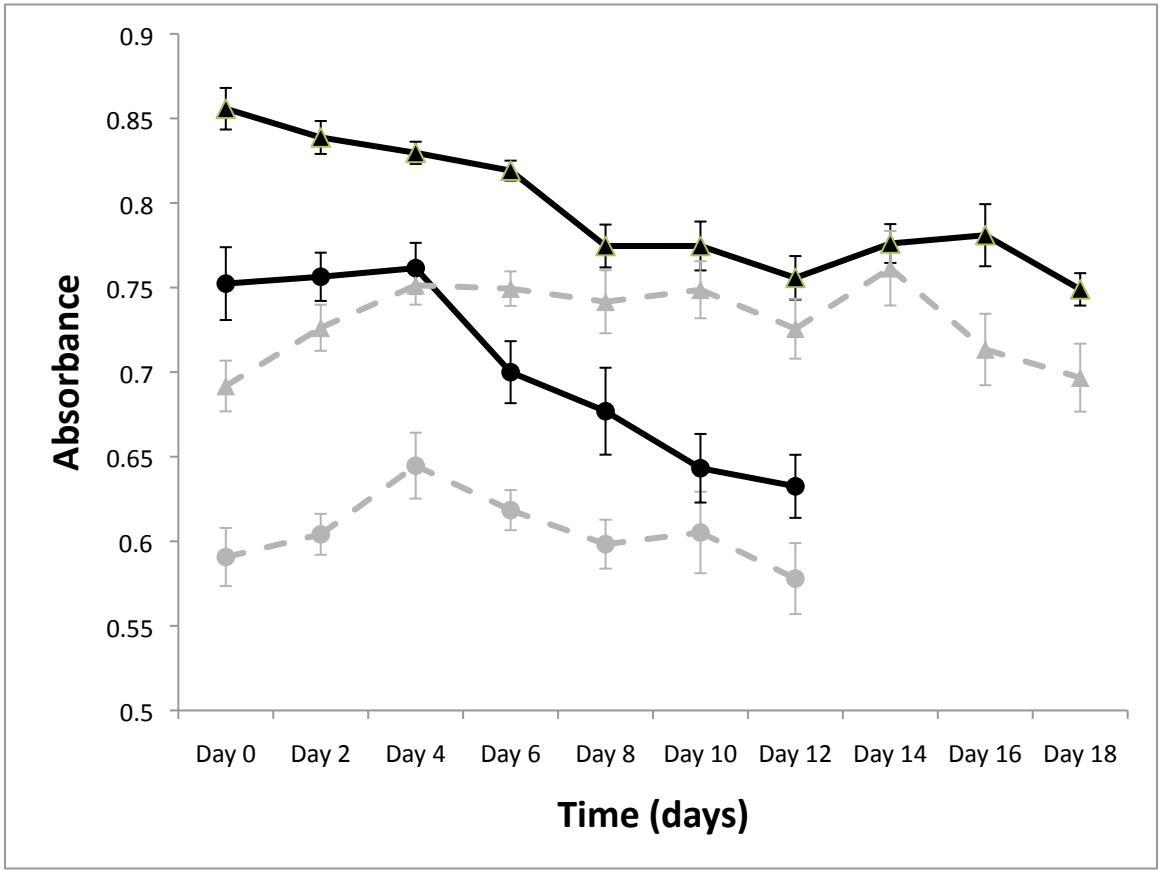


Figure 6d: Change in the ratio of 625:678 nm absorbance thru time in treatments (solid lines) and controls (dashed lines) for replicates of *G. salicornia* (triangles) and *G. coronopifolia* (circles). Mean + SE, n=10.

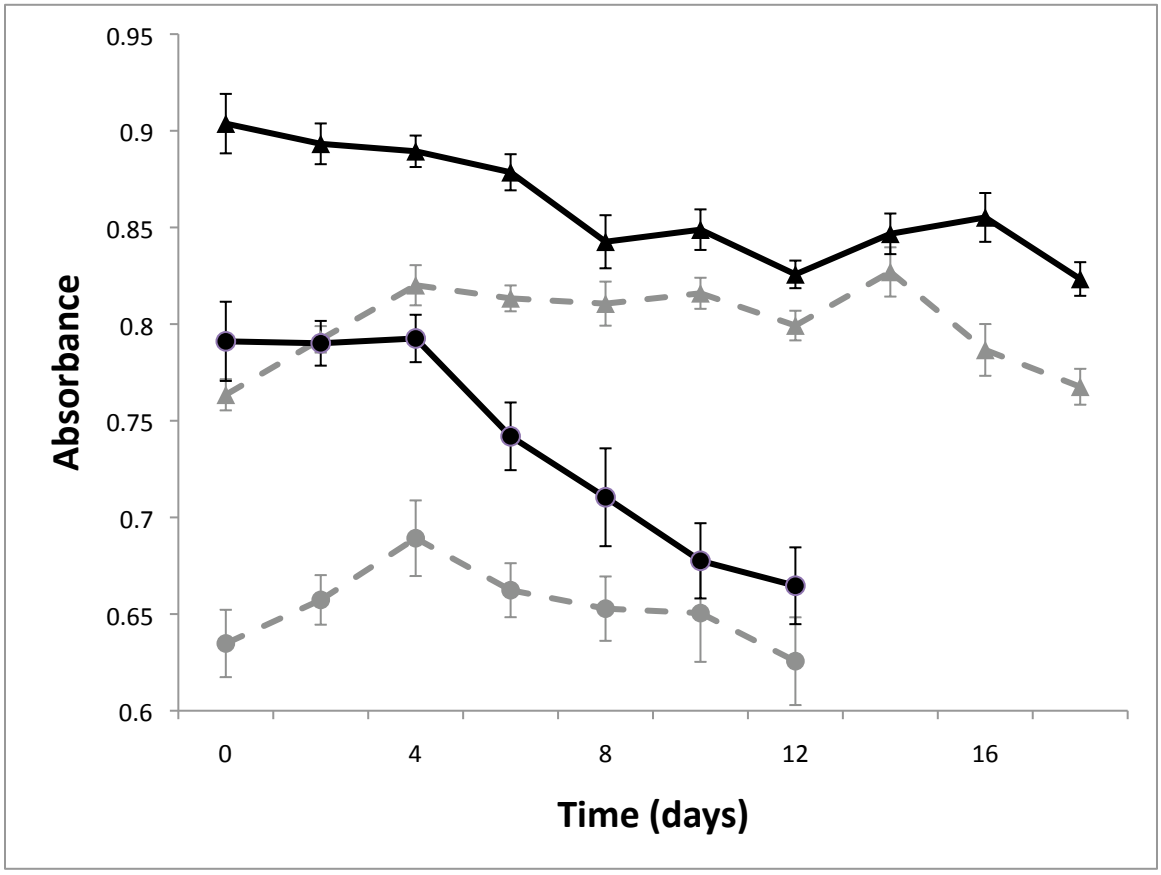


Figure 6e: Change in the ratio of 568:678 nm absorbance thru time in treatments (solid lines) and controls (dashed lines) for replicates of *G. salicornia* (triangles) and *G. coronopifolia* (circles). Mean + SE, n=10.

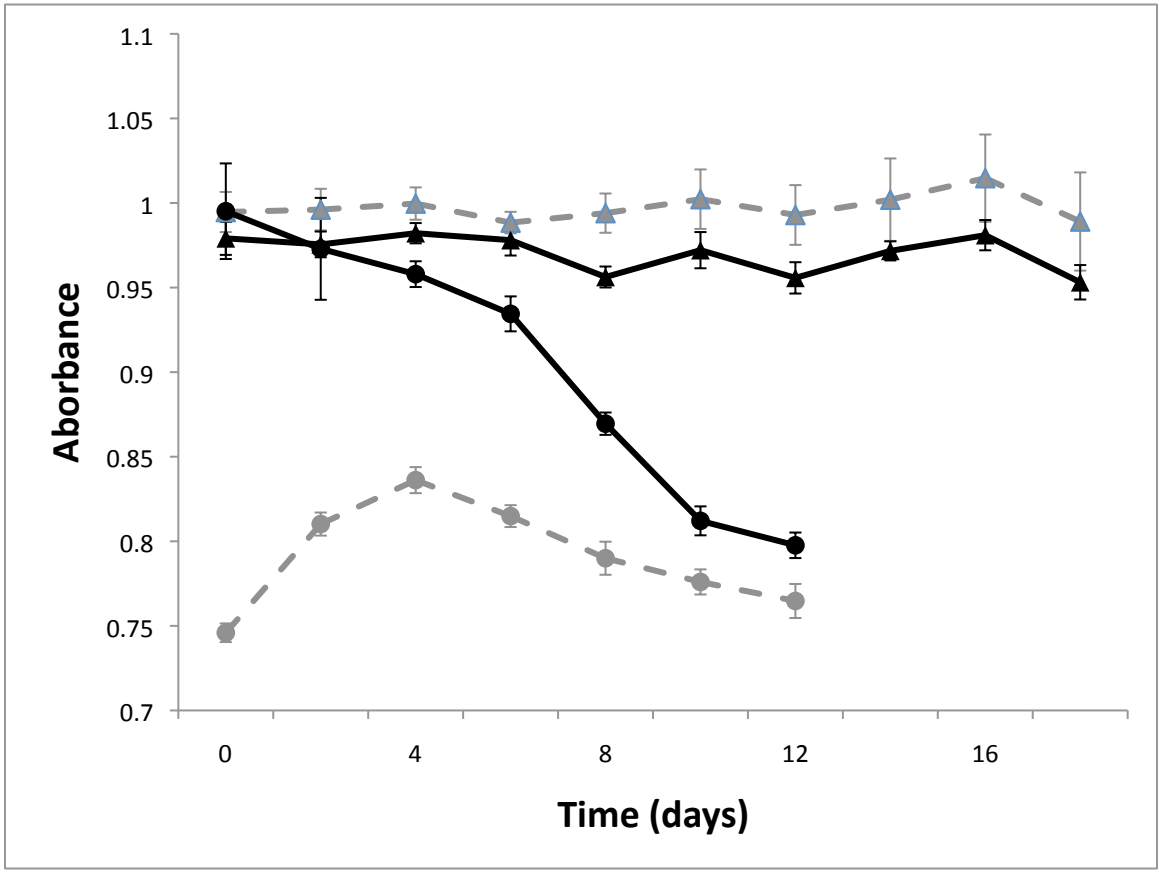


Figure 6f: Change in the ratio of 540:678 nm absorbance thru time in treatments (solid lines) and controls (dashed lines) for replicates of *G. salicornia* (triangles) and *G. coronopifolia* (circles). Mean + SE, n=10.

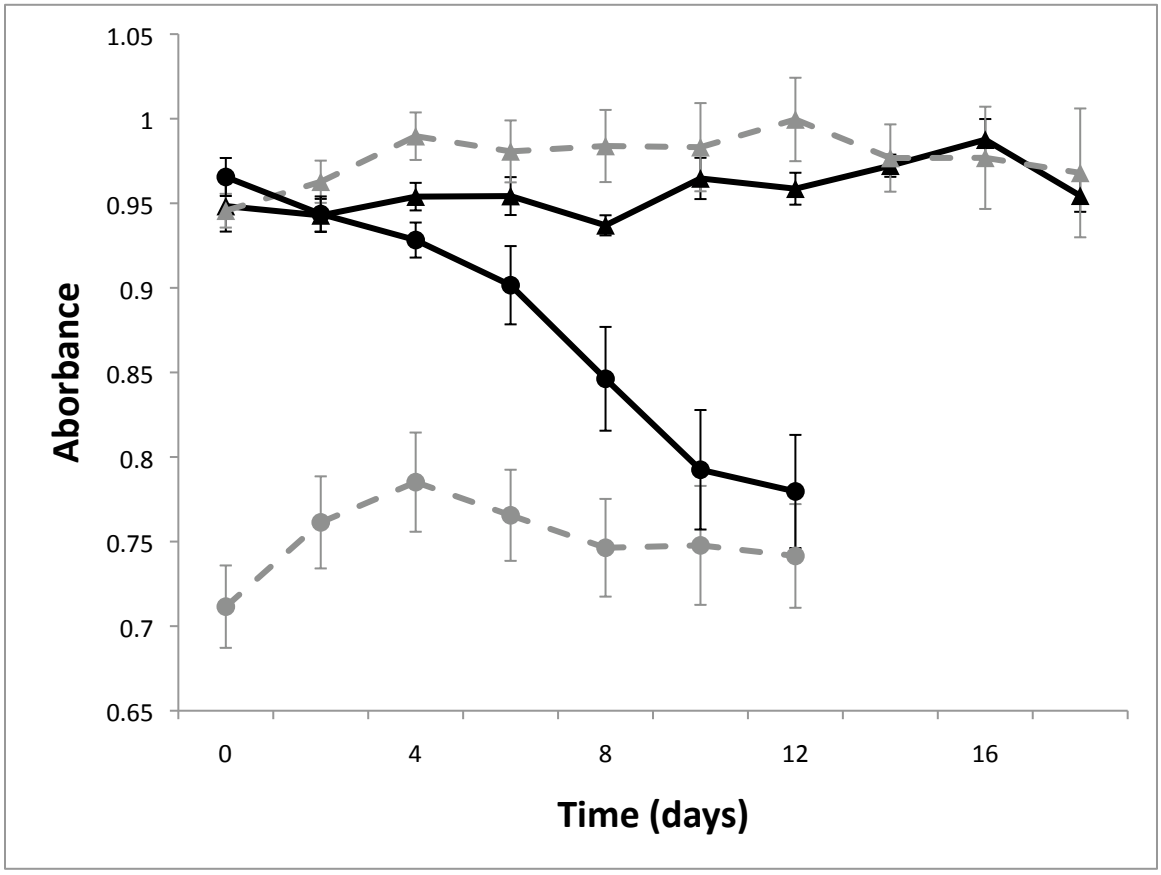


Figure 6g: Change in the ratio of 495:678 nm absorbance thru time in treatments (solid lines) and controls (dashed lines) for replicates of *G. salicornia* (triangles) and *G. coronopifolia* (circles). Mean + SE, n=10.

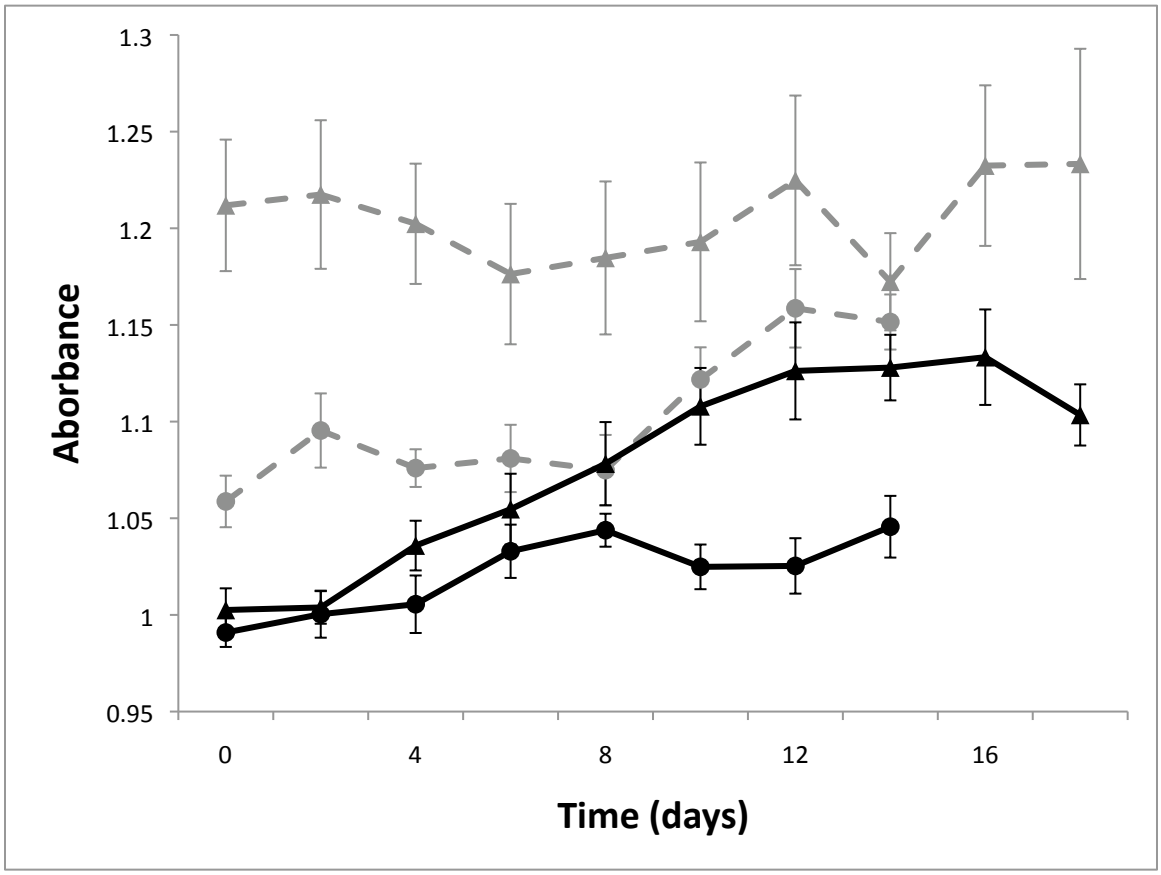


Figure 6h: Change in the ratio of 440:678 nm absorbance thru time in treatments (solid lines) and controls (dashed lines) for replicates of *G. salicornia* (triangles) and *G. coronopifolia* (circles). Mean + SE, n=10.

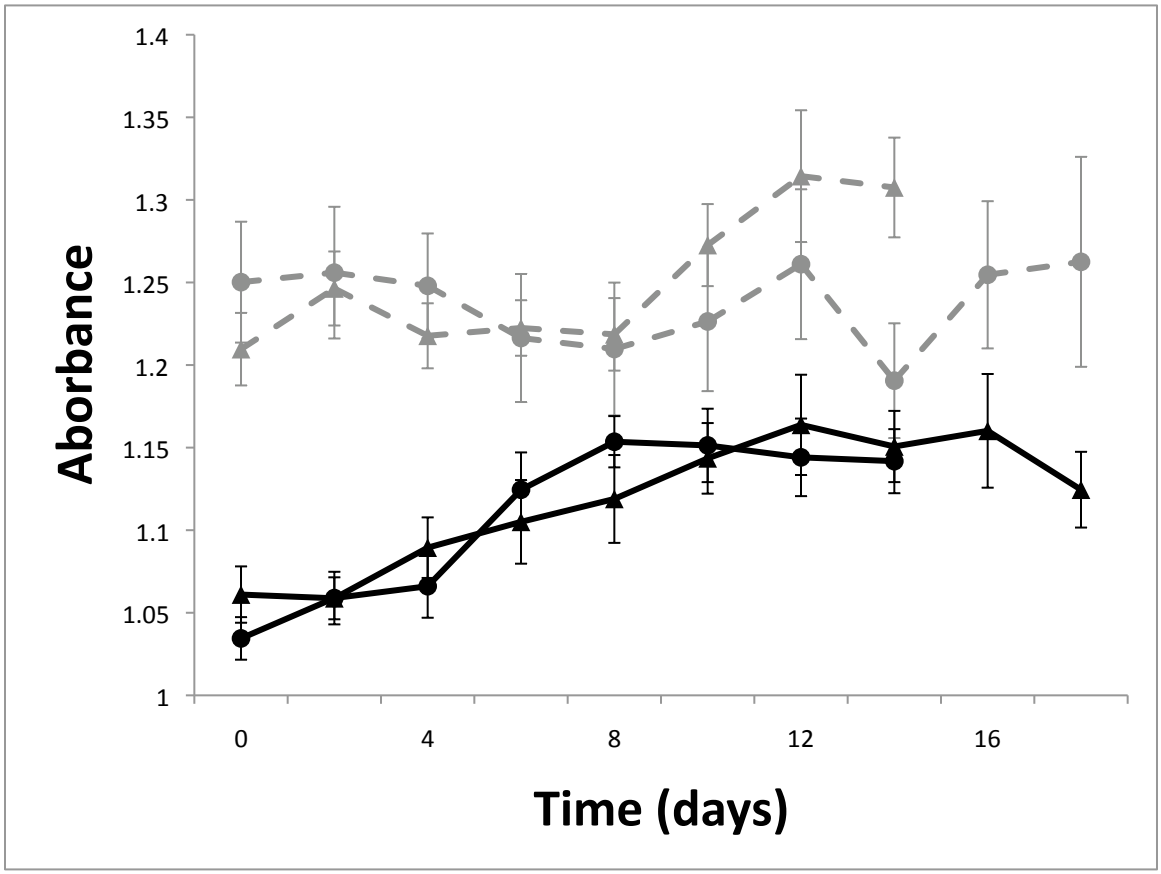


Table 2.1: Summary of repeated measures ANOVA results for the differences between light regime treatments and controls days (light) and measurement days (time). Significance is given as ** (P<0.001) and * (P<0.05).

| | | $rETR_{max}$ | | | $E_k$ | | | 625:678nm | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Light | Time | Interaction | Light | Time | Interaction | Light | Time | Interaction |
| *G. coronopif olia* | Sun → Shade | 28.317 ** | 6.754 * | 7.758 ** | 39.824 ** | 20.526 * | 17.185 ** | 59.585 ** | 17.238 ** | 10.727 ** |
| | Shade → Sun | 12.729 * | 7.068 ** | 3.141 * | 19.255 * | 8.099 ** | 3.092 * | 15.575 * | 16.833 ** | 8.480 ** |
| *G. salicornia* | Sun → Shade | 39.545 ** | 5.180 ** | 5.253 ** | 35.527 ** | 12.521 ** | 15.568 ** | 47.459 ** | 22.638 ** | 10.526 ** |
| | Shade → Sun | 25.824 ** | 9.324 ** | 4.003 * | 60.160 ** | 4.714 ** | 3.720 * | 19.258 ** | 5.639 ** | 6.439 ** |

| | | 568:678nm | | | 540:678nm | | | 495:678nm | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Light | Time | Interaction | Light | Time | Interaction | Light | Time | Interaction |
| *G. coronopif olia* | Sun → Shade | 44.017 ** | 36.550 ** | 32.589 ** | 42.825 ** | 48.045 ** | 34.456 ** | 6.958 * | 1.555 | 4.495 * |
| | Shade → Sun | 9.819 * | 17.021 ** | 11.116 ** | 14.535 * | 17.149 ** | 15.981 ** | 35.494 ** | 6.376 ** | 3.856 * |
| *G. salicornia* | Sun → Shade | 48.093 ** | 7.297 ** | 5.880 ** | 1.646 | 2.497 * | 0.215 | 65.745 ** | 11.484 ** | 8.504 ** |
| | Shade → Sun | 11.477 * | 2.331 | 2.331 | 1.114 | 1.569 | 1.056 | 15.143 * | 3.320 * | 3.071 * |

| | | 440:678nm | | |
|---|---|---|---|---|
| | | Light | Time | Interaction |
| *G. coronopif olia* | Sun → Shade | 29.263 ** | 5.482 * | 7.281 ** |
| | Shade → Sun | 70.003 ** | 7.047 ** | 2.447 |
| *G. salicornia* | Sun → Shade | 22.709 ** | 12.998 ** | 6.983 ** |
| | Shade → Sun | 11.535 * | 1.382 | 1.979 |

Table 2.2: Post-hoc t-test results for treatment values tested against control values. Sun→shade (i.e. sun to shade) values were tested against shade controls, shade→sun treatments were tested against sun controls. *t* values in italics, p values in roman type.

**$rETR_{max}$**

sun → shade

| | Day 0 | Day 2 | Day 4 | Day 6 | Day 8 | Day 10 | Day 12 | Day 14 | Day 16 | Day 18 |
|---|---|---|---|---|---|---|---|---|---|---|
| *G. coronopifolia* | *7.981* | *3.628* | *0.672* | *0.867* | *2.988* | *1.057* | *0.820* | - | - | - |
| | 0.000 | 0.002 | 0.510 | 0.397 | 0.008 | 0.305 | 0.423 | - | - | - |
| *G. salicornia* | *10.550* | *1.874* | *2.837* | *1.726* | *2.056* | *1.018* | *0.483* | *5.720* | *2.713* | *1.287* |
| | 0.000 | 0.077 | 0.011 | 0.102 | 0.055 | 0.322 | 0.635 | 0.000 | 0.014 | 0.214 |

shade → sun

| | Day 0 | Day 2 | Day 4 | Day 6 | Day 8 | Day 10 | Day 12 | Day 14 | Day 16 | Day 18 |
|---|---|---|---|---|---|---|---|---|---|---|
| *G. coronopifolia* | *6.991* | *3.776* | *2.808* | *0.748* | *1.374* | *0.559* | *-0.307* | - | - | - |
| | 0.000 | 0.001 | 0.012 | 0.464 | 0.186 | 0.583 | 0.762 | - | - | - |
| *G. salicornia* | *6.382* | *5.029* | *3.775* | *2.993* | *1.488* | *1.222* | *0.585* | *0.112* | *1.576* | *1.181* |
| | 0.000 | 0.000 | 0.001 | 0.008 | 0.154 | 0.238 | 0.566 | 0.912 | 0.132 | 0.253 |

**$E_k$**

sun → shade

| | Day 0 | Day 2 | Day 4 | Day 6 | Day 8 | Day 10 | Day 12 | Day 14 | Day 16 | Day 18 |
|---|---|---|---|---|---|---|---|---|---|---|
| *G. coronopifolia* | *7.344* | *2.711* | *1.490* | *1.225* | *0.891* | *0.442* | *1.726* | - | - | - |
| | 0.000 | 0.014 | 0.154 | 0.236 | 0.385 | 0.664 | 0.101 | - | - | - |
| *G. salicornia* | *10.562* | *3.158* | *2.176* | *1.379* | *0.505* | *0.897* | *0.485* | *2.898* | *1.383* | *0.598* |
| | 0.000 | 0.005 | 0.043 | 0.185 | 0.619 | 0.382 | 0.634 | 0.010 | 0.184 | 0.557 |

shade → sun

| | Day 0 | Day 2 | Day 4 | Day 6 | Day 8 | Day 10 | Day 12 | Day 14 | Day 16 | Day 18 |
|---|---|---|---|---|---|---|---|---|---|---|
| *G. coronopifolia* | *5.962* | *4.118* | *0.714* | *2.463* | *3.310* | *1.887* | *2.280* | - | - | - |
| | 0.000 | 0.001 | 0.485 | 0.024 | 0.004 | 0.075 | 0.035 | - | - | - |
| *G. salicornia* | *9.960* | *4.834* | *2.199* | *4.078* | *0.260* | *0.847* | *2.042* | *1.720* | *2.448* | *2.241* |
| | 0.000 | 0.000 | 0.041 | 0.001 | 0.798 | 0.408 | 0.056 | 0.103 | 0.025 | 0.038 |

**625:680nm**

sun → shade

| | Day 0 | Day 2 | Day 4 | Day 6 | Day 8 | Day 10 | Day 12 | Day 14 | Day 16 | Day 18 |
|---|---|---|---|---|---|---|---|---|---|---|
| *G. coronopifolia* | *-7.213* | *6.695* | *3.684* | *4.495* | *1.195* | *-1.409* | *-1.863* | - | - | - |
| | 0.000 | 0.000 | 0.002 | 0.000 | 0.248 | 0.176 | 0.079 | - | - | - |
| *G. salicornia* | *-8.281* | *6.509* | *5.145* | *4.320* | *2.951* | *-2.993* | *-4.076* | *-2.291* | *-1.375* | *-1.200* |
| | 0.000 | 0.000 | 0.000 | 0.000 | 0.009 | 0.008 | 0.001 | 0.034 | 0.186 | 0.246 |

shade → sun

| | Day 0 | Day 2 | Day 4 | Day 6 | Day 8 | Day 10 | Day 12 | Day 14 | Day 16 | Day 18 |
|---|---|---|---|---|---|---|---|---|---|---|
| *G. coronopifolia* | *0.000* | *0.000* | *0.000* | *0.002* | *0.073* | *0.407* | *0.212* | - | - | - |
| | -5.812 | 7.683 | 4.476 | 3.554 | 1.902 | -0.848 | -1.293 | - | - | - |
| *G. salicornia* | *-8.220* | *6.692* | *5.301* | *6.660* | *1.558* | *-1.514* | *-1.192* | *-0.579* | *-2.443* | *-2.317* |
| | 0.000 | 0.000 | 0.000 | 0.000 | 0.138 | 0.149 | 0.250 | 0.570 | 0.026 | 0.033 |

**568:680nm**

sun → shade

| | Day 0 | Day 2 | Day 4 | Day 6 | Day 8 | Day 10 | Day 12 | Day 14 | Day 16 | Day 18 |
|---|---|---|---|---|---|---|---|---|---|---|
| *G. coronopifolia* | *-9.001* | *4.334* | *3.457* | *1.202* | *0.491* | *1.345* | *0.715* | - | - | - |
| | 0.000 | 0.000 | 0.003 | 0.245 | 0.629 | 0.195 | 0.484 | - | - | - |
| *G. salicornia* | *-6.028* | *3.780* | *2.311* | *1.791* | *1.349* | *-1.474* | *-1.198* | *-1.626* | *-0.946* | *0.534* |
| | 0.000 | 0.001 | 0.033 | 0.090 | 0.194 | 0.158 | 0.247 | 0.121 | 0.357 | 0.600 |

shade → sun

| | Day 0 | Day 2 | Day 4 | Day 6 | Day 8 | Day 10 | Day 12 | Day 14 | Day 16 | Day 18 |
|---|---|---|---|---|---|---|---|---|---|---|
| *G. coronopifolia* | *-7.675* | *5.801* | *4.092* | *3.458* | *1.689* | *-0.635* | *-0.592* | - | - | - |
| | 0.000 | 0.000 | 0.001 | 0.003 | 0.108 | 0.533 | 0.561 | - | - | - |

**540:680nm**

sun → shade

| | Day 0 | Day 2 | Day 4 | Day 6 | Day 8 | Day 10 | Day 12 | Day 14 | Day 16 | Day 18 |
|---|---|---|---|---|---|---|---|---|---|---|
| *G. coronopifolia* | *-9.838* | *5.503* | *5.064* | *2.621* | *1.443* | *-0.005* | *-1.145* | - | - | - |
| | 0.000 | 0.000 | 0.000 | 0.017 | 0.166 | 0.996 | 0.267 | - | - | - |

shade → sun

| | Day 0 | Day 2 | Day 4 | Day 6 | Day 8 | Day 10 | Day 12 | Day 14 | Day 16 | Day 18 |
|---|---|---|---|---|---|---|---|---|---|---|
| *G. coronopifolia* | *-9.469* | *6.257* | *4.602* | *3.831* | *2.371* | *-0.897* | *-0.840* | - | - | - |
| | 0.000 | 0.000 | 0.000 | 0.001 | 0.029 | 0.382 | 0.412 | - | - | - |

**495:680nm**

sun → shade

| | Day 0 | Day 2 | Day 4 | Day 6 | Day 8 | Day 10 | Day 12 | Day 14 | Day 16 | Day 18 |
|---|---|---|---|---|---|---|---|---|---|---|
| *G. coronopifolia* | *-3.705* | *0.138* | *0.075* | *0.967* | *1.335* | *2.475* | *1.529* | - | - | - |
| | 0.002 | 0.891 | 0.941 | 0.346 | 0.199 | 0.023 | 0.144 | - | - | - |
| *G. salicornia* | *5.064* | *4.586* | *3.368* | *5.873* | *4.661* | *2.882* | *1.816* | *3.251* | *0.669* | *2.647* |
| | 0.000 | 0.000 | 0.004 | 0.000 | 0.000 | 0.011 | 0.088 | 0.005 | 0.513 | 0.018 |

shade → sun

| | Day 0 | Day 2 | Day 4 | Day 6 | Day 8 | Day 10 | Day 12 | Day 14 | Day 16 | Day 18 |
|---|---|---|---|---|---|---|---|---|---|---|
| *G. coronopifolia* | *4.437* | *4.180* | *3.960* | *2.163* | *1.546* | *4.791* | *5.362* | - | - | - |
| | 0.000 | 0.001 | 0.001 | 0.044 | 0.139 | 0.000 | 0.000 | - | - | - |
| *G. salicornia* | *5.845* | *5.428* | *4.945* | *2.989* | *2.363* | *1.865* | *1.949* | *1.464* | *2.051* | *2.108* |
| | 0.000 | 0.000 | 0.000 | 0.008 | 0.030 | 0.079 | 0.067 | 0.160 | 0.055 | 0.049 |

**440: 680nm**

sun → shade

| | Day 0 | Day 2 | Day 4 | Day 6 | Day 8 | Day 10 | Day 12 | Day 14 | Day 16 | Day 18 |
|---|---|---|---|---|---|---|---|---|---|---|
| *G. coronopifolia* | *5.587* | *3.999* | *1.009* | *1.590* | *0.754* | *1.884* | *1.271* | - | - | - |
| | 0.000 | 0.001 | 0.327 | 0.129 | 0.461 | 0.760 | 0.220 | - | - | - |
| *G. salicornia* | *5.170* | *3.403* | *2.222* | *3.684* | *2.402* | *2.090* | *1.277* | *1.703* | *0.216* | *1.676* |
| | 0.000 | 0.003 | 0.039 | 0.002 | 0.027 | 0.051 | 0.218 | 0.106 | 0.831 | 0.111 |

| shade → sun | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Day 0 | Day 2 | Day 4 | Day 6 | Day 8 | Day 10 | Day 12 | Day 14 | Day 16 | Day 18 |
| *G. coronopifolia* | *6.879* | *6.819* | *5.530* | *3.477* | *2.418* | *3.639* | *3.678* | - | - | - |
| | 0.000 | 0.000 | 0.000 | 0.003 | 0.026 | 0.002 | 0.002 | - | - | - |
| *G. salicornia* | *4.685* | *4.712* | *4.327* | *2.404* | *1.883* | *1.753* | *1.782* | *0.975* | *1.678* | *2.042* |
| | 0.000 | 0.000 | 0.000 | 0.027 | 0.076 | 0.097 | 0.092 | 0.342 | 0.111 | 0.056 |

TABLE 2.3: Days required to reach acclimation to the new light environment for sun to shade treatments as measured by the given parameter. Dashed line (-) indicates that parameter treatment did not differ from control.

| | $rETR_{max}$ | Ek | 625: 678 nm | 568: 678 nm | 540: 678 nm | 495: 678 nm | 440: 678 nm |
|---|---|---|---|---|---|---|---|
| *Gracilaria coronopif olia* | 4 | 4 | 8 | 6 | 8 | 2 | 4 |
| *Gracilaria salicornia* | 6 | 6 | 16 | 8 | – | 16 | 12 |

TABLE 2.4: Days required for shade to sun treatments to acclimate to the new light environment as measured by given parameters. Dashed line (-) indicates that parameter treatment did not differ from control, asterisk (*) indicates a failure of treatment values to equal control values at any given time point over the course of the experiment.

| | $rETR_{max}$ | Ek | 625: 678 nm | 568: 678 nm | 540: 678 nm | 495: 678 nm | 440: 678 nm |
|---|---|---|---|---|---|---|---|
| *Gracilaria coronopif olia* | 6 | 4 | 10 | 8 | 10 | * | * |
| *Gracilaria salicornia* | 6 | 6 | 8 | – | – | 14 | * |

Table 2.5: Percent change in sun to shade parameter values between measurement days. Values not recorded for parameters after acclimation was reached, or if acclimation was not reached.

| *G. salicornia* | Day 0 - 2 | Day 2 - 4 | Day 4 - 6 | Day 6 - 8 | Day 8- 10 | Day 10 - 12 | Day 12 - 14 | Day 14 - 16 |
|---|---|---|---|---|---|---|---|---|
| $rETR_{max}$ | -22.6% | -7.0% | -11.7% | — | — | — | — | — |
| $E_k$ | -42.2% | -12.3% | -3.1% | — | — | — | — | — |
| 650:678 nm | 9.7% | 7.4% | 1.0% | -0.5% | 2.4% | 0.1% | 2.0% | 2.1% |
| 625:678 nm | 7.5% | 5.2% | 0.7% | -0.3% | 2.2% | 0.1% | 1.4% | 2.1% |
| 568: 678 nm | 4.8% | 2.8% | 0.3% | 1.5% | — | — | — | — |
| 540:678 nm | — | — | — | — | — | — | — | — |
| 495:678 nm | -11.3% | -4.0% | -0.2% | -1.1% | -0.3% | -0.7% | 0.2% | -1.3% |
| 440:678 nm | -11.8% | -3.4% | 0.6% | -2.0% | -0.3% | -0.9% | — | — |

| *G. coronopif olia* | Day 0 - Day 2 | Day 2 - Day 4 | Day 4- Day 6 | Day 6 - Day 8 | Day 8- 10 | Day 10 - 12 | Day 12 - 14 | Day 14 - 16 |
|---|---|---|---|---|---|---|---|---|
| $rETR_{max}$ | -23.0% | -38.8% | — | — | — | — | — | — |
| $E_k$ | -44.0% | -39.4% | — | — | — | — | — | — |
| 650:678 nm | 12.6% | 11.1% | 2.3% | -0.7% | — | — | — | — |
| 625:678 nm | 12.5% | 10.5% | 2.2% | 0.0% | — | — | — | — |
| 568: 678 nm | 19.6% | 10.5% | 2.3% | — | — | — | — | — |
| 540:678 nm | 18.9% | 8.4% | 3.8% | 0.1% | — | — | — | — |
| 495:678 nm | -4.1% | — | — | — | — | — | — | — |
| 440:678 nm | -8.0% | -5.0% | — | — | — | — | — | — |

Table 2.6: Percent change in shade to sun parameter values between measurement days. Values not recorded for parameters after acclimation was reached, or if acclimation was not reached.

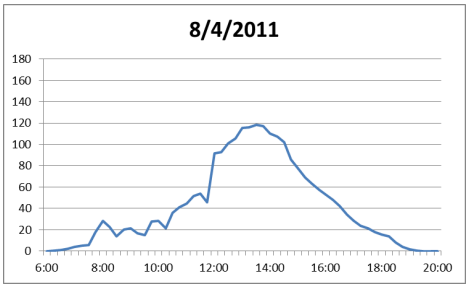
| *G. salicornia* | | Day 0 - 2 | Day 2 - 4 | Day 4 - 6 | Day 6 - 8 | Day 8- 10 | Day 10 - 12 | Day 12 - 14 | Day 14 - 16 |
|---|---|---|---|---|---|---|---|---|---|
| | $rETR_{max}$ | 7.7% | 13.8% | 17.9% | 11.6% | — | — | — | — |
| | $E_k$ | 38.6% | 14.4% | 6.0% | 15.9% | — | — | — | — |
| | 650:678 nm | -2.0% | -1.1% | -1.3% | -5.4% | — | — | — | — |
| | 625:678 nm | -7.5% | -5.2% | -0.7% | 0.3% | — | — | — | — |
| | 568: 678 nm | — | — | — | — | — | — | — | — |
| | 540:678 nm | — | — | — | — | — | — | — | — |
| | 495:678 nm | 0.1% | 3.1% | 1.8% | 2.2% | 2.7% | 1.6% | 0.2% | — |
| | 440:678 nm | — | — | — | — | — | — | — | — |

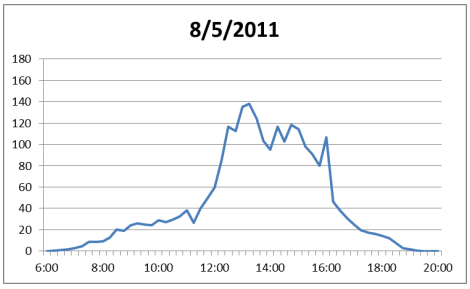
| *G. coronopif olia* | | Day 0 - Day 2 | Day 2 - Day 4 | Day 4- Day 6 | Day 6 - Day 8 | Day 8- 10 | Day 10 - 12 | Day 12 - 14 | Day 14 - 16 |
|---|---|---|---|---|---|---|---|---|---|
| | $rETR_{max}$ | 25.3% | 18.9% | 23.3% | — | — | — | — | — |
| | $E_k$ | 39.6% | 27.9% | — | — | — | — | — | — |
| | 650:678 nm | 0.5% | 0.7% | -8.8% | -3.4% | -5.2% | — | — | — |
| | 625:678 nm | -0.1% | 0.3% | -6.4% | -4.2% | -4.6% | — | — | — |
| | 568: 678 nm | -2.2% | -1.5% | -2.5% | -6.9% | — | — | — | — |
| | 540:678 nm | -2.3% | -1.6% | -2.9% | -6.1% | -6.4% | — | — | — |
| | 495:678 nm | 0.9% | 0.5% | 2.7% | 1.0% | -1.8% | — | — | — |
| | 440:678 nm | — | — | — | — | — | — | — | — |

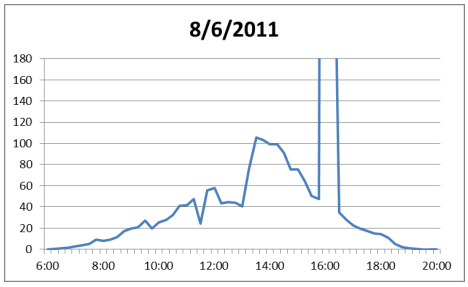
Appendix 1: Daily PAR values over the course of five trials

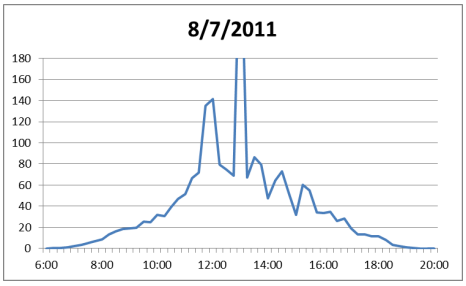
Technical difficulties inhibited recording of daily irradiance for over half of the experimental period, including all of Trial 1 (June 21-July 5) and parts of Trial 2 (Aug 1-Aug 23), Trial 3 (Aug 23-Sept 12), Trial 4 (Sept 18-Oct 8) and Trial 6 (Oct 18-Nov 13).

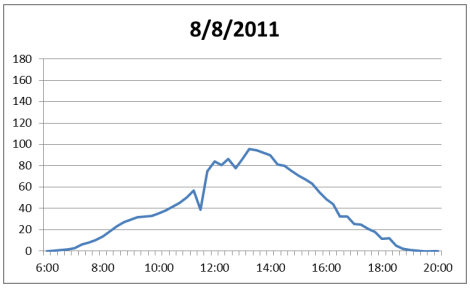
Trial 2 Shade Treatments

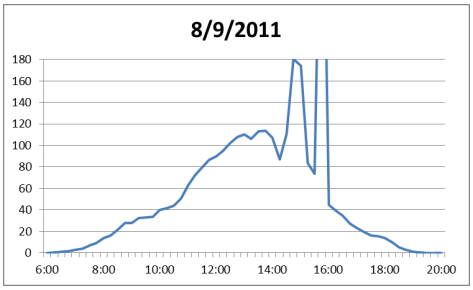


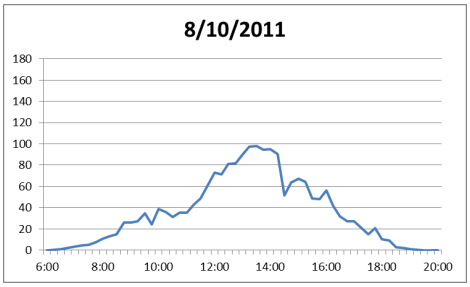



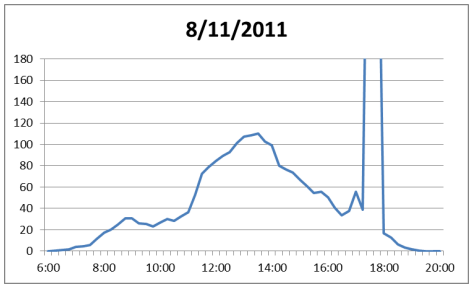



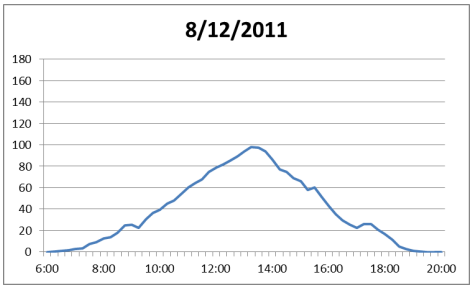



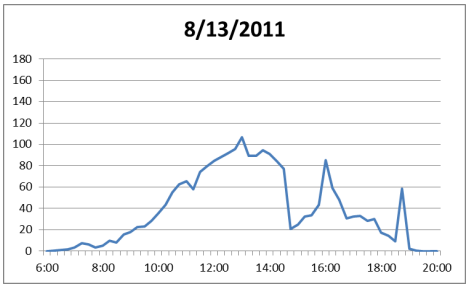



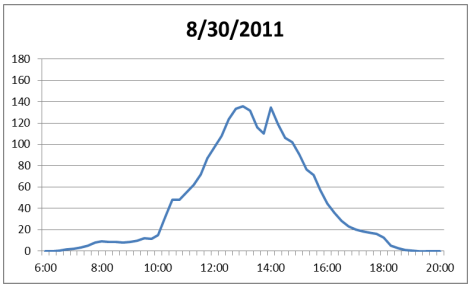



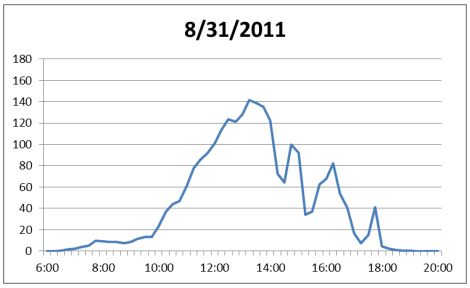



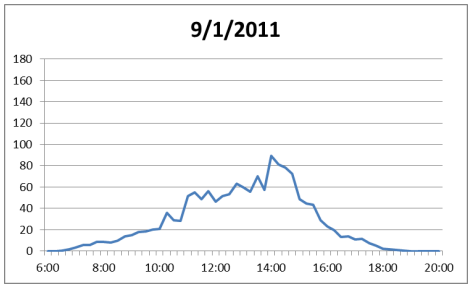



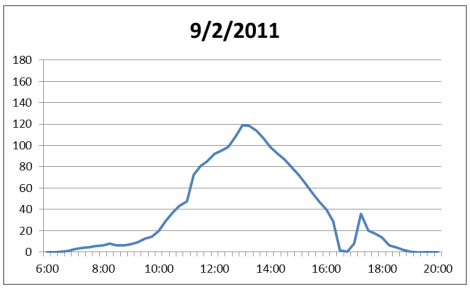



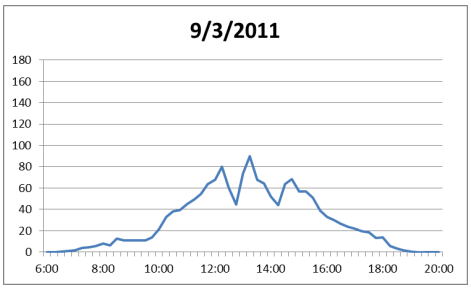
Trial 3 Shade Treatment

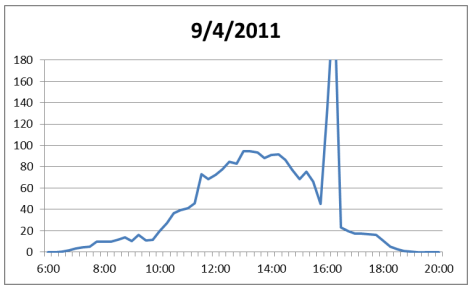


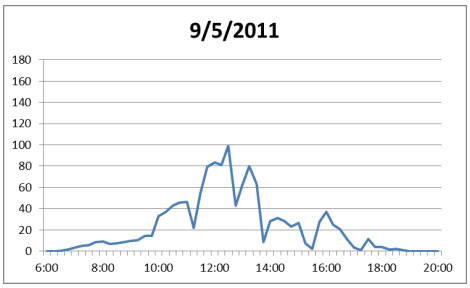



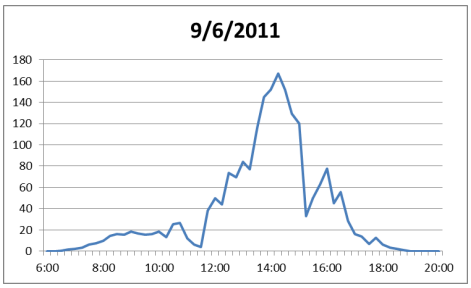



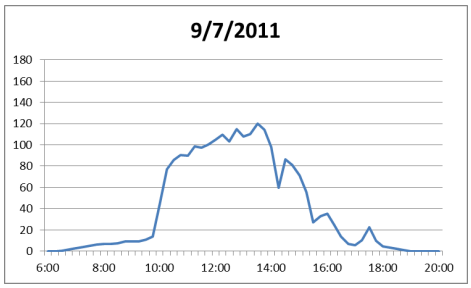



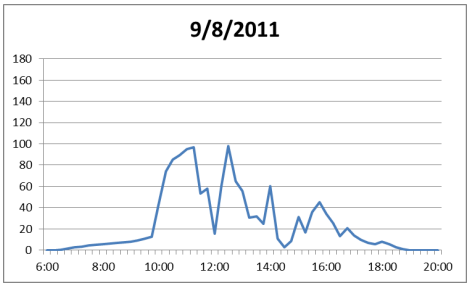



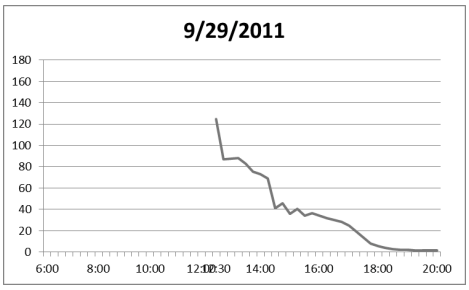



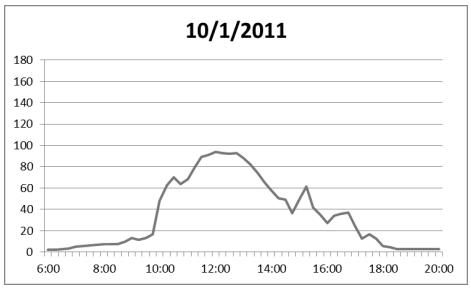



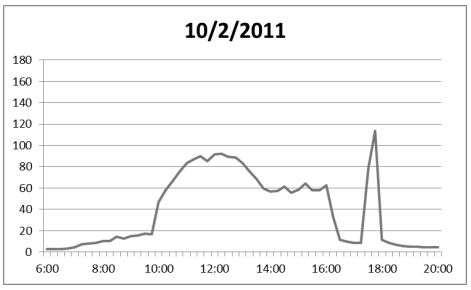



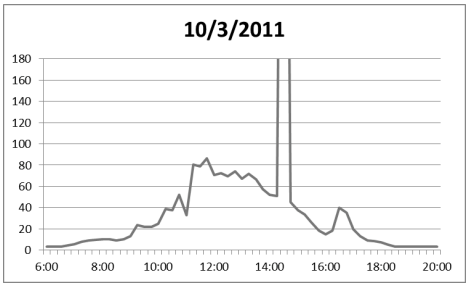



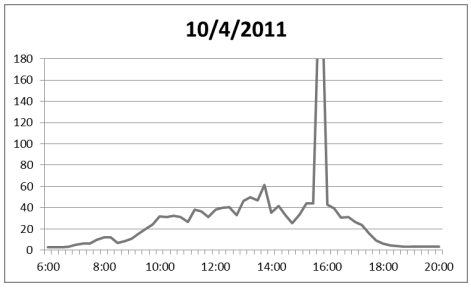
# Trial 4 Shade Treatments

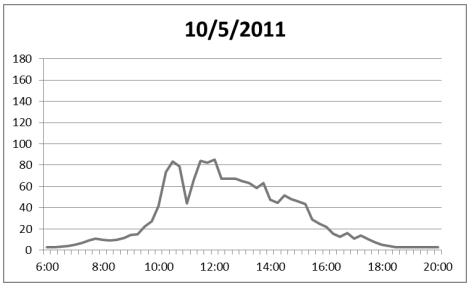


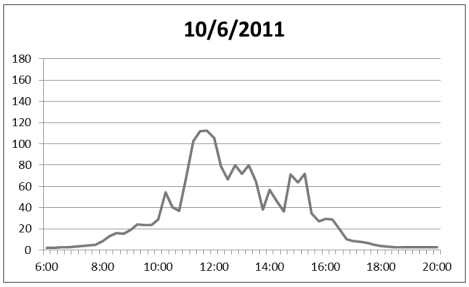



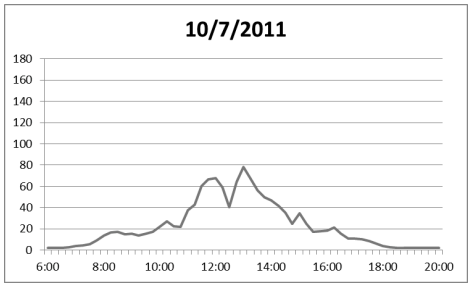



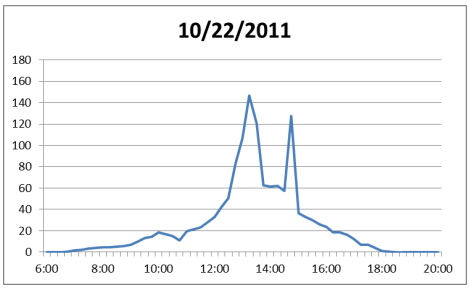



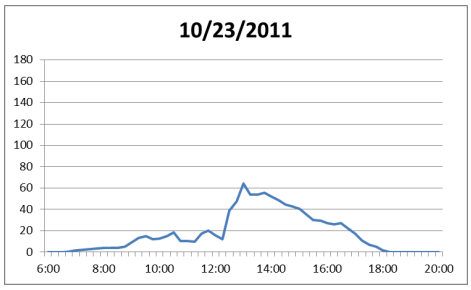



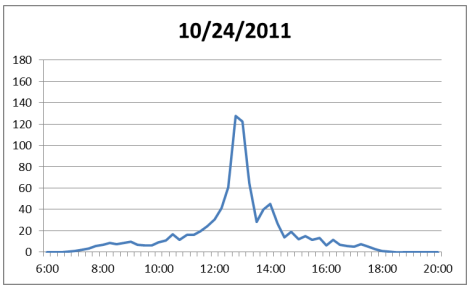



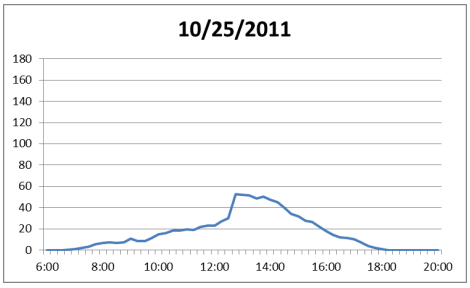



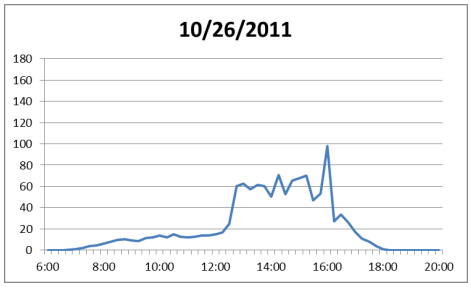
# Trial 6 Shade Treatments

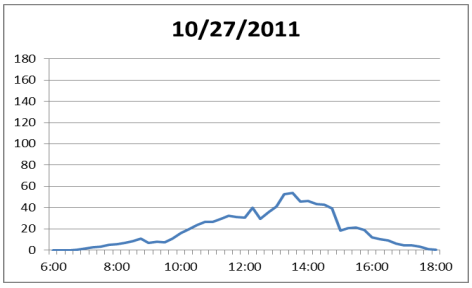


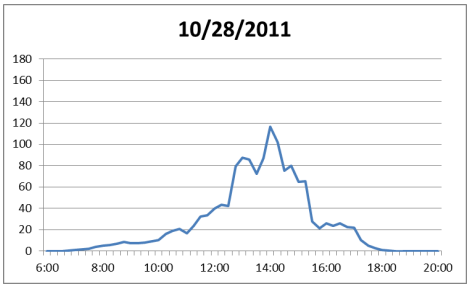



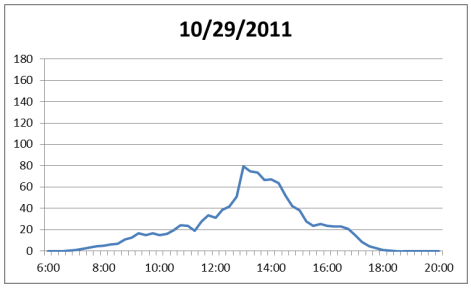



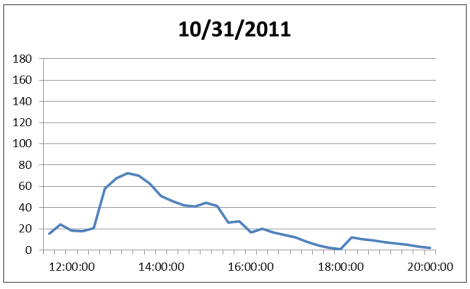



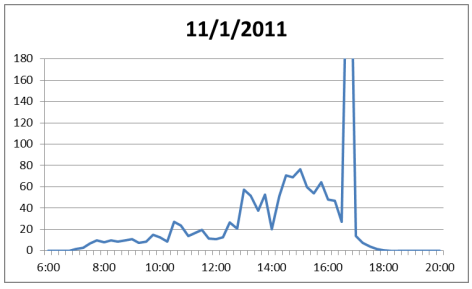



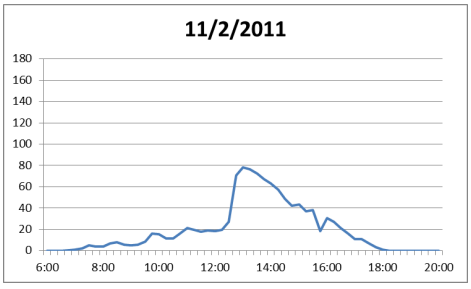



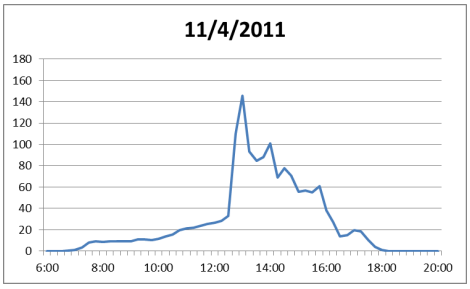



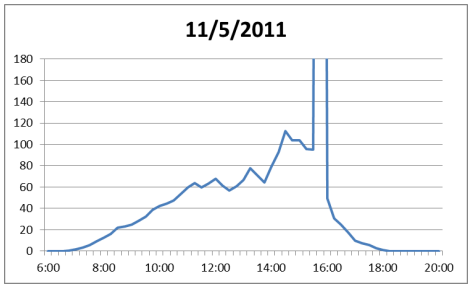



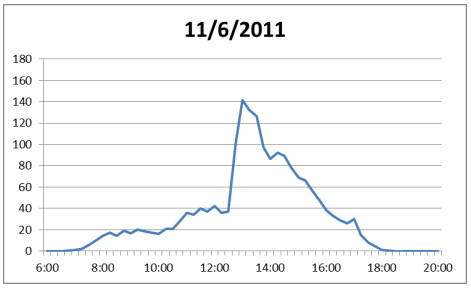



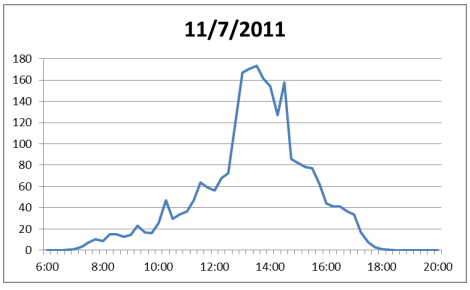



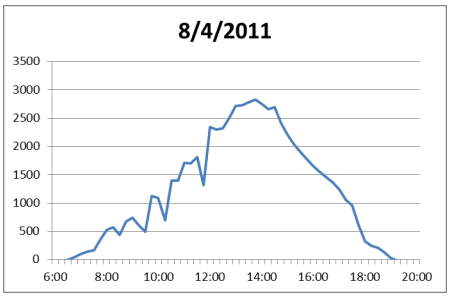



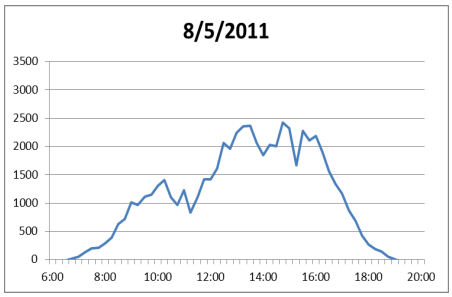



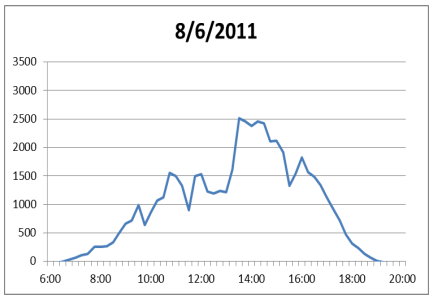



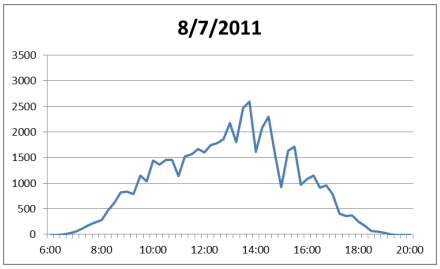



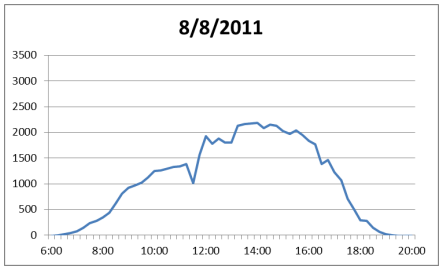
Trial 3 Sun treatments

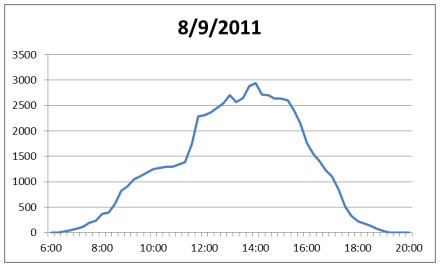


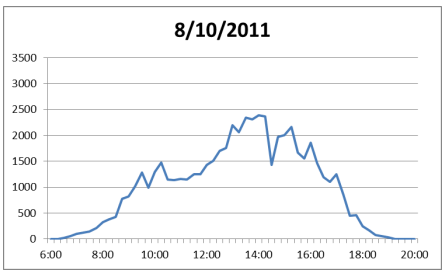



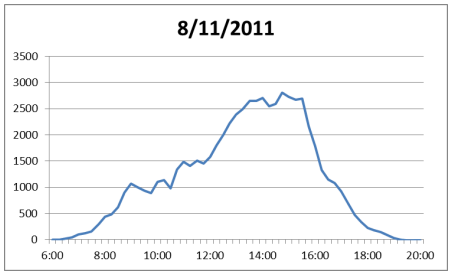



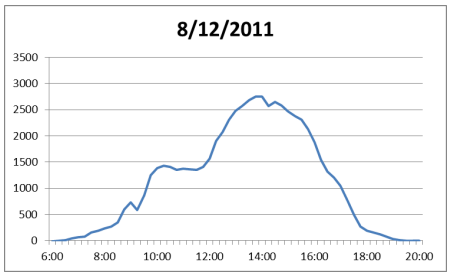



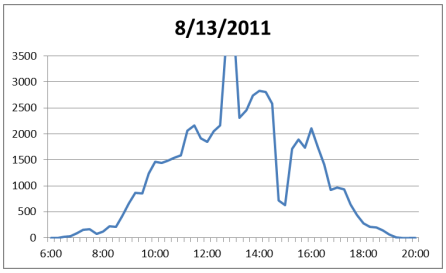



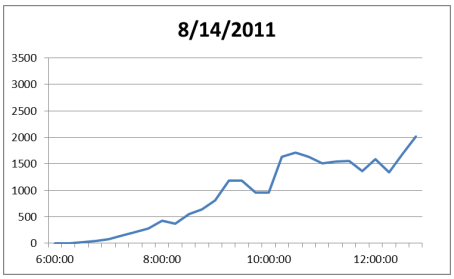



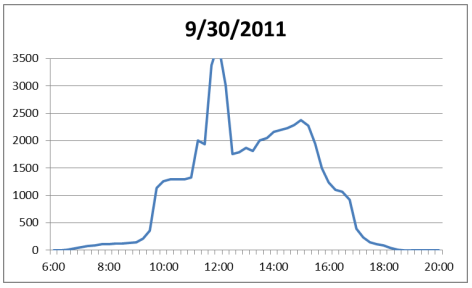



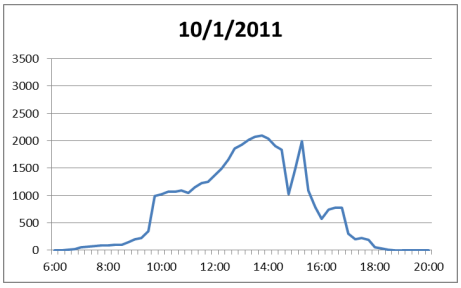



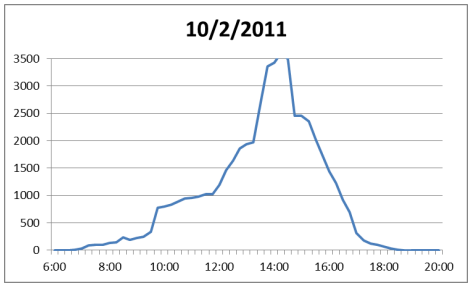



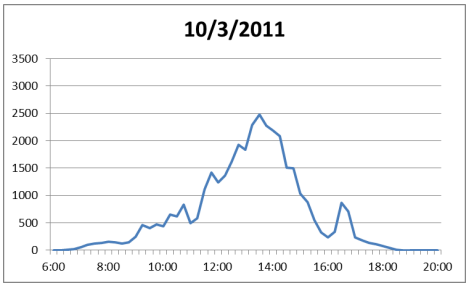



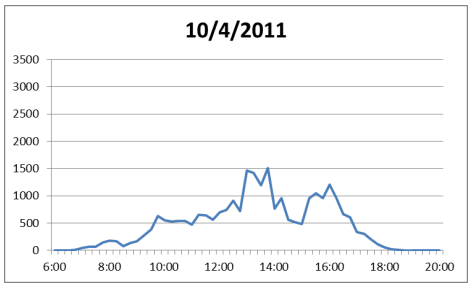
# Trial 5 Sun Treatments

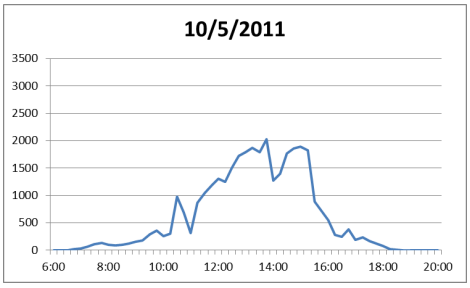


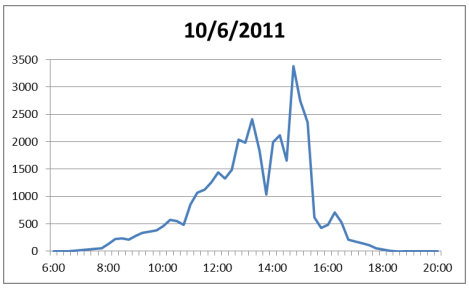



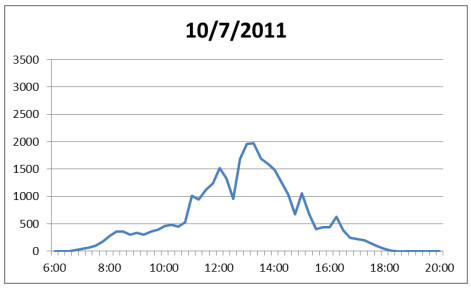



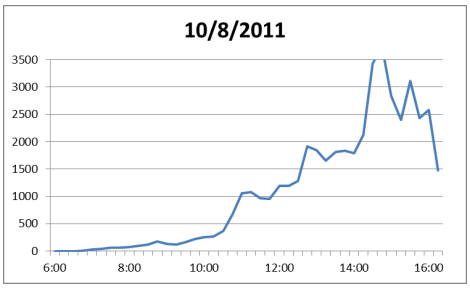



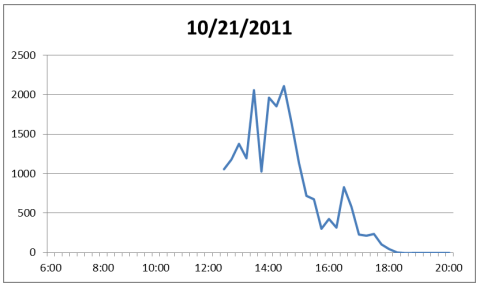



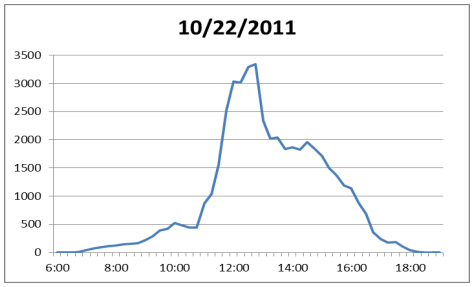



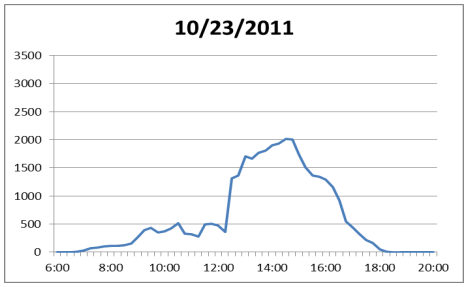



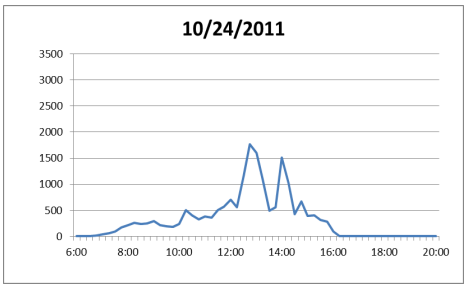



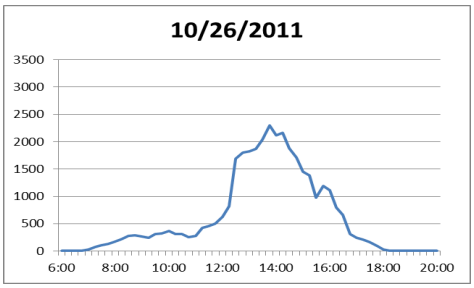
# Trial 6 Sun Treatments

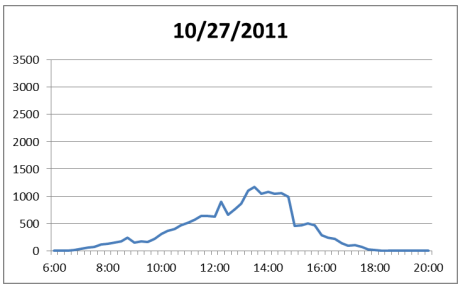


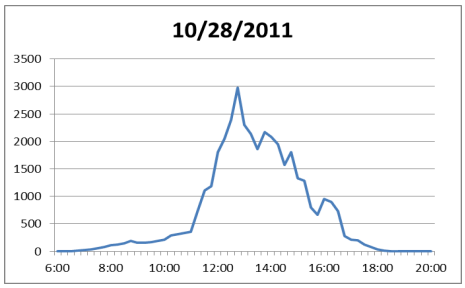



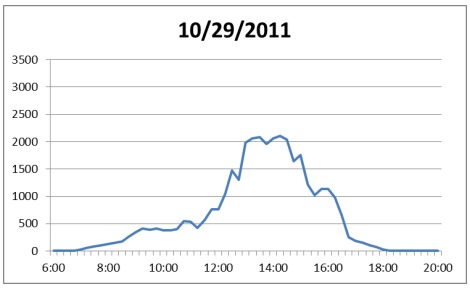



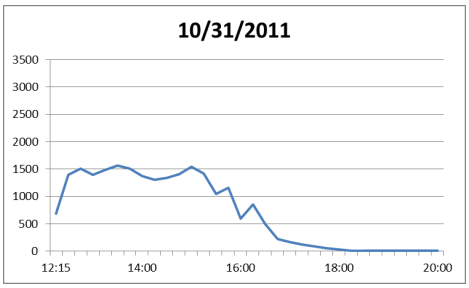



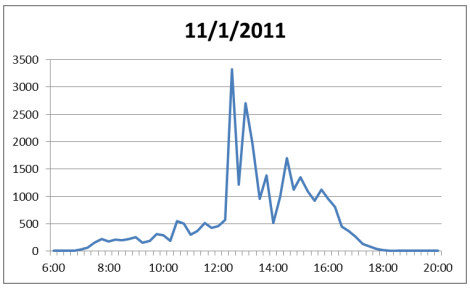



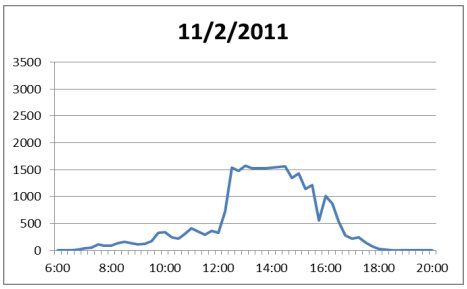



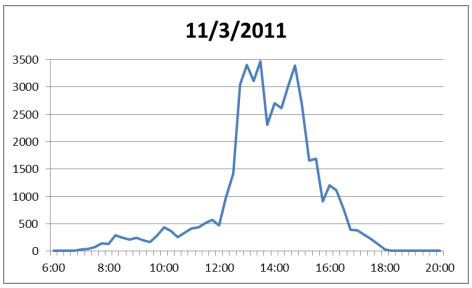



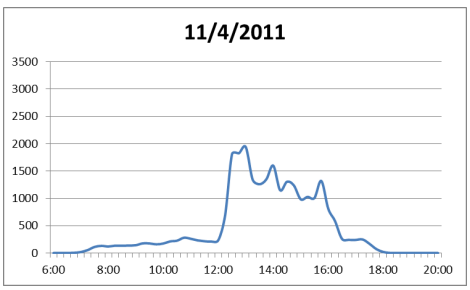



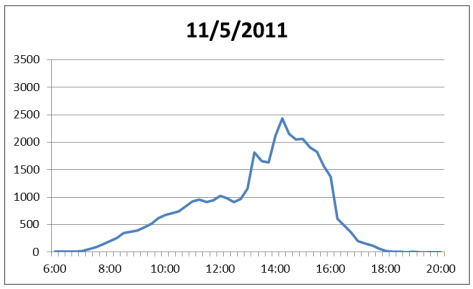



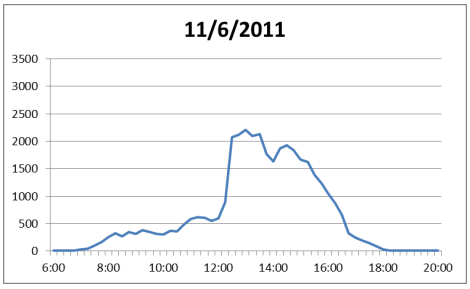



Appendix 2: *Gracilaria coronopifolia* 23S rDNA UPA sequences

Sequences identical for all *G. coronopifolia* populations at all collection locations.

>07534_UPA_Gracilaria coronopifolia_Paiko Lagoon

GCTTTACTGTAGCTTGGAATTGAATTTGGGTTTAATTTGCGCAGTATAGGTGGGAGGCAAA
GAATATATGTTTGTGGATATATATGAGCCACTAGTGAGATACCACTCTGATTAAACTAGAA
TTCTAATATTGACTGCCATAAGCTGGCCAATAGACAGTTTCAGGTGGGCAGTTTGACTGG
GGCGGTCGCCTCCTAAAAGGTAACGGAGGCGTGCAAAGGTTTCCTCAGGCTGGTCGGA
AATCAGTCGTAGAGTATAAAGGCATAAGGAAGCTTGACTGCGAGACTTACAAGTCGAGCA
GAGACGAAAGTCGGCCTTAGTGATCCGACAGTACTGAGTGGAAAGGCTGTCGCTCAACG
GATAAAAGTTA