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by

Kevin Scott Beach

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ABSTRACT

Stresses of the marine irradiance environment have resulted in a diversity of photoadaptative traits in macroalgae. As judged by in vivo absorption spectra, pigment identities in tropical macroalgae reflected the conservation of pigments within taxonomic groups. However, pigment concentrations and photosynthetic performance of tropical macroalgae were altered over very precise scales in response to irradiance stress. Specific inter- and intra-life cycle phase modifications of photosynthetic characteristics appear to contribute to the ecological success of both pioneer and perennial macroalgae.

For Ulva fasciata and Enteromorpha flexuosa, pioneer Hawaiian macroalgae, rates of photosynthesis of reproductive unicells can be equivalent to that of vegetative adults. Reproductive unicells of U. fasciata are low-light adapted compared to E. flexuosa. Concomitant with photosynthetic differences is pigment differentiation found in reproductive unicells of U. fasciata but not in cells of E. flexuosa. Spectral analyses indicated either formation of a novel-carotenoid or alteration of existing carotenoid ratios. Maintaining substantial rates of photosynthesis by achieving net-fixation at low irradiances may increase the likelihood that unicells will successfully colonize.

Ahnfeltiopsis concinna and Laurencia mederidiae, perennial Hawaiian macroalgae, exhibit marked pigment differences between canopy and understory tissues of the same thallus separated by <10 cm. Although both species decrease phycobilin concentrations and increase photoprotective pigment levels with increased irradiance, the specific phycobilins adjusted differ. Photoacclimation in A. concinna responded precisely to irradiance quantity but not quality. Concomitant with pigment adjustments was acclimation of photosynthetic performance. Laurencia mederidiae and A. concinna exhibited a sun- to shade-type acclimation over an extremely responsive scale. Understory tissues of A. concinna had higher $P_{\text{max}}$ than canopy tissues. This and other evidence indicates that canopy tissues of A. concinna were chronically photoinhibited.
Ahnfeltiopsis concinna is exposed to extreme levels of emersion stresses. Unlike other intertidal macroalgae, A. concinna did not exhibit increased temperature tolerance while desiccated. Temperature and osmolarity photosynthetic response curves demonstrated a broad range of tolerance, indicative of adaptation to high tidal elevations. Although canopy tissues of A. concinna are chronically photoinhibited, they have the physiological capacity to recover photosynthetic activity from emersion stresses more efficiently than do understory tissues from the same thallus.
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LIST OF ABBREVIATIONS

α : Quantum Efficiency

ANOVA: Analysis of Variance

APC : Allophycocyanin

CCI : Core Complex I

CCII: Core Complex II

Chl a : Chlorophyll a

Chl b : Chlorophyll b

Chl c : Chlorophyll c

dw: Dry Weight

FSW: Filtered Sea Water

fw: Fresh Weight

HPFD: High Photon Flux Density

Hsat: Hours at Saturating Irradiances

Ic : Compensation Irradiance

Ik: Saturation irradiance

LHCI : Light Harvesting Complex I

LHCII : Light Harvesting Complex II

LHPPC: Light Harvesting Pigment Protein Complex

LPFD: Low Photon Flux Density

MAAs: Mycosporine Amino Acids

MLLW: Mean Lower Low Water

PAR : Photosynthetically Active Radiation

PBS : Phycobilisome
PC : Phycocyanin
PE: Phycoerythrin
P_gmax: Maximum Rate of Gross Photosynthesis
P_max: Maximum Photosynthetic Rate
P_nmax: Maximum Rate of Net Photosynthesis
PFD: Photon Flux Density
PPFD: Photosynthetic Photon Flux Density
P:R ratio: Net Photosynthesis to Respiration Rate Ratio
PS I : Photosystem I
PS II : Photosystem II
PSU I: Size of Photosystem I Photosynthetic Unit
PSU O_2: Size of Photosystem II Photosynthetic Unit
Respir / R_d: Respiration Rate
R-PC: R-type Phycocyanin
R-PE: R-type Phycoerythrin
UV : Ultraviolet radiation (280 to 400 nm)
UV-A: Ultraviolet Radiation (320 to 400 nm)
UVACs: UV-Absorbing Compounds
UV-B: Ultraviolet radiation (280 to 320 nm)
PART I

A REVIEW OF THE LITERATURE ON THE IMPACT
OF ENVIRONMENTAL STRESSES ON PHOTOSYNTHESIS
IN MARINE MACROALGAE
Introduction

The tropical marine environment imposes a wide range of physiological stresses on resident marine algae. In these tropical environments, potential stresses such as irradiance, deiccation, and ultra-violet radiation are experienced at levels as or more extreme than those experienced by any macroalgae at higher latitudes. Irradiance can often be at levels greater than 2300 μmol quanta m\(^{-2}\) s\(^{-1}\) and may induce photoinhibition. Desiccation is caused by emergence from the marine environment which may be brief but that exposure when coupled with high air temperature (27°C) and nearly continuous winds, may combine to drastically inhibit photosynthesis and other physiological activity for potentially long periods (hours). Levels of UV-radiation in many tropical settings have recently begun to be assessed. UH Sea Grant researchers have found the amount of UV-B radiation experienced in Hawai‘i on a typical summer day is higher than levels under the ozone hole seasonally experienced at the tip of South America (S. Bogel, UH SeaGrant, pers. comm, Caldwell 1980). UV-radiation can damage the structure of DNA possibly as well as inducing physiologically harmful mutations. Because of the great capacity for physiological stress that may be experienced with the interaction of these factors, selective pressures for physiological tolerance or acclimation to these stresses may be of more importance to tropical intertidal species than temperate macrophytes. This pattern is likely even though tropical intertidal zones are relatively compressed when compared with high latitudes (Stephenson & Stephenson 1972).

The compressed vertical nature of tropical intertidal zones takes on greater ecological significance because in addition to low tidal amplitude, heightened herbivory in subtidal regions results in high levels of interspecific competition in tropical intertidal areas. Because all energy for metabolic processes is derived ultimately from photosynthesis, maximizing photosynthetic performance for the conditions under which an alga grows and reproduces is essential for competitive success in pre- and post-settlement interactions.
Without the capacity to photoacclimate or to certain environmental stresses (e.g., decreased irradiance, spatially separated life cycle phases), an alga's competitive ability may be curtailed and may ultimately be biologically outcompeted.

The model of adaptive specialization by Grime (1979) which has been considered appropriate for macroalgal communities (Kain & Norton 1990), recognizes three viable strategies: (1) exploitation, the ability to preemptively capture (exploit) limiting resources; is successful in regions of low stress (phenomena which restrict photosynthetic production (Grime 1979)) and low disturbance (partial or total destruction of plant biomass (Grime 1979)); (2) a ruderal strategy, the ability to withstand or recover from disturbance and still capture limiting resources; occurs in response to low stress and high disturbance; and (3) stress tolerance, adaptation for enduring suboptimal physiological conditions, occurs in response to high stress and low disturbance. Two of these strategies, ruderal and stress tolerance, will be explored in the context of potential photoacclimations and / or photoadaptations of two types of model algal systems. First, the photosynthetic capacity of distinct life cycle stages in two ruderal species will be explored as how they relate to spatial separation of benthic and planktonic phases. Secondly, stress tolerance will be examined as a mechanism by which perennial rhodophyte turfs persist in Hawaiian intertidal bench communities. Their photosynthetic, PAR as well as UV-absorbing pigment constituents will be contrasted with an assemblage of tropical reef macrophytes from evolutionarily diverse lineages.

**Ruderal strategists:** Similarly pigment differentiation has been noted to take place between reproductive and vegetative portions of ruderal species in the genera, Ulva and Enteromorpha. Typically, zoospore or gamete producing tissue appears as "pale yellow" to the naked eye but can be 'olive-green' in some species such as Ulva fasciata. The photosynthetic capacity of algal reproductive unicells has largely been understudied. Planktonic unicells of benthic macroalgae have commonly been reported to have brief
viability, 24 h to 11 days under laboratory conditions (Kain 1964, Hoffmann & Camus 1989, Santelices 1990). This ephemeral nature has been partially attributed to protoplastids in newly released propagules (McBride & Cole 1971, Toth 1976, Borowitzka 1978, Santelices 1990), resulting in greatly reduced photosynthetic rates (Kain 1964, Santelices 1990). In only two studies to date, net positive photosynthesis has been demonstrated in algal reproductive unicells of a macroalgal life cycle.

Planktonic stages are transient but crucial bottlenecks in macroalgal life cycles (Chapman 1984). The period of viability in planktonic life-cycle stages is enhanced when they can feed or have a yolk sac compared to non-feeding larvae (Thorson 1950, Olsen 1985). Planktonic reproductive unicells of macroalgae that are photosynthetically competent upon release could similarly extend a "window of viability", leading to increased success in settlement and recruitment. Photoadaptive strategies for particular irradiance regimes common to coastal environment may further enhance the success of reproductive unicells over those without such a capability. The photosynthetic characteristics of only a few reproductive unicells of temperate brown algae have been characterized (McLachlan & Bidwell 1978, Amsler & Neushul 1991). Other algal groups with markedly different photosynthetic pigments, life-cycle strategies, as well as latitudinal distributions remain unexamined.

Stress tolerance strategists: Stress tolerance, which has been recognized as an essential strategy for high intertidal macrophytes (Connell 1960, Smith & Berry 1986), is used to diminish competition by growing in an area where competition is reduced. This has been termed avoidance. Avoidance can be accomplished by being able to tolerate some stress or combination of stresses that most organisms cannot withstand. The high intertidal zone in tropical and temperate environments provides an area in which biological competition may be reduced because few can survive the exposure to high levels of physiological stress.
Two processes, tolerance and acclimation, allow for survival within a stressful habitat. Tolerance to a physiological stress implies the endurance of that stress without the gradual reversible phenotypic alteration of physiology for better or status quo performance while under that stress. The latter is termed acclimation (Lincoln et al. 1982). Extremes of a physiological stress, which high intertidal tropical algae may frequently experience, require either physiological tolerance for survival or acclimative ability for physiological and potentially subsequent ecological success. Because of the essential nature of photosynthesis for algal survival and the potential effect of the above stresses in tropical environments on photochemical processes, the capacity for acclimation in algal photosynthetic physiology may be integral feature of tropical intertidal existence.

The ecophysiological consequences of living in the intertidal zone have been addressed for many temperate intertidal algae (Matta & Chapman 1991, Smith & Berry 1986, Waaland et al. 1974). Studies concerned with macroalgal photoacclimation have concentrated on temperate algae and the pigment changes observed over water depth gradients in which the algae are found (Lubimenko & Tichovskaya 1928, Rhee & Briggs 1977, Ramus et al. 1976, Waaland et al. 1974), pigment differences between sun versus shade grown algae (Falkowski & Owens 1980) and differential photosynthetic ability of different parts of large macrophytes like Macrocystis and Sargassum (Sakanishi et al. 1991, Gao 1991, Aruga et al. 1990, Arnold & Manley 1985).

Macroalgal acclimations to the tropical environment have not been examined but a better understanding of intertidal community construction will be gained by better understanding the diversity of methods and extent of photoacclimation in high intertidal tropical algae. This examination is likely to expand the breadth of understanding of the photosynthetic flexibility in marine algae in general. Specifically, the study of tropical species is also likely to extend our knowledge of stress tolerance ranges and possibly
discover new mechanisms of stress tolerance that have allowed many tropical algae to be successful in this environment.

Studies by Doty (1967), Liang (1984), and Gil-Rodriguez and Haroun (1992) have directly or indirectly commented upon the diversity of pigmentation phenotypes found in many tropical red algae. Modifications in the amount and/or type of accessory pigments found in two Hawaiian algal species, Ahnfeltiopsis concinna (J. Agardh) Silva and DeCew and Laurencia mcdermidiae (J. Agardh) Abbott, seem to represent two distinct potential solutions to stresses imposed by a intertidal environment. Both species dramatically alter their pigment composition in canopy portions with respect to understory parts of the same upright thallus in the space of less than a decimeter.

In both model systems with ruderal and stress tolerant strategies these examples, ruderal chlorophytes and rhodophyte turfs, respectively, the ecophysiological implications of these pigment differentiations and the underlying mechanism remains unexplored. Understanding the consequences, methods of regulation, and relative occurrence of these photoacclimations / photoadaptations will provide insights into methods of successfully dealing with extremes in photophysiological stress, such as UV-radiation, that are becoming more influential with the precipitous degradation of the world's ozone layer and global warming.

**Photosynthetic pigments in marine macroalgae**

All marine macroalgae carry out oxygenic photosynthesis utilizing chlorophyll a (Chl a) in their reaction centers. Chl a is the only chlorophyll present in red algae; green algae posses Chl b in their antenna and Chl c is found in brown algae. Rhodophytes are unique among eukaryotes by using phycobilisomes (PBS) as photosynthetic antennae (Redlinger & Beale 1991). Many different carotenoids have been documented throughout macroalgal species (Goodwin 1980). No positive role in photosynthetic energy transduction has been adequately documented in rhodophytes to demonstrate that
carotenoids are photosynthetically active with either photosystem (see: Carotenoids). In contrast to other oxygen evolving eukaryotes, red algae do not have stacked thylakoids and also appear to lack the spatial segregation of photosystem I (PS I) and photosystem II (PS II) that is present in most plants where PS I is primarily in the stroma lamellae and PS II in the stacked grana regions (Taiz & Zeiger 1991, Gantt 1990). Chlorophytes and phaeophytes have their thylakoids stacks (grana) of 3-6 and 3's, respectively. In the following three sections, the photosynthetic pigments of marine algae will be discussed in more detail. Chlorophylls, phycobiliproteins, and carotenoids will be examined for form and function in the photosynthetic process and how these pigments relate to macroalgal ecophysiology.

**Chlorophyll:** The chlorophylls are generally defined as Mg-containing tetrapyrole molecules that function in the light harvesting and photochemical processes (Redlinger & Beale 1991). Only one form of chlorophyll, Chl a, has been assigned a photosynthetic function in red algae. Chl a is also functional in all chlorophytes and phaeophytes with the addition of Chl b and Chl c in each division, respectively. "Chlorophyll d has been reported in extracts of Gigartina but no confirmation of this pigment has been found in vivo and no functional role has been established. Even if it is not a degradation product, which is quite possible, chlorophyll d does not occur in sufficient quantity to have been designated as making a significant contribution to the action spectrum of any red alga (Gantt 1990)". Therefore, the subsequent discussion and experiments will strictly deal with Chl a as the only chlorophyll in the division Rhodophyta.

Chlorophyll(s) is (are) integral in the photosynthetic process and possesses several functions which are detailed by Redlinger and Beale (1991) in a recent review. Because of this review's comprehensive nature, much of the following discussion will be drawn from this source. "Chlorophylls can be depicted as having three separate distinct roles in photosynthesis: energy collection, energy transmission, and energy transduction."

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The bulk (99.5%) of Chl a in algae and embryophytes is involved in energy collection as an antennae pigment. The Chl a associated with PS I (P700) and PS II (P680) account for less than 0.5% of the total chlorophyll. Reaction center Chl a is the site of primary photochemistry (Gantt 1990). In contrast to embryophytes and green algae that possess large Chl a and b antennae and phaeophytes which possess large Chl a and c antennae associated with both photosystems, the bulk of chlorophyll in red algae is a major contributor to PS I with up to 80% of the Chl a associated with this complex (Wang et al. 1977). In algae with phycobilisomes (Rhodophyta and Cyanophyta), the amount of Chl a associated with light harvesting complex II (LHClI) is small (Larkum & Barrett 1983).

The absorption spectrum of Chl a is bimodal in distribution with absorption maxima at 430 and 660 nm (in ether) (Redlinger & Beale 1991). The standard Chl a absorption spectrum is in actuality the summation of the absorbance of various spectral forms of Chl a. These spectral forms, which are pigment aggregates of dissimilar energy levels (Redlinger & Beale 1991), in the light harvesting complex compose a resonance energy transfer chain which in the red algae could transfer energy principally to P700 (Lawlor 1987, French et al. 1972). The accessory chlorophylls, Chl b and Chl c, also have bimodal absorption spectra with absorption maxima at 455 and 642 nm for Chl b and 445 and 628 nm for Chl c. Shifts in the absorbance spectra for these accessory chlorophylls is the result of the replacement of a hydrogen with a oxygen atom from the Chl a structure resulting in Chl b; while Chl c lacks the phytol tail present in both Chl a and b (Rowan 1989).

Light-energy transduction by Chl a is accomplished by reaction center chlorophyll via its donation of an excited electron to phaeophytin. The sequence of subsequent redox reactions known as the Z-scheme, results in the formation of ATP and strong reductants that are utilized in the enzymatic processes of photosynthesis as well as other metabolic processes (e.g., nitrogen metabolism). "The ultimate source of electrons, in oxygenic photosynthesis, is water" (Redlinger & Beale 1991). The splitting of water by the Mn rich
DL protein associated with PS II, is the ultimate source of electrons for this series of PS II reactions. Accompanying the transfer of electrons to PS II, oxygen is also released by this reaction. The rate of oxygen evolution has been utilized as an indicator of photosynthetic rate and is a better indicator of actual photosynthetic activity than carbon assimilation because ATP and reducing power produced by the light reactions may not be exclusively used for carbon fixation (Geider & Osborne 1992, Smith et al. 1992).

**Phycobiliproteins:** The phycobiliproteins are composed of phycobilin chromophores bound to a protein complex by a cysteiny1 residue. A generic phycobilin molecule is a straight chain tetrapyrrole formed by the oxidative opening of a porphyrin ring (Goldsworthy 1991). There are three common types of phycobilins: phycocyanobilin, phycoerythrobilin and phycourobilin that are found in the red algae (Rowan 1989). Phycocyanobilin is the principle chromophore in two phycobiliproteins, allophycocyanin (APC) and C-phycocyanin (C-PC). Phycoerythrobilin is the chromophore in C-phycoerythrin (C-PE) and it occurs in combination with phycocyanobilin in R-phycocyanin (R-PC) and with phycourobilin in R-phycoerythrin (R-PE) (Goldsworthy 1991). The vast array of different functional forms of phycobiliproteins is attributable to differential arrangement of these chromophores in the alpha and beta polypeptide subunits in the PCs and APCs and an additional gamma subunit sometimes found in forms of PE (Gantt 1990). The phycobiliproteins are arranged into a macromolecular complex known as a hycobilisome (PBS), being bound together by colorless linker polypeptides that ensure proper orientation of the phycobiliprotein in this extrinsic membrane assembly (Gantt 1990). PBSs have been found to be hemi-discoidal, hemi-ellipsoidal or hemi-spherical in shape (Gantt 1990) and generally consist of core APC with an array of rod-like structures protruding from that core. The rods consist of PC and PE with the former being closer to the core (Goldsworthy 1991). This arrangement allows for the transfer of excitation energy from shorter wavelength / higher energy absorbing PE to adjacent PC molecules.
that are energetically "downhill" in terms of characteristic absorption wavelengths. Subsequently excitation energy passes to the core and eventually to PS II (Lobban et al. 1985). Transfer of excitation energy can occur between the PBS and PS I but only with the closure of PS II and 'spillover' from PS II to PS I by mechanism(s) still largely undescribed (Gantt 1990, but see Satoh and Fork 1986).

The particular light absorption properties of different phycobiliproteins vary within the division Rhodophyta; different absorption maxima and shoulders may occur and depend upon the chromophore and polypeptide configuration (see Rowan 1989). In general, the phycobiliproteins principally absorb in the green to yellow (450-650 nm) portions of the visible light spectrum, absorbing a region of photosynthetically active radiation (PAR) that is not extensively used by Chl a. Generally, PE primarily absorbs light in the shorter, higher energy 450-550 nm region, PC in the 525-625 nm range and APC in the 575-650 nm region. The wavelength overlap, coupled with the structure as discussed above provide the funnel necessary for favorable "downhill" resonance energy transfer and subsequent energy transfer to PS II (Taiz & Zeiger 1991).

Another overall effect from the usual abundance of phycobiliprotein in most red algae is to give the algal thalli a reddish color, hence the divisional designation Rhodophyta. Many investigators have noted exceptions to this generalization however, even in non-tropical genera like Chondrus, Gelidium, Gracilaria, Mastocarpus, Gigartina (Rhee & Briggs 1977, Ramus et al. 1976, Waaland et al. 1974, Lubimenko & Tichovskaya 1928, pers. obs.). "This variation in pigmentation is particularly striking in red algae where specimens of a given species may be pale yellow-green when found in the intertidal and deep purple when gathered from 10 to 20 m in depth" (Waaland et al. 1974). This change in color has been attributed to a increase in the ratio of phycobiliproteins to Chl a (Ramus et al. 1976) and has been shown to be driven by changes in ambient irradiance (Ramus 1983, Rhee & Briggs 1977, Waaland et al. 1974). Changes in irradiance
wavelength with increasing water depth may also drive this physiological shift (Ramus et al., 1976). The ecophysiological implications of increasing accessory pigment concentration versus Chl a concentration is to acclimate the alga to a photosynthetically challenging environment, optimizing photosynthetic capacity to ambient irradiance, in a pattern analogous to shade vs. sun acclimations studied in many terrestrial systems.

All of the studies mentioned above involve an examination of temperate algae and their pigmentation over extensive spatial gradients; no such studies of any scale have focused on any tropical alga, where the amount and type of irradiances impacting a thallus in the intertidal zone is potentially more dynamic than most other habitats. Many "red" algae in the tropics are noted as having non-red portions of their thalli (Gil-Rodriguez & Haroun 1992, Liang 1984). Some Hawaiian rhodophytes appear to increase phycobiliprotein content but over a greatly reduced scale. Instead of over meters of population distribution as discussed above, subtropical algae drastically alter pigment content within the centimeters of an individual thallus. This modification appears to have been taken to its extreme in species such as Ahnfeltiopsis concinna and the green ecotype of Laurencia nidifica. Even with the highly compressed intertidal zone found in Hawai'i that greatly limits the potential for extensive vertical distribution, these algae have developed the capacity to spatially alter their pigmentation within a <10cm distance.

**Carotenoids:** The carotenes, α- and β-carotene and the corresponding xanthophylls, lutein and zeaxanthin are the usual carotenoid pigments of red algae (Goodwin 1980). Phaeophytes are characterized by the presence of fucoxanthin while siphonoxanthin and siphonein are characteristic of several orders of chlorophytes (Rowan 1989). Liang (1984) also reports that antheraxanthin is found more frequently than reported earlier in her survey of 30 Hawaiian rhodophytes. Several recent reviews (Goodwin 1991, Rowan 1989, Goodwin 1980) have thoroughly addressed the structure, function and evolution of carotenoids in plant as well as animal systems. The following
discussion focusing on types of carotenoids in red algae and their possible functions is largely excerpted from these reviews. Carotenoids are tetraterpenes and consist of 8 isoprenoid units. Over 500 carotenoids are known but all are related in their structure and their synthesis to lycopene. The carotenoids are of two types: the carotenes which are hydrocarbons and the xanthophylls which are carotenoid molecules containing at least one molecule of oxygen. The carotenoids of algae and more derived plants are found in pigment-protein complexes. In angiosperms, β-carotene is associated with the Core Complex I (CCI) of PS I and the Core Complex II (CCII) of PS II with one molecule in the former and seven in the latter (normalized per pair of cytochrome b molecules). Leaf xanthophylls are associated with the light harvesting complexes of both PS I (LHCI) and PS II (LHClII).

Chloroplastic carotenoids reportedly have two functions in photosynthetic organisms: 1) photoprotection, and 2) light harvesting via excitation energy transfer (Larkum & Barret 1983). Photoprotection is accomplished by carotenoids by a triplet–triplet exchange reaction with photogenerated triplet chlorophyll ($^{3}\text{Chl}^*$). The action of triplet state chlorophyll on ground state oxygen produces a singlet oxygen radical that can be lethal to biological systems because of its oxidizing ability. $^{3}\text{Chl}^*$, produced by the further excitation of singlet state chlorophyll ($^{1}\text{Chl}^*$), is returned to its ground state via triplet-triplet energy exchange with a carotenoid. The triplet carotenoid ($^{3}\text{Car}^*$) decays to the ground state by dissipating the energy over the conjugated and unconjugated carbon double bonds releasing excess energy harmlessly as heat. For carotenoids to be effective photoprotectors, their triplet-triplet exchange rate must be faster than the rate of $^{3}\text{Chl}^*/^{3}O_2$ interaction; that is indeed the case. The speed of the triplet-triplet exchange rate is attributable to the very close proximity of these molecules to LHCI and LHClII. Carotenoids found in red algae primarily absorb irradiance between 400-500 nm (Taiz & Zeiger 1991) with peak absorbances between the short wavelength peak of Chl a and PE.
Light harvesting and energy transfer to either PS I or PS II by carotenoids has been demonstrated by in vivo fluorescence excitation spectra in several divisions of photosynthetic organisms. Oquist et al. (1980) demonstrated the photosynthetic role of \( \beta,\beta \)-carotene associated with PS I in *Pisum sativum* (pea), *Triticum aestivum* (wheat), and *Scenedesmus obliquus* (Chlorophyta). Ulva japonica, a deep water chlorophycean alga was found to utilize siphonaxanthin as a light harvesting pigment for PS II (Kageyama et al. 1977). Both fucoxanthin and peridinin have energy transfer efficiencies up to 100% in the Chl a/c pigment protein complex of brown algae and diatoms, and the Chl a complex of dinoflagellates, respectively (Siefermann-Harms 1985, Song et al. 1976). Owens et al. (1987) demonstrated the photosynthetic light harvesting function of violaxanthin in *Nannochloropsis* spp. and concluded that violaxanthin is the major light harvesting pigment in the Eustigmatophyceae. Alberte and Andersen (1986) further demonstrated use of carotenoids in photosynthesis by documenting energy transduction by antheraxanthin to PS II in *Chrysoaphera magna*, a chromophyte alga. The function of these carotenoids was determined with the use of in vivo fluorescence excitation spectra and except for action spectra based on O\(_2\) evolution, this is the principal way to accurately assess pigment light harvesting function.

What should be noted in the above review is the lack of phycobiliprotein possessing photoautotrophs. Goodwin (1991) states that "considerable evidence exists for the efficient transfer of energy from carotenoids to chlorophylls in both PS I and PS II in algae, including the red algae and the cyanobacteria." Gantt (1990) in her review of pigmentation and photoacclimation in red algae, states that "the contribution of specific carotenoids to photosynthesis is not clear, except for \( \beta \)-carotene, which is active in PS I and probably also in PS II. Larkum and Weyrauch (1977) examined the photosynthetic action spectra of *Griffithsia monilis* and attributed part of the spectra (400-480 nm) to a possible carotenoid function. But the +/- 5nm band width used to generate the action spectra and the overlap of
the phycobiliproteins and Chl a action spectra with this region provide less than compelling evidence for a photosynthetically functional role for carotenoids in red algae. Goedheer (1969) discusses the transfer of energy from carotenoids to chlorophyll in the red alga *Porphyridium cruentum* and concludes that in red algae energy transfer from beta-carotene to chlorophyll occurs in PS I exclusively. The pigment protein complexes of *P. cruentum* have been shown to posses β-carotene in both CCI and CCII (Redlinger & Gantt 1983) but in Goedheer's examination of this alga's fluorescence emission spectrum, he used fragments of the chloroplasts that are washed free of phycobiliproteins. Goedheer stated that "it proved to be difficult to remove PE from the chloroplasts to such an extent that the band of PE was completely absent from the absorption spectrum", also polar solvents were utilized in extraction of the carotenoids from the sonicated chloroplast fragments. This method can produce large structural and spectral changes (Searle & Wessels 1978) that are not attributable just to the loss of carotenoids. With these complications, the role of carotenoids in energy transfer in red algae remains tentative. To unequivocally resolve the dilemma of the function of different carotenoids found in red algae, a system that lessens the confounding effects of the phycobiliproteins offers a novel means by which the basic arrangement of the pigment-protein complexes can be examined.

**Photoacclimation and photoadaptation**

Photoacclimation refers to the expression of the photosynthetic related adjustments that an organism can make to its light environment within the limits of its genome, whereas alterations in the genome that effect the photosynthetic process is more accurately designated photoadaptation (Gantt 1990). Several ecophysiological modifications are shared between photoacclimation and photoadaptation. Most algal studies that have examined photoadaptation have actually examined photoacclimation (Vonshak & Guy 1992, Cota & Sullivan 1990, Falkowski & Owens 1980, for an exception see Kuebler et al. 1991.). The responses (acclimations and adaptations) of photosynthetic organisms to
decreasing irradiance or shade have been the focus of extensive research on herbaceous plants, woody trees and marine algae (Kozlowski et al. 1991, Friend 1984, Boardman 1977, Rhee & Briggs 1977, Waaland et al. 1974). Shade-tolerant plants generally have lower respiration rates and consequently lower light compensation points for photosynthesis ($I_c$) than shade intolerant plants. Shade tolerant plants also have lower saturation thresholds for photosynthesis ($I_k$). At subsaturating irradiances, shade grown plants typically have a higher quantum efficiency ($\alpha$) than sun grown plants, whereas at saturating irradiances, high-light grown plants generally have a higher maximum rate of photosynthesis ($P_{max}$) than low light grown plants (Kozlowski et al. 1991). As seen in comparisons between canopy and subcanopy plants in terrestrial forests (Kozlowski et al. 1991), there is an increase in photosynthetic pigment levels in many macroalgae with decreased irradiance levels found with increasing water depth (Rhee & Briggs 1977, Ramus et al. 1976, Waaland et al. 1974, Lubimenko & Tichovskaya 1928).

The mechanism(s) responsible for these differences has been characterized for some terrestrial plants, macroalgae as well as phytoplankton (Kozlowski et al. 1991, Ramus 1981). Two possibilities, discussed by Lobban et al. (1985), can account for photosynthetic differences between high light and low light acclimated plants. "An alga may acclimate by increasing the number of photosynthetic units (PSUs), or by increasing the size of each PSU (Lobban et al. 1985)." The studies that have examined seaweeds to determine if they modify either the size or number of PSUs (Ramus 1981) have assumed that they can determine this by an examination of the characteristics of the net performance estimated via a now standard photosynthesis versus irradiance curve (P vs. I curve) of acclimated individuals of the same species (Lobban et al. 1985, Ramus 1981). In fact, the same characteristics of the P vs. I curve that change with an increasing antennae size ($I_k$, $I_c$, $\alpha$, but not $P_{max}$) should change in the same manner with increasing number of PSUs without increasing the number of Z-scheme components. $P_{max}$ may not be higher as
predicted by Ramus (1981) with an increase in the number of PSU's because of other rate limiting steps beyond the light harvesting role of the PSU. An evaluation of the adjustments made by photosynthetic organisms is best accomplished by the direct determination of PS I and PS II sizes (see Cunningham et al. 1989). This type of evaluation has not been extensively used to evaluate adjustments to high or low light levels in macroalgae. The capacity to photoacclimate within a life cycle phase or photoadaptation between life cycle phases may enhance the fitness of an individual by facilitating substantial rates of productivity even in the presence of less than ideal environmental conditions. In tropical regions with elevated temperatures and PAR and UV-irradiances, the capacity for precise photoacclimative responses or phase specific photoadaptations may be crucial for macroalgae in particular stress filled niches.

**Environmental factors affecting intertidal algae**

Dawson (1966) evaluated the factors that need to be considered when studying a marine community (Dawes 1980). These factors fall into the four categories: Physical, Chemical, Biological and Dynamic factors. The interaction within and among these general categories are complex and are not easily separable for experimental examination. Several aspects of these interactions may profoundly influence the photosynthetic physiology of intertidal marine macrophytes. Irradiance, effects of emersion from the marine environment, and factors enhancing the potential severity of these two potential stresses will be discussed in the following sections in the context of their impact on algal ecophysiology.

**Irradiance:** Irradiance is the single most important physical factor affecting oxygenic photosynthesizers. Without the sun's energy most if not all forms of primary production would cease. In general, the wavelengths of irradiance reaching the earth's surface range from 280 nm (UV-B) to 800 nm (infrared). The total intensity and spectral quality of energy are affected by the solar angle and atmospheric conditions. The quantity
and quality of irradiance is further altered when it reaches the air-water interface by reflection at the interface and absorption/scattering in the water column (Jerlov 1968). The quantity of irradiance decreases in the water column with increased depth. The quality of irradiance also changes, narrowing from full spectrum PAR (400-700 nm) to either green, in coastal waters, or blue, in oceanic waters, portions of the PAR spectrum (Jerlov 1976). The rate of these two attenuations depends upon the type of water (Jerlov 1976). The impact of this attenuation on the algal light harvesting is to drive acclimations to shade conditions by: increasing the accessory pigment to Chl a ratio, decreasing dark respiration rate, decreasing $P_{\text{max}}$, and increasing $\alpha$ and $I_k$ (see Photoacclimation) (Rhee & Briggs 1977, Ramus et al. 1976, Brown & Richardson 1968, Lubimenko & Tichovskaya 1928).

Life cycles of benthic seaweeds are characterized by a planktonic gamete or spore phase. Planktonic reproductive unicells may face a range of irradiance regimes before settlement. Upon release several important hydrodynamic processes influence the distribution of these reproductive unicells. Surface currents sweep cells away from the sites of potential settlement and / or other compatible gametes can reduce the probability of settlement (Roughgarden et al. 1988). With the capability of satisfying metabolic and motility demands by efficient photosynthesis, the temporal window for fusion and / or settlement may be extended. This possibility could increase the ecological range of a particular population and would increase potential for outcrossing among at least local populations. Downwelling currents would similarly alter zoospore and gamete distribution. Reproductive unicells that have the capacity to reach $P_{\text{max}}$ at lower irradiance levels (e.g., have a low $I_C$ and $I_k$) and have the capability to harvest the quality of irradiance present in turbid coastal waters may prosper in these conditions while those requiring higher irradiance levels to reach $I_C$ and $I_k$ may not remain viable when transported away to depth from near-surface waters.
Epilithic seaweeds grow in exceptionally dynamic and diverse light environment. The continuous ebb and flow of tides have a profound effect on the quality and quantity of irradiance reaching seaweeds (Lobban et al. 1985). Periodically, intertidal algae are emerged in full sun light and subsequently submerged into an environment with reduced irradiance quantity and quality. The extent of the impact of this stress on the alga is dependent upon its position relative to the mean low tide level (in Hawai’i: mean lower low water) as well as presence of shading factors such as a similarly or differently pigmented canopy. The capacity to respond to changes in the irradiance environment and to tolerate the extremes of emersed irradiance levels and/or tidal induced/self-shading may give certain macroalgae a selective advantage in intertidal environments.

Emersion: Intertidal algae are subjected to periodic emersion from the ocean. The duration of exposure to air is directly effected by the period and amplitude of the tide, the extent of non-tidal water movement and the position of the alga on the shore. The tidal range in the Hawaiian Islands is small with spring tides never exceeding +1.0 m above mean lower low water (Armstrong 1983). The extent of non-tidal water movement, long/short period swell, and surface chop is highly variable in the Hawaiian Islands (Magruder 1977) and can completely negate the exposure effects of a low tide (pers. obs.).

The primary effect of algal exposure to air is the loss of water from algal tissues. This gradient in osmotic potential occurs even with air relative humidities as high as 96%. The loss of water from algal tissues in temperate latitudes alters normal photophysiological function from which the ability to recover is correlated with vertical position in the intertidal zone (Smith & Berry 1986), as well as directly determining upper distributional limits of lower and mid intertidal species and ultimately species with high distributional limits (Smith & Berry 1986, Dring & Brown 1982).

Magruder (1977a) proposes that the upper limit of Ahnfeltiopsis concinna is controlled by desiccation. His observations of uppermost fronds being killed by periods of
very small waves, neap tides, and no cloud cover support this surmise. The ability and mechanisms of high intertidal algal survival in response to a desiccation event in a high light (including UV-irradiance) subtropical environment is an unaddressed area of macrophyte photoecophysiology. An understanding of the macroalgal responses to high PAR and UV levels will provide critical insight into a stress response that may become more influential in coming times.

**Organisms of primary interest**

Species of the weedy genera *Ulva* and *Enteromorpha* are generally the first macroalgae to colonize open substrate and are considered troublesome fouling organisms. This ecological role is attributed to their simple adult morphologies and to their fecundity. With the formation of propagules, *Ulva* and *Enteromorpha* species typically undergo a change in pigmentation (Haxo and Clendenning 1953). The margins of *U. fasciata*, a common Hawaiian pioneer alga, appear dark green to olive in coloration; while the apices of *E. flexuosa* appear light-green to yellow. The pigmentation of reproductive unicells in these two examples is clearly different than the vegetative adult tissues (Smith 1947). Propagule release resulting from a sporic meiosis life cycle may occur daily for species in low latitude populations. For *Ulva* spp., 20 to 60% of overall biomass is allocated monthly to reproduction depending upon season (Smith 1947, Niesembaum 1988). Spore release in intertidal *Ulva* and *Enteromorpha* species is driven by tidal / lunar rhythms (Smith 1947, Christie & Evans 1962, Sawada 1978). Reproductive unicells of *E. intestinalis* can remain motile for up to eight days after release if subjected to continuous irradiance and agitation; reduction in the irradiance period corresponds to a shortened period of motility (Jones & Babb 1968). Even low rates of photosynthesis by spores or gametes of *Ulva* and *Enteromorpha* species could extend both their viability and dispersal range while subsequently contributing to the success of these species over taxa with shorter-lived or physiologically less-competent cells. With planktonic phases as a crucial
bottleneck in macroalgal life cycles (Chapman 1984), adaptations to a planktonic niche however brief may contribute to the ecological success of pioneer species such as _U. fasciata_ and _E. flexuosa_.

_Ahnfeltiopsis concinna_ (J. Agardh) Silva and DeCew in the order Gigartinales, is a perennial intertidal alga that grows almost exclusively on intertidal basalt benches on the high islands of the Central and Eastern Pacific (Doty & Santos 1973, Magruder 1977a). The life cycle of _A. concinna_ was determined by Magruder (1977b) to involve an alternation of heteromorphic generations with up to 15 cm upright gametophytes and crustose tetrasporophyte. The tetrasporophyte grows in the intertidal zone (Magruder 1977a 1977b) while the gametophyte is the highest growing conspicuous macroscopic alga on prehistoric steep basalt shores in the Hawaiian Islands (Doty 1967). Gametophytes of _Ahnfeltiopsis concinna_ recruit on newly formed basalt substrate as early as four and a half years after the initial lava flow replacing the pioneer algae _Cladophora_ spp., _Enteromorpha_ spp. and _Hinksia breviarticulata_ (Doty 1967).

_Ahnfeltiopsis concinna_ has been recorded from all the high Hawaiian islands (Bishop Museum collections) and was collected as early as 1876 by H.N. Mosley on the Challenger Expedition. Dickie, (1876) made the first observation as to the pigment modification that is found in _A. concinna_. He, as well as M.S. Doty, note "that this bunchy "red" alga often forms a yellow-brown canopy on the rocks, not unlike that formed by _Fucus_ or _Pelvetia_ on North American and European shores" (Doty & Santos 1973, Doty 1967, Dickie 1876). The yellow appearance of the canopy portions this alga can be attributed to one or some combination of the four carotenoids, two carotenes, α-carotene and β-carotene, and two xanthophylls, lutein and zeaxanthin, documented in _A. concinna_ by Liang (1984). Unlike the canopy portions of the thallus, the understory portions of _A. concinna_ are characteristically red to black (pers. obs.) in appearance presumably attributable to a prevalence of phycobiliproteins.
Laurencia mcdermidiae (J. Agardh) Abbott, in the order Cerami ales is one of at least fifteen species of Laurencia in Hawaiian Islands (McDermid 1988a). Laurencia mcdermidiae was considered by Saito to be an ecotype of L. nidifica. According to McDermid (1988b), the green ecotype of L. nidifica lacks lenticular thickenings and possesses different biochemical compounds than L. nidifica. For these reasons, McDermid (1988b) suggests that Laurencia sp. "green" be recognized as separate species. Recently this change was made by Dr. I. A. Abbott (Abbott, in prep). Laurencia mcdermidiae is distributed throughout the high Hawaiian Islands with collection records from Kaua‘i, O‘ahu, Moloka‘i, Lana‘i (Bishop Museum collections), Maui (Abbott, pers. comm.) and Hawai‘i (pers.obs.) and is found in the lower intertidal zone intermingled with other turf algae (pers.obs.). This habitat is similar to that occupied by Laurencia viridis in the Macaronesian Archipelago (Gil-Rodriguez & Haroun 1992). Laurencia mcdermidiae is one of seven species of Laurencia from around the world, that have been observed to possess a green color in at least the canopy portions of its thalli (Gil-Rodriguez & Haroun 1992).

The understory stoloniferous portions of this alga, as with A. concinna, conform to more "typical" red algal pigmentation by presumably being phycoerythrin rich. With this seemingly not uncommon modification in the distribution of the photosynthetic accessory pigments, it is surprising to find a complete lack of research addressing the ecological function of such marked photosynthetic acclimations.

The pigment dichotomies in A. concinna, L. mcdermidiae, U. fasciata and E. flexuosa remain uninvestigated in terms of their causes, their distribution in respect to position in the intertidal zone, the role it plays in the overall photosynthetic and competitive performance of these algae, and the role of the individual pigments in relation to their position in the thallus as well as in the photosynthetic machinery.

Research objectives

The proposed research has three primary components: 1) Evaluation of the in vivo
absorbance characteristics from 280 to 700 nm and UV-absorbing compounds of select intertidal macrophytes, 2) Examination of the photosynthetic capacity of different life cycle stages, specifically planktonic and benthic phases, in Ulva fasciata and Enteromorpha flexuosa, 3) Analysis of the ecophysiological implications of microclimate differences between canopy and understory tissues of A. concinna and L. mcdermidiae.

The objective of the first component is to provide information on the spectral characteristics and photoadaptive responses for several common tropical reef macrophytes. This survey will develop a baseline of comparison to which other tropical macrophytes can be compared as well as allowing comparison between temperate and tropical macroalgae (Smith and Alberte 1994).

To accomplish the second objective I seek to evaluate aspects of the photosynthetic and respiratory competence of different stages in the life cycle of Ulva fasciata and Enteromorpha flexuosa. Specifically, I address the following questions: (1) What physiological changes resulted from the formation of reproductive unicells from vegetative cells? (2) Are gametes and zoospores similar in terms of photosynthetic performance and respiratory demands? (3) Do closely allied genera from similar physical environments produce unicells that are physiologically similar? (4) How do the photosynthetic performances of these subtropical unicells compare to that reported for unicells from other macroalgae? (5) Does the apparent photoadaptation by these unicells indicate overall adaptive strategy(ies) that may contribute to patterns in algal community composition and development?

The third component has four objectives. (1) To evaluate the possible differences in the photosynthetic attributes of the species of interest in terms of the net photosynthesis vs. irradiance curves, in vivo absorbance, sizes of PS I and PS II, and photosynthetic pigment composition of canopy versus understory portions of the A. concinna and L. mcdermidiae. (2) To examine and relate potential differences to the differential spatial
distribution of these species over gradients of irradiance and emersion stresses present in the intertidal zone. Differences detected in objectives 1 and 2 will be evaluated experimentally to determine causality. (3) To evaluate the significance intra-individual canopy effects on the photoacclimation responses of understory portions of *A. concinna*. (4) To determine the ability of *A. concinna* to tolerate emersion stresses in relation to canopy and understory portions of the thallus.

**Hypothesis**

The subsequent research was designed to address the following hypothesis:

1) Photosynthetic properties do not differ between nor within life cycle phases of select subtropical macroalgae.
Literature cited


PART II

IN VIVO ABSORBANCE SPECTRA AND UV-ABSORBING COMPOUNDS
IN TROPICAL REEF MACROALGAE
Abstract

In vivo spectra of tropical macroalgal absorbance were obtained for the first time, providing a baseline for future comparisons. The first examination of macroalgal tissue absorbances of UV-irradiance was performed. A marked consistency in in vivo and fourth-derivative maxima in the visible spectra region was observed from 400 to 750 nm for species within specific taxonomic divisions. In vivo spectral absorbances and extract characteristics from 280 to 400 nm indicated that concentrations of UV-absorbing compounds (UVACs) were higher among rhodophytes when compared to other algae. Fourth-derivative spectra resolved a consistent number of maxima between 280-400 nm but at variable positions, suggesting heterogeneity in the type and/or pools sizes of UVACs among the taxa examined here. Potentially photodestructive, UV-irradiance may be preemptively captured by UVACs as indicated by the relationship between in vivo and extract characteristics. In vivo absorbance characteristics provide a quick and accurate gauge to the UV-shielding capacity of primary producers from tropical latitudes.
Introduction

In vivo absorbance and fourth derivative spectra have been applied to many algae as a tool to examine pigment diversity in macro- and micro-algae (Owens et al. 1987, Smith & Alberte 1994, Part III & IV). Tropical macrophytes are evolutionarily diverse primary producers that occupy regions receiving the highest global levels of photosynthetically active radiation (PAR) and ultra-violet (UV) irradiance (Caldwell et al. 1989, Hader 1993). This epilithic algal community plays an essential role in the trophodynamics of coral reef communities (Klump & McKinnon 1992), where they contribute a large proportion of net primary productivity (Odum & Odum 1955, Marsh 1976, Klump & McKinnon 1989). Research in primary productivity of tropical macrophytes has focused on: 1) fundamental responses to irradiance, 2) production rates and 3) quantification of accessory pigments (Klump & McKinnon 1992, Littler & Littler 1992, Titlyanov et al. 1992). Although in vivo absorbance spectra have great utility in the detection of novel pigments and stress responses (Smith & Alberte 1994, Part III & VI), investigations of this kind for tropical reef taxa are lacking. Considering reports of degradation of coral reef communities (Smith & Buddemeier 1992), non-invasive rapid measures of reef organism responses to thermal, eutrophication and/or irradiance stresses may provide insight into responses of essential community components. In this regard, baseline data as in vivo absorption spectra from a diverse assemblage of healthy primary producers is essential.

Increasing levels of UV-irradiance has been a primary concern in polar regions (Helbling et al. 1992) but typical levels of UV-irradiance in tropical regions are elevated at least two-fold compared to higher latitudes (Caldwell et al. 1989). With this strong selective pressure, extant tropical primary producers (e.g., macroalgae, phytoplankton, corals) have adapted to long-term conditions of elevated PAR and UV-irradiances and are likely to possess mechanism(s) to minimize photo-induced damage (Hazzard 1993, Kinzie 1993, Shick et al. 1995, Hunter & Smith, in review). Mycosporine-like amino acids
(MAAs) in some marine organisms, scytonemin in select cyanobacteria, and flavonoids in terrestrial plants are current examples of photoprotective compounds (Caldwell et al. 1983, Dunlap & Chalker 1986, Garcia-Pichel & Castenholz 1991, Karentz et al. 1991). Until now UV ACs detection has been based on extract quantification, even though spectral features of intact thalli differ substantially from those of extractable pigments in the visible spectrum from the same tissues (Haxo & Blinks 1950, Kirk 1983, Smith & Alberte 1994).

Presentation of UV ACs in living cells will have a direct effect on their ability to preemptively capture UV-irradiance and on their role as likely photoprotectors. For instance, microspectrometry revealed the presence of MAAs exclusively in the chloroplasts of Porphyra vezoensis (Yoshida & Sivalingam 1970). Additionally, scytonemin occurs in the extracellular sheaths of some cyanobacteria (Garcia-Pichel & Castenholz 1991).

Here, I addressed several important aspects of the photoadaptive responses for tropical reef macrophytes to provide a baseline to which other tropical macrophytes can be compared. This technique also provides a method with utility in assessing pigment responses of a diverse assemblage of primary producers such as corals (Hunter & Smith, in review.), phytoplankton (Johnsen et al. 1994) and macroalgae (Smith & Alberte 1994, Part III). In this investigation, distinct in vivo absorbance and fourth derivative signatures from 400 to 700 nm were observed among the divisions of algae examined. A marked consistency of spectra within algal divisions was discerned. Extractable levels of UV ACs were directly correlated to in vivo absorption maxima from -317.5 to 345 nm in all macroalgal divisions. UV ACs concentrations were highest in rhodophytes; these levels may aid in this division's success in tropical irradiance-rich environment.

**Material and methods**

**Sample collection and handling:** One representative of each of the species of interest (Figures 2.1-2.11) was collected on 8 Oct. 1994 at Koloko, O'ahu, Hawai'i, USA. These taxa represent a selection of algal ridge and lagoonal epilithic macroalgae common in
Hawaiian coastal regions (Magruder and Hunt 1979, Smith 1992). Thalli were immediately transferred to the laboratory and cleaned of macroscopic epiphytes. The thalli were rinsed and stored in 0.2 μm filtered seawater (FSW) at room temperature (23°C) under 50 μmol quanta m⁻² s⁻¹. In vivo spectral analyses were performed within 3 hours of collection. Samples were then blotted dry and frozen (-20°C) prior to methanol: tetrahydrafuran extraction (see below).

In vivo absorption spectral analyses: In vivo absorption spectra were obtained using a Shimadzu UV Vis-2101 spectrophotometer with a 150 mm integrating sphere attachment (Shimadzu) on algal tissues 1.3 cm by 3.0 cm in dimension following procedures detailed in Part III & IV with light source change (tungsten to deuterium) wavelength at 360 nm. Depending upon the morphology of the species studied, whole pieces of foliose thalli (Padina japonica, Ulva fasciata, Dictyosphaeria versluysii), 'leaflet' portions (Sargassum polyphyllum, Melanomansia glomerata) or many upright axes placed in parallel orientation to create a non-overlapping layer of similarly aged and pigmented vegetative tissue (Acanthophora spicifera, Caulerpa racemosa, Chaetomorpha antennina, Hinksia breviarticulata, Chnoospora minima) were sampled.

UVACs extraction: UV-absorbing compounds, chlorophylls and carotenoids were extracted with (80:20 v:v) methanol : tetrahydrafuran following minor modifications of Kinzie (1993). Extract absorbance spectra were measured with a Shimadzu UV Vis-2101 spectrophotometer in 1.0 cm quartz cuvettes after baseline corrections to solvents.

Results

In vivo PAR absorption and fourth-derivative spectra in macroalgae: All of the following pigment identities are based on maxima described by Haxo and Blinks (1950), French et al. (1972), Daley (1990), Smith and Alberte (1994), references therein, and similar extract maxima from Rowan (1989).
Chlorophyta. The chlorophyte in vivo absorption spectra fell into two types. The spectra either had a pronounced absorbance shoulder from 500 to 600 nm (Caulerpa racemosa and Dictyosphaeria versluysii) (Figures 2.1a & 2.2a) or had a sharp decrease in absorbance from 500 to 520 nm (Chaetomorpha antennina and Ulva fasciata) (Figures 2.3a and 2.4a). The two spectral types were additionally resolved by fourth-derivative analyses. The derivative maxima from 500 to 520 nm in C. racemosa and the maxima at 508 and 550.5 nm in D. versluysii were attributed to siphonein and/or siphonoxanthin, known components in the Caulerpales and Siphonoclad-group of the Cladophorales (Figures 2.1b & 2.2b). No such maxima were detected in U. fasciata nor C. antennina (Figures 2.3b & 2.4b). All of the in vivo absorbance spectra had characteristic red Chl a maximum at ~677.5 nm and a Chl a Soret maximum at ~439 nm (Figures 2.1a-2.4a). The fourth derivative spectra resolved spectral components at ~683, ~621.5, ~439 and 411 to 418.5 nm attributable to Chl a (Figures 2.1b-2.4b). In vivo maxima for Chl b were visualized at ~473 and ~653 nm (Figures 2.1a-2.4a). The in vivo maximum for Chl b in the Soret region shifted to ~486 in C. racemosa and D. versluysii (Figures 2.1a & 2.2a). The derivative maxima ascribed to Chl b were at ~649.5 and from 463.5 to 467 nm (Figures 2.1b-2.4b). The derivative maxima between 488 and 498 nm were most likely ascribable to β,β carotene (Figures 2.1b-2.4b). Derivative maximum at ~547 nm in U. fasciata and C. antennina was attributed to Chl b and / or carotenoid (Figures 2.3b & 2.4b).

Phaeophyta. The brown algae selected for this survey demonstrated similar abilities to absorb irradiance, vis a vis in vivo absorption and fourth-derivative spectra (Figures 2.5-2.8). Chl a was visualized by distinct maxima at ~675, and ~439 nm in vivo, and at ~684, ~440, and ~414.5 nm in the fourth-derivatives (Figures 2.5-2.8). In vivo absorption spectra of Hinksia breviarticulata, Padina japonica and Sargassum polyphyllum resolved two maxima attributable to Chl c at ~633.5 and 462 nm (Figures 2.6a-2.8a) while Chnoospora minima had only one discernible Chl c maximum at 634.5 nm (Figure 2.5a).
The fourth derivative resolved two Chl ε specific maxima at ~637 and ~466 nm in all four species examined (Figures 2.5b-2.8b). In vivo maxima at ~493 and ~587 nm were primarily contributed by fucoxanthin (Figures 2.5a-2.8a). Fourth-derivative maxima from 490 to 590 nm were attributed principally to fucoxanthin which is known to have three minor maxima (Friedmann & Alberte 1984, 1986) and possibly to other minor carotenoids (Figures 2.5b-2.8b). *Chloospora minima* was distinct among the phaeophytes examined in that the fourth-derivative demonstrated extensive peak splitting from 350 to 590 nm (Figure 2.5b). Whereas *P. japonica*, *S. polyphyllum* and *H. breviarticulata* had three major maxima from 490 to 590 nm (Figures 2.6b-2.8b), *C. minima* had 5 maxima (Figure 2.5). A ~668 nm maximum (663.5 in *C. minima*) was detected and was ascribed to a satellite band of Chl a (Rowan 1989, Iglesias-Prieto et al. 1993) (Figures 2.5b-2.8b).

**Rhodophyta.** *Acanthophora spicifera, Hypnea musciformis, and Melanomansia glomerata* exhibited similar in vivo absorption and fourth-derivative spectra (Figures 2.9-2.11). Two in vivo absorbance maxima were ascribed to Chl a at ~678 and 437 nm while the fourth-derivative resolved similar Chl a maxima at 687 and 442 nm (Figures 2.9-2.11). In the fourth-derivative analyses, Chl a maxima at 414 nm and a satellite band at ~633 nm shared with R-phycocyanin (R-PC) were also detected (Figures 2.9b-2.11b). Maxima between ~625 and ~633 nm were ascribed to R-PC in the in vivo and fourth-derivative spectra, respectively (Figures 2.9-2.11). In vivo absorbance maxima of ~539 and 567 nm were attributed solely to R-phycoerythrin (R-PE) while ~494 nm maximum was contributed to by both R-PE and minor carotenoids (Figures 2.9a-2.11a). Fourth-derivative analyses resolved an in vivo absorbance maximum at ~494 nm (Figures 2.9a-2.11a) into both a ~465.5 nm carotenoid maximum and ~500 nm R-PE maximum (Figures 2.9b-2.11b). Additionally, fourth-derivative maxima at ~539 and 570 nm were ascribed to R-PE (Figures 2.9b-2.11b). Maxima at ~667, ~617 and ~595.5 nm were also detected (Figures 9b-11b). As among the phaeophytes fourth-derivative spectra, the ~667 nm
maximum was determined to be a satellite band of Chl a (Rowan 1989, Iglesias-Prieto et al. 1993). The ~595.5 nm maximum had two potential accessory pigment components: R-PC and APC, while the ~617 nm maximum was ascribed to R-PC (Rowan 1989).

Characterization of UV-absorption in tropical macrophytes: All of the intact macroalgae examined absorbed irradiance in the range from 280 to 400 nm to an extent approximately equaling or surpassing comparative PAR absorbances levels (Figures 2.1a-2.11a). In vivo UV-absorbance spectra were similar among most of the macrophytes examined with an absorbance shoulder from 317.5 to 345 nm. Methanol : tetrahydrafuran extraction of UV ACs revealed prominent absorption maximum between 315-337 nm in most of the macrophytes examined (Figures 2.1a-2.11a). There was a high degree of similarity observed between in vivo absorption maxima and respective extract absorption maxima (Figures 2.1a-2.11a), although maxima detected after extraction were bathychromically shifted approximately 5-20 nm in comparison to maxima detected via in vivo absorption. After extraction, maxima corresponding to in vivo maxima from 334 to 345 nm in red algae showed pronounced increases in absorbance, even when normalized to Chl a 680 nm absorbance levels (Figures 2.9a-2.13a). This increase was not observed for absorption maxima from 317 to 345 nm in extracts from brown algae (Sargassum polyphyllum, Chnoospora minima, Padina japonica) nor among the green algae (Figures 2.2a-2.5a, 2.7a, 2.8a) except Caulerpa racemosa (Figure 2.1a).

Among all the macroalgae examined, fourth-derivative analyses revealed the single in vivo UV-absorbing maximum from 317.5 to 345 nm to have at least two components with maxima ranging from 317.5 to 328 nm and 335.5 to 367 nm (Figures 2.1b-2.11b). The exact position of these maxima was varied (Figures 2.1b-2.11b). The putative identity of these components was ascribed to MAAs (Karentz et al. 1991, Sivalingam et al. 1974). In addition to these maxima, peaks at ~305 and 370 to 385 nm were also revealed by the fourth derivative analyses (Figures 2.1b-2.11b). In Caulerpa racemosa, and Acanthophora
spicifera (Figures 2.1a & 2.9a), a prominent 360 to 381 nm in vivo absorbance shoulder was detected. Extraction spectra also resolved a maximum in this region in these species (Figures 2.1a & 2.9a).

Correspondence of in vivo absorption and extracts of UVACs: To evaluate the utility of in vivo absorption spectra in the assessment of relative UVACs concentration, in vivo absorbance and extract signatures were compared via regression for 11 common reef macroalgae. This signature was ratio of UVACs absorbance maxima to the ~680 nm Chl a absorbance maxima for both in vivo and extract absorbance spectra. A highly significant positive linear relationship was detected between in vivo and extract absorbance parameters (p<0.0005, ANOVA) (Figure 2.12).

**Discussion**

In vivo absorbance and fourth derivative spectra from 280 to 750 nm have been reported here for tropical reef macroalgae for the first time. These benchmark spectra exhibit a high degree of consistency in PAR and UV-absorbance characteristics within algal divisions when determined for algae from similar light and nutrient histories. This study is the first to demonstrate a possible in vivo role of UVACs in the light budget of primary producers. In vivo absorbance spectra present a powerful non-invasive probe into the photosynthetic pigment constituents of tropical and temperate macroalgae (Smith & Alberte 1994) but also appear to have great utility in determining UV-absorbance and relative UVACs concentrations.

**In vivo absorbance spectra in PAR region of irradiance spectrum:**

In vivo PAR absorbance of tropical chlorophytes corresponded closely to those of temperate greens (Smith & Alberte 1994). As in Smith and Alberte (1994), two distinct in vivo absorbance patterns were detected: 1) increased absorbance from 500 to 600 nm attributed to siphonein / siphonoxanthin (Figures 2.1a & 2.2a), and 2) markedly decreased
absorbance from 500 to 520 nm (Figures 2.3a & 2.4a). Comparison of the fourth-derivative also revealed this dichotomy.

In addition to kelps examined by Smith and Alberte (1994) four additional orders were examined in this study (Ectocarpales, Scytosiphonales, Dictyotales and Fucales). Overall, PAR in vivo absorbance and fourth-derivative spectra showed high degrees of similarity among the species examined (Figures 2.5-2.8) as well as with members of the temperate Laminariales. Two main distinctions separate these studies. First, two additional fourth derivative maxima at ~668 and 616.5 nm that were present in all the tropical species examined (Figures 5b-8b) were not resolved by Smith and Alberte (1994). This may be attributable to the 0.5 nm band pass utilized in this study in contrast to the 2-3 nm bandpass utilized by Smith and Alberte (1994), which potentially increased resolution for derivative analyses of this study. Additionally, the fourth-derivative spectrum of Chnoospora minima demonstrated extensive peak splitting from 400 to 560 nm even though the acquisition procedures were identical among all specimens of this study. This observation warrants further investigation into at least the carotenoid composition of this species.

Rhodophyte taxa from the Gigartinales and Ceramiales had markedly similar in vivo absorption and 4th derivative spectra indicating the expected conserved composition of the accessory pigments in these species. The fourth derivative spectra for rhodophytes were more highly resolved from 600 to 670 nm than in Smith and Alberte (1994) (see above discussion). With this level of enhanced resolution, I were able to discern maxima appropriate to APC and additional R-PC maxima. The detection of these maxima are likely to aid in tracking photoacclimative and nutrient induced changes in phycobilisome composition in novel and non-invasive ways (Part VI & VII).
In vivo absorbance and extract spectra in UV Region of irradiance spectrum:

In vivo absorbances from 280 to 400 nm of the select macroalgae closely corresponded to methanol : tetrahydrafuran extract absorbance characteristics from 280 to 400 nm (Figures 2.1a-2.11a). This observation substantiates previous work relating the absorbance of extracted UVACs to an absorption role in intact tissues (Sivalingam et al. 1974, Carreto et al. 1990, Sivalingam & Nisizawa 1990).

Although the positions of in vivo absorbance maxima were similar among the species examined with an absorbance maximum between 320-340 nm, the magnitude of UV-absorbance and the characteristics of methanol: tetrahydrafuran extracts differed among rhodophytes, phaeophytes and chlorophytes as seen by Sivalingam et al. (1974). The rhodophytes examined had the greatest relative concentration of UVACs as judged by the in vivo absorbance ratio of the UVACs specific absorbance peak to the red Chl a peak (1.17 in reds vs. 1.01 and 1.02 in greens and browns, respectively). The ability to pre-emptively absorb UV-irradiance to a greater extent in rhodophytes may aid in their apparent ecological success at tropical latitudes where the ratio of number of red to brown algal species is reportedly over 4. In temperate areas, the ratio of red to brown algal species is about 2.4 (Kain & Norton 1990).

Among the Chlorophytes, an apparent decrease of extractable UVACs in relation to Chl a content may be attributable to: 1) increased levels Chl a in the antennae complex of chlorophytes compared to other algal lineages, 2) presence of UVACs that were not extracted by the solvent system and / or, 3) may reflect a general lack of UV-protective substances in these Chlorophytes. Ulva fasciata (Figure 2.4a) exemplifies this trend of lower UVACs extract absorbance coupled with high in vivo UV-absorbance in the UV-B region. For Ulva spp. significant photoinhibition (Henley et al. 1992), thallus bleaching and tissue loss occurs during low tide exposure (CMS, pers. obs.). The rapid growth rate and fecundity of this alga may indirectly serve as a way to compensate for vulnerability to
UV-irradiance, thereby circumventing metabolic costs associated with UVACs synthesis as might be expected in longer lived macrophytes.

Fourth derivative spectra from 280 to 400 nm had a consistent number of maxima but their exact position varied. This variability may represent different individual or combinations of MAA pools in the macroalgae of this study. Karentz et al. (1991) has detected up to 7 distinct MAAs in Antarctic rhodophytes. Until the identity of the UVACs in the species examined here, fourth derivative (280-400 nm) analysis remains the best measure of functional pool identity available.

Conclusions

This study establishes a baseline to which other spectra from tropical macrophytes can be compared. The consistency of the position of absorbance maxima within algal divisions and between temperate (Smith & Alberte, 1994) and tropical taxa demonstrates the conserved nature of the pigment protein complexes within algal divisions. The 280 to 400 nm portions of the in vivo absorption spectra are the first of their kind and demonstrate marked absorbance of UV-irradiance by macrophytes.

With the use of in vivo absorption spectra, UVACs concentrations can be accurately and quickly assayed (Figure 2.12). The utility of this non-invasive in vivo comes from the ability to assess: 1) stress responses at the level of fully functional pigment pools in a variety of primary producers, 2) to predict UV-resistance, and 3) to gain an efficient and comprehensive analysis of PAR and UV-stress resistance of different growth stages, life cycles phases, as well as population or community components. This technique is likely to allow researchers to more accurately identify stress-sensitive developmental or life cycle periods of species / strains that may be adversely impacted by enhanced stress levels.

UVACs may play an essential role as photoprotective agents in tropical rhodophytes. Although the mechanism(s) of energy dissipation by UVACs remains unresolved, in vivo detection of UVACs and potential identification of pools provides
convincing evidence that UVACs have an impact on the energy budget of intact algal thalli. Enhanced levels of UVACs in rhodophytes compared to other algal divisions may impart a selective advantage to these taxa exposed to the stresses of the tropical light regime.
Literature cited


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Figure 2.1. A) In vivo (solid circles) and methanol:tetrahydrafuran extract (open diamonds) absorbance spectrum and B) fourth-derivative of in vivo absorption spectrum of Caulerpa racemosa
Figure 2.2. *Dictyosphaeria versluysii* (see description Figure 2.1).
Figure 2.3. Chaetomorpha antenina (see description Figure 2.1).
Figure 2.4. *Ulva fasciata* (see description Figure 2.1).
Figure 2.5. *Chnoospora minima* (see description Figure 2.1).
Figure 2.6. *Hinksia breviarticulata* (see description Figure 2.1).
Figure 2.7. *Padina japonica* (see description Figure 2.1).
Figure 2.8. *Sargassum polyphyllum* (see description Figure 2.1).
Figure 2.9. *Acanthophora spicifera* (see description Figure 2.1).
Figure 2.10. *Hypnea musciformis* (see description Figure 2.1).
Figure 2.11. *Melanamansia glomerata* (see description Figure 2.1).
Figure 2.12. Relationship of in vivo absorption ratio of MAA: Chl a maxima to the corresponding extract absorption ratio in subtropical macroalgae. Equation of the least squares regression line: $y = -7.58 + 8.61x$, $R^2 = 0.779$, $H_0$: $b_1 = 0$, $p < 0.0005$. 
PART III

PHOTOSYNTHESIS IN REPRODUCTIVE UNICELLS OF
ULVA FASCIATA DELILE AND ENTEROMORPHA FLEXUOSA (WULFEN) J.
AGARDH: IMPLICATIONS FOR ECOLOGICAL SUCCESS
Abstract

Photosynthetic performance of adult vegetative tissues is modified following the formation of free-swimming reproductive unicells by Ulva fasciata and Enteromorpha flexuosa. Comparisons of photosynthetic performance of adult haploid or diploid thalli versus gametes or zoospores (respectively), yield the following generalizations: (1) $P_{\text{max}}$ for motile cells (e.g., gametes of Ulva fasciata and zoospores of Enteromorpha flexuosa) can be equivalent to $P_{\text{max}}$ of appropriate adult tissues when expressed on a Chl $a$ or Chl $a+b$ basis; (2) respiration rates for all motile cells were 1.5 to 6 fold higher than rates for adult thalli when normalized to Chl; (3) as expected with increasing respiration rates, $I_c$ and $I_k$ also increased for all motile cell types; (4) chlorophyll $a:b$ ratios were lower in all motile cell types; (5) $P:R$ ratio were 3.3 to 7.7 fold lower in zoospores of Ulva fasciata and Enteromorpha flexuosa, respectively. Photosynthetic performances of settled zoospores of Ulva fasciata were more similar to the vegetative parent than to the free-swimming zoospores in terms of $I_k$ and $P_{\text{max}}$ even with altered Chl $a:b$ ratios. An unexpected finding was the increased in vivo absorbance in the 490 to 550 nm range for reproductive tissues of Ulva fasciata, only. This absorbance increase suggests a potential photoadaptation. This coupled with alteration of the Chl $a:b$ ratio, could allow reproductive unicells of Ulva fasciata to harvest light more readily for photosynthesis as part of the plankton when compared to zoospores of Enteromorpha flexuosa. Such a difference between motile cells may enhance the competitive ability and subsequent ecological success of one over the other. High rates of $P_{\text{max}}$ have the selective advantages of subsidizing high respiratory costs associated with motility in reproductive unicells while still allowing for the rapid germination and growth upon eventual attachment to the substrate. Comparably, species such as late successional kelps do not show this pattern and may represent a different photoadaptive strategy.
Introduction

Photosynthetic capacity by planktonic reproductive unicells of many macroalgae is poorly known yet may reveal a diversity of exploitative strategies (sensu Grace 1990) and resulting ecological adaptations to the planktonic habitat, as has been found for life cycle phases of terrestrial plants (Grime 1979, Tilman 1982, Grace 1990). These models have been applied to growth of planktonic algae (Tilman 1977) as well as to photosynthetic and morphological features of adult macroalgae (Littler & Littler 1984), but no link has been made previously to possible strategies among algal reproductive units. The model of adaptive specialization by Grime (1979) which has been considered appropriate for macroalgal communities (Kain & Norton 1990), recognizes three viable strategies: (1) exploitation, the ability to preemptively capture (exploit) limiting resources, is successful in regions of low stress (phenomena which restrict photosynthetic production (Grime 1979)) and low disturbance (partial or total destruction of plant biomass (Grime 1979)); (2) a ruderal strategy, the ability to withstand or recover from disturbance and still capture limiting resources, occurs in response to low stress and high disturbance; and (3) stress tolerance, adaptation for enduring suboptimal physiological conditions, occurs in response to high stress and low disturbance. Given the great diversity of exploitative strategies present among adult phases of life cycles, one might expect some diversity in strategies to be present among planktonic stages of macroalgal life cycles as well.

Planktonic stages are transient but crucial bottlenecks in macroalgal life cycles (Chapman 1984). The period of viability in planktonic life-cycle stages is enhanced when they can feed or have a yolk sac compared to non-feeding larvae (Thorson 1950, Olsen 1985). Planktonic reproductive unicells of macroalgae that are photosynthetically competent upon release could similarly extend a "window of viability", leading to increased success in settlement and recruitment. Photoadaptive strategies for particular light regimes common to coastal environment may further enhance the success of reproductive unicells
over those without such a capability. The photosynthetic characteristics of only a few reproductive unicells of temperate brown algae have been characterized (McLachlan & Bidwell 1978, Amsler & Neushul 1991), and other algal groups with markedly different photosynthetic pigments, life-cycle strategies, as well as latitudinal distributions remain unexamined.

Planktonic unicells of benthic macroalgae have commonly been reported to have brief viability, 24 h to 11 days under laboratory conditions (Kain 1964, Hoffmann & Camus 1989, Santelices 1990). This ephemeral nature has been partially attributed to protoplasts in newly released propagules (McBride & Cole 1971, Toth 1976, Borowitzka 1978, Santelices 1990), resulting in greatly reduced photosynthetic rates (Kain 1964, Santelices 1990). In only two studies to date, net positive photosynthesis has been demonstrated to take place in algal reproductive unicells. These are zoospores of four genera characterized by an heteromorphic alternation of generations and sporic meiosis (Amsler & Neushul 1991) and Fucus serratus gametes, an intertidal alga with gametic meiosis (McLachlan & Bidwell 1978). In both cases photosynthetic capacity is markedly lower than benthic macroalgal rates (Littler & Arnold 1982).

Species of the weedy genera Ulva and Enteromorpha are generally the first macroalgae to colonize open substrate and are considered troublesome fouling organisms. This ecological role is attributed to their simple adult morphologies and to their fecundity. Propagule release resulting from a sporic meiosis life cycle (Figure 3.1) may occur daily (H. Shin pers. obs.) for species in low latitude populations. In Ulva spp. 20 to 60% of overall biomass is allocated monthly to reproduction depending upon season (Smith 1947, Niesembaum 1988, H. Shin pers. obs.). Spore release in intertidal Ulva and Enteromorpha species is driven by tidal / lunar rhythms (Smith 1947, Christie & Evans 1962, Sawada 1978). Reproductive unicells of E. intestinalis can remain motile for up to eight days after release if subjected to continuous irradiance and agitation; reduction in the
irradiance period corresponds to a shortened period of motility (Jones & Babb 1968). Even low rates of photosynthesis by spores or gametes of *Ulva* and *Enteromorpha* species could extend both their viability and dispersal range while subsequently contributing to the success of these species over taxa with shorter-lived or physiologically less-competent reproductive cells.

I sought here to evaluate aspects of the photosynthetic and respiratory competence of different stages in the life cycle of *Ulva fasciata* and *Enteromorpha flexuosa* (Figure 3.1). Specifically, I asked (1) What physiological changes resulted from the formation of reproductive unicells from vegetative cells? (2) Are gametes and zoospores similar in terms of photosynthetic performance and respiratory demands? (3) Do closely allied genera from similar physical environments produce reproductive unicells that are physiologically similar? (4) How do the photosynthetic performances of these subtropical unicells compare to that reported for unicells from other macroalgae? (5) Does apparent photoadaptation by these unicells indicate overall adaptive strategy(ies) that may contribute to patterns in algal community composition and development?

**Materials and methods**

Reproductive and vegetative thalli of intertidal *Ulva fasciata* Delile and *Enteromorpha flexuosa* (Wulfen) J. Agardh were collected at Ka'ala'wai and Kailua, O'ahu, Hawai'i, respectively, from 19 July to 15 October, 1993. Individuals were washed free of macroscopic epiphytes and maintained separately in 0.2 \( \mu \)m² filtered seawater (FSW) at 25 °C under a 16:8 h light-dark cycle (50 \( \mu \)mol quanta \( \cdot \) m\(^{-2}\) \cdot \) s\(^{-1}\), provided by fluorescent lamps).

**In vivo absorption and 4th derivative spectra analyses:** In vivo absorption spectra were obtained using a Shimadzu UV Vis-2100 spectrophotometer with a 150 mm Shimadzu integrating sphere attachment on algal tissues of dimensions 1.3 cm by 3.0 cm. Spectra were obtained by placing a non-overlapping layer of vegetative or gamete /
zoospore bearing tissue, which had gently been blotted dry, between two glass slides. The spectral scan (>60 s in duration) were acquired at room temperature and normalized to 0 at 750 nm. The spectrum sampling interval was set at 0.2 nm. The 4th derivative spectra were generated with the algorithms provided by the manufacturer's software PC revision 2.2 with a lambda of 16. By performing spectral analyses on intact tissues and not comparing intact tissues with dense reproductive unicell suspensions, differences in the 'package effect' between the reproductive and vegetative were minimized but could not be eliminated because of differential compartmentalization between vegetative cells and the reproductive unicells resulting from meiotic or mitotic divisions. Typically the reproductive unicells were released within 0.5 hr of spectral analysis, confirming the reproductive maturity of the tissues analyzed.

**Release and handling of motile cells for physiological measurements:** Zoospores from fertile thalli of *U. fasciata* or *E. flexuosa* were released synchronously by rehydration of partially desiccated thalli within 6 hours of field collection of the adult material. Care was taken to examine only motile gametes or zoospores from single individuals within 0.5 h of release. For post-settlement photosynthetic measurements, zoospores were induced to settle onto 2.0 cm² nytex mesh utilizing a low photon flux (25 μmol quanta m⁻² s⁻¹) white light, provided by a dissecting lamp, focused on the mesh. Zoospores were allowed to settle onto the mesh for 3 h and then physiologically evaluated after a brief rinse to facilitate removal of non-attached zoospores. Three hours of settlement time on glass slides produced >99% zoospore settlement with flagellar detachment.

Gamete formation in *U. fasciata* was similarly induced in previously vegetative thalli by a 12 h partial submergence in 100 ml FSW. A synchronous release of gametes was initiated by completely rehydrating the thalli. Syngamy was minimized by maintaining and running trials on isolated individual thalli and their gametes.
Densities of gametes and zoospores were maintained between $2.5 \times 10^6$ to $3.0 \times 10^7$ cells ml$^{-1}$ for photosynthesis and respiration measurements. Shelf shading and reproductive unicell settlement was minimized by a high but non-damaging stirring speed. Unicells were evaluated microscopically post photosynthetic trials for visible cell damage (disruption, etc.). A 0.5 ml aliquot of each cell suspension was preserved in 4.0 % buffered formalin at the beginning of each trial for subsequent determination of cell density.

During the period of this study I was unable to obtain sufficient numbers of *E. flexuosa* gametes or settled zoospores for characterization.

**Physiological measurements of adult thalli:** Vegetative tissue disks (0.37 cm$^2$) of *U. fasciata* thalli were sampled with a cork borer from apical regions just below the marginal reproductive tissue. Vegetative samples (0.25 cm$^2$) from *E. flexuosa* were obtained from the apical vegetative tissue located below apical sporogenous zone. Preliminary microscopic evaluation was utilized to differentiate vegetative and reproductive tissues. Vegetative samples were attached to a modified chamber seal of the oxygen electrode (Smith & Berry 1986) by nytex mesh and maintained in FSW under 50 µmol quanta m$^{-2}$ s$^{-1}$.

**Photosynthesis and respiration measurements:** Net photosynthesis was measured as oxygen exchange using a water jacketed Clark-type oxygen electrode (Rank Brothers, Cambridge UK). Temperature was maintained at 25±1°C via a Neslab RTE-110 temperature controlled water bath. Illumination was provided by a Kodak Ektagraphic III E slide projector with 300 W Tungsten Halogen lamp and photon flux were varied from 0 to 2500 µmol quanta m$^{-2}$ s$^{-1}$ with neutral density filters. To minimize carbon limitation the FSW was augmented to 20 mM NaHCO$_3^-$ above ambient levels in vegetative and reproductive unicell trials. Chl concentrations were determined utilizing N,N-dimethylformamide extraction (Moran & Porath 1980) and a Hewlett Packard 5428 A
Diode array spectrophotometer. Calculations were based on the equations of Inskeep and Bloom (1985).

Photosynthetic parameters of compensation irradiance ($I_C$), quantum efficiency ($\alpha$) and maximum photosynthetic rate ($P_{\text{max}}$) were determined by linear regression of the linear light-limited and linear light-saturated portions of the photosynthesis versus irradiance curves. Saturation irradiance ($I_K$) was estimated by the irradiance at the intersection of the above mentioned lines. Respiration was determined in the dark.

Statistical analyses: The subsequent statistical analyses were carried out using Minitab version 8.2 (Minitab Inc.). Normality was assessed with a test equivalent to Shapiro-Wilk test with an $\alpha$ of 0.05 (Minitab Reference Manual, release 8 1991). When normally distributed, comparisons of gametophyte to gametes, sporophyte to zoospores and sporophyte to settled zoospores were made with pairwise t-tests (Zar 1984). If normality was in question either a pairwise Wilcoxon test (when data were symmetrical) or a pairwise Sign test (when symmetry was in question) were used (Zar 1984).

Results

Gametophyte & gametes physiological features: When normalized to Chl $a+b$, values for respiration, $I_C$, $I_K$, the ratio of $P_{\text{max}}$ (net) to respiration (P:R ratio) and Chl $a+b$ ratio for gametes of $U$. fasciata were significantly different to those of gametophytes (Table 3.1). Gametes of $U$. fasciata attained rates of $P_{\text{max}}$ and $\alpha$ similar to those of haploid thalli (Table 3.1).

Sporophyte & zoospore physiological features: Adult sporophytes of $U$. fasciata differed physiologically from zoospores in that Chl $a+b$ specific $I_C$, $I_K$ and respiration rates were significantly higher for zoospores than for parental tissues (Table 3.2). The value of $\alpha$, $P_{\text{max}}$ and P:R ratio were all markedly lower for zoospores of $U$. fasciata when compared with adults (Table 3.2) but P:R ratio remained greater than one.
Photosynthesis and respiration by zoospores of *E. flexuosa* differed from comparable processes by adult sporophytes in ways similar to those observed for *U. fasciata* (Table 3.3), yet the magnitude of change in the transition from adult thallus to zoospores appeared to differ between *U. fasciata* and *E. flexuosa* (Tables 3.2 & 3.3). Respiration rates, \( I_c \) and \( I_k \) were three-fold, six-fold and three-fold higher, respectively, for zoospores of *E. flexuosa* when compared with zoospores of *U. fasciata*. The value of \( \alpha \) and P:R ratio were 21-fold and three-fold lower, respectively, for zoospores of *E. flexuosa* when compared with zoospores of *U. fasciata*. The P:R ratio for zoospores of *E. flexuosa* was 0.58. The photophysiological responses of adult thalli of *E. flexuosa* were similar to *U. fasciata* adults (Tables 3.2 & 3.3).

**Sporophyte & recently settled zoospore physiological features:** Rate of respiration and \( I_c \) of recently settled zoospores of *U. fasciata* was significantly greater than that observed in adult thalli (Table 3.2). The P:R ratio decreased significantly in the settled zoospores state compared to the vegetative state. The values of \( I_k \) and \( P_{\text{max}} \) of settled zoospores for *U. fasciata* were indistinguishable from adult thalli (Table 3.2). Median value of \( \alpha \) of settled zoospores was significantly less than that of the adult thalli on a pairwise basis (Table 3.2).

Comparisons between vegetative tissue and reproductive unicells were reported on a Chl a+b basis only. Similar differences were seen on a Chl a basis except for \( \alpha \). Because of the pronounced differences in Chl a+b ratios between stages, Chl a+b was chosen as a more reliable measure on which to base photosynthetic parameters in this study. For ease of future comparisons \( P_{\text{max}} \) rates normalized to Chl a, fresh weight, dry weight, and surface area are reported in Table 3.4. On account of the high reproductive cell densities required for this type of physiological evaluation self-shading in the oxygen electrode was a concern. No significant relationship was found relating cell density and \( I_c \) or \( I_k \) for *U. fasciata* and *E. flexuosa* (data not shown). This suggests that within the order
of magnitude range of cell densities used that there were no artifacts caused by self-shading. On account of this the physiological differences seen between different reproductive unicell types are genuine. In regard to the differences between vegetative and reproductive states the most marked change is the augmented respiration rates of reproductive unicells with concomitant increases in $I_C$ and $I_K$, because this is determined in the dark self-shading was not considered to be an overriding factor in these results.

**In vivo absorption features and 4th derivative spectra analysis:** Both *in vivo* absorption and fourth derivative spectra of *U. fasciata* and *E. flexuosa* were in close agreement with data from *U. expansa* and *U. taeniata* (Smith & Alberte 1994). *In vivo* absorption spectra of sporophytes and gametophytes of *U. fasciata* exhibited consistent changes in pigmentation with reproductive unicell formation (Figures 3.2a & b). The relative magnitudes of the red absorption peaks at 680 (Chl a) and 650 nm (Chl b) and Soret bands revealed the elevated Chl b content of reproductive tissues compared to vegetative tissues (Figures 3.2a & b) confirming the results of pigment extraction (Tables 3.1 & 3.2). The 490 to 540 nm region of the spectra revealed an increase in absorption by both zoospore and gamete producing tissue compared with parental tissue.

Fourth derivative deconvolution of this spectral region demonstrated that the 490 nm peak of both gametophyte and sporophyte thalli of *U. fasciata*, attributed to carotenoid absorption (Smith & Alberte 1994), had shifted 12 nm to longer wavelengths (Figs. 3.2a & b) and may represent the presence of a novel pigment or an increase in the content of a carotenoid species in these reproductive tissues. The identifying the specific changes in carotenoid pigment composition from vegetative to reproductive tissues in *U. fasciata* is being actively investigated by H.W. Shin.

**In vivo** spectral features of vegetative sporophyte and zoospore producing thalli of *E. flexuosa* exhibited virtually identical spectra with a consistent but less pronounced change than *U. fasciata* in the relative magnitudes of the absorption peaks at 680 and 650.
nm (Figure 3.2c). Pigment analysis confirmed that reproductive tissue had a lower Chl a:b ratio than vegetative tissue (Table 3.3). The 4th derivative spectra of vegetative and reproductive *E. flexuosa* were identical (Figure 3.2c) and did not exhibit a change in 490 nm spectral region as seen in *U. fasciata* (Figures 3.2a & b).

**Discussion**

Reproductive unicells of *U. fasciata* and *E. flexuosa* demonstrated substantial rates of photosynthesis in contrast to those observed in kelp zoospores (Amsler & Neushul 1991). Gametes and zoospores showed elevated rates of respiration when compared with adult thalli, which might be attributable to increased metabolic demands of motility. Both I_C and I_K were found to increase in all cases, while P_max was depressed only in the case of zoospores of *U. fasciata*. Overall, I observed similar results for (1) in vivo absorption spectra for gamete and zoospore producing thalli of *U. fasciata*, (2) 4th derivative spectra of gamete producing and zoospore producing thalli of *U. fasciata*. Yet photosynthetic performance of *U. fasciata* gametes and zoospores differed in their physiological divergence from parental thalli collected at the same site. While vegetative gametophytes and sporophytes were similar in all their photosynthetic attributes, gametes of *U. fasciata* demonstrated values for P_max and α were equal to that of vegetative gametophytes. Zoospores of *U. fasciata* did not reveal similar rates or efficiencies as vegetative sporophytes.

Although the trends of photophysiological change with zoospore formation are similar for both species, they differed by the magnitude of change detected. The respiration rate and subsequently I_C and I_K were all modified to a greater degree with zoospore formation in *E. flexuosa* than in *U. fasciata*. These changes may be solely attributed to increased respiration rates but for both species' zoospores α was also markedly altered (200-fold decrease in *E. flexuosa*). A decrease in α is usually interpreted as a decreased efficiency of the photosynthetic apparatus. This may occur at the site of carbon fixation.
and / or with modification in the size and / or number of photosynthetic units in photosystem I and / or photosystem II. The difference in these photosynthetic parameters resulted in a marked difference in the daily net carbon balance between the zoospores of these species (calculated as in Matta & Chapman 1991). Zoospores of *U. fasciata* have a net positive carbon balance (+6.6 µmol C fixed·mg Chl a+b⁻¹·day⁻¹ with a *H*_sat of 10 h) while zoospores of *E. flexuosa* were markedly negative (-1318 µmol C fixed·mg Chl a+b⁻¹·day⁻¹ with the same photoperiod).

The difference in the photosynthetic performance between zoospores of *E. flexuosa* and *U. fasciata* may be attributable to the observed changes in the relative pigment composition of these two algae. Both species decreased their Chl a:b ratio with zoospore formation but this change occurred to a lesser degree in *E. flexuosa*. *Ulva fasciata* appeared to have also produced an increased relative amount of carotenoids in zoospore producing portions of the sporophyte. These pigment changes may result in a broadening of the absorbed photosynthetically active radiation (PAR) into the blue-green region for *U. fasciata* zoospores and gametes. This is the light quality typical of coastal waters (Jerlov 1968).

Upon release several important hydrodynamic processes influence the distribution of reproductive unicells. Surface currents away from the sites of potential settlement and / or other compatible gametes can reduce the probability of settlement (Roughgarden et al. 1988). With the capability of satisfying metabolic and motility demands by efficient photosynthesis, the temporal window during which fusion and / or settlement may be accomplished could be extended. This possibility could increase the ecological range of a particular population and would increase potential for outcrossing among at least local populations. Downwelling currents would similarly alter zoospore and gamete distribution. Reproductive unicells that have the capacity to reach *P*_max at lower irradiance levels (e.g., have a low *I*_c and *I*_k) and have the capability to harvest the quality of light.
present in turbid coastal waters may prosper in these conditions while those requiring higher irradiance levels to reach $I_C$ and $I_k$ may not remain viable when transported away from near-surface waters. Overall, this relative low-light photoadaptation ($I_k$: 172 μmol quanta m$^{-2}$ s$^{-1}$ (U. fasciata) vs. 620 μmol quanta m$^{-2}$ s$^{-1}$ (E. flexuosa)) would allow the reproductive unicells of U. fasciata to exploit light more successfully in their planktonic habitat compared to unicells of E. flexuosa.

Within 3 h of settlement, the photophysiology of U. fasciata zoospores adjusted to a state closer to the vegetative adult thalli than that of free-swimming zoospores. Upon settlement respiration and $I_C$ were only slightly elevated above values of the parental thalli. At the same time the values of $P_{\text{max}}$ and $I_k$ were indistinguishable from parental thalli. Alpha ($\alpha$) remained lower than $\alpha$ of parental thalli but was greater than $\alpha$ observed for zoospores of U. fasciata supporting the hypothesis that the shift seen between vegetative adults and zoospores were not solely caused by motility. With settlement, the metabolic demands of motility are lost but other metabolic process associated with attachment may contribute to the slightly elevated respiration levels. All of these changes took place without an immediate alteration of the Chl $a:b$ ratio to levels associated with vegetative tissues.

Even with the limited number of species that have been examined in this manner, four kelp species (Amsler & Neushul 1991), one fucoid alga (McLachlan & Bidwell 1978), and two ulvalean species (this study), some comparisons can be made regarding photosynthetic and respiration rates by reproductive unicells in these two groups. (Direct comparisons to McLachlan and Bidwell 1978 are not possible because of the different techniques and scope of questions addressed by the different workers.) Compensation irradiance ($I_C$), $I_k$, $P_{\text{max}}$ (cell$^{-1}$ and Chl $a$ $^{-1}$), Chl $a$ / cell, and respiration are markedly lower for these select phaeophyte algae when compared to U. fasciata and E. flexuosa (Table 3.5). This disparity may reflect evolutionary differences. Additionally, the light
environment seen by sporophylls of temperate lower intertidal to subtidal kelps (Reed & Foster 1984) is substantially different to that of the irradiance field for subtropical Ulvallean algae. The above-mentioned photosynthetic differences are proposed to be a selective response by reproductive unicells, implemented at the level of photosynthetic features, for increased success in coastal ecological niches. Physiological studies of plants adapted to deeply shaded terrestrial habitats have suggested evolutionary mechanisms of conserving energy such as low respiratory rates may be important in maintaining a carbon balance (Grime 1979). The reproductive unicells of kelps and other late successional species may benefit from a low-light tolerant strategy in that by being adapted to low-light conditions, reproductive unicells may persist in the environment in which they are released and settle. Pioneer species can attribute part of their ecological success to a persistent pool of motile cells that can rapidly colonize cleared or new substrate (Doty 1967). A longer lived pool of motile cells that can contribute to their own metabolic demands insures this success. This Ulvallean strategy exploits light to enhance the success of weedy benthic algae as pioneer species.

Conclusions

Contrary to prior generalizations, some reproductive unicells can rapidly attain photosynthetic rates equal to those observed in adult thalli. The selective advantage of photosynthetic capacity and photoadaptation in algal reproductive unicells for colonization, adult zonation and individual competitive ability remain largely unexplored. These are however important steps in establishment of an algal community that may be markedly influenced by the photosynthetic potential exhibited by these important early settlers. The photosynthetic potential differences observed between different free-swimming stages of U. fasciata and E. flexuosa suggest that evolutionarily and ecologically allied species can have markedly different photoadaptive strategies. The photophysiological differences observed between reproductive unicells of opportunistic Ulvallean algae and kelps
characteristic of late successional communities are proposed to be representative of
exploitative and stress tolerant exploitation strategies, respectively. These strategies may be
seen in planktonic unicells of many benthic algae with similar macroalgal niches and adult
exploitation strategies.
Literature cited


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Table 3.1 Comparison of *Ulva fasciata* Gametophyte and Gamete P vs. I curve parameter means. Standard errors of the means are in parentheses. Pairwise comparisons of P vs. I parameters between life cycle stages are T-tests unless otherwise noted.

<table>
<thead>
<tr>
<th>Life Cycle Stages</th>
<th>n</th>
<th>R (^a)</th>
<th>I(_c) (^b)</th>
<th>I(_k) (^b)</th>
<th>(\alpha) (^c)</th>
<th>P(_{\text{max}}) (^a)</th>
<th>P:R ratio</th>
<th>Chl (a+b) ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gametophyte</td>
<td>12</td>
<td>0.55</td>
<td>27.1</td>
<td>162.9</td>
<td>1.17x10(^{-2}) m</td>
<td>1.84</td>
<td>4.78</td>
<td>1.72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.10)</td>
<td>(3.1)</td>
<td>(12.5)</td>
<td>(0.22)</td>
<td>(0.92)</td>
<td>(0.06)</td>
<td></td>
</tr>
<tr>
<td>Gametes</td>
<td>12</td>
<td>1.16</td>
<td>99.5</td>
<td>288.3</td>
<td>9.8x10(^{-3}) m</td>
<td>1.62</td>
<td>1.44</td>
<td>1.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.10)</td>
<td>(7.0)</td>
<td>(22.6)</td>
<td>(0.22)</td>
<td>(0.16)</td>
<td>(0.06)</td>
<td></td>
</tr>
<tr>
<td>Gametophyte vs.</td>
<td>12</td>
<td>0.61</td>
<td>-72.4</td>
<td>-125.4</td>
<td>1.8x10(^{-3}) m</td>
<td>0.22</td>
<td>3.34</td>
<td>0.35</td>
</tr>
<tr>
<td>Gamete pairwise</td>
<td></td>
<td>(0.11)</td>
<td>(5.6)</td>
<td>(21.3)</td>
<td>(0.23)</td>
<td>(0.89)</td>
<td>(0.04)</td>
<td></td>
</tr>
<tr>
<td>mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

p-value           | 0.0002 * | <0.0000* | <0.0000*   | 0.073 w ND    | 0.37 ND        | 0.0016 *       | <0.0000 * |

\(a\): \(\mu\)mol \(O_2\cdot mg\ Chl \(a+b\)\(^{-1}\cdot min^{-1}\)  
\(b\): \(\mu\)mol quanta \(m^{-2} \cdot s^{-1}\)  
\(w\): Pairwise Wilcoxon Test  
\(m\): median  
\(c\): \((\mu\)mol \(O_2\cdot mg\ Chl \(a+b\)\(^{-1}\cdot min^{-1}\))/ (\mu\)mol quanta \(m^{-2} \cdot s^{-1}\)  
ND: No difference  
*: significant difference  
\(n\): number of samples  
R: Respiration rate
Table 3.2 Comparison of *Ulva fasciata* Sporophyte, Zoospore and Settled Zoospore P vs. I curve parameter means. Standard errors of the means are in parentheses. Pairwise comparisons of P vs. I parameters between life cycle stages are T-tests unless otherwise noted.

<table>
<thead>
<tr>
<th>Life Cycle Stages</th>
<th>n</th>
<th>R</th>
<th>I&lt;sub&gt;c&lt;/sub&gt;</th>
<th>I&lt;sub&gt;k&lt;/sub&gt;</th>
<th>α</th>
<th>P&lt;sub&gt;max&lt;/sub&gt;</th>
<th>P:R ratio</th>
<th>Chl α:b ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporophyte</td>
<td>21</td>
<td>0.49</td>
<td>22.1</td>
<td>133.2</td>
<td>1.71x10^-2</td>
<td>2.04</td>
<td>5.33</td>
<td>1.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.08)</td>
<td>(3.2)</td>
<td>(9.5)</td>
<td>(2.07x10^-3)</td>
<td>(0.22)</td>
<td>(0.60)</td>
<td>(0.03)</td>
</tr>
<tr>
<td>Zoospores</td>
<td>13</td>
<td>0.80</td>
<td>61.5</td>
<td>172.6</td>
<td>1.14x10^-2</td>
<td>1.18</td>
<td>1.58</td>
<td>1.30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.16)</td>
<td>(6.4)</td>
<td>(9.8)</td>
<td>(1.84x10^-3)</td>
<td>(0.16)</td>
<td>(0.11)</td>
<td>(0.03)</td>
</tr>
<tr>
<td>Settled Zoospores</td>
<td>8</td>
<td>0.50</td>
<td>25.0</td>
<td>123.3</td>
<td>1.83x10^-2</td>
<td>1.83</td>
<td>3.83</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.04)</td>
<td>(1.7)</td>
<td>(11.50)</td>
<td>(0.15)</td>
<td>(0.45)</td>
<td>(0.02)</td>
<td></td>
</tr>
<tr>
<td>Sporophyte vs. Zoospore pairwise mean</td>
<td>13</td>
<td>0.32</td>
<td>-34.3</td>
<td>-26.9</td>
<td>8.63x10^-3</td>
<td>1.28</td>
<td>2.74</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.15)</td>
<td>(6.9)</td>
<td>(11.4)</td>
<td>(0.35)</td>
<td>(0.57)</td>
<td>(0.05)</td>
<td></td>
</tr>
</tbody>
</table>

p-value  
0.028 * 0.0002 * 0.019 * 0.026 * 0.0018 * 0.0002 * <0.0000 *

| Sporophyte vs. Settled Zoospore pairwise mean | 8  | 0.10 | -11.4        | -8.7         | 2.56x10^-3 | 0.13         | 3.14       | 0.36          |
|                                               |    | (0.03)| (5.6)       | (25.3)       | (0.16)      | (1.07)      | (0.02)     |               |

p-value  
0.005 * 0.0010 * 0.37 ND 0.0352 s * 0.22 ND 0.011 * <0.0000 *

\( a: \mu\text{mol O}_2/\text{mg Chl }a + b/\text{min}^{-1} \)  
\( b: \mu\text{mol quanta m}^{-2}\text{ s}^{-1} \)  
\( c: (\mu\text{mol O}_2/\text{mg Chl }a + b/\text{min}^{-1})/(\mu\text{mol quanta m}^{-2}\text{ s}^{-1}) \)  
\( m: \text{median} \)  
\( s: \text{Pairwise Sign Test} \)  
\( \text{ND: No difference} \)  
\( *: \text{significant difference} \)  
n: number of samples  
R: Respiration rate
Table 3.3 Comparison of *Enteromorpha flexuosa* Sporophyte and Zoospore P vs. I curve parameter means. Standard errors of the means are in parentheses. Pairwise comparisons of P vs. I parameters between life cycle stages are T-tests unless otherwise noted.

<table>
<thead>
<tr>
<th>Life Cycle Stages</th>
<th>n</th>
<th>R</th>
<th>I_c</th>
<th>I_k</th>
<th>α</th>
<th>P_max</th>
<th>P:R ratio</th>
<th>Chl a:b ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sporophyte</td>
<td>5</td>
<td>0.44</td>
<td>32.8</td>
<td>195.6</td>
<td>1.28×10^{-2}</td>
<td>2.00</td>
<td>4.70</td>
<td>1.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.46)</td>
<td>(6.6)</td>
<td>(15.2)</td>
<td>(1.03×10^{-3})</td>
<td>(0.09)</td>
<td>(0.48)</td>
<td>(0.04)</td>
</tr>
<tr>
<td>Zoospores</td>
<td>5</td>
<td>2.49</td>
<td>393.8</td>
<td>620.2</td>
<td>6.28×10^{-4}</td>
<td>1.30</td>
<td>0.58</td>
<td>1.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3.56)</td>
<td>(78.9)</td>
<td>(75.3)</td>
<td>(7.32×10^{-5})</td>
<td>(0.27)</td>
<td>(0.17)</td>
<td>(0.06)</td>
</tr>
<tr>
<td>Sporophyte vs. Zoospore pairwise mean</td>
<td>5</td>
<td>2.05</td>
<td>-361.0</td>
<td>-424.6</td>
<td>1.22×10^{-2}</td>
<td>0.70</td>
<td>4.12</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.35)</td>
<td>(77.7)</td>
<td>(86.1)</td>
<td>(1.03×10^{-3})</td>
<td>(0.35)</td>
<td>(0.60)</td>
<td>(0.04)</td>
</tr>
</tbody>
</table>

p-value | 0.0021 * | 0.0049 * | 0.0039 * | 0.0001 * | 0.058 ND | 0.0011 * | 0.001 * |

\(a: \mu\text{mol} \text{ O}_2 \cdot \text{mg Chl a+b}^{-1} \cdot \text{min}^{-1}\) for Chl a + b

\(b: \mu\text{mol quanta m}^{-2} \text{ s}^{-1}\)

ND: No difference

*: significant difference

\(c: (\mu\text{mol O}_2 \cdot \text{mg Chl a+b}^{-1} \cdot \text{min}^{-1}\) for Chl a + b) / (\mu\text{mol quanta m}^{-2} \text{ s}^{-1})\)

n: number of samples

R: Respiration rate
Table 3.4. Maximum photosynthetic rates of vegetative tissues normalized to Chl a, fresh weight, dry weight, and surface area. Stand error or the mean is in parentheses below each mean.

<table>
<thead>
<tr>
<th></th>
<th>$P_{\text{max}}^a$</th>
<th>$P_{\text{max}}^b$</th>
<th>$P_{\text{max}}^c$</th>
<th>$P_{\text{max}}^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ulva fasciata</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gametophyte (n=12)</td>
<td>3.41 (0.39)</td>
<td>3.02 (0.30)</td>
<td>14.23 (1.41)</td>
<td>31.25 (3.78)</td>
</tr>
<tr>
<td>sporophyte (n=21)</td>
<td>3.36 (0.39)</td>
<td>4.10 (0.34)</td>
<td>19.34 (1.61)</td>
<td>19.50 (1.33)</td>
</tr>
<tr>
<td><strong>Enteromorpha flexuosa</strong></td>
<td>(n=5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sporophyte</td>
<td>2.81 (0.310)</td>
<td>0.49 (0.41)</td>
<td>3.94 (0.36)</td>
<td>ND</td>
</tr>
</tbody>
</table>

$^a$: $\mu$mol O$_2$·mg Chl a$^{-1}$·min$^{-1}$  
$^b$: $\mu$mol O$_2$·g fresh weight$^{-1}$·min$^{-1}$  
$^c$: $\mu$mol O$_2$·g dry weight$^{-1}$·min$^{-1}$  
$^d$: $\mu$mol O$_2$·cm$^{-2}$·min$^{-1}$  
ND: not determined
Table 3.5 Comparison of P vs. I parameters for reproductive unicells of subtropical Ulvales and temperate Laminariales. 2.0 ml sample volume assumed for photosynthesis and respiration determinations for kelps (C. Amsler pers. comm.) fg Chl a/cell estimated from Figure 2 (Amsler & Neushul 1991).

<table>
<thead>
<tr>
<th>Source</th>
<th>P&lt;sub&gt;max&lt;/sub&gt;</th>
<th>P&lt;sub&gt;max&lt;/sub&gt;</th>
<th>R</th>
<th>I&lt;sub&gt;c&lt;/sub&gt;</th>
<th>I&lt;sub&gt;k&lt;/sub&gt;</th>
<th>fg Chl a/cell</th>
<th>Source</th>
</tr>
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<tr>
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<td>0.58</td>
<td>2.79</td>
<td>0.59</td>
<td>100</td>
<td>288</td>
<td>300</td>
<td>this study</td>
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<tr>
<td>gametes</td>
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</tr>
<tr>
<td>Ulva fasciata</td>
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<td>2.09</td>
<td>0.86</td>
<td>62</td>
<td>173</td>
<td>753</td>
<td>this study</td>
</tr>
<tr>
<td>zoospores</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Enteromorpha</td>
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<td>2.51</td>
<td>0.98</td>
<td>394</td>
<td>620</td>
<td>243</td>
<td>this study</td>
</tr>
<tr>
<td>flexuosa zoospores</td>
<td></td>
<td></td>
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<td>0.098</td>
<td>0.27</td>
<td>46</td>
<td>59</td>
<td>27</td>
<td>Amsler &amp; Neushul 1991</td>
</tr>
<tr>
<td>pyrifera zoospores</td>
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<tr>
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<td>0.0038</td>
<td>47</td>
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<td>58</td>
<td>Amsler &amp; Neushul 1991</td>
</tr>
<tr>
<td>luetkeana zoospores</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Laminaria</td>
<td>0.0047</td>
<td>0.039</td>
<td>0.0040</td>
<td>32</td>
<td>41</td>
<td>120</td>
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<tr>
<td>Pterogophora</td>
<td>0.012</td>
<td>0.038</td>
<td>0.0045</td>
<td>23</td>
<td>65</td>
<td>337</td>
<td>Amsler &amp; Neushul 1991</td>
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<td>californica zoospores</td>
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<td></td>
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<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

a: fmol O<sub>2</sub>·cell<sup>-1</sup>·min<sup>-1</sup>  
b: µmol O<sub>2</sub>·mg Chl a<sup>-1</sup>·min<sup>-1</sup>  
c: µmol quanta m<sup>-2</sup>·s<sup>-1</sup>  
R: Respiration rate
Figure 3.1. Typical sporic meiosis life cycle of *Ulva* and *Enteromorpha*. A: male and female gametophyte, B: biflagellate gamete, C: sporophyte, D: quadriflagellate zoospore.
Figure 3.2. Mean In vivo absorption and 4th derivative spectra of vegetative and reproductive tissues of A) Ulva fasciata gametophyte (n=5), B) Ulva fasciata sporophyte (n=8) and, C) Enteromorpha flexuosa sporophyte (n=7).
A gamete producing organism was compared to a vegetative organism. The gamete producing organism showed a significant increase in absorbance at certain wavelengths compared to the vegetative organism. The 4th derivative of the absorbance curves for both organisms also showed distinct patterns, with the gamete producing organism having a sharper peak at a specific wavelength.
PART IV

MICROSCALE ACCLIMATION IN PIGMENTATION
Abstract

Microscale (<10 cm) changes in the in vivo absorbance features and quantitative pigment extracts were observed among microsites in the intertidal turf algae Ahnfeltiopsis concinna (J. Ag.) Silva et DeCew and Laurencia mcdermidiae (J. Ag.) Abbott. Canopy tissues of A. concinna had significantly reduced levels of phycoerythrin, phycocyanin and allophycocyanin compared to understory tissues; whereas canopy tissues of L. mcdermidiae reduced levels of only phycoerythrin compared to understory tissues. These alterations coupled with enhanced carotenoid levels in canopy tissues compared to understory tissues indicated a pattern of remarkably sensitive photoacclimation over the <10 cm axes for turf forming algae.

This microscale variation in the in vivo UV-absorbance in turfs of A. concinna and L. mcdermidiae was directly related to the relative amount of extractable UV-absorbing compounds. Canopy tissues of these turf algae absorbed a significantly higher amount of UV-radiation than understory tissues in lab assays, increasing the likelihood that less UV-radiation reaches understory tissues. An in vivo absorption signature at 345 nm appears to provide a method to quickly and accurately gauge the UV-shielding capacity of primary producers even at remarkably fine ecological scales.

Microscale pigment adjustments to tropical PPFD and UV-environment by intertidal turfs are promoted by thalli densities that self-shade the understory portions of the same axes. The additional capacity, highly-responsive biochemical adjustments that result in marked canopy-understory distinctions, may be crucial for macroalgal tolerance of physiological stresses associated with tropical intertidal zones.
Introduction

Tropical primary producers inhabit latitudes with highest global levels of photosynthetic photon flux density (PPFD) and ultra-violet (UV) radiation (Caldwell et al. 1989, Hader 1993). Tropical terrestrial plants have adapted to high photon flux densities (HPFD) with a plethora of morphological, anatomical, and biochemical modifications (Hatch & Slack 1966, Yamamoto 1979, Caldwell et al. 1983, Demmig-Adams 1990, Melcher et al. 1994). Macroalgal photosynthesis is typically saturated at less than 15-25% of full sun levels compared to ~40% and 80% of full sun levels that saturate HPFD grown C3 and C4 plants, respectively (Boardman 1977, Luning 1981, Parts III & V). HPFD would appear to be especially problematic for intertidal macroalgae where desiccation and thermal stresses are also encountered during emersion into an PPFD rich environment. Sophisticated anatomical adaptations to water and PPFD stresses such as Kranz anatomy are overall genetically constrained among morphologically simple macroalgae compared to terrestrial plants. With this limitation, the genetic capacity for wide-ranging physiological acclimation may be essential for intertidal and shallow reef algae commonly exposed to PPFD ~8-fold higher than needed to saturate photosynthetic machinery.

Primary productivity of tropical macroalgae have been surveyed for fundamental responses to PPFD and quantification of accessory pigments (Littler & Littler 1992, Titlyanov et al. 1992) across intertidal to subtidal distributions. Among rhodophyte taxa, unusual variations from typical pigmentation of reddish color, have been noted in genera such as Chondrus, Gelidium, Gracilaria, Gigartina, Laurencia (Lubimenko & Tichovskaya 1928, Waaland et al. 1974, Ramus et al. 1976, Rhee & Briggs 1977, Gil-Rodriguez & Haroun 1992, KSB & CMS pers. obs.). Changes in red algal pigmentation coupled with a shift from sun- to shade-type physiological state are most pronounced when contrasting individuals from opposite ends of tidal distributions regardless of latitude (Waaland et al. 1974, KSB & CMS pers. obs.). Pigmentation changes in red algae are attributed to an
increase in the ratio of Chl a to phycobiliproteins (Ramus et al. 1976) and has been shown to be driven by changes in ambient PPFD (Waaland et al. 1974, Rhee & Briggs 1977, Ramus 1983). This ecophysiological acclimation strategy, the optimization of photosynthetic capacity to ambient PPFD, is analogous to the better known shade vs. sun acclimations of terrestrial plants. For macroalgae of higher latitudes, this change is usually detected over extensive spatial gradients = their tidal distribution (meters). However, some red algal turfs in tropical latitudes have narrow vertical distributions as well as non-red apical tissues (Doty 1967, Liang 1984, Gil-Rodriguez & Haroun 1992). These modifications appear to be at an extreme in tropical turf species such as Ahnfeltiopsis concinna and Laurencia mcdermidiae whose canopy tissues have obvious alterations in pigmentation relative to understory tissues of the same axis. Within the compressed tidal zone found in Hawai‘i, these algae appear to have the capacity to adjust their photosynthetic constituents on a substantially sensitive scale.

Not only is the tropical environment rich in PPFD, it is also a UV-rich habitat. The occurrence of extractable UV-absorbing compounds (UVACs), primarily mycosporine-like amino acids (MAAs), scytonemin, and flavonoids from an evolutionarily diverse assemblage of primary producers has been well documented (Sivalingam et al. 1974, Dunlap & Chalker 1986, Carreto et al. 1990, Karentz et al. 1991, Ziska et al. 1992, Garcia-Pichel et al. 1993). The extent to which such compounds can be detected in vivo and vary spatially in macroalgae has only recently been addressed (Part II).

UV-radiation impacts primary productivity in a wide array of organisms including phytoplankton (Hebling et al. 1992), macroalgae (Wood 1989), zooxanthellae (Kinzie 1993), and terrestrial plants (Sullivan & Teramura 1989). Just as HPFD is associated with sun-plant acclimations, many primary producers exposed to high levels of UV-radiation increase concentrations of UVACs (e.g., S-320, S-334, flavonoids) (Wood 1989, Ziska et al. 1992, Kinzie 1993). The interception of UV-radiation by UVACs in primary producers
may partially pre-empt UV-induced damage to the D1 protein of photosystem II (Yerkes et al. 1989), Calvin cycle enzymes (Dohler 1984, Strid et al. 1991), and nucleic acids (Peak & Peak 1983), reduce the potential for UV-induced photoinhibition (Cullen & Lesser 1991) as well as mitigate total crop losses (Teramura & Sullivan 1993).

Increasing levels of UV-radiation have been a primary concern in polar regions (Helbling et al. 1992) even though typical levels of UV-radiation to which tropical algae are subjected are at least two-fold higher than at higher latitudes (Caldwell et al. 1989). With this strong, persistent selective pressure, it follows that extant tropical primary producers have adapted to long-term conditions of elevated PPFD and UV-radiation and are likely to possess mechanism(s) to minimize photon flux density (PFD)-induced damage (Hazzard 1993, Shick et al. 1995). MAAs in some marine organisms and flavonoids in terrestrial plants are current examples of putative photoprotective compounds (Robberecht et al. 1980, Caldwell et al. 1983, Karentz et al. 1991).

In the following investigations (parts I and II), I evaluated the extent of in situ pigment and physiological adjustments in model tropical turf algae to better understand the importance of acclimation strategies for these primary producers. The following models were chosen. 1) Ahnfeltiopsis concinna is the highest intertidal macroalga on basalt lava flows in the Hawaiian Islands (Doty 1967) and typically occurs in distinct monospecific stands on basalt benches in both shaded and unshaded habitats (KSB pers. obs.). 2) Laurencia mcdermidiae is part of a dense turf community composed of up to 30 species of macroalgae (McDermid 1988a). Laurencia mcdermidiae is typically on the seaward-most edge of both limestone and basalt benches but is not found in shaded habitats (KSB pers. obs.). In part I, I identify adjustments in photosynthetic accessory and photoprotective pigments within stands of A. concinna and L. mcdermidiae. In Part II, I identify adjustments in physiological capacity in response to changes in PFD and explore the
coupling between pigmentation and physiological changes in *A. concinna* and *L. mcedermidiae*.

In part I, I specifically asked: 1) How do pigments, both photosynthetic accessory and photoprotective, vary over the height of a thallus in these diminutive turfs?; and 2) How do qualitative *in vivo* and quantitative extract measures compare in evaluating these pigments and their potential modifications?

**Materials and methods**

**Microsite definitions and comparison scheme:** In order to address the adjustments made within a thallus and over the distribution of *A. concinna* and *L. mcedermidiae*, a number of tissue types were compared. I first defined the following tissue types (Figure 4.1): 1) canopy: tissues 1.0 cm in length sampled 1.0 cm below apical meristem from the center of unshaded regions, 2) understory: tissues 1.0 cm in length sampled 1.0 cm above basal attachment from the center of unshaded regions, 3 & 4) high-photon flux density (HPFD) and low-photon flux density (LPFD): tissues 1.0 cm in length sampled 1.0 cm below apical meristem from permanently unshaded and shaded regions, respectively, and 5 & 6) peripheral and central: tissues 1.0 cm in length sampled 1.0 cm above basal attachment from the edge and center regions of unshaded turfs, respectively (Figure 4.1). Secondly, the following tissue types were compared: 1) canopy vs. understory tissues, 2) tissues from HPFD vs. LPFD, and 3) tissues from peripheral vs. central regions. Comparisons 2 and 3 could not be performed for *L. mcedermidiae* because these microsites did not occur in the range of habitats available on O'ahu. Sites sampled for canopy vs. understory, peripheral vs. central, and HPFD tissues for HPFD vs. LPFD comparisons had a relief similar topographically and lacked any overhangs or basalt formations that may have shaded turfs from direct diurnal exposure to the sun. Sites sampled for LPFD tissues in LPFD vs. HPFD comparison were all chosen purposely from regions underneath overhangs or within depressions (>10 cm) of the basalt bench and did not receive any
direct sunlight during the course of a day. Canopy and HPFD tissues as well as understory and central tissues were by the above definition identical, but independent samplings were performed for each comparison except for phycobilin concentrations (see Statistical Analyses below).

**PPFD at microsites:** Incident PPFD was measured for the above mentioned microsites from sunrise to noon on several days in Sept. 1994. Incident PPFD at microsites was related by linear regression to incident PPFD upon canopy tissues of *A. concinna* and *L. mcdormidiae*. PPFD was measured with a Li-Cor LI-192SA cosine quantum sensor with two methodologies: 1) Microsite PPFD was measured at 15 min intervals on one of 5 randomly selected individuals of *A. concinna* and *L. mcdormidiae*; 2) for incident understory PPFD of *A. concinna*, mean PPFD was also recorded every minute via a Li-Cor LI-1000 data logger and compared to incident canopy PPFD recorded in an identical manner. Five 'individuals' of *A. concinna* were monitored in this second manner for ~1 hour per stand. (Note: comparison of the regression relationships of understory vs. canopy PPFD for *A. concinna* generated by each method were not statistically different (data analysis not shown). From these data, the percent extinction of canopy / HPFD PPFD at microsites 1-3 was determined.

**Sample collection and handling:** *Ahnfeltiopsis concinna* (Rhodophyta, Gigartinales) and *L. mcdormidiae* (Rhodophyta, Ceramiales) were collected between 1 Aug. 1994 and 4 Feb. 1995 at Makapu'u and Kaloko, O'ahu, Hawai'i. Thalli were immediately transferred to the laboratory and cleaned of macroscopic epiphytes. The thalli were rinsed and stored in 0.2 µI filtered seawater (FSW) at room temperature (23°C) under 50 µmol quanta m-2 s-1. **In vivo** spectral analyses were performed within 3 hours of collection. Samples for methanol: tetrahydrofuran (MTHF) extraction (see below) were then blotted dry and frozen (~20 ºC). An independent set of replicated samples was utilized
for Chl a, carotenoid and phycobilin extraction. Photosynthetic accessory pigment extraction was performed within 12 h of sample collection.

**Photosynthetic pigment quantification:** Chl a concentration was determined using N,N-dimethylformamide (DMF) extraction (Moran & Porath 1980) and a Hewlett Packard 5428 A Diode array spectrophotometer. Calculations were based on the extinction coefficient of Inskeep and Bloom (1985). Chl a concentration was normalized to tissue fresh weight (fw) and dry weight (dw). The relative concentrations of total carotenoids in canopy and understory tissues of *A. concinna* were also determined from the DMF extracts. The ratio of 482 nm extract absorbance maxima to the Chl a 664 nm maxima were used as a measure of carotenoid concentration normalized to Chl a content.

Concentrations of individual phycobiliproteins, R-phycoerythrin (R-PE), R-phycocyanin (R-PC) and allophycocyanin (APe), were determined for replicate fresh samples. Phycobilin type (e.g., B-PE vs. R-PE) was determined via the relative position of both in vivo and extract absorbance maxima (data not shown). Phycobilins were extracted at 5 °C (unless otherwise noted) with NaPi buffer (pH: 7.0). Tissues were frozen in liquid nitrogen and homogenized with a pre-chilled mortar and pestle. This homogenate was centrifuged for 10 min at 3200 rpm. The supernatant was passed through a Celite column buffered with NaPi buffer to remove residual Chl a (Kursar & Alberte 1983). Samples were centrifuged for 10 min at 1500 xg and absorbance measured in a Hewlett Packard 5428 A Diode Array spectrophotometer. Concentrations were determined on a fw basis using the equations of Kursar and Alberte (1983). Additional extraction of pellet formed after the initial centrifugation yielded ≤6% of phycobiliproteins from the initial extraction (data not shown).

**Analyses of in vivo absorption spectra:** In vivo absorption spectra were obtained using a Shimadzu UV Vis-2101 spectrophotometer with a 150 mm integrating sphere attachment (Shimadzu) on algal tissues 1.3 cm by 3.0 cm in dimension using procedures
detailed in Part III. Spectra were obtained by first gently blotting vegetative tissues and then orienting tissues to display a 1.3 x 3.0 cm region for absorbance analysis. These tissues were aligned with the sample window at the rear of the integrating sphere. Many upright axes were placed in parallel orientation to create a non-overlapping layer of similarly aged and pigmented vegetative tissue. Spectral scans from 280 to 750 nm (400 to 750 nm for HPFD, LPFD, peripheral and central tissues) were acquired in under one minute at room temperature with a lamp changeover wavelength at 360 nm. Spectrum sampling intervals were set at 0.5 nm with a fast scan speed and normalized to zero at 750 nm. Baseline spectra were acquired using the same window components without algal tissue. Fourth derivative spectra were generated from in vivo absorbance spectra with the Savitsky-Golay algorithm provided by the manufacturer's software utilizing a λ of 16 nm.

Percent transmittance of canopy tissues of *A. concinna* were assessed using the same methodologies as detailed above and in Part III but tissues were placed in the front of the integrating sphere in the solid sample holder manufactured by Shimadzu. One hundred percent transmittance was established by acquiring the baseline in transmittance mode with the solid sample holder and support materials in optical path.

**Extraction of UV-absorbing compounds:** UV-absorbing compounds, Chl a and carotenoids were extracted from canopy and understory tissues of *A. concinna* and *L. mcdonaldii* following Kinzie (1993) with minor modifications. Frozen algal tissue (0.50 g (±0.02 g)) was placed in a pre-chilled mortar and pestle and repeatedly ground in liquid nitrogen into a fine powder followed by the addition of 5.0 ml of chilled methanol : tetrahydrofuran solvent (MTHF) (80:20 v:v). The components used in extraction were rinsed with 5.0 ml of solvent until clear of all visible pigment. This resulted in a total extract volume of 10.0 ml. Tubes were capped, shaken vigorously and placed on ice for 2 hrs. All steps were carried out in dim light. After 2 hrs samples were again shaken and then centrifuged for 10 min at high speed. The supernatant was diluted with MTHF to a
total volume of 20.0 ml and measured spectrophotometrically from 200 to 750 nm. Measurements were performed on a Shimadzu UV Vis-2101 spectrophotometer with a 0.5 nm sampling interval in a standard 1.0 cm quartz cuvette.

**Statistical analyses:** Statistical analyses were carried out using Minitab version 8.2 (Minitab Inc.). Normality was assessed with a test equivalent to the Shapiro-Wilk test with an α of 0.05 (Minitab Reference Manual, 1991). When normally distributed, comparisons of 1) R-PE, R-PC, APC, Chl a concentrations, 2) in vivo absorption peak ratios and extract peak ratios of microsites in *A. concinna* and *L. mcedermidiae* were made with one-sided pairwise t-tests. Comparisons of phycobilin concentrations from HPFD vs. LPFD and central vs. peripheral tissues were made in a non-pairwise manner on account of the independence of samples from each microsite.

The relationship of 1) in vivo absorbance UVACs maxima (normalized to red Chl a maxima) to corresponding extract absorption maxima in *A. concinna* and *L. mcedermidiae* was tested by linear regression. Normality of the regression residuals was tested as mentioned above. Comparisons of the slopes and y-intercepts of regression lines were made as detailed by Zar (1984). Bonferonni corrections were performed on the α's for tests involving *A. concinna* and *L. mcedermidiae* when the same data were used for multiple tests.

**Results**

**Microsite characterization:** *In situ*, understory tissues of *A. concinna* received PPFD at levels 0.3% of canopy PPFD while fully hydrated (Figure 4.2a). Similarly, understory tissues of *L. mcedermidiae* on average experienced ~12% of the PPFD incident on canopy tissues of the same axes (Figure 4.2b). Peripheral and LPFD microsites of *A. concinna* experienced only 71% and 48% of the incident canopy PPFD, respectively (Figures 4.2c & d).
Comparison of photosynthetic pigments at microsites: No differences were detected in Chl a concentration on a fw or dw basis between canopy and understory tissues of _A. concinna_ (Table 4.1). _Laurencia mcdermidiae_ had markedly higher Chl a:fw ratio in understory tissues compared to canopy tissues (Table 4.2). Chl a concentrations in central tissues were indistinguishable from those of peripheral tissues in _A. concinna_ on both a fw and dw basis (Table 4.3). Contrary to this pattern, LPFD tissues of _A. concinna_ possessed higher concentrations of Chl a on a fw and dw basis when compared to HPFD tissues (Table 4.4).

Phycobilin concentrations and PE:PC ratio were altered in distinctly different patterns for _A. concinna_ when compared with _L. mcderrmidiae_. The relative amount of R-PE, R-PC and APC in canopy, HPFD, and peripheral tissues compared to understory, central and LPFD tissues, respectively, were reduced in _A. concinna_ (Tables 4.1, 4.3, 4.4). (Note: R-PC was reduced in canopy tissues compared to understory tissues of _A. concinna_ but the difference was not statistically significant (Table 4.1)). Canopy tissues of _L. mcderrmidiae_ only differed in the concentration of R-PE when compared with understory tissues (Table 4.2). The ratio of PE:PC was consistent in the different microsites of _A. concinna_ (Tables 4.1, 4.3, 4.4) but was reduced in the canopy tissues of _L. mcderrmidiae_ when compared with understory tissues (Table 4.2).

_Ahnfeltiopsis concinna_ increased relative carotenoid concentration in canopy relative to understory tissues (Table 4.5). Relative carotenoid concentrations were not examined in _L. mcderrmidiae_ via extraction (but see Discussion).

**Microsite variation for in vivo 400 to 750 nm absorbance:** The positions of in vivo absorption and fourth derivative maxima in _A. concinna_ and _L. mcderrmidiae_ were nearly identical to those of other tropical rhodophytes from similar light environments (Part II) and similar but more highly resolved than fourth derivative spectra from temperate rhodophytes.

The relative magnitude of in vivo absorbance maxima differed among microsites in these turf forming algae (Figures 4.3a, 4.4a, 4.5). Canopy, HPFD and peripheral tissues of *A. concinna* had markedly reduced absorbances between 500 and 650 nm, including maxima specific for R-PE (546 and 568 nm) and R-PC (625 nm) when contrasted to understory, LPFD and central tissues, respectively (Figures 4.3a & 4.5). Canopy tissues of *L. mcdermidiae* had reduced absorbances between 500 to 580 nm when compared to understory tissues, including maxima specific for R-PE (542 and 572 nm) only (Figure 4.4a). Both turfs increased the relative absorbance ratio of ~440:680 nm in canopy tissues compared to understory tissues; in contrast only *A. concinna* increased the ~495:680 nm absorbance ratio from understory to canopy tissues (Table 4.5). Fourth derivative spectra were nearly identical when comparing canopy and understory tissues of *A. concinna* or *L. mcdermidiae* (Figures 4.3b & 4.4b). Canopy tissues of *A. concinna* transmitted a greater proportion of light between 525 and 660 nm compared to other wavelengths between 280 to 700 nm.

**In vivo UV-absorption and fourth-derivative spectra:** Both turf species absorbed radiation between 280 and 400 nm to an extent approximately equaling or surpassing comparative 400 to 750 nm absorbances (Figures 4.3a & 4.4a). In vivo UV-absorbance spectra were similar among both species with an absorbance maxima at ~345 nm. MTHF extraction of UVACs revealed prominent absorption maximum between 328 and 333.5 nm (Figures 4.3a & 4.4a). Similar maxima were observed for in vivo absorption and extract absorption spectra (Figures 4.3a & 4.4a), although maxima detected after extraction were shifted approximately 15 nm to shorter wavelengths in comparison to maxima detected via in vivo absorption.
Fourth-derivative analyses revealed that the single in vivo UV-absorbing maximum at ~345 nm has 3 to 5 components with maxima ranging from ~305, 319.5 to 323, ~340, 354 to 356.5, and 370 to 385 nm (Figures 4.3b & 4.4b). The putative identity of these components was ascribed to MAAs (Sivalingam et al. 1974, Karentz et al. 1991). For L. mcdermidiae tissues, a prominent 360 to 381 nm shoulder was detected via in vivo absorbance spectroscopy and confirmed with spectra of extracts (Figure 4.4a).

**Microsite variation as revealed by in vivo UV-absorbance:** Because canopy tissues of both thalli are quite effective in absorbing UV-wavelengths and attenuating PPFD in situ (see above), intact understory tissues of A. concinna and L. mcdermidiae are likely to have less incident UV-radiation than canopy tissues. This probable difference in exposure to UV-radiation in the field was clearly paralleled by sharp increases in in vivo absorbances around 370 nm in canopy tissues of A. concinna (Figure 4.3a). This 370 nm signature was present in all canopy tissues examined and was lacking in all understory tissue samples of A. concinna (Figure 4.3a). In contrast, L. mcdermidiae lacked a definitive spectral signature but increased UV-absorbance was manifested as higher, overall absorbances from 280 to 400 nm in canopy tissues compared to understory tissues (Figure 4.4a).

For A. concinna, canopy tissues were found to have a significantly higher concentration of UVACs than understory tissues as measured by both the in vivo absorption ratio (345:680 nm) and extraction absorption ratio (334: 666 nm of the UVACs specific absorption maximum to the Chl a maximum) (Table 4.5). Laurencia mcdermidiae was found to similarly alter its distribution of UVACs (Table 4.5).

**Correspondence of in vivo absorption and extracts of UV-absorbing compounds:** To evaluate the utility of in vivo absorption spectra in the assessment of relative UVACs concentration, the ratios of in vivo UVACs maxima to the red Chl a maxima from canopy and understory tissues of A. concinna and L. mcdermidiae were regressed on corresponding ratios from extractions. Highly significant, positive relationships between
in vivo and extract ratios were observed in A. concinna (Figure 4.6a) and L. mcedermidiae (Figure 4.6b). The slopes of these relationships (Figure 4.6) were markedly different (F=14.44, df:2,37, p<0.0005) with the slope for A. concinna relationship being significantly greater than the relationships for L. mcedermidiae. The y-intercepts of the regression lines however were not statistically different (F=2.63, df:2,39, p=0.08). The normalized 375.5 nm maximum in L. mcedermidiae was also consistently greater in canopy tissues when compared to understory tissues (Figure 4.4a).

**Discussion**

Associated with PPFD reaching 2300 μmol quanta m⁻² s⁻¹ and typical UV-radiation surpassing 'ozone-hole' conditions found at higher latitudes (Caldwell et al. 1989, Hader 1993), intertidal rhodophyte turfs have genetic capacities to precisely regulate photoprotective and photosynthetic accessory pigments at scales previously not identified. The sensitivity of these acclimation responses allows coordinated adjustment of carotenoids, phycobilins, and UVACs contents over distances <10 cm in response to rapid attenuation of PFD. Intertidal macroalgae are exposed to PPFD 8-fold greater than saturation irradiances, but are typical shade acclimated, C-3 primary producers. With this in mind, the capacity to precisely regulate and coordinate a diversity of biochemical responses to HPFD stresses is likely to provide a set of essential adaptations for tidal existence, especially when one considers the apparent genetic constraints against sophisticated anatomical adaptations such as pubescence or Kranz anatomy found in some terrestrial plants. Two distinct patterns were apparent when contrasting A. concinna and L. mcedermidiae. Ahnfeltiopsis concinna acclimates to HPFD by decreasing all phycobilin pigment beds while concomitantly augmenting carotenoid and putative MAA levels. Laurencia mcedermidiae acclimates to HPFD by decreasing only PE, maintaining high levels of PC and APC, while concomitantly increasing levels of carotenoids, putative MAAs and additionally UV-absorbing terpenoids.
Regulation of synthesis and turnover of photosynthetic accessory and protective pigments takes place at a remarkably fine scale (centimeters) along axes of tropical rhodophyte turfs. The in vivo differences in phycobilin and carotenoid pigmentation in these tropical turfs parallel changes seen in other turf and non-turf rhodophytes such as *Mastocarpus papillatus* and *Porphyra perforata* from high and low intertidal locations (Smith & Alberte 1994) as well as *Chondrus crispus* from shallow and deep subtidal tissues (Rhee & Briggs 1977). Differences in extract quantification of phycobilin pools between microsites corresponded to differences in high-PPFD vs. low-PPFD grown *Gracilaria* sp. and *Griffithsia pacifica* (Waaland et al. 1974, Beer & Levy 1983). These striking modifications observed in *A. concinna* and *L. mcdermidiae* in phycobilin, carotenoid and UVACs levels are believed to be promoted by the functional morphology of turfs which produces a markedly different light microclimate in understory tissues of turfs compared to the external canopy (Glynn 1965). Increased phycobilin concentration as an photoacclimation response to decreased PPFD, as well as increased carotenoid levels in response to increased, potentially photoinhibitory irradiances, are well documented among temperate algae (Lubimenko & Tichovskaya 1928, Brown & Richardson 1968, Waaland et al. 1974, Ramus et al. 1976, Rhee & Briggs 1977, Falkowski & LaRoche 1991), but occur only over large spatial gradients.

*Ahnfeltiopsis concinna* and *L. mcdermidiae* possess the ability to precisely control levels of biochemical constituents (Note: Laboratory experiments where canopy and understory tissues of *A. concinna* were exposed to experimental PPFD regimes support photoacclimation as the mechanism controlling the in situ pigment differentiation between microsites (Parts VI & VII)). Sensitive photoacclimation responses, coupled with the functional morphology of a turf may play an essential role in minimizing the impact of excess PPFD and UV-radiation as well as desiccation stress.
Ahnfeltiopsis concinna and L. mcdermidiae have both been noted as having unusual canopy pigmentation (Doty 1967, McDermid 1988a). The canopy tissues of most specimens of A. concinna are typically yellow-orange to the naked eye while the canopy of L. mcdermidiae, previously referred to as Laurencia sp. 'green' (McDermid 1988b), has a blue-green appearance. The understory tissues of these turfs are red to black (pers. obs.).

In vivo absorbance studies quickly and accurately substantiate these casual observations and additionally strongly correlate (Pearson correlation coefficient: 0.798) to extract quantification of the modifications in R-PE, R-PC and carotenoid contents in these differentially pigmented tissues (analysis not shown). Similar pigment changes seen when comparing peripheral vs. canopy as well as understory vs. LPFD tissues demonstrates that the observed pigmentation changes did not result from the age of the tissue but instead were caused by an acclimation to the ambient light environment.

The differential decreases in absorbance maxima observed between canopy tissues of A. concinna and L. mcdermidiae demonstrates some of the diversity in photoacclimative strategies possible among tropical turfs. Whereas A. concinna decreased levels of R-PE, R-PC and APC in similar proportions in response to HPFD, L. mcdermidiae decreased levels of R-PE only. Phycobilin modifications although distinct in these two turfs, accomplish similar outcomes. Reduction in levels of photosynthetic accessory pigment are part of the acclimation to "sun" conditions. In this acclimation, nitrogen bound into phycobilins and linker polypeptides which were needed to optimize photon capture in light limiting conditions are likely to be incorporated into Z-scheme, RUBISCO and dark reaction components (Osmond 1994) to optimize ATP production and carbon fixation.

As suggested by differences in in vivo absorption and transmittance spectra of canopy vs. understory tissues of A. concinna, the canopy alters the quantity and potentially the quality of light incident on understory tissues as observed with far-red enrichment in terrestrial forest understories (Nobel 1991). Understory tissues may experience long
periods of enriched levels of green wavelengths for the few photons that pass through canopy tissues. Thus, the canopy enhances light harvesting by transmitting predominately the quality of light the phycobilisome absorbs most efficiently. Enhanced green light levels may also enhance R-PE synthesis as C-PE synthesis was enhanced in *Fremyella diplosiphon* (Cyanophyta) (Oelmuller *et al.* 1988). The marked differences in UV-absorption between canopy and understory tissues of *A. concinna* (Figure 1a) and *L. mcdermidiae* (Figure 3a) are likely to also have direct relationships to the quantity of UV-radiation incident on the basal tissue of these turf algae. Thallus densities inhibit an average of ≥95 and 90% incident PPFD from reaching understory tissues of *A. concinna* and *L. mcdermidiae*, respectively. UVACs of canopy tissues seem likely to protect understory tissues from photodamage by pre-emptive UV-capture.

The pigment identities appear to be conserved in canopy and understory tissues of *A. concinna* or *L. mcdermidiae*. Peak splitting revealed in fourth derivative spectra, as has been observed for novel carotenoids in high intertidal *Mastocarpus papillatus* (Smith & Alberte 1994), or novel fourth derivative maxima were not observed in *A. concinna* nor *L. mcdermidiae*. Equivalent carotenoid maxima at 466.5 nm were seen in both sampling tissues for both species (Figures 4.3b & 4.4b). The increased in vivo absorbance at ~495 nm in canopy tissues of *A. concinna* may be the result of identical carotenoids varying in pool sizes. The carotenoids present in *A. concinna* are lutein, zeaxanthin, α-carotene, and β,β-carotene (Liang 1984). Assignment of specific fourth derivative maxima to specific carotenoids is complicated by the contribution of R-PE to fourth derivative spectra and the similarity of the absorbance maxima of these pigments. Although not indicated by the 495:680 nm ratio, the overall in vivo absorbance spectrum indicates carotenoid concentration were higher in canopy tissues of *L. mcdermidiae*. Because Chl a concentrations were higher in understory tissues of *L. mcdermidiae* when determined on a fw basis (Table 4.2) and R-PE specific peaks were reduced in canopy tissues relative to
understory tissues (Table 4.5), it follows that the height of the 495 nm R-PE/carotenoid peak in canopy tissues must be augmented with increased carotenoid levels (Figure 4.3). Specific carotenoids identities in L. mcdernidiae remain uninvestigated. The role of carotenoids in quenching of triplet state Chl a and subsequent dissipation of excess energy as heat is well established (Goodwin 1991). The violaxanthin-cycle via zeaxanthin has not been demonstrated among rhodophytes (Demmig-Adams 1990, Young 1991) while a light harvesting function for many carotenoids in one or both light harvesting complexes in rhodophytes remains unclear (Gantt 1990).

As the highest intertidal macroalgae on Hawaiian intertidal benches (Doty, 1967) (mean height +1.1 m above MLLW, Beach, unpublished data), A. concinna is exposed to a more severe combination of PFD (UV and photosynthetically active radiation) and desiccation stress than experienced by lower intertidal macrophytes such as L. mcdernidiae (mean height +0.3 m above MLLW, Beach, unpublished data). The ability to concentrate high levels of UVACs in the canopy tissues of an A. concinna coupled with increases in levels of photoprotective carotenoids may aid in A. concinna's success and longevity in this stressful niche. Different regression relationships of extractable UVACs concentration to in vivo maxima ratios were detected in this study (Figures 4.6). In A. concinna, the in vivo absorbance spectra does not reflect the extractable concentration of UVACs to the extent that is seen in L. mcdernidiae. If in vivo absorbance characteristics reflected equivalent extractable amounts in both species, then in vivo absorbance should be much greater from 280 to 400 nm for A. concinna than for L. mcdernidiae. The packaging of UVACs and cellular organization in the thallus may differ in these species or the ease of extraction of UVACs may also differ. Repeated methanol:tetrahydrafuran extraction of the algal pellet indicated that >95% of the UVACs were extracted via our method. For accurate in vivo prediction of relative UVACs concentration, individual species must be characterized at this time rather than relying solely on absorbance maxima.

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Maxima for \textit{in vivo} absorbance at 381 nm in \textit{L. mcdermidiae} may be related to secondary metabolites known to have roles in herbivory and other biological deterrences. Members of the genus \textit{Laurencia}, such as \textit{L. mcdermidiae}, are known producers of a vast array of terpenoid-like compounds (Fenical 1983). \textit{Corps en cerise} contained within the cortical cells of \textit{L. mcdermidiae} (McDermid 1988b) are the site of storage of halogenated secondary metabolites (Feldmann \\& Feldmann 1950). These highly refractive globular inclusions appear to affect the absorbance of UV-A radiation, at least in \textit{L. mcdermidiae}. Although not held to be the primary role for this class of secondary metabolites, these compounds may have a role in absorbance of UV-A radiation by the canopy tissues.

Pigment based acclimation is highly responsive to the ambient PFD environment for \textit{A. concinna} and \textit{L. mcdermidiae}. Broad spectrum pigment-based acclimations and adaptations of the photosynthetic apparatus are evident among algae in several ways: 1) Clearly, macro- and microalgae harvest light via a diversity of photosynthetic accessory pigments (e.g., fucoxanthin, peridinin, siphonoxanthin, siphonein, phycoerythrin, phycocyanin, allophycocyanin, violaxanthin, Chl \textit{c1+c2}) (Rowan 1989); their lack of development or loss in the Charophyte to Traechophyte lineages suggests a strong selective pressure in the marine environment to maintain pigment diversity; 2) Relative modifications of pigment constituents are also greater in algae, whereas in the common garden plants \textit{Veronica} spp., Chl \textit{a:b} ratios range from 0.8 to 1.8 in when grown in different light regimes (Dale \\& Causton 1992), and have been reported between 2.5 to \textasciitilde2.8 in other terrestrial plant samples from LPFD to HPFD regimes, respectively (Anderson \\& Osmond 1987, Terashima \\& Evans 1988, Bjorkman 1981, Barber 1985). This up to 125\% intra-generic increase is small compared to \textit{Ulva rotundata} where Chl \textit{a:b} ratio was adjusted from 1.6 to 4.5, a 281\% change, depending upon total daily PFD (Henley \\& Ramus 1989). A genetic basis for a broadness of biochemical responses is strongly
supported by an 84% and 189% increase in levels of R-PE and 250% and 244% increase in UVACS within an individual thallus of A. concinna and L. mcdermidiae, respectively.

Intertidal turfs appear to require a broad genetic basis for the capacity to acclimate to both HPFD induced stresses found in canopy tissues and LPFD in self-shaded understories. Engelmann's Chromatic Adaptation hypothesis continues to be supported in that all algae living under light limitation must find a way to harvest the "green-window" in which the bimodal absorption spectrum of Chl a is ineffective (Luning 1981). Expression of different light harvesting constituents such as siphonein over depth gradients (Kageyama et al. 1977), between life cycle stages (Part III) and the ubiquitous presence of 490 to 600 nm absorbing photosynthetic accessory pigments in deep water macrophytes (Littler et al. 1986) suggests an evolutionary selection for the ability to respond to short and/or long term challenges of the marine light environment. Luning (1981) suggests that differential sensitivity towards HPFD and UV-radiation may be a major factor in controlling algal distributions especially in high intertidal regions. I propose that persistence in the high intertidal regions also requires additional coordinated synthesis of photoprotective pigments coupled to the absorption spectra of Chl a, nucleotides, and amino acids thereby reducing the potential for disruption of cell functions.

Conclusions

Detailed examination of in vivo absorbance and fourth derivative spectra over the 280 to 750 nm range have been obtained for tropical rhodophyte turfs for the first time. The relationship between in vivo and extract measures of pigment abundance demonstrated the ability of the in vivo absorption spectra to characterize differences in level of photosynthetic accessory pigments of specimens from different photoacclimative states. This predictive power coupled with the ability to assay relative UVACs concentration provides a powerful tool in the study of ecophysiology for primary producers.
Microscale acclimation to tropical PFD levels is manifest in high intertidal turfs by striking changes at the level of the pigment bed. Within an individual thallus (<10 cm) phycobilin, carotenoid and UVACs levels are precisely adjusted in response to changes in PFD. Rhodophyte turfs respond to increased (potentially photoinhibitory) PPFD and UV-radiation by increasing level of putative photoprotective pigments (carotenoids and UVACs) while concomitantly decreasing nitrogen-costly phycobilins. Two distinct strategies accomplish this in A. concinna and L. mcdermidiae. Ahnfeltiopsis concinna reduces levels of all phycobilins while L. mcdermidiae maintains PC and APC levels while reducing PE with exposure to HPFD. Both turfs increase levels of carotenoids and MAAs but only L. mcdermidiae increases absorbance of UV-A radiation with higher levels of terpenoid-like compounds. Continued study of this spatially precise physiological control is likely to reveal cell regulation that rivals terrestrial plant adaptations to light and water stresses.
Literature cited


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Table 4.1. Comparison of relative photosynthetic pigment content for canopy vs. understory tissues of *Ahnfeltiopsis concinna*. Standard errors of the means are in parentheses below the means. Chl \( a:fw \) ratio & Chl \( a:dw \) ratio: mg/g. R-PE, R-PC and APC: mg/g (fw). *: significant difference, ND: no difference

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Canopy</th>
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<th>Canopy vs. Understory pairwise mean</th>
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<td>APC</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>15</td>
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<td>4.0x10^{-3} (1.0x10^{-3})</td>
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<tr>
<td></td>
<td>15</td>
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<td>4.1x10^{-2} (1.0x10^{-2})</td>
<td>0.071 ND</td>
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Table 4.2. Comparison of relative photosynthetic pigment content for canopy and understory tissues of *Laurencia mcdormidiae*. Standard errors of the means are in parentheses below the means. Chl a:fw ratio & Chl a:dw ratio: mg/g. R-PE, R-PC and APC: mg/g(fw). *: significant difference, ND: no difference

<table>
<thead>
<tr>
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<th>Canopy vs. Understory pairwise mean</th>
<th>p-value</th>
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<td>Chl a:fw ratio</td>
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Table 4.3. Comparison of relative photosynthetic pigment content for peripheral vs. central tissues of *Ahnfeltiopsis concinna*. Standard errors of the means are in parentheses below the means. Chl \(a/fw\) ratio & Chl \(a/dw\) ratio: mg/g. R-PE, R-PC and APC: mg/g(fw). NA: not applicable, *: significant difference, ND: no difference

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<td>Chl (a/dw) ratio</td>
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Table 4.4. Comparison of relative photosynthetic pigment content for HPFD vs. LPFD apices of *Ahnfeltiopsis concinna*. Standard errors of the means are in parentheses below the means. Chl a:fw ratio & Chl a:dw ratio: mg/g. R-PE, R-PC and APC: mg/g(fw). NA: not applicable, *: significant difference, ND: no difference.

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<td>0.024 (0.002)</td>
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Table 4.5. Mean ratios of in vivo and extract absorbance by different pigment maxima normalized to red Chl a maxima for canopy and understory regions of rhodophyte turfs. n=8, standard error of the mean in parenthesis below mean, pairwise difference: canopy - understory, *: significant difference, e: extract ratio, ND: no difference, NS: not sampled

<table>
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<tr>
<th>Pigment maxima range</th>
<th>Ahnfeltiopsis concinna</th>
<th>Laurencia mcdonaldiæ</th>
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<td>understory</td>
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<tr>
<td>PE: Chl a (568:680 nm)</td>
<td>0.755 (0.024)</td>
<td>0.956 (0.006)</td>
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<td>Carotenoid &amp; PE: Chl a (495:680 nm)</td>
<td>1.125 (0.018)</td>
<td>1.000 (0.004)</td>
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<td>Carotenoid : Chl a (482:664 nm)e</td>
<td>2.13 (0.13)</td>
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<td>PC: Chl a (625:680 nm)</td>
<td>0.754 (0.012)</td>
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<td>Soret: Red Chl a (440:680 nm)</td>
<td>1.180 (0.020)</td>
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<td>UVACs: Chl a (334:666 nm)e</td>
<td>12.00 (1.93)</td>
<td>4.80 (0.79)</td>
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</table>
Figure 4.1. Microsites in a model stand of macroalgal turf. LPFD, canopy, and HPFD tissues: apical. Peripheral, understory and central tissues: basal.
Figure 4.2. Relationship of incident PPFD on canopy / HPFD microsites to PPFD incident on a) A. concinna understory, b) L. mederntidae understory, c) A. concinna peripheral, and d) A. concinna LPFD microsites. Equation of least-squares regression lines: a) $y = 1.39 + 0.003x$, $R^2 = 0.36$, $H_a: b_1 \neq 0$, $p < 0.0005$, b) $y = -3.45 + 0.122x$, $R^2 = 0.92$, $H_a: b_1 \neq 0$, $p < 0.0005$, c) $y = -31.15 + 0.709x$, $R^2 = 0.88$, $H_a: b_1 \neq 0$, $p < 0.0005$, d) $y = -17.27 + 0.483x$, $R^2 = 0.70$, $H_a: b_1 \neq 0$, $p < 0.0005$. 

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Figure 4.3. A) In vivo (open symbols) and methanol:tetrahydrafuran extract (solid symbols) absorbance of canopy (circles) and understory (diamonds) tissue and percent transmittance (open squares (canopy tissues, only)) of *A. concinna*. B) Fourth derivative of canopy (circles) and understory (diamonds) of in vivo absorption spectra of *A. concinna*. Spectra are the mean absorbances / derivatives of 8 individuals.
Figure 4.4. A) In vivo (open symbols) and methanol:tetrahydrafuran extract (solid symbols) absorbance of canopy (circles) and understory (diamonds) tissue of L. mcdermidiae; B) Fourth derivative of canopy (circles) and understory (diamonds) of in vivo absorption spectra of L. mcdermidiae. Spectra are the mean absorbances / derivatives of 8 individuals.
Figure 4.5. A) *In vivo* absorbance of central (diamonds) and peripheral (circles) tissues of *A. concinna*; B) *In vivo* absorbance of LPFD (diamonds) and HPFD (circles) tissue of *A. concinna*. Spectra are the mean absorbances / derivatives of 10 individuals.
Figure 4.6 A) Relationship of ratio for mycosporine amino acid: Chl a (345 : 680 nm) in *vivo* absorption maxima to the corresponding extract absorption ratio (334 : 666 nm) in *Ahnfeltiopsis concinna*. Equation of the least squares regression line: $y = -34.6 + 37.3x$, $R^2 = 0.542$, $H_0: b_1 \neq 0$, $p=0.001$. B) Relationship of ratio for mycosporine amino acid: Chl a (337 : 680 nm) in *vivo* absorption maxima to the corresponding extract absorption ratio (334 : 666 nm) in *Laurencia mcedermidiae*. Equation of the least squares regression line: $y = -9.47 + 11.1x$, $R^2 = 0.62$, $H_0: b_1 \neq 0$, $p<0.0005$. 
Abstract

Parameters of photosynthesis vs. irradiance curve vary markedly between microsites along the axis of the tropical intertidal red algae, Ahnfeltiopsis concinna (J. Ag.) Silva et DeCew and Laurencia mcdormidiae (J. Ag.) Abbott. Differences in photosynthetic performance between canopy and understory tissues indicate that L. mcdormidiae exhibited a 'typical' sun to shade acclimation but over the space of less than 10 cm. Respiration, I_C, I_K, and P_max values significantly decreased in understory tissues relative to the canopy tissues of L. mcdormidiae, while PS I size (PSU I) significantly increased. Quantum efficiency was unchanged. Ahnfeltiopsis concinna in contrast exhibited decreased respiration, I_C, and I_K values in understory tissues while α increased in the understory relative to canopy tissues. Unusually, the canopy tissue values of P_max for A. concinna were significantly decreased while PSU O2 increased relative to understory tissues. These are potential indicators of a high degree of irradiance stress in canopy tissues of this, the highest intertidal alga in Hawaian coastal zones. Acclimation to high PAR and UV-irradiance especially in tropical regions appears an essential mechanism(s) for stress resistance and persistence for intertidal algae. Algal turfs acclimate at microscales in part fostered by their dense stands that create sharp irradiance gradients, as well as adjust physiologically at the canopy and stand periphery as mechanisms for stress tolerance and substantial photosynthetic performance.
Introduction

Photoacclimation refers to the expression of photosynthetic adjustments that primary producers can accomplish to changes in ambient photosynthetic photon flux density (PPFD) without genetic changes; genetic alterations that permanently alter photosynthetic process are more accurately designated photoadaptations (Gantt 1990). Several ecophysiological modifications are shared between photoacclimation and photoadaptation strategies. The responses (acclimations and adaptations) of photosynthetic organisms to decreasing light intensity have been extensively researched for herbaceous plants, woody trees and marine algae (Boardman 1977, Rhee & Briggs 1977, Ramus 1983, Friend 1984, Kozlowski et al. 1991).

Shade-tolerant primary producers generally have lower respiration rates and consequently lower light compensation points for photosynthesis ($I_c$) than sun-tolerant primary producers. Shade-tolerant primary producers also require less PPFD to saturate photosynthesis ($I_k$). At subsaturating PPFD, shade-grown primary producers typically have a higher quantum efficiencies ($\alpha$) than sun-grown primary producers (Kok Effect). At saturating PPFD sun-grown primary producers generally have higher maximum rates of net photosynthesis ($P_{\text{max}}$) than shade-grown primary producers (Kozlowski et al. 1991). Comparison of these $P$ vs. $I$ parameters between angiosperms and macroalgae reveals that most macroalgae are comparitively shade-adapted. High-PPFD grown C3 and C4 plants generally have saturation irradiances at ~40 and 80% of full sun whereas high intertidal macroalgae saturate at ~15 to 25% (Boardman 1977, Luning 1981, Part III). Additionally, $P_{\text{max}}$ values are 3-fold and 30-fold higher in average C-3 terrestrial plants and C-4 plants like sugar cane, respectively, compared to Macroystis pyrifera (Arnold & Manely 1985, Boardman 1977, Nobel 1991, Littler & Littler 1992, Part III). With the perspective that M. pyrifera is one of the most productive macroalgae known, it would appear that macroalgae have a limited photosynthetic capacity when compared to terrestrial counterparts. With this
performance differential and the knowledge that macroalgae have a limited capacity for anatomical adaptations (e.g., Kranz anatomy), how do intertidal macroalgae tolerate exposure to irradiance levels well in excess of photosynthetic requirements? Answering this question is central to understanding macroalgal distribution in intertidal regions.

The ecophysiological consequences of living in the intertidal zone have been examined for many intertidal algae (Dring & Brown 1982, Smith & Berry 1986, Matta & Chapman 1991, Henley et al. 1992). Studies concerned with acclimation to irradiance have concentrated on physiological changes observed over large-scale, depth gradients (Lubimenko & Tichovskaya 1928, Waaland et al. 1974, Ramus et al. 1976, Rhee & Briggs 1977), pigment differences between sun versus shade grown algae (Falkowski & Owens 1980), optimization of accessory pigment levels (Henley 1989) and differential photosynthetic ability of dissimilar parts of large macrophytes such as species of *Macrocystis* and *Sargassum* (Arnold & Manley 1985, Aruga et al. 1990, Gao 1991, Sakanishi et al. 1991).

The tropical PPFD exceeds 2300 μmol quanta m⁻² s⁻¹ and UV-radiation levels are twice as high as temperate latitudes. This combination poses a potential lethal stress to intertidal macroalgae (Hader 1993, KSB unpublished data). Microscale regulation of pigment levels in tropical rhodophyte turfs, manifested as adjustments in levels of phycobilins, carotenoid(s) and UV-absorbing compounds (UVACs), parallels observed photoacclimation responses by temperate macrophytes but over a remarkably sensitive scale (centimeters) (Part IV). Tropical intertidal habitats (characterized by narrow tidal amplitude) are characterized by a complex mosaic of 30 turf species and limited free-space (McDermid 1988). Precise physiological control at an extremely responsive scale may play an essential role in the physiologically mediated competition for space and irradiance by these competitors.

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The following experiments were designed to evaluate the photosynthetic performance of the intertidal rhodophytes, *Ahnfeltiopsis concinna* and *Laurencia mcdermidiae* in the microsites examined in Part IV. Specifically I asked: 1) Does photosynthetic performance vary within the stand structure and over the distribution of these tropical turfs?; 2) Is the pigment differentiation present between microsites indicative of differential photosynthetic performance? 3) How does this scale of change in photosynthetic performance compare to other ecosystems?

**Methods and materials**

**Sites and sampling:** *Ahnfeltiopsis concinna* and *L. mcdermidiae* were haphazardly sampled from 1 June 1993 to 1 July 1995 at Makapu' u and Koloko, O'ahu, Hawai'i. Heights of individuals above mean lower low water (MLLW) were determined using standard surveying methods utilizing a field transit. Heights were based on heights of USGS benchmarks above mean lower low water (MLLW). Individuals sampled for physiological evaluation were cleaned of epiphytes and maintained separately in 500 ml of 0.2 μm filtered seawater (FSW) at 23-26°C under 400 μmol quanta m⁻² s⁻¹ and ambient day length. Photosynthetic responses of macroalgae were performed within 24 hours of field collection. Previous investigations indicated detectable increase in respiration (wound respiration) after tissue manipulation (data not shown).

Sampling of tissues from microsites (canopy & understory, peripheral & central, high photon flux density (HPFD) & low photon flux density (LPFD)) followed procedures and specifications of Part IV. Samples were attached to modified chamber seal (Smith & Berry 1986) by nytex mesh and maintained in FSW under 50 μmol quanta m⁻² s⁻¹ just prior to physiological measurements. Paired samples (e.g., canopy and understory tissues from the same individual) were evaluated at the same time to minimize differences attributable to tidal and/or circadian induced physiological rhythms.
Photosynthesis and respiration measurements: Net photosynthesis was measured from 20 to 50 mg fresh weight (fw) pieces of algal tissues as increases in oxygen concentration using a temperature controlled, water jacketed, Clark-type oxygen electrode (Rank Brothers, Cambridge UK) using methods detailed in Part III. FSW augmented with 20 mM NaHCO₃ (pH 8.3 buffered with HEPES) was used in all trial to minimize carbon limitation during the duration of each trial. A minimum of 10 PPFD levels (0-2000 μmol quanta m⁻² s⁻¹) were utilized to generate each P vs. I curve. These included a minimum of 6 limited and 4 post-saturation levels. Photosynthesis versus irradiance curves were calculated on fw, chlorophyll a (Chl a), and dry weight (dw) bases. All biomass measurements were performed on a Sartorius A-200-S Analytic balance. Dry weights were determined after Chl a extraction (as in Part IV) of intact tissues and subsequent drying at 60 °C for 72 hr.

Quantum efficiency (α) and rates of maximal net photosynthesis (P max) were determined by non-linear regression using the model of Smith (1936): P = \( P_{\text{max}} \cdot \alpha \cdot I / (P_{\text{max}}^2 + (\alpha \cdot I)^2)^{1/2} \), as suggested by Nelson and Siegrist (1987) for tropical macrophytes. Non-linear regressions were performed via Systat version 5.2.1. Mean R² for the 96 P vs. I curves was: 0.936 (± 0.0133 se). Saturation irradiance (I k) was calculated by: I k = P max / α. Respiration was determined by averaging the initial dark treatment with a dark treatment at the end of trial. Compensation irradiance (I c) was calculated by: I c = Respiration / α.

Photosynthetic unit characteristics: Photosystem II (PS II) unit size based on the concentration of PS II reaction centers relative to Chl a, termed PSU O₂, was determined from tissues from microsites. PSU O₂ was calculated by dividing Emerson and Arnold numbers by 4 (Kursar & Alberte 1983). Emerson and Arnold numbers were acquired via oxygen yield per unit saturating flash at a flash rate of 1000 min⁻¹. Variable flash rates were assessed prior to experiments and 1000 flashes per minute was found to be sufficient.
to allow complete trap re-opening before a subsequent saturating flash. Chl a was quantified as in Part IV.

Photosystem I (PS I) unit size, termed PSU I, was quantified from tissues from light microsites according to the methods of Shiozawa et al. (1974). PSU I determinations are based on the 697 nm induced bleaching of 1% Triton-solubilized PS I enriched membranes (Kursar & Alberte 1983). PS I enriched membranes were prepared by Polytron grinding of tissues and high speed (10,000x g) refrigerated centrifugation. Samples were kept at 5°C and in darkness until just prior to spectrophotometric assessment using a Hewlett Packard 5428 A Diode array spectrophotometer in kinetics mode with a 1.0 s integration.

**Fluorescence emission spectra**: Room temperature fluorescence emission spectra were obtained from newly collected canopy and understory portions of *A. concinna*. Emission spectra from 540 to 750 nm were generated with actinic excitation irradiance set at 495 ± 0.5 nm in a Photon Technology International Quanta Master-2 Luminescence Spectrometer. Fluorescence emission measured at 1 nm intervals from 540 to 750 nm with a scan speed of 5.0 nm s⁻¹ using Felix 1.0. Algal tissues were place at a 45° angle to both the excitation and emission monochrometers. Algae were exposed to 750 μmol quanta m⁻² s⁻¹, 25 °C and 34 ppt FSW for 10 min just prior to obtaining fluorescence emission spectra.

**Relationship of in vivo absorption spectra and P vs. I curve parameters**: In vivo absorption spectra from 400 to 750 nm were obtained using methods in Part III on canopy and understory tissues of *A. concinna* and *L. mecermidiae*. P vs. I curves were obtained as discussed above from the same tissues samples used in assessment of in vivo absorption spectra. The relationship of in vivo absorption maxima (normalized to ~680 nm maxima) to photosynthetic parameters was determined via linear regression (see Statistical comparisons below).
Statistical comparisons: Statistical analyses were carried out on physiological parameters using Minitab version 8.2 (Minitab Inc.). Normality was assessed with a test equivalent to Shapiro-Wilk test with an $\alpha$ of 0.05 (Minitab Reference Manual, release 8 1991). When normally distributed, comparisons of canopy vs. understory, peripheral vs. central, and HPFD vs. LPFD tissues were evaluated with one sided pairwise t-tests (Zar 1984). If normality was in question, then either a pairwise one-sided Wilcoxon test (when data were symmetrical) or a pairwise one-sided Sign test (when symmetry was in question) was used. Regression analyses were carried out on canopy or understory photosynthetic parameters over the vertical distribution of individuals in the intertidal zone. Assessment of the relationship between in vivo absorption characteristics and $P$ vs. $I$ curve parameters were performed with normal linear regression. All regression analyses were performed after evaluation of the normality and equivalency of variance residuals. Bonferroni corrections were performed on the $\alpha$'s for cases where data was used in more than one comparison.

Statistical inaccuracies that result from normalization routines for photosynthetic parameters over multiple variables and tests performed on parameters with other parameters used in their calculation were a concern. Several points warrant these type of tests. 1) The ecophysiological trends being described in this study are complex. 2) Normalization to only one basis may be ecologically misleading. 3) Parameters such as $I_K$ and $I_C$ have marked consequences on primary production and competition (Dennison & Alberte 1982, Zimmerman et al. 1995).

Results

Intertidal distribution: At Makapu'u, individuals of *A. concinna* ranged in height from 0.92 to 1.34 m above MLLW. At Makapu'u and Koloko, *L. mcdormidae* was distributed from 0.01 to 0.78 m above MLLW with the majority of individuals found from 0.2 to 0.4 m above MLLW.
Photosynthesis and respiration for canopy and understory tissues: For *A. concinna*, respiration rates, $I_c$ and $I_k$ increased in canopy tissues compared to understory tissues. Canopy tissues attained rates of $P_{\text{max}}$ similar to those of understory tissues on a fw and dw basis but $P_{\text{max}}$ decreased significantly in canopy tissues compared to understory tissues on a Chl a basis (Table 5.1). P:R ratio was lower in canopy tissues compared to understory tissues. The value of $\alpha$ decreased for canopy tissues relative to understory tissues (Table 5.1).

Canopy tissues of *L. mcdarmidiae* differed physiologically from understory tissues in that Chl a content, fw, and dw specific respiration, $P_{\text{max}}$, $I_c$ and $I_k$ were all significantly higher in canopy tissues when compared with understory tissues (Table 5.2). Unlike *A. concinna*, $P_{\text{max}}$ for *L. mcdarmidiae* was higher in canopy tissues when compared to understory tissues (Table 5.2). The values of $\alpha$ and the P:R ratio did not differ between canopy and understory tissues (Table 5.2).

Photosynthetic unit sizes of PS I and PS II differed in their apparent manner of adjustment from understory to canopy portion for *A. concinna* and *L. mcdarmidiae* (Table 5.3). *Laurencia mcdarmidiae* increased the size of both PS I and PS II in understory tissues relative to canopy tissues, however, this difference was only significant for PSU I. In contrast, the size PS II was larger in canopy relative to understory tissues of *A. concinna*. The ratio of PS I to PS II was decreased in understory tissues of both species relative to canopy values.

Both canopy and understory tissues of *A. concinna* had similar fluorescence emission maxima when excited with 495 ± 5 nm light (Figure 5.1). Based on emission maxima identities in Fork and Oquist (1981), Oquist and Fork (1982), and Smith (1983), the following pigments were designated as the source of emission maxima: PE: ~579 nm, PC: ~650 nm, APC: ~680 nm, PSII Chl a: ~710 nm, PSI or PSII Chl a: 730 nm (Figure 5.1). The relative height of emission maxima differed between canopy and understory
tissues. Understory tissue emission was highest from Chl a, intermediate from PC and lowest from PE while the reverse was true of canopy tissues. The ratio of 710 : 579 nm maxima was significantly different between canopy and understory tissues (pairwise t-test, n= 10, mean: -1.228±0.499, t=-2.46 , p= 0.018).

Photosynthesis and respiration in Ahnfeltiopsis concinna peripheral and central tissues: Rates of respiration in peripheral tissues of A. concinna were significantly increased compared to central tissues. This difference was apparent on dw and fw bases but not on a Chl a basis (Table 5.4). The value of I_c decreased significantly only on a fw basis for peripheral tissues compared to central-stand (Table 5.4). Values of I_k (based on Chl a) decreased significantly for peripheral compared to central tissues. Mean value of α for peripheral tissues was significantly less than that of central tissues on a fw basis but not on a dw or Chl a basis (Table 5.4). The values of P:R ratio and P_max of peripheral tissues were indistinguishable from central tissues (Table 5.4).

Photosynthesis and respiration in Ahnfeltiopsis concinna: HPFD and LPFD tissues: Absolute rates of respiration (Chl a and dw basis) and I_c were significantly increased for HPFD compared to LPFD tissues. P_max was significantly decreased in HPFD tissues relative to LPFD tissues. The value of α when calculated on a Chl a basis, was markedly depressed in HPFD tissues contrasted with LPFD tissues (Table 5.5). With markedly elevated P_max and α in LPFD tissues, the difference between I_k of HPFD (315.1 umol quanta m^-2 s^-1) and LPFD tissues (287.6 umol quanta m^-2 s^-1) was not significant. HPFD tissues had a lower P:R ratio than LPFD tissues when those ratios were calculated on a normalized to Chl a basis.

Evaluation of photosynthetic parameters over vertical distribution: Regression analysis of all previously described photosynthetic parameters of canopy and understory tissues of A. concinna and L. medermidiae over their vertical position in the intertidal zone revealed no significant relationships.
Relationship of in vivo absorption spectra characteristics to photosynthetic performance: The ~540 nm R-PE specific maxima (normalized to ~680 nm maxima) was selected as a predictive measure of photosynthetic performance because this maximum had the greatest degree of change when comparing canopy and understory tissues of both L. mcdermidiae and A. concinna (see Part IV). Alterations in photosynthetic performance similar to those observed above were seen in this independent assessment of canopy and understory tissues. For tissues of A. concinna and L. mcdermidiae, highly significant negative relationships were detected between the R-PE maximum and values of $I_k$ (Figure 5.2a). For A. concinna tissues, a significant positive relationship of the R-PE maximum to values of $\alpha$ was seen (Figure 5.2b). For L. mcdermidiae tissues, a negative relationship between R-PE maximum and $P_{\text{max}}$ was detected (Figure 5.2c).

Discussion

Scale of photoacclimation & constraints on marine primary producers:

Photoacclimation occurs over a markedly responsive scale in tropical turf species, as exhibited by Ahnfeltiopsis concinna and Laurencia mcdermidiae. Canopy photoacclimation is driven by excess PPFD while understory photoacclimation (< 10 cm below) is driven by marked gradient in irradiance, self-shading and probable selective enhancement of spectral distribution for understory PPFD. This distinct shift in acclimation state from sun to shade coupled with the stress tolerant morphology of turfs allows these species to inhabit high intertidal regions (A. concinna) and to compete with a up to 30 other species of turfs (L. mcdermidiae) even though stress levels of tropical tidal environment are rigorous and space is limiting.

The extent of intrathallus photoacclimation in these tropical turfs is comparable to macroscale (meters) changes in temperate marine plants over their vertical distribution or in terrestrial plant forest canopies and understories. Laurencia mcdermidiae increased $P_{\text{max}}$ 152% (Chl a basis) from understory to canopy tissues. In the seagrass Zostera marina,
Dennison and Alberte (1984) measured a 47% increase in $P_{\text{max}}$ (Chl a basis) from deep (-375 μmol quanta m$^{-2}$ s$^{-1}$, peak PPFD) to shallow subtidal (-825 μmol quanta m$^{-2}$ s$^{-1}$, peak PPFD) sites. Contrasting upper intertidal versus subtidal populations of Colpomenia peregrina (Phaeophyta), Matta and Chapman (1990) observed a 166 and 134% increase in daily $P_{\text{max}}$ in high intertidal macrophytes during summer and winter, respectively. Contrary to this, A. concinna had a 15% reduction in $P_{\text{max}}$ (Chl a basis) in canopy relative to understory tissues. In Porphyra perforata, a similar reduction (44%) in $P_{\text{max}}$ (surface area basis) was observed between high vs. low intertidal populations (Smith 1983). This similarity is not unexpected as both occupy the most elevated (above MLLW) niche in their respective localities and at this elevation both experience a severe combination of stresses caused by prolonged emersion (Smith & Berry 1986, Part VIII). $P_{\text{max}}$ in marine macrophytes increases with increased PPFD caused by higher vertical position in the water column or decreased intra- or inter-individual shading. With further increases in exposure to emersion related stresses, decreases in $P_{\text{max}}$ are observed.

Terrestrial forests present a similar gradient in PPFD as among canopy/understory regions. Forest understories may receive only 0.5-2% of incident canopy PPFD (Chazdon & Fetcher 1984) compared to <0.3-10% in these rhodophyte turfs. In general, terrestrial forest canopies have saturation irradiances 100-1000% higher than shade-tolerant understory plants (Bohning & Burnside 1956, Boardman 1977). Canopy tissues of L. mcedermidiae and A. concinna exhibit higher values of $I_k$, 108% and 173%, respectively, relative to understory tissues. The above comparisons emphasize the responsiveness of A. concinna and L. mcedermidiae to alterations in PPFD. These diminutive turfs undergo similar ranges of photoacclimation as seen over much greater scales among other primary producers. Physiological adjustments at this fine scale are unparalleled in eukaryotic systems and may represent a crucial adaptation to intertidal tropical PPFD.
Although the direction of physiological changes is the same for terrestrial and marine systems there appears to be constraints on $P_{\text{max}}$ among marine primary producers. Maximum rates of photosynthesis in marine algae and seagrasses are at best 1/3 the rate found in typical terrestrial C-3 plants (Drew 1979, Dennison & Alberte 1984, Arnold & Manley 1985, Dawes 1987, Fourquean & Zieman 1991, Nobel 1991, Perez & Romero 1992, Littler & Arnold 1982). This perspective strongly suggests that there are selection pressures to maximize the number of hours per day that $P_{\text{max}}$ is achieved rather than high $P_{\text{max}}$ rates. Episodic supply of nutrients and/or variability of PPFD attenuation associated with coastal zones may provide this selection pressure. These questions emphasize the process differences between terrestrial and marine ecosystems but remain largely unaddressed.

**Diversity in acclimation strategies: a comparison of tropical turfs:** Characterization of physiological parameters for *A. concinna* and *L. mcdermidiae* revealed different ecophysiological strategies implemented at the level of the photosynthetic apparatus. *Laurencia mcdermidiae* conforms to the typical sun-shade acclimation as seen on large vertical scales in terrestrial plant canopies (Kozlowski et al. 1991). Over the vertical extent of a turf (~5.0 cm), *L. mcdermidiae* lowered both respiration and $P_{\text{max}}$ rates in understory tissues relative to canopy tissues. This resulted in a consistent P:R ratio in canopy and understory tissues while $I_k$ and $I_c$ similarly lowered in understory tissues. Typically, $\alpha$ is augmented in terrestrial plants acclimated/adapted to deeply shaded environments (Kozlowski et al. 1991) but $\alpha$ appeared to be constrained in this acclimation response by *L. mcdermidiae*. Understory tissues of *L. mcdermidiae* additionally augmented concentrations of Chl $a$ (fw basis) (Part IV). This photoacclimation coupled with increased concentrations of phycoerythrin (Part IV), and increasing the size of both PSUs, facilitated maximal photosynthetic performance over the entire thallus. This was accomplished while compensating for the changes in PPFD and spectral quality that take place in dense turf.
communities. Microscale photoacclimation in L. mcdermidiae is ecologically more important to the competitive ability of this turf alga than adjustments over the vertical distribution of this species. Intertidal benches in Hawai'i are broad flat regions with less of a differential in PPFD than turfs create for themselves. Over L. mcdermidiae's narrow intertidal distribution, no physiological changes were related to the vertical position of individuals above MLLW.

Physiological changes from canopy to understory tissues of A. concinna did not fully conform to expected models of sun to shade acclimation. On fw and dw bases, P max was equivalent for tissues in both microsites, while on a Chl a basis P max was surprisingly higher in understory tissues than canopy tissues. Not surprising because they are related measures, P:R ratios were also augmented in understory tissues relative to canopy tissues. Additionally, as with shade acclimation, respiration rate, I c and I k were all lower but α was augmented in understory tissues. Reduced production by the canopy tissues are likely to be the result of physiological stresses encountered as the highest intertidal alga on Hawaiian basalt benches (Doty 1967). Fluorescence emission spectra from canopy and understory tissues indicate that energy transfer from phycobilin components to PSII reaction center is less efficient in canopy tissues than in understory tissues. In unstressed tissues such as understory tissues of A. concinna, elevated Chl a emission indicates efficient transfer of excitation energy from the phycobilin components of the antennae complex to the reaction center (Fork & Oquist 1981). This was not observed in canopy tissues of A. concinna. Enhanced PE and PC emission indicates an uncoupling of excitation energy transfer between these light harvesting components and reaction centers. Uncoupling of PE and PC components differs from the response of P. nereocystis to desiccation. Desiccation intolerant P. nereocystis manifested this stress with greatly enhanced APC fluorescence relative to Chl a (Smith et al. 1986).
Phenological factors can be ruled out as an explanation for this unexpectedly low canopy production. Comparison of central vs. peripheral and HPFD vs. LPFD tissues strongly suggest that age was not a factor affecting the photosynthetic physiology of *A. concinna*. If with aging *A. concinna* altered the composition and functionality of the photosynthetic apparatus, one would expect similar apical to basal changes regardless of microsite. Yet, similar differences were observed between peripheral vs. central and HPFD vs. LPFD tissues as were seen in the comparison of canopy vs. understory tissues. This coupled with similar physiological responses for similar PPFD at different microsites strongly suggests that canopy tissues of *A. concinna* are responding to changes in irradiance levels. Observed physiological changes were the result of stress responses inherent with intertidal existence in habitats with the highest levels of PPFD, UV-radiation, and desiccation tolerated by any macroalga, possibly world-wide.

Additionally, contrasting HPFD vs. LPFD to the canopy vs. understory comparison revealed the physiological consequences of emersion in a HPFD environment (Tables 1 & 5). Sampling for HPFD vs. LPFD comparison was performed after a series of days with high tidal amplitude and low wave height. HPFD tissues had a P:R ratio of 1.33. Similar tissues from canopy vs. understory comparison were sampled after a less stressful period with reduced tidal amplitude and increased wave height. Canopy tissues had a P:R ratio of 3.20. Furthermore, LPFD tissues sampled during the 'stressful' period had the greatest P$_\text{max}$ and P:R ratio observed for *A. concinna* during the entire period of this study.

Given these observations, canopy tissues of *A. concinna* appear to be chronically photoinhibited (Osmond 1994), given the current definition as reduced oxygen evolution or carbon fixation. The degree of photoinhibition was variable in *A. concinna* and associated with tidal exposure. Chronic photoinhibition in canopy tissues of *A. concinna* was manifest at several levels: 1) depression in $\alpha$ and P$_\text{max}$ compared to understory and low-
light tissues; 2) increased apparent PSU O2 size in canopy tissues, and 3) enhanced PE and PC fluorescence emission. The values of photosynthetic unit size in this study are similar to values obtained by Greene and Gerard (1990) for Chondrus crispus. Replicate trials with lower flash rates (PSU O2), various Triton-X-100 concentrations (PSU I) and Ulva fasciata as an alternate system supported the accuracy of these measures. PSU sizes for Ulva fasciata were similar to reported values for other Chl a + b containing organisms (data not shown) (Myers & Graham 1971, Ley & Mauzerall 1982, Fisher et al. 1989). PSU O2 estimates were normalized to total Chl a concentration. There are distinct pools of functional and non-functional of PS II units (Osmond 1994) in chloroplasts. Only a subset of the entire pool will be measured as functional by O2 flash yield. It follows that bulk-Chl a associated with LHPPC similarly associated with non-functional reaction centers would artificially inflate estimates of PSU size. These determinations do not distinguish 'photochemically non-functional' Chl a from functional Chl a in estimates PSU size. Photoinhibition in late spring through early summer in mangrove canopies has been associated with periods of high incident irradiance, elevated leaf temperatures coupled with salinity stress (Cheeseman 1994). Periods of excessive physiological stress, especially synergistic combinations of stresses such as UV- and photosynthetically active radiation (PAR), plus osmotic stress, and elevated temperatures have detrimental impacts on primary production in many fringe environments (e.g., high intertidal macrophytes, estuarine communities, etc.).

Increased concentration of carotenoids and UVACs in canopy tissues are a likely mechanism to mitigate damaging stress of tropical irradiance environment and insure that perenniating basal attachments remain viable. Even with these possible mechanisms of non-photochemical quenching, extensive tissues bleaching as seen in Gastroclonium coulteri (Hodgson 1980) occurs in exposed tissues of A. concinna and L. mcdermidiae on days of low tidal / wave amplitude and full sun (Magruder 1977, KSB pers. obs.). The
impact of photoinhibition, UV-stress and desiccation are reduced in understory tissues because a turf’s functional morphology reduces tissue temperatures, water loss and incident PPFD (Part VIII).

Photophysiological performance is tightly coupled to the levels of photosynthetic accessory pigment concentration in both A. concinna and L. mcdermidiae. This is not surprising based on research examining the pigmentation adjustments by many plants to sun and shade environments (Rhee & Briggs 1977, Falkowski & Owen 1980, Matta & Chapman 1991). Here I extend these concepts by establishing the utility of in vivo absorption spectra as a rapid, precise estimate of photoacclimation states. Zimmerman et al. (1995) and Dennison and Alberte (1984) established the applicability of period of saturating irradiance (H_{Sat}) to modeling rates primary productivity. With sufficient calibration, in vivo absorption spectra can rapidly approximate I_k as seen in Figure 5.2a, a parameter essential in determining H_{Sat}. Precise modeling of population productivity can now readily account for changes in photoacclimative states that naturally occur over temporal or spatial gradients.

Conclusions

Photoacclimation from sun- to shade-type physiological states occurs at exceptionally diminutive scales within individual tissues of probably many tropical rhodophyte turfs. These changes are comparable to photoacclimation responses over entire intertidal to subtidal distributions in temperate marine macrophytes and from canopies to understories in terrestrial forests. Self-shading resulting from the functional morphology of a turf promotes photoacclimation which allows for maximal productivity over the extent of the turf thallus. Responsiveness of the photosynthetic apparatus appears to be particularly essential in marine primary producers which should be collectively viewed as shade adapted in comparison to C3 and C4 terrestrial counterparts. Persistence of intertidal macrophytes with incident PPFD well in excess (≥ 5-fold) of photosynthetic demands
appears to require biochemical adaptability. Photosynthetic adjustments in many tropical turfs are tightly coupled to significant alterations in levels of phycobilin and photoprotective pigments. Increased levels of carotenoids and UVACs in canopy tissues of rhodophyte turfs are likely to be photoprotective responses to high levels of PPFD, UV-radiation and desiccation stress experienced by high intertidal macroalgae.
Literature cited


Table 5.1. Comparison of P. vs. I parameters of canopy vs. understory tissues of *Ahnfeltjopsis concinna*. Standard errors of the means are in parentheses below the means. Photosynthesis and respiration rates: μmol O₂·mg Chl a·s⁻¹·min⁻¹ for Chl a, μmol O₂·g fresh weight⁻¹·min⁻¹ for fw, μmol O₂·g dry weight⁻¹·min⁻¹ for dw. Irradiance levels for all analyses: μmol quanta m⁻² s⁻¹. P:R ratio: net photosynthesis / respiration, m: median, w: Wilcoxon Test, *: significant difference, ND: no difference

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Table 5.2. Comparison of P vs. I parameters of canopy vs. understory tissues of *Laurencia mcdermidiae*. Standard errors of the means are in parentheses below the means. Photosynthesis and respiration rates: $\mu$mol O$_2$·mg Chl $^{-1}$·min$^{-1}$ for Chl $a$, $\mu$mol O$_2$·g fresh weight$^{-1}$·min$^{-1}$ for fw, $\mu$mol O$_2$·g dry weight$^{-1}$·min$^{-1}$ for dw. Irradiance levels for all analyses: $\mu$mol quanta m$^{-2}$·s$^{-1}$. P:R ratio: net photosynthesis / respiration, *: significant difference, ND: no difference

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</tr>
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Table 5.3. Comparison of photosynthetic unit size of PSII and PSI (PSU O₂ & PSU I, respectively) in canopy and understory tissues of *Ahnfeltiopsis concinna* and *Laurencia mcdermidia*. Standard errors of the means are in parentheses below the means. Ratios based on mean PSU values. NA: not applicable, *: significant difference, ND: no difference

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Table 5.4. Comparison of P vs. I parameters for peripheral vs. central tissues of *Ahnfeltiopsis concinna*. Standard errors of the means are in parentheses below the means. Photosynthesis and respiration rates: \( \mu \text{mol O}_2 \text{mg Chl a}^{-1}\text{min}^{-1} \) for Chl a, \( \mu \text{mol O}_2 \text{g fresh weight}^{-1}\text{min}^{-1} \) for fw, \( \mu \text{mol O}_2 \text{g dry weight}^{-1}\text{min}^{-1} \) for dw. Irradiance levels for all analyses: \( \mu \text{mol quanta m}^{-2} \text{s}^{-1} \). P:R ratio: net photosynthesis / respiration, \( ^m \): median, \( ^w \): Wilcoxon Test, *: significant difference, ND: no difference

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Table 5.5. Comparison of P vs. I parameters for HPFD and LPFD Apices of *Ahnfeltjöpsis concinna*. Standard errors of the means are in parentheses below the means. Photosynthesis and respiration rates: \( \mu \text{mol O}_2 \cdot \text{mg Chl a}^{-1} \cdot \text{min}^{-1} \) for Chl a, \( \mu \text{mol O}_2 \cdot \text{g fresh weight}^{-1} \cdot \text{min}^{-1} \) for fw, \( \mu \text{mol O}_2 \cdot \text{g dry weight}^{-1} \cdot \text{min}^{-1} \) for dw. Irradiance levels for all analyses: \( \mu \text{mol quanta m}^{-2} \cdot \text{s}^{-1} \). P:R ratio: net photosynthesis / respiration, \( m \): median, \( w \): Wilcoxon Test, *: significant difference, ND: no difference.

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Figure 5.1. Mean room temperature fluorescence emission spectra of canopy (open circles) and understory (closed circles) of *Ahnfeltiopsis concinna* (*n*=10). Spectra normalized at ~579 nm maxima to facilitate comparisons.
Figure 5.2. Regression relationship of $-540(2):680$ nm in vivo absorbance maxima ratio with A) $I_k$ (dw basis), B) $\alpha$ (Chl a basis), and C) $P_{\text{max}}$ (Chl a basis). Open symbols: Laurencia mcdormidae; Closed symbols: Ahnfeltiopsis concinna. A) A. concinna: $y=618.7-529.3x$, $R^2=0.57$; L. mcdormidae: $y=1210.9-1128.4x$, $R^2=0.56$. B) A. concinna: $-0.154+0.289x$, $R^2=0.70$. C) L. mcdormidae: $y=6.68*10^{-5}-6.60*10^{-5}x$, $R^2=0.70$. The slope of each relationship was significantly different than 0, judged at an $\alpha$ of 0.001.
PART VI

EXPERIMENTAL ANALYSIS OF PHOTOACCLIMATION POTENTIAL
Abstract

Differentiation of in situ pigment profiles in the turf forming alga Ahnfeltiopsis concinna is a photoacclimation response to self shading. Analysis of changes in pigment and photosynthetic performance in response to reciprocal transplants conducted in a marine greenhouse revealed the capacity of canopy and understory tissues to acclimate to changes in the intensity of photosynthetically active radiation. When exposed to low-PPFD, canopy tissues acclimated by increasing phycobilin while decreasing carotenoid and UV-absorbing compound specific absorbance, thereby becoming similar to pigmentation of in situ understory tissues. Understory tissues exposed to high-PPFD altered the same pigment constituents but in the opposite manner of canopy tissues treated to low-PPFD levels. Complete acclimation to the reciprocal pigmentation state required 20 days.

Acclimation to the reciprocal pigmentation and photosynthetic state was faster for catabolic than for anabolic processes. Distinct pigment beds altered at different rates. Phycoerythrin and UV-absorbing compounds adjusted at faster rates than carotenoids for example. Changes in absorbance over time of phycobilin-specific-maxima indicated that the rate of alteration of phycoerythrin levels was greater than the rate of change in either phycocyanin or allophycocyanin pools. The differential kinetics of distinct phycobilin pools maintained a similar PE:PC ratio throughout the photoacclimation process regardless of acclimation route to high- or low-PPFD conditions.

Increased photosynthetic rates in tissues treated with low-PPFD compared to high-PPFD strongly suggests that even high intertidal algae are shade-adapted primary producers compared to terrestrial plant counterparts. The capacity for fine scale regulation of the levels of photoprotective compounds and photosynthetic accessory pigments permits this algal turf to tolerate terrestrial irradiance levels incident on canopy tissues while simultaneously maximizing PPFD harvesting in deeply-shaded understories.
Introduction

The genetic capacity for fine scale adjustments of photoprotective and photosynthetic accessory pigments has been proposed as essential for ecological success at high tidal elevations for macroalgae under tropical irradiances (Parts IV & V). Emersion exposes intertidal macroalgae to irradiances of which less than one-quarter is needed to saturate photosynthesis (Nobel 1991, Part III). The shade-type physiology which is characteristic of many algae would pre-dispose intertidal macroalgae to observed photoinhibition and bleaching (Hodgson 1981) if mechanisms to ameliorate these effects were not in place. Chlorophyll triplet state quenching carotenoids, xanthophyll cycle, D1 repair cycle, mycosporine amino acids (MAAs), and antioxidants are examples of putative stress reducing agents (Demming-Adams 1990, Goodwin 1991, Karentz et al. 1991, Foyer & Harbinson 1994, Osmond 1994). In addition to excess irradiances experienced by algal canopies, algal turfs also experience greatly diminished irradiances in their understories (Part IV). Therefore an individual exists in both a sun and shade environment but over a space of <10 cm. The capacity to precisely allocate resources to either photoprotect or harvest irradiance is essential for continued performance when presented with such a varied irradiance field.

Ahnfeltiopsis concinna is an intertidal turf that has the highest intertidal distribution on pre-historic basalt lava flows in the high Hawaiian islands (Doty 1967). In this niche, canopy tissues of this rhodophyte experience irradiances in excess of 2300 µmol quanta m^{-2} s^{-1} upon emersion. The turf morphology of A. concinna attenuates incident irradiances, exposing understory tissues to \( \geq 0.2\% \) canopy irradiance values (Part IV). Precise physiological control coupled with the functional morphology of a turf that creates a understory microclimate reducing emersion stresses seems likely mechanism to allow more elevated than usual distribution with relaxed competition characteristic of lower intertidal areas (McDermid 1988, Smith 1992, Part IV).
Intact stands of *A. concinna* have been shown previously to alter relative levels of pigment constituents over the extent of an individual axis (<10 cm) (Part IV). Canopy tissues have enhanced levels of carotenoids and UV-absorbing compounds (UVACs) coupled with reduced levels of light harvesting phycoerythrin (PE), phycocyanin (PC) and allophycocyanin (APC) compared to understory tissues. This pigment differentiation is tightly coupled to physiological shifts from sun- to shade-type physiological states. Canopy tissues of *A. concinna* have increased respiration rates, saturation and compensation irradiances as well as depressed quantum efficiencies compared to understory tissues (Part V). These microscale changes are correlated with alterations in ambient irradiance caused by self-shading (Glynn 1965, Part V). Parts IV and V contend that the above *in situ* conditions are the result of a fine scale photoacclimation response by this alga. In the following investigation, I examine the capacity of this alga to alter pigment constituents and photosynthetic capacity by transplanting canopy and understory tissues into simulated reciprocal irradiance regimes.

Acclimation to sun and shade by primary producers has been well documented at several physiological levels (Boardman et al. 1974, Owens & Esaias 1976, Boardman 1977). In red algae, the effects of irradiance on plastid structure, pigment composition, photosynthetic unit size and photosynthetic performance have been examined (Waaland et al. 1974, Ramus et al. 1976, Rhee & Briggs 1977, Jahn et al. 1984, Kursar & Alberte 1984). The scope of these studies is typically focused on one facet of the myriad changes that take place in a photoacclimation response. The consensus from these works is that phycobilin concentrations increase with decreased irradiance, concomitantly photosynthetic performance shifts from a sun- to a shade-state indicated by depressed $P_{\text{max}}$ and increased quantum efficiency. Although the endpoints of these adjustments are well characterized, the kinetics of these changes have received limited attention (Jahn et al. 1984). Are
carotenoids, distinct phycobilins MAAs adjusted at similar rates? Do pigment based and photosynthetic performance based alterations occur in tandem?

Addressing these and other questions has been partially hindered by the destructive nature of pigment extraction and the difficulty of quantifying phycobilin, chlorophyll and carotenoid constituents in one tissue sample. In vivo absorption spectra however provide a non-invasive probe into the photosynthetic accessory and photoprotective pigment constituents of primary producers (Johnsen et al. 1994, Smith & Alberte 1994, Hunter & Smith, in review, Parts II-IV). Maxima detected in in vivo absorption spectra that are specific for discreet pigments have been shown to significantly correlate with extractable amounts of the same pigments (Part IV). In the following examination, I use in vivo absorption spectra to monitor photoacclimation at the level of the bulk pigments in the same tissues over time. By investigating irradiance-induced modifications of bulk pigments and characterizing photosynthetic performance before and after laboratory-based reciprocal transplants, I addressed the following questions: 1) Is the in situ pigment differentiation found between canopy and understory portions of A. concinna a photoacclimation response?, 2) At what rate are distinct photoprotective and photosynthetic accessory pigments modified when placed in different PPFD regimes?, 3) Are distinct pigments modified in similar or independent manners?, 4) Does the extent of adjustments in bulk harvesting pigments reflect the extent of acclimation in photosynthetic performance as judged by oxygen evolution?

Method and materials

Assessment of photoacclimation rates: The ability of the canopy and understory portions of A. concinna to acclimate their pigmentation and photosynthetic physiology was assessed by culturing tissues in one of three photosynthetic photon flux density (PPFD) regimes (mean maximum daily irradiance: 18 (low), 250 (medium), and 850 (high) μmol
quanta m\(^{-2}\) s\(^{-1}\)) for 8 days in an culture chamber placed in a temperature controlled greenhouse (Note: The PPFD environment of the greenhouse is free of UV-radiation and therefore this experiment only examined the effect of changes in levels of photosynthetically active radiation (PAR)). High, medium and low-PPFD treatments corresponded to irradiance levels less than saturation irradiance (<\(I_k\), =\(I_k\), and >\(I_k\), respectively, for canopy tissues while corresponded to irradiance levels <\(I_k\), >\(I_k\), and >>\(I_k\), respectively, for understory tissues (Part V). PAR was continuously monitored during two experimental periods between 29 May 1995 - June 6 1995 (Period 1) and 9 Sept. 1995 - 17 Sept. 1995 (Period 2) in the culture chamber with a LiCor Underwater UWQ quantum cosine sensor. Mean irradiance greater than 1.0 \(\mu\)mol quanta m\(^{-2}\) s\(^{-1}\) was sampled at 5 min intervals and stored in a LiCor LI-1000 data logger. Treatment irradiance were maintained at the above levels during each experimental period by varying the number of layers of shade cloth affixed to the clear plastic lid of the culture chamber.

During Period 1, in vivo absorption spectra were acquired from 400 to 750 nm. During Period 2, in vivo absorbance spectra were acquired from 280 to 400 nm. On Day 0 freshly collected canopy and understory tissues were excised and attached to white plastic plates with cable ties. An equal number of canopy and understory individuals were randomly assigned to one of three PPFD treatments. In Period 1, a total of 6 samples in the high-PPFD treatment and 10 in the medium-PPFD and low-PPFD treatments were tested. In Period 2, a total of 6 samples in the high- and low-PPFD treatments and 10 in the medium-PPFD treatment were tested.

The water temperature of the culture chamber was maintained between 25 and 29 °C via Neslab RTE-110 circulating water-bath cooled water-jacket. The temperature of the chamber increased and decreased with parallel changes in incident irradiance. The variation in temperature in the chamber was less than that experienced by A. concinna during a typical daily tidal cycle (KSB pers. obs.). Salinity was monitored daily with a Reichert

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temperature compensated refractometer and adjusted to 34 ppt with distilled water. Salinity
varied between 33 and 36 ppt during the duration of the experiment. The chamber was
continuously aerated and the chamber media (20 L of 0.2 μm filtered seawater) was
completely replaced every other day. Each day 10.0 ml of a 1.0 mM NaNO₃ / 1.0 mM
Na₂HPO₄·7H₂O nutrient solution was added to the chamber to minimize nutrient
limitation that may have otherwise hampered photoacclimation responses.

Every other day, beginning at the day of sample collection (Day 0), an in vivo
absorption spectrum (400 to 750 nm for period 1, 280 to 400 nm for period 2) was
acquired from each sample. Samples were simultaneously removed from the chamber,
blotted dry removing any epiphytic diatoms and cyanobacteria, and spectra obtained.
Sample order for acquisition of in vivo absorbance spectra was random. In vivo
absorption spectra were acquired using the methods detailed in Parts II-IV, using a lamp
changeover wavelength of 290 nm. Finally, in vivo absorption spectra were normalized to
1.0 at 680 nm maxima after spectra acquisition.

On Day 8 of the first experimental period, photosynthetic performance of three
canopy and three understory samples from both high-PPFD and low-PPFD treatments
were assessed via methods described in Parts III and V. The photosynthetic parameters
obtained were compared with in situ values (Part V) to estimate the extent of acclimation to
new PPFD regimes.

Statistical analyses: Simple linear regression of in vivo absorbance maxima ratios
versus time was carried out using Minitab version 10 Xtra (Minitab Inc.). Normality of
regression residuals was assessed with a test equivalent to Shapiro-Wilk test with an α of
0.05 (Minitab Reference Manual, 1991). ANOVA analysis of regression relationships
tested whether the slopes of the least squares regression lines differed significantly from
zero.
Results

Day 0 in vivo absorbance characteristics: Canopy tissues of A. concinna had markedly reduced absorbances between 500 and 650 nm, including maxima specific for PE (540 and 568 nm) and PC (625 nm) when contrasted to understory tissues (Figure 6.1). Ahnfeltiopsis concinna had increased carotenoid levels in canopy tissues relative to understory tissues. This was manifest as increased absorbances at both 440 and 490 nm absorbance maxima in canopy tissues compared to understory tissues (Figure 6.1). Canopy tissues had higher UV-absorbances than understory tissues. Canopy tissues also had a sharp increase in in vivo absorbances at 370 nm (Figure 6.1). Understory tissues had a largely uniform in vivo absorbance from 280 to 680 nm.

Photoacclimation responses of canopy and understory tissues:

Phycobilin components. Canopy tissues increased their relative absorbances from 525 to 650 nm in response to the altered PPFD regimes (Figure 6.2). The rates of increase in absorbance at the 540 nm maximum in canopy tissues exposed to irradiances i) <I_k and ii) =I_k were greater than the rate of increase with exposure to irradiances >I_k (Figure 6.3a). PE specific maxima (540 and 568 nm) had the greatest magnitude of increase compared to 625 nm PC specific maximum as well as increases at 650 nm attributed largely to APC (Figures 6.4a).

Understory tissues decreased their relative absorbances between 525 and 650 nm with exposure to irradiances >>I_k compared irradiances <I_k or >I_k (Figure 6.5). Tissues treated with irradiances <I_k maintained similar PE specific absorbance as judged by the position of the 540 nm maximum throughout the period of this study, whereas absorbance when exposed to irradiances >I_k was variable over time (Figure 6.3b). As with canopy tissues, the greatest rate of change in understory tissues was observed in PE specific 563 nm maximum when contrasted with alterations at 625 and 650 nm (Figure 6.4b).
Carotenoid components. Absorbance increased from 420 to 500 nm in both canopy and understory tissues in the high-PPFD treatment (Figures 6.6a & 6.7a). Canopy tissues in treated with irradiances <I_k or =I_k reduced absorbance over time at carotenoid and Chl a-carotenoid specific maxima at 495 and 440 nm, respectively (Figures 6.6a,b & 6.8a). As observed for phycobilin-specific-absorbance, understory tissues exposed to irradiances <I_k did not alter carotenoid levels. Carotenoid specific absorbance at 495 nm maximum in the medium-PPFD treatment decreased significantly (Figures 6.7b,c & 6.8b).

UV-absorbing components. Levels of UVACs increased over time in the high-PPFD treatment in both canopy and understory tissues as judged by absorbance from 280 to 400 nm as well as by absorbance at 342 nm maximum (Figures 6.9a, 6.10a, 6.11). Medium- and low-PPFD treatments resulted in a decrease in UV-absorbance in canopy tissues while understory tissues only decreased UV-absorbance in the medium PPFD-treatment (Figures 6.9b,c, 6.10b,c & 6.11).

Comparisons between photoacclimation processes: Two perspectives were used in comparing the rates of photoacclimation responses: 1) the change over time in the position of in vivo absorbance maxima (judged by maxima position normalized to Chl a 680 nm absorbance), 2) the relative extent of acclimation of canopy tissues placed in low-PPFD and understory tissues placed in high-PPFD (judged by the percentage change to the reciprocal Day 0 state). The mean difference between Day 0 state of canopy and understory tissues was defined as 100%.

Comparison of pigment kinetics: Absolute absorbances of UVACs (342 nm maximum) and PE (540 nm maximum) changes at similar rates when canopy tissues were placed in low-PPFD or understory tissues were placed in high-PPFD (Figure 6.12). Absolute absorbance of carotenoids (495 nm maximum) was altered to a lesser degree when compared to PE and UVACs (Figure 6.12). Contrary to the similar absolute rates of change of PE and UVACs, levels of PE as judged by the 568 nm maximum, adjusted more
quickly in both canopy low-PPFD and understory high-PPFD treatments than other phycobilisome components (Figure 6.4). The relative percentage of change to the reciprocal pigmentation state differed between pigment-beds and tissue types (Table 6.1). Phycoerythrin specific maxima at 540 and 568 nm were altered to the greatest relative extent while maxima with a carotenoid contribution (440 and 495 nm) had the least percentage change for both transplant extremes. Additionally, comparison of the relative degree of pigment state change by the end of the study revealed that high-PPFD treated understory tissues had progressed further to the reciprocal state than did low-PPFD treated canopy tissues (Table 6.1). The pooled mean change of in vivo pigment ratios for high-PPFD treated understory tissues was 44.5% while low-PPFD treated canopy tissues achieved only a 32.2% change (Table 6.1).

**Photosynthetic performance acclimation:** Canopy tissues treated with low-PPFD and understory treated with high-PPFD both adjusted photosynthetic performances to their respective PPFD treatments in a manner consistent with acclimation to shade and sun treatments, respectively (Table 6.2). Canopy tissues treated with irradiances <\(I_k\) experienced decreases in \(I_k\) and compensation irradiance while understory tissues treated with irradiances >\(I_k\) increased these irradiance thresholds in contrast to pre-treatment tissues. \(P_{\text{max}}\) was markedly elevated in both tissue types treated with low-PPFD compared to pre-treatment and high-PPFD treatments states (Table 6.2). Pre-treatment understory tissues and understory tissues treated with irradiances <\(I_k\) had similar photosynthetic performance characteristics. Pre-treatment canopy tissues and canopy tissues treated with irradiances >\(I_k\) differed physiologically with respiration rates and \(I_k\) increased as well as \(P_{\text{max}}\) and P:R ratio reduced in high-PPFD treated tissues.

**Pigment vs. photosynthetic acclimation:** The degree of change in phycobilin specific absorbance did not reflect the degree of change observed in photosynthetic performance (Table 6.1). Alterations in values of \(I_c\) and \(I_k\) were greater in canopy tissues
treated with low-PPFD compared to understory tissues treated with high-PPFD. The percentage change of phycobilin pigmentation in canopy tissues treated with irradiances \(< I_k \) was less than the percentage change in \( I_c \) and \( I_k \) while the opposite was observed in understory tissues treated irradiances \( \geq I_k \).

**Discussion**

Differentiation in pigmentation between canopy and understory portions of *A. concinna* is the result of intra-thallus photoacclimation to irradiance most likely associated with the morphology of this turf forming alga. When transplanted to simulated reciprocal PPFD environments, canopy and understory tissues acclimate by altering levels of distinct pigment pools such that canopy tissues ultimately resemble understory tissues and vice-versa. The responsiveness of the photosynthetic constituents in this high intertidal algae to changes in ambient irradiance suggests that disturbances that alter ambient irradiance at microsites (herbivory, canopy bleaching) can be readily accommodated. Augmented photosynthetic rates for tissues in low-PPFD treatments compared with high-PPFD treatments emphasizes that this alga is best thought of as shade-adapted. This capacity to markedly enhance levels of photoprotective compounds in specific microsites permits this species to inhabit a region where *A. concinna* regularly experiences high levels of terrestrial irradiance.

Conversion of canopy tissue to an understory phycobilin-rich state or vice-versa required on average \(~20 \text{ days}\). Photoacclimation occurred more quickly for adjustments involving catabolic rather than anabolic processes. Phycobilin degradation in understory tissues treated with high-PPFD was faster than phycobilin synthesis in canopy tissues treated with low-PPFD. Photosynthetic performance was altered to a greater extent in canopy tissues treated with low-PPFD than in understory tissues treated with high-PPFD. Conversion of photosynthetic performance from a sun- to shade-state, as in canopy tissues treated with low-PPFD involves down-regulation and degradation of photosynthetic
enzymes such as ribulose 1,6 bisphosphate carboxlyase oxygenase (RUBISCO) with concomitant synthesis of LHPPC (Boardman et al., 1977). Conversion of photosynthetic performance from a shade- to sun-state, as in understory tissues treated with high-PPFD involves synthesis of photosynthetic enzymes such as RUBISCO and degradation of LHPPC size (Boardman et al., 1977).

The absolute rate of change in pigment levels appeared to be conserved for PE and UVACs. In both synthesis and degradation of these pigments, the rates of acclimation were strikingly similar. Carotenoids levels altered at a slower rate. Maintenance of carotenoids which a primarily carbon skeletons may be energetically less costly than phycobilisome components and MAAs both of which require a substantial nitrogen investment. If nitrogen is more limiting than carbon in process of photoacclimation, tighter regulation of components rich in nitrogen would be favored while maintaining luxury levels of carotenoids may not incur significant additional cost.

Contrary to the similarity of the absolute rate of change of PE and UVACs, specific phycobilin components were altered at different rates. Degradation or synthesis of PE was more rapid when compared to changes in levels of PC and APC. The enhanced rate of change in levels of PE allows for the maintenance of the ratio of PE:PC during photoacclimation. In field collections of both canopy and understory tissues, the PE:PC ratio was maintained at 1.68 regardless of irradiance levels (Part IV).

As seen in previous studies, alterations in pigment levels in A. concinna responded to changes in ambient irradiance (Waaland et al., 1974, Rhee & Briggs 1977, Jahn et al., 1984). Phycoerythrin levels as judged by the 540 nm maximum increased when canopy tissues were transferred to decreased irradiance levels and the extent of increase paralleled the extent of change in irradiance. Exposure to high-PPFD treatments caused marked degradation of PE and other phycobilin components. Carotenoid levels, as judged by 495 nm maximum, were maintained in canopy tissues exposed to high-PPFD but, with
decreasing irradiance, carotenoid levels also decreased. Carotenoid levels in understory tissues were maintained in medium-PPFD, decreased in low-PPFD and augmented in high-PPFD. UVACs, as judged by the 342 nm maximum, responded to irradiance levels in similar manners in both canopy and understory tissues, high-PPFD treatments caused an increase in levels of UVACs over time while low- and medium-PPFD treatments caused a reduction in levels of UVACs. Increased concentrations with increased irradiance strongly support a photoprotective role for carotenoids and UVACs.

In vivo absorption measures provided a non-invasive probe into pigment responses of _A. concinna_ when exposed to altered PPFD environments. Insight into the physiological state and recent PPFD history of individuals can be gained by monitoring maxima specific for discreet pigments. Similar benefits of monitoring reflectance at 531 nm has been proposed for terrestrial plant systems (Gamon et al., 1993). This reflectance signature is directly related to quantum efficiency and state of the protective violaxanthin-cycle in _Helianthus annuus_ (Gamon et al., 1990, 1992). With higher pigment diversity in algae compared to terrestrial plants, algal spectral data are more complex but this work and work by others (Owen et al., 1987, Smith & Alberte 1994, Parts II & IV) strongly suggests that by monitoring a few select maxima, the photoacclimative states of primary producer can be readily assessed. With this type of assay, an spectrally assessed index of photosynthetic performance for marine primary producers appears feasible. High-resolution, spectral assays may have novel application in efforts to model coastal zone productivity. Marine algae exhibit great diversity in pigment types and adaptive strategies. Production estimates based only on Chl a (Strong 1974, Mittenzwey et al., 1992) are limited in their abilities to assess benthic macroalgae (Kirk 1994).

MAAs have been proposed by numerous researchers to be in vivo, photoprotective compounds that absorb UV-B and UV-A radiation (Sivalingam et al., 1974, Karentz et al., 1991, Garcia-Pichel et al., 1993). Limited evidence demonstrates the function of these
compounds except for their correlated increase with increased UV-levels (Kinzie 1993, Shick et al. 1995) and their absorbance of UV-radiation for intact macroalgae as judged by in vivo absorption spectra (Parts II & IV). Increases in levels of MAAs associated with high levels of PAR-only has been previously demonstrated for dinoflagellates, such as *Alexandrium excavatum* and *Prorocentrum micans* (Carreto et al. 1989) and now in a red alga. Increased levels of putative MAAs may be a general response to increased irradiance load (Carreto et al. 1989). In coastal zones, PAR and UV-levels typically increase and decrease in parallel over short time scales (Caldwell 1989, Hader 1993). Do similar rates of change in distinct pigment pools suggest that enhancement of levels of MAAs share a common regulatory mechanism with other photoprotective compounds such as carotenoids? If MAAs are specifically UV-photoprotective compounds, should it be expected that the addition of UV-A and/or UV-B will further enhance accumulation of MAAs beyond that of only high levels of PAR? These questions still need to be addressed for macroalgae in order to further elucidate the role of MAAs.

Conclusions

The tropical rhodophyte *Ahnfeltiopsis concinna* responds directly to altered irradiance regimes by precisely regulating the levels of photoaccessory phycobilins, photoprotective carotenoids and putative MAAs in tandem with irradiance loads. This capacity results in several benefits: 1) persistence of canopy tissues when exposed to high intertidal irradiance levels, 2) maximized photosynthetic performance in deeply shaded understory tissues, 3) maintaining substantial productivity in vastly different microclimates separated by >10 cm. *In vivo* absorption spectra allowed for the simultaneous examination of changes in major light harvesting pigments from 280 to 750 nm in the same tissue sample over time. These spectra revealed the differences in the rate of change of particular pigments as well as the maintenance of a constant PE:PC ratio throughout an acclimation response principally by changes in PE accumulation / degradation. The faster rate of
catabolic processes during acclimation compared to anabolic processes strongly suggests that one or more of the signal transduction, novel protein synthesis or placement into thylakoid membranes steps is a slower biochemical process than the degradation of photosynthetic constituents.
Literature cited


Table 6.1 Mean percentage change to opposite state in \textit{in vivo} pigmentation and photosynthetic states of canopy low-PPFD and understory high-PPFD treated tissues. \% change = \((\text{initial state (Day 0) - final experimental state (Day 8)}) / \text{difference between canopy and understory state (Day 0)}) \times 100.

<table>
<thead>
<tr>
<th></th>
<th>Canopy Low-PPFD</th>
<th>Understory-High PPFD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soret: Red Chl (\delta) (440:680 nm)</td>
<td>20.0</td>
<td>23.8</td>
</tr>
<tr>
<td>APC: Chl (\delta) (650:680 nm)</td>
<td>22.6</td>
<td>44.1</td>
</tr>
<tr>
<td>PC: Chl (\delta) (625:680 nm)</td>
<td>21.6</td>
<td>42.9</td>
</tr>
<tr>
<td>PE: Chl (\delta) (568:680 nm)</td>
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<tr>
<td>PE: Chl (\delta) (540:680 nm)</td>
<td>37.4</td>
<td>78.3</td>
</tr>
<tr>
<td>Carotenoid &amp; PE: Chl (\delta) (495:680 nm)</td>
<td>26.8</td>
<td>11.0</td>
</tr>
<tr>
<td>UVACs : Chl (\delta) (342:680 nm)</td>
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<td>44.6</td>
</tr>
<tr>
<td>(I_c)</td>
<td>83.0</td>
<td>58.0</td>
</tr>
<tr>
<td>(I_k)</td>
<td>78.0</td>
<td>68.0</td>
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Table 6.2. Comparison of P. vs. I Parameters of Canopy vs. Understory tissue of *Ahnfeltiopsis concinna*. Standard errors of the means are in parentheses below the means. Photosynthesis and respiration rates: μmol O₂·g fresh weight⁻¹·min⁻¹. Irradiance levels for all analyses: μmol quanta m⁻²·s⁻¹. P:R ratio: net photosynthesis / respiration.

<table>
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<td>n respir.</td>
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<td>3</td>
<td>3</td>
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<tr>
<td>(0.002)</td>
<td>(0.011)</td>
<td>(0.004)</td>
<td>(0.002)</td>
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<tr>
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<tr>
<td>(37.8)</td>
<td>(30.6)</td>
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<td>1.4x10⁻³</td>
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<td>(0.006)</td>
<td>(0.008)</td>
<td>(0.042)</td>
<td>(0.008)</td>
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<td>(0.09)</td>
<td>(0.51)</td>
<td>(0.71)</td>
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Figure 6.1. Mean (n=11) In vivo absorption spectra of canopy (squares) and understory (circles) tissues of Ahnfeltiopsis concinna. Spectra acquired on day 0 of the second experimental period. Note: 680 nm Chl a maxima normalized to 1.0 in all spectra.
Figure 6.2. 525-700 nm in vivo absorption spectra of canopy tissues exposed to a) high-PPFD, b) medium-PPFD, and c) low-PPFD treatments over a period of 8 days. Day 0: solid circles, Day 2: open circles, Day 4: solid squares, Day 6: open squares, Day 8: solid triangles.
Figure 6.3. Changes in 540:680 nm ratio for a) canopy and b) understory tissues exposed to low-PPFD (solid circles), medium-PPFD (open squares), and high-PPFD (open diamonds) treatments. Regression relationships: canopy low: $y = 0.644 + 0.0135x$, $R^2 = 0.699$, $H_0: b_1 = 0$, $p=0.000$, canopy medium: $y = 0.730 + 0.138x$, $R^2 = 0.594$, $H_0: b_1 = 0$, $p=0.000$, canopy high: $y = 0.671 + 0.006x$, $R^2 = 0.281$, $H_0: b_1 = 0$, $p=0.042$, understory low: $y = 0.952 - 0.0012x$, $R^2 = 0.053$, $H_0: b_1 = 0$, $p=0.268$, understory medium: $y = 0.963 - 0.002x$, $R^2 = 0.069$, $H_0: b_1 = 0$, $p=0.206$, understory high: $y = 0.921 - 0.014x$, $R^2 = 0.821$, $H_0: b_1 = 0$, $p=0.000$. 
Figure 6.4. Changes in PE (568 nm)(solid squares), PC (625 nm)(open circles) and APC (650 nm)(solid circles) absorption for a) low-PPFD treated canopy and b) high PPFD treated understory tissues. Regression relationships: canopy PE: \( y = 0.595 + 0.018x, R^2 = 0.639, H_a: b_1 \neq 0, p=0.000 \), canopy PC: \( y = 0.608 + 0.007x, R^2 = 0.608, H_a: b_1 \neq 0, p=0.000 \), canopy APC: \( y = 0.602 + 0.007x, R^2 = 0.655, H_a: b_1 \neq 0, p=0.000 \), understory PE: \( y = 0.935 - 0.016x, R^2 = 0.886, H_a: b_1 \neq 0, p=0.000 \), understory PC: \( y = 0.867 - 0.009x, R^2 = 0.484, H_a: b_1 \neq 0, p=0.004 \), understory APC: \( y = 0.857 - 0.010x, R^2 = 0.478, H_a: b_1 \neq 0, p=0.004 \).
Figure 6.5. *In vivo* absorption spectra from 525 to 700 nm of understory tissues exposed to a) high-PPFD, b) medium-PPFD, and c) low-PPFD treatments over a period of 8 days. Day 0: solid circles, Day 2: open circles, Day 4: solid squares, Day 6: open squares, Day 8: solid triangles.
Figure 6.6. In vivo absorption spectra from 420 to 500 nm of canopy tissues exposed to a) high-PPFD, b) medium-PPFD, and c) low-PPFD treatments over a period of 8 days. Day 0: solid circles, Day 2: open circles, Day 4: solid squares, Day 6: open squares, Day 8: solid triangles.
Figure 6.7. *In vivo* absorption spectra from 420 to 500 nm of understory tissues exposed to a) high-PPFD, b) medium-PPFD, and c) low-PPFD treatments over a period of 8 days. Day 0: solid circles, Day 2: open circles, Day 4: solid squares, Day 6: open squares, Day 8: solid triangles.
Figure 6.8. Changes in 495:680 nm ratio for a) canopy and b) understory tissues exposed to low-PPFD (solid circles), medium-PPFD (open squares), and high-PPFD (open diamonds) treatments. Regression relationships: canopy low: $y = 1.32 + 0.009x$, $R^2 = 0.149$, $H_a: b_1 \neq 0$, $p=0.057$, canopy medium: $y = 1.18 - 0.012x$, $R^2 = 0.322$, $H_a: b_1 \neq 0$, $p=0.003$, canopy high: $y = 1.29 + 0.011x$, $R^2 = 0.381$, $H_a: b_1 \neq 0$, $p=0.014$, understory low: $y = 1.01 + 0.0001x$, $R^2 = 0.0$, $H_a: b_1 \neq 0$, $p=0.924$, understory medium: $y = 1.00 - 0.003x$, $R^2 = 0.276$, $H_a: b_1 \neq 0$, $p=0.007$, understory high: $y = 0.994 + 0.006x$, $R^2 = 0.328$, $H_a: b_1 \neq 0$, $p=0.026$. 
Figure 6.9. *In vivo* absorption spectra from 280 to 400 nm of canopy tissues exposed to a) high-PPFD, b) medium-PPFD, and c) low-PPFD treatments over a period of 8 days. Day 0: solid circles, Day 2: open circles, Day 4: solid squares, Day 6: open squares, Day 8: solid triangles.
Figure 6.10. *In vivo* absorption spectra from 280 to 400 nm of understory tissues exposed to a) high-PPFD, b) medium-PPFD, and c) low-PPFD treatments over a period of 8 days. Day 0: solid circles, Day 2: open circles, Day 4: solid squares, Day 6: open squares, Day 8: solid triangles.
Figure 6.11. Changes in 342:680 nm ratio for a) canopy and b) understory tissues exposed to low-PPFD (solid circles), medium-PPFD (open squares), and high-PPFD (open diamonds) treatments. Regression relationships: canopy low: $y = 1.24 - 0.024x$, $R^2 = 0.493$, $H_a: b_1 \neq 0$, $p=0.004$, canopy medium: $y = 1.21 - 0.021x$, $R^2 = 0.386$, $H_a: b_1 \neq 0$, $p=0.001$, canopy high: $y = 1.08 + 0.025x$, $R^2 = 0.609$, $H_a: b_1 \neq 0$, $p=0.000$, understory low: $y = 0.98 - 0.007x$, $R^2 = 0.153$, $H_a: b_1 \neq 0$, $p=0.150$, understory medium: $y = 0.999 - 0.011x$, $R^2 = 0.394$, $H_a: b_1 \neq 0$, $p=0.001$, understory high: $y = 0.983 + 0.015x$, $R^2 = 0.624$, $H_a: b_1 \neq 0$, $p=0.000$. 
Figure 6.12. Absolute change in PE (540 nm, solid lines), carotenoid (495 nm, large dashed lines), and UVACs (342 nm, small dashed lines) in low-PPFD treated canopy tissues and high-PPFD treated understory tissues. To facilitate comparisons of the rate of change the reciprocal of the in vivo absorbance maxima which decreased with time are plotted in conjunction with maxima that increased with time. Solid circles: 540 nm canopy low, $y = 0.64 + 0.014x, R^2 = 0.70, H_a: b_1 \neq 0, p > 0.000$, open circles: 495 nm understory high, $y = 0.99 + 0.004x, R^2 = 0.328, H_a: b_1 \neq 0, p = 0.026$, solid squares: 342 nm understory high, $y = 0.98 + 0.015x, R^2 = 0.624, H_a: b_1 \neq 0, p < 0.000$, open squares: 540 nm understory high$^{-1}$, $y = 1.08 + 0.020x, R^2 = 0.819, H_a: b_1 \neq 0, p > 0.000$, solid triangles: 495 nm canopy low$^{-1}$, $y = 0.76 + 0.005x, R^2 = 0.151, H_a: b_1 \neq 0, p = 0.055$, open triangles: 342 canopy low$^{-1}$, $y = 0.81 + 0.017x, R^2 = 0.508, H_a: b_1 \neq 0, p = 0.003$. 

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PART VII

EFFECTS OF UV-RADIATION ON PHOTOACCLIMATION
Abstract

The rates of acclimation of distinct photoprotective and photosynthetic accessory pigments were not changed by exposure to either UV-B (280 to 320 nm) or UV-A (320 to 400 nm) irradiances when compared to PAR (400 to 700 nm) irradiances for the tropical rhodophyte Ahnfeltiopsis concinna. Similar response of carotenoids and MAAs to different substantially wavelengths ranges strongly suggests that the levels of these potentially photoprotective compounds respond to increases in over all photon flux density and not to the specific wavelengths they are thought to protect against. The difference between phycobilin acclimation responses of canopy and understory tissues reflects the response of sun- and shade-acclimated tissues moved into a similar irradiance environment. Changes in phycobilin specific absorbance over time was strongly associated with daily integrated irradiance.
Introduction

Ultra-violet (UV) radiation impacts primary production by a wide array of organisms: phytoplankton (Hebling et al. 1992), macroalgae (Wood 1989), zooxanthellae (Kinzie 1993), and terrestrial plants (Sullivan & Teramura 1989). Typical levels of UV-radiation to which tropical algae are subjected are at least two-fold higher than at higher latitudes (Caldwell et al. 1989). With this strong, persistent selective pressure, it follows that extant tropical primary producers are likely to have adapted some mechanism to survive long-term conditions of elevated photosynthetic photon flux density (PPFD) and UV-radiation. Mechanism(s) to minimize photon flux density (PFD)-induced damage have been reported for a diatom and coral (Hazzard 1993, Shick et al. 1995). Photoprotective mechanisms combating UV-radiation stress in primary producers may partially pre-empt UV-damage to the D1 protein of photosystem II (Yerkes et al. 1989), Calvin cycle enzymes (Dohler 1984, Strid et al. 1991), and nucleic acids (Peak & Peak 1983), reduce the potential for UV-induced photoinhibition (Cullen & Lesser 1991) as well as mitigate total crop losses (Teramura & Sullivan 1993).

One means of pre-empting UV-damage is by its absorbance by non-photosynthetic compounds. Not surprisingly, UV-absorbing compounds (UVACs), primarily mycosporine amino acids (MAAs), scytonemin, and flavonoids have been identified from an evolutionarily diverse assemblage of primary producers has been well documented and may represent photoprotective compounds (Sivalingam et al. 1974, Dunlap & Chalker 1986, Carreto et al. 1990, Karentz et al. 1991, Ziska et al. 1992, Garcia-Pichel & Castenholz 1991). The extent to which such compounds can be detected in vivo and vary spatially in macroalgae has only recently been addressed (Parts II & IV). There has been considerable research into the response of UVACs to changes in irradiance quantity and quality in several primary producer and symbiotic systems (Wood 1989, Carreto et al.)
1989, Kinzie 1993, Maegawa et al. 1993, Shick et al. 1995, Part VI). No clear consensus has emerged as to the trigger that enhances synthesis of UVACs.

Individually photosynthetically active radiation (PAR), UV-A and UV-B radiation have been proposed as triggers of UVACs synthesis (Wood 1989, Carreto et al. 1989, Kinzie 1993, Part VI). For *Eucheuma striatum* (red alga) and *Montipora verrucosa* (coral), increases in UV-irradiance were paralleled by increases in extractable levels of MAAs (Wood 1989, Kinzie 1993). *Acropora microphthalma* (coral) and red algal communities increased levels of UVACs with decreased depth (Maegawa et al. 1993, Shick et al. 1995), concomitant with increases in both photosynthetically active radiation (PAR) and UV-irradiance. The dinoflagellates, *Alexandrium excavatum* and *Prorocentrum micans* and the red alga *Ahnfeltiopsis concinna*, all increased levels of UVACs with increased PAR irradiances in laboratory experiments (Carreto et al. 1989, Part VI). Levels of UVACs have been demonstrated to vary from apical to basal portions of several tropical rhodophytes (Wood 1989, Part IV). In summary, two stimuli appear to stimulate accumulation of UVACs: high levels of PAR and/or UV-radiation. These stimuli are not necessarily mutually exclusive. Because two evolutionarily related taxa *E. striatum* and *A. concinna* responded to different cues with increased UVACs levels, there appears to be a diversity in responses to even this single mechanism.

*Ahnfeltiopsis concinna* is a tropical intertidal turf and has been the model of several studies examining adaptations to tropical stresses associated with high-tidal-elevations and tropical latitudes. *Ahnfeltiopsis concinna* occurs as a perennial ecological dominant at high-tidal-elevations on pre-historic basalt lava flows in the high Hawaiian islands (Doty 1967). In this niche, canopy tissues of this rhodophyte most likely experience the highest irradiances of any macroalga in the Hawaiian islands; however the morphology of *A. concinna* attenuated irradiances to $\geq 0.2\%$ canopy values in the turf's understory (Part IV). In response to this microscale gradient, intact stands of *A. concinna* markedly alter relative
levels of pigment constituents within the extent of an individual thallus (<10 cm) (Part IV). Canopy tissues had enhanced levels of photoprotective pigments coupled with reduced levels of phycobilins when compared with understory tissues. This pigment differentiation is tightly coupled to physiological shifts from sun- to shade-acclimated physiological states. Canopy tissues of *A. concinna* had increased respiration rates, saturation and compensation irradiances as well as depressed quantum efficiencies when compared with understory tissues (Part V). These microscale changes are correlated with alterations in the irradiance environment caused by self-shading (Glynn 1965, Parts IV & VI). Parts IV-VI have shown in laboratory experiments that the above *in situ* conditions are the result of a fine scale photoacclimation of this alga to alterations in levels of PAR.

Given that alterations in distinct pigments of *A. concinna* were driven by alteration in levels of PAR and that *in situ* alterations in levels of PAR typically accompany identical changes in UV-B and UV-A from 280 to 400 nm, the response of pigments of *A. concinna* was determined under 3 irradiance wavelength regimes (PAR-only, PAR+UV-A, PAR+UV-A+UV-B). This experiment allowed for the examination of the responses of *A. concinna* to UV-radiation stresses associated with tropical existence and to elucidate the pigment-based responses to distinct wavelengths of irradiance. I specifically asked: 1) How do pigments, both photosynthetic accessory and photoprotective respond to different irradiance wavelengths?; 2) Do canopy and understory tissues have similar or distinct responses to these irradiance wavelengths? 3) Do the responses of *A. concinna* to irradiance wavelength differ from that of irradiance intensity?

**Method and materials**

*Assessment of photoacclimation rates:* The ability of the canopy and understory portions of *A. concinna* to acclimate their pigmentation to different wavelengths of irradiance was assessed by culturing tissues in one of three light regimes for 18 days in a temperature controlled culture chamber. Irradiance treatments were of equivalent amounts
of PAR but differed in amounts of >400 nm irradiance (Figure 7.1). Tissues in treatment 1 were exposed to wavelengths ≤400 nm (PAR-only), treatment 2 tissues were exposed to wavelengths ≤320 nm (PAR+UV-A), and treatment 3 tissues were exposed to wavelengths ≤280 nm (PAR+UV-A+UV-B). Wavelength treatments were obtained with the use of 100% clear Acrylic, Mylar and Aclar filters for treatments 1-3, respectively (Figure 7.1). These materials were used to filter ambient sunlight. PAR was continuously monitored during the experimental periods between 17 October 1995 - 3 November 1995 in the plastic culture chamber with a LiCor Underwater UWQ quantum cosine sensor (Figure 7.2). Culture chamber conditions were as described in Part VI but plastic chambers were placed outside. Mean irradiance greater than 1.0 μmol quanta m⁻² s⁻¹ was sampled at 5 min intervals and stored in a LiCor LI-1000 data logger.

In vivo absorption spectra were acquired from 280 to 750 nm as detailed in Part VI. On Day 0 freshly collected canopy and understory tissues were excised and attached to white plastic plates with cable ties. An equal number of canopy and understory individuals were randomly assigned to one of three light treatments. A total of 6 samples in treatment 1 and 2 with 10 samples in treatment 3 were utilized.

The water temperature of the culture chamber was maintained between 24 and 30 °C via Neslab RTE-110 circulating water-bath cooled water-jacket. The temperature of the chamber increased and decreased with parallel changes in incident irradiance. The variation in temperature in the chamber was less than that experienced by A. concinna during a typical daily tidal cycle (KSB pers. obs.). Salinity was monitored daily with a Reichert temperature compensated refractometer and adjusted to 34 ppt with distilled water. Salinity varied between 33 and 37 ppt during the duration of the experiment. The chamber was continuously aerated and the chamber media (20 l of 0.2 μm filtered seawater (FSW)) was completely replaced every other day. Each day 10.0 ml of a 1.0 mM NaNO₃ / 1.0 mM
Na₂HPO₄*7H₂O nutrient solution was added to the chamber to minimize nutrient limitation that may have otherwise hampered photoacclimation responses.

Every other day, beginning at the day of sample collection (Day 0), an in vivo absorption spectra from 280 to 750 nm was acquired from each sample. Samples were simultaneously removed from the chamber, blotted dry removing any epiphytic diatoms and cyanobacteria, and spectra obtained. Sample order for acquisition of in vivo absorbance spectra was random. In vivo absorption spectra were acquired using the methods of Part VI using a lamp changeover wavelength of 290 nm. In vivo absorption spectra were normalized to 1.0 at 680 nm post spectra acquisition. The ratio of pigment bed specific in vivo absorption maxima normalized to 680 nm absorbance maxima for chlorophyll a (Chl a) was used as a measure of changes in composition of the pigment composition of A. concinna. To examine the extent of change in individual pigments, these ratios were converted into percentages with the Day 0 ratio assigned a value of 100%.

Statistical analyses: Values were compared statistically using a three-factor repeated measures ANOVA experimental design (Britting & Chapman 1993, Howell 1992, Eberhardt & Thomas 1991). Analysis was performed using Systat 5.2.1. Tissue type (microsite), irradiance treatment, and time were the main factors. Days since the beginning of the experiment was the factor in which in vivo absorbance spectra were measured repeatedly on individuals for 18 days and was regarded as the repeated measures factor. A posteriori least significant difference Tukey's test were used to evaluate the difference within factors judged significant by the three-factor ANOVA.

Results

Irradiance treatment effects: There was no effects of different irradiance wavelength treatments on any of the pigment beds examined (342 nm: UVACs, 440 nm: Chl a+carotenoids, 495: phycoerythrin (PE)+carotenoids, 540 nm: PE, 568 nm: PE, 625 nm: phycocyanin (PC)) (Figures 7.3 & 7.4, Tables 7.1-7.6). Pigment bed identities are based

**Differential microsite responses:** Canopy and understory tissues of A. concinna differed in the capacity to alter levels of photosynthetic accessory pigments (Figure 7.4, Tables 7.4-7.6) (e.g., phycobilins) but not photoprotective compounds (Figure 7.3, Tables 7.1-7.3) (carotenoids and UVACs). Understory tissues decreased levels of PE and PC as judged by positions of in vivo absorbance maxima in all irradiance treatments while phycobilin levels in canopy tissues initially increased and then decreased over time (Figure 7.4).

**Photoacclimation response over time:** Daily integrated irradiance varied over the experimental period (Figure 7.2). Daily integrated irradiance remained at moderate levels until Day 7, on Day 7 irradiance decreased, remaining low for 5 days and then elevated to the highest levels measured during the experimental period. Carotenoid and UVACs levels either decreased or remained constant in both tissues types for the first 8 days of the experiment, after which levels increased (Figure 7.3). This increase was most pronounced after Day 14. The maximum value out of all the single degree of freedom (1-9 order) polynomial contrasts and statistical significance (p≥0.0005) of the F-statistic for the first order (linear) relationship revealed that the increases in absorbance over time for 342, 440 and 495 nm maxima were highly significant. Understory tissues steadily decreased phycobilin specific absorbance at 540, 568 and 625 nm over the course of the experiment (Figure 7.4). As with carotenoids and UVACs, single degree of freedom contrasts strongly suggested that the first order (linear) decreases were highly significant for each phycobilin maximum (p≥0.0005). Phycobilin levels responded in a reciprocal manner compared to carotenoids and UVACs in canopy tissues of A. concinna in that levels either remained constant or increased for the first 10 days and then decreased as judged by in vivo absorbance maxima (Figure 7.4).
Discussion

Photoprotective and photosynthetic accessory pigments did not respond differently to changes in irradiance wavelength in *A. concinna*. UV-B and UV-A radiation did not enhance accumulation of putative UV-photoprotective UVACs while increased irradiance, regardless of wavelength from 280 to 700 nm, stimulated production of UVACs. Similar responses of the levels of carotenoids and UVACs to irradiance wavelength and changes in irradiance strongly suggest an similar mechanism for enhancement in levels of potentially photoprotective compounds to increased irradiance levels. As increases in PAR and UV-radiation are typically coupled, a similar photoacclimation response is not surprising. Changes in phycobilin composition were the result of changes in irradiance for shade- and sun-acclimated understory and canopy tissues, respectively, to moderate irradiance (mean daily quantum irradiance = 830 μmol quanta m\(^{-2}\) s\(^{-1}\)) as well as precise responses to daily fluctuations in irradiance. Phycobilin absorption decreased throughout the course of this study in understory tissues. Mean daily quantum irradiance was consistently higher than understory tissues value of saturation irradiance (I_0) at 108 μmol quanta m\(^{-2}\) s\(^{-1}\) (Part V). Increases in phycobilin absorbance in canopy tissues occurred at mean daily quantum irradiance levels twice that of canopy tissues values of I_0 at 297 μmol quanta m\(^{-2}\) s\(^{-1}\) (Part V) while decreases in phycobilin absorbance occurred at mean daily quantum irradiance levels ~3-fold higher than I_0.

The difference in responses to UV-irradiance by *A. concinna* and *E. striatum* may reflect the different irradiance regimes in these two experiments or may reflect a truly different response in these two rhodophytes in the order Gigartinales to irradiance stresses. Although irradiance values were not reported for culture conditions by Wood (1989), the extractable concentration of UVACs from UV+ treated tissues were higher than those in situ or in UV- treatments, indicating that irradiance in the UV+ treatments was higher than normally experienced by *E. striatum*. Irradiances in this study were lower than ambient
low tide conditions experienced by canopy tissues but much higher than ambient conditions for understory tissues of *A. concinna* (Part IV). Both experiments (Wood 1989 & this study) had similar duration's of 20 and 18 days, respectively. If enhanced levels of UV-radiation separately contribute to UVACs accumulation beyond that of enhanced PAR, a difference should have been detected between wavelength treatments at least in understory tissues of *A. concinna*. This was not observed in the 18 days of this study. Additionally, carotenoid and UVACs levels in *A. concinna* underwent similar changes with similar treatments. Therefore it appears that regulation of UVACs levels in *A. concinna* is controlled by irradiance load and not irradiance wavelength as in *E. striatum* (Wood 1989).

The response of phycobilin constituents to changes in irradiance has been previously addressed (Part VI). This experiment further substantiates earlier findings but also shows the exceptionally responsive nature of the phycobilin pigmentation in *A. concinna* to daily fluctuations in irradiance. Transferring deeply shaded understory tissues ($I_k = 108 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) to moderate irradiance (mean daily quantum irradiance = 830 \mu mol quanta m^{-2} s^{-1}) conditions caused a decrease in the in vivo absorption maxima specific to the phycobilins PE and PC within 4 days. These changes acclimated tissues to high irradiance conditions and potentially liberates nitrogen for other physiological processes (Osmond 1994). Alterations in maxima for specific phycobilin maxima in canopy tissues was highly dependent upon integrated daily irradiance. With moderate to low irradiance levels (mean daily quantum irradiance from 375 to 805 \mu mol quanta m^{-2} s^{-1}), PE and PC levels increased (Day 0-10) and with increased irradiance (mean daily quantum irradiance 1000 \mu mol quanta m^{-2} s^{-1})(after Day 13), concentrations of these components markedly decreased.

A diversity of physiological responses to stress appears to be the rule rather than the exception in red algae. *Ahnfeltiopsis concinna* and *Laurencia mcdermidiae* differed in adjustments of specific phycobilisome constituents, the type of UVACs altered, and
adjustments of photosynthetic performance in response to high irradiance (Parts IV & V). Enhanced temperature tolerance of photosynthesis with desiccation occurred in high intertidal *Porphyra perforata* but not in *A. concinna* (Smith 1983, Part VIII). Enhanced irradiance load or irradiance wavelength were triggers for augmenting levels of UVACs in *A. concinna* and *E. striatum*, respectively (Wood 1989, this study).

**Conclusions**

Irradiance load between 280-700 nm not irradiance wavelength stimulates production of UVACs in *A. concinna*. Carotenoid and UVACs levels respond to changes in irradiance in similar manners suggesting the enhancement of both putative photoprotective compounds may be part of a similar cascade of biochemical events in response to an high irradiance stimulus. Regulation of levels of phycobilin constituents precisely track levels of daily integrated irradiance in *A. concinna*. This promptly acclimates tissues to irradiance conditions allowing for efficient use of excitation energy as well as nutrients. The distinction between responses of *A. concinna* to physiological stress compared to other rhodophyte algae emphasizes the diversity of mechanisms this ancient group of primary producers have developed via natural selection and argues that stress physiology models based on one model system are of limited utility.
Literature cited


Table 7.1 Treat of individual effects and their interactions on the percent change of 342:680 nm in vivo absorbance ratio in *A. concinna*. 100% = Day 0 in vivo absorbance state. *: significantly different.

<table>
<thead>
<tr>
<th>Source</th>
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Table 7.2 Treat of individual effects and their interactions on the percent change of 440:680 nm in vivo absorbance ratio in *A. concinna*. 100% = Day 0 in vivo absorbance state. *: significantly different.

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Table 7.3. Treat of individual effects and their interactions on the percent change of 495:680 nm in vivo absorbance ratio in *A. concinna*. 100% = Day 0 in vivo absorbance state. *: significantly different.

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Table 7.4 Treat of individual effects and their interactions on the percent change of 540:680 nm in vivo absorbance ratio in *A. concinna*. 100% = Day 0 in vivo absorbance state. *:* significantly different.

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Table 7.5 Treat of individual effects and their interactions on the percent change of 568:680 nm in vivo absorbance ratio in *A. concinna*. 100% = Day 0 in vivo absorbance state. *: significantly different.

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<tr>
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Table 7.6. Treat of individual effects and their interactions on the percent change of 625:680 nm in vivo absorbance ratio in *A. concinna*. 100% = Day 0 in vivo absorbance state. *: significantly different.

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Figure 7.1. Percent transmittance of filters used to create irradiance treatments 1-3.

Treatment 1: clear acrylic (solid line), transmittance cut off between 390-400 nm (PAR-only). Treatment 2: mylar (dashed line), transmittance cut off at ~320 nm (PAR+UV-A). Treatment 3: aclar (dotted line), transmittance cut off at <280 nm (PAR+UV-A+UV-B).
Figure 7.2. Integrated daily PAR irradiance within experimental treatments. Irradiance levels not recorded on Day 0 or Day 18. Line = Least-squares regression relationship, $R^2$: 0.734.
Figure 7.3. Percent change in in vivo absorbance maxima over time. 345 nm: UVACs, 440 nm: Chl a+carotenoids, 495 nm: carotenoids+PE. Treatment 1 (PAR-only): canopy (solid diamonds), understory (open diamonds); treatment 2 (PAR+UV-A): canopy (solid squares), understory (open squares); treatment 3 (PAR+UV-A+UV-B): canopy (solid circles), understory (open circles). Least-squares regression lines are not significantly different between irradiance treatments.
Figure 7.4. Percent change in \textit{in vivo} absorbance maxima over time. 540 nm: PE, 568 nm: PE, 625 nm: PC. Treatment 1 (PAR-only): canopy (solid diamonds), understory (open diamonds); treatment 2 (PAR+UV-A): canopy (solid squares), understory (open squares); treatment 3 (PAR+UV-A+UV-B): canopy (solid circles), understory (open circles). Least-squares regression lines significantly different between canopy versus understory tissues.
PART VIII

ECOPHYSIOLOGY OF A TROPICAL RHODOPHYTE IV:
IMPACT OF EMERSION STRESSES ON AHNFEITIOPSIS CONCINNA

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Abstract
The capacity to recover photosynthetic activity from emersion stresses varied between microsites separated by <10 cm in the tropical turf forming alga *Ahnfeltiopsis concinna* (J. Ag.) Silva et DeCew. Canopy tissues which are regularly exposed to a greater range of irradiance, temperature and osmotic stresses than understory tissues were physiologically impacted to less of a degree by these stresses alone or in combination compared to understory tissues. This, the first multivariate analysis of physiological recovery from emersion stresses for any macroalgae, revealed the synergistic effects of high irradiance and temperature stresses while suggesting that enhanced temperature tolerance upon desiccation is not a universal feature of macroalgae at high tidal elevations. Net photosynthesis was enhanced by 20% water loss or exposure to 2150 mOsm media compared to fully immersed state. Osmolarities between 200-3500 mOsm had a minimal negative impact on net photosynthesis. Temperature optimum for net photosynthesis was 33°C while the upper threshold was 40°C. These lines of evidence suggest that the physiology of this alga is well adapted to existence at high tidal elevations while additionally possessing the capacity to precisely acclimate to microclimate conditions. This diminutive physiological gradient emphasizes the differences in environmental pressures between high tidal amplitude, low irradiance temperate regions and low tidal amplitude, high irradiance tropical regions. In general, these pressures result in similar conspicuous photoacclimative and stress tolerance adaptations but over vastly different spatial scales.
Introduction

Causes of global patterns of epilithic intertidal zonation (Stephenson & Stephenson 1972) has been an area of intensive investigation (Connell 1961, Menge 1976, Lubchenco 1980, Underwood 1980, Dring & Brown 1982, Smith & Berry 1986). In general, macroalgal persistence at low tidal elevations has been attributed to enhanced competitive abilities (Lubchenco & Menge 1978) while existence at high tidal elevations has been ascribed to physiological adaptation(s) to emersion stresses (Connell 1961, Dring & Brown 1983, Smith et al. 1986, Bose et al. 1988, Britting & Chapman 1993).


Increased temperature typically associated with emersion increases maximal photosynthetic and dark respiration rates with a Q10 of ~ 2.0. Photosynthetic capacity reaches a temperature optimum beyond which photosynthetic rates rapidly decline (Davison 1991). In Porphyra perforata, a temperate rhodophyte, temperatures beyond 25°C negatively impacted photosynthesis (Smith and Berry 1986). Temperature optima and high temperature thresholds vary over latitudinal as well as seasonal gradients (Smith & Berry 1986, Davison 1991, Pakker et al. 1995).

Physiological responses to increased irradiance while algae are immersed have been well characterized with approximately linear increases in photosynthetic rates until carbon acquisition becomes limiting (Sukenik et al. 1987) and a maximum rate of photosynthesis
is attained. With continued increases in irradiance, photoinhibition may result from photoprotection and/or photodamage to photosystem II (PS II) (Henley 1993, Osmond 1994). The extent of photoinhibition can be synergistically exacerbated beyond the detrimental effects of high irradiance levels in combination with other stresses (Henley et al. 1991). Emerged rates of photosynthesis have received less attention (but see Matta & Chapman 1995).

Physiological adaptations to emersion related stresses have been extensively examined among species of the red algal genus *Porphyra* (Oquist & Fork 1982, Satoh et al. 1983, Smith & Berry 1986, Smith et al. 1986, Herbert & Waaland 1988, Bose et al. 1988). Tolerance of high temperatures increases with tissue water loss in *P. perforata*. Under stress conditions the photosynthetic apparatus undergoes transition from State I (Fork & Satoh 1986), where the harvesting complex typically associated with PSII (LHPPC II) transfers excitation energy to the reaction center of photosystem II (PS II), to State II (Fork & Satoh 1986) where irradiance harvested by LHPPC II is directed to photosystem I (PS I). Further desiccation causes an unusual transition from State II to State III. State III dissipates absorbed irradiance without fluorescence emission from either PS I or PS II. *Porphyra* is the only photoautotroph in which State III has been observed (Fork & Satoh 1986). Morphological modifications that take place during water loss of *Porphyra*’s sheet-like thallus appears essential for proper configuration of cell and membrane components, allowing for state transitions and rapid recovery of photosynthesis upon rehydration (Oquist & Fork 1982). Considering the high surface area to volume ratio of *Porphyra*, the ability of some species to tolerate a period of up to 2 months without immersion during daylight hours is an impressive example of stress tolerance (Smith 1983). Other functional morphologies are also successful at high tidal elevations (Britting & Chapman 1993). Related morphologies have been shown to have different capacities to recover from desiccation stress (Dudgeon et al. 1993).
Turf morphologies have a direct impact on the physical microclimate experienced by different portions of the same thallus (e.g., canopy vs. understory tissues). Glynn (1965) demonstrated decreased temperatures in the interior of stands of *Endocladia* compared to air or substrate temperatures. The tropical rhodophyte turf, *Ahnfletiopsis concinna* stands 15 cm upright and is the highest intertidal macrophyte on pre-historic basalt lava flows in the high Hawaiian islands (Doty 1967, KSB pers. obs.). Photosynthetically active radiation (PAR) can be markedly attenuated by dense turfs of *A. concinna* with canopy irradiances being reduced to less than 0.2% of incident levels (Part III). Turf morphologies promote marked intra-thallus photoacclimation at the level of pigments and photosynthetic performance at tropical altitudes (Parts III & IV).

*Ahnfletiopsis concinna* photoacclimates to high-irradiance by increasing levels of photoprotective carotenoids and putative mycosporine amino acids (MAAs) while concomitantly reducing levels of phycobilins (Part V). Canopy tissues of *A. concinna* are chronically photoinhibited in the field with understory tissues from the same individuals having at least equivalent rates of photosynthesis (Part IV). Physiological differentiation between microsites of turfs may also extend to emersion stress tolerance. Precise physiological acclimation to stresses associated with microsites may facilitate ecological dominance of *A. concinna* at high tidal elevations.

The ability to rapidly recover photosynthetic activity from stresses imposed by emersion, in contrast to the ability to maintain net photosynthesis (Matta & Chapman 1995), has been correlated to persistence at high tidal elevations (Dring & Brown 1983, Smith & Berry 1986). Given this and that the emersion related stresses: temperature, osmotic and irradiance occur in tandem in the natural environment, the following investigation presents the first multifactorial analysis of the impact of these emersion stress on physiological recovery. Additionally, this investigation examines the impact of a turf...
morphology on stress levels and acclimation to emersion stress in canopy versus understory tissues of *A. concinna*.

Specifically, we addressed the following questions: 1) What levels of desiccation and thermal stress are experienced by microsites of *A. concinna* in the field?, 2) What is the immediate impact of and the impact on recovery from emersion stresses on dark respiration (R_d), maximum net photosynthetic rate (P_{n_{max}}), maximum gross photosynthetic rate (P_{g_{max}}) and net maximum photosynthetic rate to dark respiration rate ratio (P:R ratio)?, 3) How do univariate and multivariate examinations of the physiological impact of emersion stresses compare?, 4) Do canopy and understory tissues of *A. concinna* differ in their stress recovery capacities?

**Methods and materials**

**Microsite environmental conditions:** Microclimate conditions and tissues of *A. concinna* collected for experimental analysis were sampled at Makapu'u and Kaloko on the island of O'ahu, Hawai'i, U.S.A (21°27' lat., 158° 28' long.). Microclimate conditions were sampled on mostly sunny days with moderate tradewinds. These conditions are typical year round at this site (KSB pers. obs.).

Throughout the following analyses, canopy and understory microsites were as previously defined (Part III). Emerged canopy and understory temperatures of *A. concinna* were determined on several sunny days between Sept. 1994 and June 1995. Microsite temperature was determined with an Omega HH-25TC Digital Thermometer with a type-T Cu-CuNi Thermocouple. Temperatures of canopy, understory tissues and concomitant air temperature were recorded for each individual examined.

Relative water content (RWC) of emerged canopy and understory tissue of *A. concinna* was determined using the methods of Smith and Berry (1986). $\text{RWC} = \left(\frac{\text{sample field weight} - \text{sample oven dry weight}}{\text{sample fully hydrated weight} - \text{sample oven dry weight}}\right) \times 100$. Sampling occurred between 1 May and 15 June 1995 over tidal cycles
reaching maximum lower low water (-0.12 m MLLW) and with low wave height (≤ 0.6 dm) (KSB pers. obs.). This sampling during periods of mostly sunny days.

**Osmotic and temperature response curves:** *Ahnfeltiopsis concinna* was collected on 15 and 22 May 1995 for analysis of physiological responses to temperature and salinity, respectively. Physiological response to temperature was measured by determining O₂ evolution / consumption rates in response to temperatures from 5 to 45 °C in 5°C increments utilizing the methods of Part II. Each measurement had saturating irradiance (750 μmol quanta m⁻² s⁻¹) preceded by a 5 min dark period. The rates at 25°C with 1050 mOsm 0.2 μm filtered sea water (FSW) were established as 100%, as these were the typical immersed conditions experienced by this alga in the field during the time of collection. Temperature changes were regulated via a Neslab RTE-110 circulating water bath and were obtained in 3.0 min.

Physiological response to salinity was measured by determining O₂ evolution / consumption rates (Part II) in response to osmolarities from 200 to 7550 mOsm at 25°C. FSW of different osmolarities was generated via either dilution of 1050 mOsm FSW with NANOpure filtered type I water or addition of NaCl. Treatment media was augmented with 20 mM NaHCO₃ to minimize carbon limitation and pH adjusted to 8.3 (buffered with HEPES). Osmolarities of treatment waters were determined with a Precision systems μOsmette freezing point depression micro-osmometer (model # 5004). μOsmette was calibrated with previously unopened Westcor 100, 290 and 1000 mOsm standards. Treatment media were stored at experimental temperatures. Transition time between treatments was <30 s. Osmotic treatments were imposed in either a progressively increasing or decreasing manner.

**Recovery from desiccation (air drying):** *Ahnfeltiopsis concinna* was collected between 15 Sept. through 15 Oct. 1994 and maintained as previously described (Part IV). All physiological trials were performed within 24 h of sample collection. Canopy and
understory tissues were excised, quickly blotted to remove excess water, and fresh weight (FW) determined. Excised tissue was then attached to a modified chamber seal (Smith & Berry 1986) and placed in FSW under dim irradiance (50 μmol quanta m⁻² s⁻¹). Samples equilibrated for 15 min post handling before pre-treatment $P_n^{\text{max}}$ and $R_d$ were determined (see below). Care was taken during handling to minimize time emersed and impact of pre-evaluation desiccation.

Pre-treatment $P_n^{\text{max}}$ and $R_d$ rates were determined via changes in $O_2$ concentration as previously described (Part II). Dark respiration rates were determined from average of oxygen consumption rates in the dark before and after saturating irradiance (750 μmol quanta*m⁻²*s⁻¹) treatment to determine $P_n^{\text{max}}$.

Desiccation treatments to obtain different RWC varied between 2 and 45 min during which the tissue was blotted to remove any excess water, exposed to saturating irradiance (750 μmol quanta*m⁻²*s⁻¹) and gentle air currents. Gentle air currents were supplied by a 6" diameter variable speed fan set on low and positioned 2.0' from the samples. After a defined period of drying, tissue was re-weighed to determine treatment dry weight and then placed in aerated FSW under 50 μmol quanta*m⁻²*s⁻¹ for a 1 h recovery. Recovery of $R_d$ and $P_n^{\text{max}}$ were then determined. After recovery measurements, samples were dried at 60°C for 72 h and oven dry weight determined.

Effects of multiple emersion stresses on physiological recovery: Individuals of A. concinna were collected between 19 June 1995 through 8 Sept. 1995 for multivariate analysis of the effects of hyperosmotic, temperature and PAR irradiance stresses on algal physiology. Individuals were maintained as previously described and samples were processed within 36 h of collection.

Recovery of $P_n^{\text{max}}$ and $R_d$ after exposure to osmotic, temperature and irradiance stresses was assessed via the methods of Smith and Berry (1986). In brief, pre-treatment $P_n^{\text{max}}$ and $R_d$ were assessed at 25 °C, 1050 mOsm and 750 μmol quanta*m⁻²*s⁻¹, and

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contrasted to rates measured after 0.5 h recovery from 1 of 27 experimental treatments (see below). Post-treatment physiological rates were measured under conditions identical to pre-treatment assessment. Recovery was in the dark at ambient sea water temperature (25°C) and osmolarity (1050 mOsm). The difference between post- and pre-treatment rates were converted to a percentage via: recovery % = (((post-pre)/pre)*100) and statistical analyses performed (see below).

Three treatment levels of irradiance (0, 1150, 2000 μmol quanta m⁻² s⁻¹), osmolyte (1050, 3125 and 10,050 mOsm) and temperature (25, 30, 35°C) resulted in 27 treatment combinations. Temperature, osmolarity and irradiance levels were obtained as previously described (see above and Part II). Additionally, canopy and understory tissues from each individual were examined; ultimately resulting in 54 combinations of experimental factors. Canopy and understory tissues from an individual were randomly assigned to identical treatments and run side-by-side to minimize variation. Three replicates of each treatment combination were performed resulting in 162 samples.

Statistical analyses: Statistical analyses were carried out using Minitab version 10xtra (Minitab Inc.) and Systat 5.2.1. Comparison of canopy versus understory emersed thallus temperatures and RWC was performed with a pair-wise and non-pairwise t-test, respectively. Normality of these data was assessed with a test equivalent to the Shapiro-Wilk test with an α of 0.05 as well as by the linearity of normal quantile-quantile plot (Minitab Reference Manual, 1991).

For the univariate experiments, the response of canopy and understory tissue Rd, Pn_max and P:R ratio to temperature, salinity as well as recovery from desiccation was determined via regression. Normality of each regression’s residuals were tested as described above. Comparisons of the slopes and Y-intercepts of select regression lines were made as detailed by Zar (1984). Because non-linear responses to osmolarity and temperature occurred, comparisons were made by either transformation of data (e.g.,
temperature vs. respiration curve, x's transformed to $x^2$) or comparison of linear relationship of data either side of relationship maxima (e.g., temperature / osmolarity vs. $P_{\text{n max}}$, temperature / osmolarity vs. P:R ratio).

Four-way ANOVA was used to differentiate the physiological responses of canopy and understory tissues to osmotic, temperature and irradiance treatments. Normality of residuals was judged as above. Interaction effects were considered significant only if at least one of the individual factors were independently significant. A posteriori. Tukey's comparisons were used to detect specific differences in factors that were judged to be significant by the ANOVA ($p \leq 0.05$).

Results

Microclimate environmental conditions: Emerged thallus temperature and RWC significantly differed between canopy and understory tissues. Mean air temperature during the periods of measurement was 23.5 °C. Canopy tissues were on average 6.3°C over ambient air temperature. Understory tissue were on average 3°C over ambient air temperature. This canopy vs. understory temperature differential was significant ($n=50$, $t=3.26$, $p=0.001$). Maximum canopy temperature measured was 35°C, 10°C higher than concurrent air temperature. The lowest RWC measured for canopy and understory tissues were 18 and 37%. These measures coincided with maximum low water for that day's tidal cycle. Over the ebb and flood tides during which this work was performed the mean RWC was 65.7 and 76.2 % for canopy and understory tissues, respectively. This differential extent of desiccation was significant ($n=54$, $t=-2.65$, $p=0.009$).

Osmotic and temperature response curves: $P_{\text{n max}}$ of both canopy and understory tissues of $A$. concinna were altered in similar manners in their immediate response to changes in osmolarity (Figure 1a). Maximum level of $P_{\text{n max}}$ was observed at 2150 mOsm (~2x sea water salinity). $P_{\text{n max}}$ fell slightly below the pre-defined 100% level with osmolarity decreases below 1050 mOsm and was depressed below 50% of ambient rate by
osmolarities above 5500 mOsm (Figure 1a). The effect of differing osmolarities on canopy and understory Rd revealed no discernible relationship (Figure 1b). Subsequently, the relationship of osmolarity to the P:R ratio was unclear (Figure 1c).

Canopy and understory tissues of A. concinna did not differ in their immediate response of either rates of Pn_max nor Rd to changes in temperature (Figure 2a & b). Pn_max was reduced 75% in the transition from 25 to 15°C; respiration was reduced 50% over the same temperature range (Figure 2a & b). The temperature at which maximum level of Pn_max occurred ranged from 30 to 34°C. Rates of Pn_max decreased rapidly over the range 39 to 43°C (Figure 2a). P:R ratio was highest at 20°C and decreased with both increased and decreased temperature in a similar manner in both canopy and understory tissues (Figure 2c).

Recovery from desiccation (air drying): The percent recovery of Pn_max one hour after rehydration was more complete in canopy than in understory tissues (Figure 3a). A parallel relationship between recovery of Pn_max and RWC as indicated by significantly different y-intercepts (df: 21, t=3.03, p=0.0072) was observed. Pn_max recovered to 50% pre-treatment rates after a reduction to 33% RWC in canopy tissues and at 54% RWC for understory tissues. Recovery of P:R ratio of canopy tissue was impacted to less of a degree by desiccation compared to understory tissues (Figure 3c). The slopes of these relationships were not significantly different (df 6, t=1.1602, p=0.189) (Figure 3c). P:R ratio was reduced to 0 after desiccation to 25% RWC for understory tissue while canopy tissue had to be desiccated to 17% RWC to experience the same reduction. Rates of Rd were similar in canopy and understory tissues in their ability to recover from desiccation (Figure 3b). Rates of Rd were double pre-treatment rates after reduction to 20% RWC following 1 h recovery for both tissue types.

Multivariate analysis of emersion stresses on physiological recovery: Four-way ANOVA were performed on the percent recovery of Rd, Pn_max, P:R ratio and Pg_max on
the factors temperature, osmolarity, irradiance and microsite. As was seen above with the immediate response to osmotic stress by $R_d$ (Figure 1b), rates of $R_d$ were significantly effected by both temperature and irradiance but not by differing osmotic levels (Table 1). Rates of $R_d$ were elevated by the 35°C as well as 2000 μmol quanta*m$^{-2}$*s$^{-1}$ treatments compared to lower temperatures and irradiances. There was also a significant interaction between temperature and irradiance as well as irradiance and microsite on recovery of $R_d$ (Table 1). Recovery of $R_d$ was less than expected as predicted by the impacts of irradiance and temperature alone. Rates of $R_d$ were augmented in canopy tissues with exposure to 2000 μmol quanta m$^{-2}$ s$^{-1}$ to a greater degree than dark exposed tissues or similarly illuminated understory tissues. $P_n$ max and P:R ratio were both significantly effected by osmolarity with the 10,050 mOsm treatment reducing $P_n$ max and P:R ratio to a greater extent than 1050 or 3125 treatments (Tables 1). There was also a significant interaction of irradiance and microsite as well as irradiance and osmolarity on $P_n$ max and P:R ratio, respectively. $P_n$ max recovered to a greater percentage than expected with exposure to 2000 μmol quanta m$^{-2}$ s$^{-1}$ than similarly treated understory or dark treated tissues. Osmolarity, temperature, irradiance and microsite were all highly significant principle effects on $P_E$ max (Table 1). 10,050 mOsm osmolyte, and 2000 μmol quanta*m$^{-2}$*s$^{-1}$ irradiance both reduced recovery of $P_E$ max compared to the lower salinities and irradiances. Recovery of $P_E$ max at both 30 and 35°C was reduced compared to 25°C. $P_E$ max of canopy tissues was more resilient to the combination of irradiance, temperature and osmotic stresses than understory tissues.

**Discussion**

This, the first multivariate analysis of emersions stress on physiological recovery in an intertidal alga, was able to elucidate response differences between tissues separated by <10 cm but occurring in vastly different microclimates. Photosynthesis in canopy tissues of *A. concinna* was more resilient in its recovery from emersion than identical processes in
understory tissues from the same axis. This experimental approach also assessed the interaction of emersion stresses on distinct physiological processes. Irradiance and tissue microsite, irradiance and osmolarity, as well as irradiance and temperature all had significant interactions for at least one physiological parameter. The combined effects of these environmentally coupled emersion stresses on the physiology and ultimately the competitive ability of algae from high tidal elevations can not be and were not anticipated from an univariate approach.

The functional form of the algal turf, *A. concinna*, ameliorates the impact of emersion stresses on understory tissues by creating a benign understory microclimate. Canopy tissues are exposed to a greater range of temperatures, osmotic conditions and irradiances (Part III) than understory tissues. Canopy tissues acclimated to these stresses are capable of more rapid physiological recovery of $P_{\text{g max}}$ than understory tissues after exposure to identical combinations of emersion stresses. Algae at high tidal elevations and at tropical latitudes are exposed to high levels of UV, PAR, temperature and desiccation stress. A turf morphology localizes emersion stresses to a specific thallus region while deeply shading understory tissues. The capacity to acclimate precisely to microscale changes in the light environment may maximize photosynthetic performance over the diminutive axis. As canopy tissues are chronically photoinhibited even with marked increases in photoprotective compounds (Parts III & IV), *A. concinna* may not thrive at high tidal elevations without the functional form of a turf and precise acclimation capacity to numerous stresses.

The impact of emersion stresses on distinct physiological processes were elucidated in a more realistic manner by simulating these stresses in tandem. $R_d$ had a significant interaction between irradiance and microsite as well as irradiance and temperature. The microsite / irradiance interaction was manifest as augmented rates of $R_d$ in canopy tissues compared to understory tissues; the rate difference was greater at 2000 μmol quanta m$^{-2}$ s$^{-1}$.
1 than in the dark. The interaction of irradiance and temperature was expressed as greater than expected recovery rates of Rd. Recovery rates of \( R_d \) increased with increased temperature as was seen in the univariate analysis but \( R_d \) was additionally augmented with exposure to high-irradiance. With exposure to 2000 \( \mu \text{mol quanta m}^{-2} \text{s}^{-1} \) and 35 °C, \( R_d \) was increased to a greater extent than could be attributed by the additive effects of temperature and irradiance, independently. Increased \( R_d \) with the combination of temperature and irradiance may be the result of enhanced photodamage to photosynthetic components and/or increased rates of photorespiration caused by (1) excess irradiance, (2) increased reaction rates for enzymes of the light and dark reactions combined with carbon limitation at the site of fixation. Augmented \( R_d \) in the light has been previously recognized (Matta and Chapman 1991) but this and more importantly the interaction of elevated temperature and irradiance influence estimates of \( H_{\text{Sat}} \). If rates of \( R_d \) increased to a greater degree with the interaction of stressful environmental conditions compared to estimates based on univariate measures, the photoperiod of saturating irradiance (\( H_{\text{Sat}} \)) would be diminished. Productivity estimates based on this parameter are likely to be be more reliable with a comprehensive understanding of physiological responses to coupled environmental stresses.

Insight into light harvesting efficiency of understory tissues was gained by detection of a significant interaction between irradiance and microsite on \( P_n^{\text{max}} \). Understory tissues had greater recovery of \( P_n^{\text{max}} \) when treated with 2000 \( \mu \text{mol quanta m}^{-2} \text{s}^{-1} \) compared to canopy and dark treated tissues. The interaction between irradiance and microsite suggest that photosynthetic processes require a induction period in the light to achieve maximum rates. This period of induction for photosynthesis appears to be greater in canopy tissues than in understory tissues. Similar requirement differences have been observed between forest canopy and understory plants separated by many meters (Pearcy 1990). In an irradiance limited environment such as an algal turf or forest understory,
capacity to harvest and utilize the majority of photons that are incident on a primary 
producer is essential to the organism's energetics; whereas canopies experiencing luxury 
levels of irradiance can achieve sufficient amounts of carbon fixation even with a slower 
initial physiological response.

As seen in univariate experiments by Britting and Chapman (1993) and Pearson and 
Davison (1994), osmotic induced desiccation of A. concinna had a no predictable effect on 
Rd even when coupled with naturally co-occurring emersion stresses. Different results 
were obtained with air and osmolyte induced desiccation. These difference were as 
follows: 1) Significant effect of desiccation in air but not via osmolyte on recovery Rd, 2) 
differences between canopy and understory recovery of Pn_max and P:R ratio from air 
desiccation but not in osmolyte desiccation. The preceeding discrepancies between air and 
osmolyte induced desiccation suggest several explinations. First among these possibilities, 
osmotic-desiccation may have effects on Rd that do not accurately simulate an emersion 
event. Potential artifactual response to NaCl induced desiccation could have confounded 
subtle physiological trends involving Rd and parameters which include Rd in their 
determination (Pn_max, P:R ratio). Temperature, irradiance, osmolyte and microsite were 
significant factors on recovery of Pn_max but not Pn_max. Air induced desiccation did not 
have these effects in univariate experiments but similar variability in Rd was observed 
immediately upon immersion and up to 12 h after air drying in E. muricata (Britting & 
Chapman 1993). A second possibility is that this discrepancy may in fact reveal an 
adaptation to high NaCl levels in high intertidal macralgae that are exposed for long 
durations compared to brief (2-4 hr) exposures like A. concinna. These possibilities 
warrent future comparisons between air and osmolyte induced desiccation.

In contrast to Smith (1983) and Matta and Chapman (1995), temperature tolerance 
was not augmented by desiccation in A. concinna. No significant interactions of 
temperature and osmolyte were detected. The presence of this physiological feature in high
tidal *P. perforata* (rhodophyte) and mid to low tidal *C. perigina* (phaeophyte) and lack of this stress tolerance mechanism in either canopy or understory tissues of high intertidal *A. concinna* (rhodophyte) suggests that this mechanism has evolved multiple times and that there is diversity of physiological strategies in coping with existence at high tidal elevations.

Another proposed mechanism for coping with high tidal existence is enhanced photosynthetic capacity with partial tissue dehydration (Bell 1993, Britting & Chapman 1993). Although photosynthetic rates were not monitored immediately upon rehydration the response of $P_{\text{Nmax}}$ to increasing osmolarities and the percent recovery of $P_{\text{Nmax}}$ after air desiccation suggest that slight desiccation enhances immediate and recovery rates of $P_{\text{Nmax}}$ in canopy but not understory tissues of *A. concinna*. $P_{\text{Nmax}}$ was ~110% pretreatment rates 1 h after desiccation to 80% RWC. Similarly, $P_{\text{Nmax}}$ was ~125% 1050 mOsm rates when tested at 2150 mOsm. The capacity to maintain or enhance photosynthetic rates over a range of desiccation states may contribute significantly to an intertidal alga's carbon budget (Matta & Chapman 1995) and may prove to be essential in determining ecological dominance at high tidal elevations.

*Ahnfeltiopsis concinna*’s capacity to precisely acclimate to changes in UV and PAR (Parts III-V), temperature and osmotic stresses reveals three important aspects of macroalgal ecology. 1) Persistence at high tidal elevations requires a myriad of stress tolerance mechanisms. These mechanisms are manifest as: a) modifications in levels of photoprotective carotenoids, fluorescence from phycobilin antennae pigments, putative mycosporine amino acids (Parts III-V), b) the functional morphology of intact stands, c) capacity for State II and State III transitions (Fork & Satoh 1986), d) capacity to acclimate to emersion stresses allowing for augmented capacity for physiological recovery following an emersion event. This type of acclimation may include the induction and synthesis of desiccation and heat shock-like proteins (Ahmad & Hellebust 1993, Close & Lammers 264
1993), changes in the fatty acid composition (Sukenik et al. 1993), and possibly the synthesis and regulation anti-oxidant levels (Demming-Adams 1990, Van Camp et al. 1994).

The second concept emerging in algal physiological ecology is that adaptations by macroalgae to perennial existence at high tidal elevations are diverse. *Mastocarpus pappilatus* and *A. concinna* both increase carotenoid concentrations substantially with increased elevation while *P. perforata* does so to much less of a degree (Smith & Alberte 1994). *Porphyra perforata* is the only known primary producer to undergo State III transition (Fork & Satoh 1986). Enhanced temperature tolerance with desiccation is not a universal trait. Whereas temperature tolerance in temperate macroalgae is best correlated with biogeographic distribution (Smith & Berry 1986), changes in temperature tolerance in tropical macroalgae is best correlated with elevation in the intertidal zone (Pakker et al. 1995). Clearly, without the examination of numerous model systems from various latitudes and tidal elevations, we will be left with a narrow perspective as how macroalgae thrive at high tidal elevations. Only with a comprehensive examination can we begin to gain an understanding of the physiological and biochemical diversity present in this group of highly stress tolerant primary producers.

Lastly, the primary scale of acclimation in temperate to tropical areas changes from vertical position (m) to intra-thallus position (em), respectively. In general, temperate coastal zone have larger tidal amplitudes than areas at lower latitudes. Additionally, irradiance (both UV and PAR) levels increase with decreases in latitude. Broad gently sloping intertidal benches, characteristic of the Hawaiian Islands, expose macroalgae to these irradiances and tropical temperatures with the ebb of the tide. Although, this exposure is brief compared to some temperate high intertidal elevations irradiance and temperature are considerably elevated at tropical latitudes. This dichotomy in physical environments has led to similar conspicuous adaptations to emersion and irradiance stresses.
over two different scales. The capacity to recover photosynthetic activity in temperate phaeophytes was best correlated with tidal elevation (Dring & Brown 1982) whereas for _A. concinna_, similar changes took place over the <10 cm axis. Photoacclimation to tidal gradients undergoes similar changes in scale (Smith and Alberte 1994, Part V). (Note: The author's belief that acclimations and adaptations to stresses associated with the intertidal zone occur over both intertidal and intra-thallus scales in both temperate and tropical coastal zones but the ones being discussed here are the most conspicuous gradients in each environment.)

**Conclusions**

Perennial dominance at high tidal elevations is facilitated by the functional morphology of the algal turf _Ahnfeltiopsis concinna_. Canopy tissues, although chronically photoinhibited, exhibit the capacity to more rapidly recover photosynthetic activity from emersion stresses than understory tissues from the same individual. Multivariate analysis of the impact of emersion stresses revealed the synergistic effects of irradiance and temperature as well as microsite and irradiance on the recovery of physiological activity. Univariate analysis of emersion stresses cannot reveal these interactions and since these stresses occur in tandem, multivariate experiments more realistically simulate emersion events. Potential artifacts of osmotic desiccation were revealed by analysis of distinct physiological processes (R_d, P^n max, P^g max ) and contrasting these trends with the results of air desiccation.

Perennial dominance at high tidal elevations in both temperate and tropical coastal zones requires adaptations to the emersion stresses of temperature, irradiance and desiccation. In general, the gradients over which these stresses vary are distinct between these latitudes. Acclimation to emersion stresses are most pronounced when contrasting high vs. low elevation populations separated by meters in temperate regions while similar stress gradients and adaptations occur over the extent of an individual thallus in tropical
algal turfs. Although there are inherent similarities in environmental gradients between these systems, the adaptations to combat emersion stresses are diverse and no one model system is like to have wholly encompassed this diversity.
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Table 8.1. Test of individual effects and their interactions on the percent recovery of dark Respiration to pre-treatment rates (100% = equivalent to pre-treatment rate) in *A. concinna.*

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272
Table 8.2. Test of individual effects and their interactions on the percent recovery of $P^n_{\text{max}}$ to pre-treatment rates (100% = equivalent to pre-treatment rate) in *A. concinna*. *:* significantly different.

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Table 8.3. Test of individual effects and their interactions on the percent recovery of P:R ratio to pre-treatment rates (100% = equivalent to pre-treatment rate) in *A. concinna*. *: significantly different

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Table 8.4. Test of individual effects and their interactions on the percent recovery of $P_{\text{Emax}}$ to pre-treatment rates (100% = equivalent to pre-treatment rate) in *A. concinna*. *: significantly different

<table>
<thead>
<tr>
<th>Source</th>
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<th>DF</th>
<th>Mean-Square</th>
<th>F-ratio</th>
<th>P</th>
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</tr>
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Figure 8.1. Percent change in $P_{n,\text{max}}$ to changes in osmolarity from 1050 mOsm for canopy (open circles) and understory (closed circles) tissues of *A. concinna*. $P_{n,\text{max}}$ at 1050 mOsm was designated as 100%. n=5.
Figure 8.2. Percent change in $R_d$ to changes in osmolarity from 1050 mOsm for canopy (open circles) and understory (closed circles) tissues of *A. concinna*. $R_d$ at 1050 mOsm was designated as 100% . n=8.
Figure 8.3. Percent change in P:R ratio to changes in osmolarity from 1050 mOsm for canopy (open circles) and understory (closed circles) tissues of *A. concinna*. P:R ratio at 1050 mOsm was designated as 100%. n=5.
Figure 8.4. Percent change in $P_{n_{\text{max}}}$ to changes in temperature from 25°C for canopy (open circles) and understory (closed circles) tissues of *A. concinna*. $P_{n_{\text{max}}}$ at 25°C was designated as 100%. n=5.
Temperature (°C)

P_{max} (% of 25°C rate)
Figure 8.5. Percent change in Rd to changes in temperature from 25°C for canopy (open circles) and understory (closed circles) tissues of *A. concinna*. Rd at 25°C was designated as 100% . n=5.
Figure 8.6. Percent change in P:R ratio to changes in temperature from 25°C for canopy (open circles) and understory (closed circles) tissues of *A. concinna*. P:R ratio at 25°C was designated as 100%. n=5.
Figure 8.7. Percent recovery of $P_{\text{max}}^n$ in canopy (open circles) and understory (closed circles) tissues of *A. concinna* to pre-desiccation rate after air desiccation to measured relative water contents. Recovery rate measured after 1 h of recovery in 1050 mOsm FSW, at 25°C at 750 μmol quanta m$^{-2}$ s$^{-1}$. n=12.
Figure 8.8. Percent recovery of P:R ratio in canopy (open circles) and understory (closed circles) tissues of *A. concinna* to pre-desiccation rate after air desiccation to measured relative water contents. Recovery rate measured after 1 h of recovery in 1050 mOsm FSW, at 25°C at 750 μmol quanta m⁻² s⁻¹, n=5.
Figure 8.9. Percent recovery of $R_d$ in canopy (open circles) and understory (closed circles) tissues of *A. concinna* to pre-desiccation rate after air desiccation to measured relative water contents. Recovery rate measured after 1 h of recovery in 1050 mOsm FSW, at 25°C at 750 μmol quanta m$^{-2}$ s$^{-1}$. n=6.
PART IX

SYNTHESIS
The physiological challenges of the marine irradiance environment have resulted in a diversity of photoadaptive traits in macroalgae. The diversity of photosynthetic and photoprotective pigments in macroalgae and the pigment homogeneity of the chlorophyll \( a/b \) (Chl \( a/b \)) bearing Traecheophytes strongly suggests that irradiance is a powerful selective pressure in the marine environment. Maximum rates of photosynthesis (\( P_{\text{max}} \)) in marine algae and seagrasses are at best 1/3 the rate found in typical terrestrial C-3 plants. This perspective strongly suggests that there are selection pressures to maximize the number of hours per day that \( P_{\text{max}} \) is achieved rather than high \( P_{\text{max}} \) rates. Variability of irradiance associated with coastal zones may provide this selection pressure. Tropical latitudes present an extreme in irradiance levels of the form of photosynthetically active (PAR) and ultra-violet radiation (UV). Because photosynthesis for tropical macroalgae is typically saturated at irradiance levels <15% full sun, photoadaptive responses by these macroalgae may be essential for their ecological success.

As judged by in vivo absorption and fourth derivative spectra, pigment identities in tropical macroalgae reflect the conserved nature of algal pigments within algal divisions and classes. In a survey of eleven species of tropical macroalgae, a marked consistency in absorbance over the visible range 400 to 750 nm was observed among species within an evolutionary lineage.

The Chl \( a \) and \( b \) bearing chlorophytes fell into two in vivo absorption spectra types. The spectra either had a pronounced absorbance shoulder from 500 to 600 nm as in Caulerpa racemosa and Dictyosphaeria versluysii or had a sharp decrease in absorbance from 500 to 520 nm as in Chaetomorpha antennina and Ulva fasciata. These two spectral types were additionally resolved by fourth-derivative spectral analyses. The derivative maxima from 500 to 520 nm in \( C. \) racemosa and the maxima at 508 and 550.5 nm in \( D. \) versluysii were attributed to siphonein and/or siphonoxanthin, known components in the
Caulerpales and Siphonoclad-group of the Cladophorales. No such fourth derivative maxima were detected in \textit{U. fasciata} or \textit{C. antennina}.

Phaeophytes demonstrated similar abilities to absorb irradiance via Chl $a/c$ and fucoxanthin light harvesting pigment protein complex of this division. Chl $a$ was visualized by distinct absorbance maxima at $-675$, and $-439$ nm in \textit{vivo}, and at $-684$, $-440$, and $-414.5$ nm in the fourth-derivative spectra analyses. \textit{In vivo} absorption spectra of \textit{Hinksia breviiarticulata}, \textit{Padina japonica} and \textit{Sargassum polyphyllum} resolved two maxima attributable to Chl $c$ at $-633.5$ and $462$ nm while \textit{Chnoospora minima} had only one discernible Chl $c$ maximum at $634.5$ nm. The fourth derivative analyses resolved two Chl $c$ specific maxima at $-637$ and $-466$ nm in all four species examined. \textit{In vivo} absorbance maxima at $-493$ and $-587$ nm were primarily contributed by fucoxanthin. Fourth-derivative maxima between $490-590$ nm were attributed principally to fucoxanthin which is known to have three minor maxima.

Chl $a$/phycobilin bearing rhodophytes had distinct spectral signatures ascribable to this division. \textit{Acanthophora spicifera}, \textit{Hypnea musciformis}, and \textit{Melanomansia glomerata} exhibited two \textit{in vivo} absorbance maxima were ascribed to Chl $a$ at $-678$ and $437$ nm while fourth-derivative resolved similar Chl $a$ maxima at $687$ and $442$ nm. \textit{In vivo} absorbance maxima of $-539$ and $567$ nm were attributed solely to R-phycoerythrin (R-PE) while a $-494$ nm maximum was contributed to by both R-PE and minor carotenoids. The fourth-derivative analyses resolved \textit{in vivo} absorbance maximum at $-494$ nm into both a $-465.5$ nm carotenoid maximum and $-500$ nm R-PE maximum. Absorbance maxima between $-625$ and $-633$ nm were ascribed to R-phycocyanin (R-PC) in the \textit{in vivo} absorbance and fourth-derivative spectral analyses, respectively. The $-595.5$ nm maximum had two potential accessory pigment components: R-PC and allophycocyanin (APC), while the $-617$ nm maximum was ascribed to R-PC.
In vivo absorption spectra from 280 to 400 nm documented substantial UV-absorbance by macroalgae for the first time. In vivo and extract absorbance characteristics from 280 to 400 nm indicated that concentrations of UV-absorbing compounds (UVACs) were higher among rhodophytes when compared to other algae. Fourth-derivative spectral analyses resolved a consistent number of maxima from 280 to 400 nm but at variable positions, suggesting heterogeneity in the type and/or pools sizes of UVACs. Potentially photodestructive, UV-irradiance may be pre-emptively captured by UVACs as indicated by the relationship between in vivo and extract characteristics. In vivo absorbance characteristics provide a quick and accurate gauge to the UV-shielding capacity of primary producers.

Even with the marked consistency of pigment identities with in algal divisions and classes, pigment concentrations and photosynthetic performance of tropical macroalgae are adjusted over very precise scales in response to irradiance stresses. Specific inter- and intra-life cycle phase modifications of photosynthetic and stress tolerance characteristics appears to contribute to the ecological success of both pioneer and perennial intertidal macroalgae.

Ulva fasciata and Enteromorpha flexuosa are pioneer components of Hawaiian intertidal communities. In these species, the rates of photosynthesis for newly released reproductive unicells can be equivalent to that of the vegetative adult on a chlorophyll basis. Photosynthetic characteristics of zoospores and gametes of U. fasciata clearly indicate that this species planktonic phases are low-light adapted compared to those of E. flexuosa. Concomitant with this performance difference is a pigment differentiation that takes place with reproductive unicell formation in U. fasciata that does not occur in E. flexuosa. Zoospore and gamete producing tissues of U. fasciata absorb a greater quantity of irradiance from 500 to 550 nm than does vegetative or E. flexuosa reproductive tissues. Fourth derivative analysis of in vivo absorbance spectra further indicate either the formation
of a novel carotenoid or alteration the ratio of pre-existing carotenoid assemblage in the reproductive tissues compared to vegetative tissues of *U. fasciata*. Contrasting the photosynthetic characteristics of the reproductive unicells of pioneer versus late successional macroalgae strongly suggests that adaptation to high irradiance conditions allows reproductive unicells of pioneers to remain in the plankton for extended periods. This may increase the overall likelihood that a pioneer unicell will colonize opened substrate given that their overall representation in the plankton pool will be greater and colonization is a lottery process.

In addition to photoadaptations in likely response to differential irradiance stresses characteristic of distinct niches, tropical perennials must endure changes in irradiance environment that result from self-shading, tidal and seasonal fluxes. *Ahnfeltiopsis concinna* is the highest elevation perennial macroalga on pre-historic basalt benches in the high Hawaiian islands while *Laurencia mcdermidiae* grows year round at a lower tidal elevation as a part of a mixed turf assemblage. Each turf species exhibits a pronounced differentiation in pigmentation between canopy and understory tissues separated by <10 cm. Deeply shaded understory tissues of both species are phycobilin-rich and appear to the unaided eye red to black in pigmentation. Canopy tissues are exposed to high irradiance levels. Pigment acclimation differs between canopy tissues of *A. concinna* and *L. mcdermidiae*, although both species decrease phycobilin concentration and increase levels of carotenoids and putative mycosporine amino acids with increased irradiance. The specific phycobilins that are adjusted differ. *Ahnfeltiopsis concinna* maintains a similar PE:PC ratio in both canopy and understory tissues by decreasing PE and PC in canopy tissues in concert. *Laurencia mcdermidiae* only alters levels of PE in response to changes in irradiance.

*Ahnfeltiopsis concinna* precisely acclimates its photosynthetic accessory and photoprotective pigmentation in response to levels of PAR but not to distinct irradiance
wavelengths of UV-B (280 to 320 nm) or UV-A (320 to 400 nm) compared to PAR. Specifically, production putative-mycosporine amino acids, which are currently thought of as UV-photoprotective compounds, was not stimulated by ultraviolet radiation but by increased levels of photosynthetically active radiation. Responses of putative-mycosporine amino acids (MAAs) absorbance maxima were similar to changes in carotenoid specific absorbance, indicating a potential for a common signal induction mechanism for both responses. Phycobilin constituents of *A. concinna* were demonstrated to be precisely modified in response to integrated daily irradiance. This capacity was manifest as a change in *in vivo* absorbance characteristics delayed no more than a 48 h from increases or decreases of irradiance.

Concomitant with photoacclimation changes in pigmentation at a microscale in *A. concinna* and *L. mcdermidiae*, were adjustments in photosynthetic performance. Both *Laurencia mcdermidiae* and *A. concinna* demonstrated a typical sun- to shade-type acclimation response but at an unprecedentedly responsive scale. Canopy tissues of *L. mcdermidiae* had elevated rates of respiration and photosynthesis coupled with increased saturation and compensation irradiances compared to understory tissues. Similar changes were observed from understory to canopy tissues in *A. concinna* but understory tissues had higher rates of maximum photosynthesis than canopy tissues on a chlorophyll basis. This evidence along with elevated fluorescence emission from PE and PC compared to chlorophyll indicated a stress induced uncoupling of the phycobilisome components. Maximum rates of photosynthesis for any tissues of *A. concinna* observed in tissues from a stress sheltered microsite, further indicated that canopy tissues of *A. concinna* are chronically photoinhibited.

The distinct shift in acclimation state from sun to shade coupled with the stress tolerant morphology of turfs allows these species to inhabit high intertidal regions (*A. concinna*) and to compete with a up to 30 other species of turfs (*L. mcdermidiae*) even
though stress levels of tropical tidal environment are rigorous and space is limiting. The extent of intra-thallus photoacclimation in tropical turfs is comparable to macroscale (meters) changes in temperate marine plants over their vertical distribution and in terrestrial plant forest canopies and understories. Laurencia mcdermidiae increased $P_{\text{max}}$ 152% (Chl $a$ basis) from understory to canopy tissues. In the seagrass Zostera marina, a 47% increase in $P_{\text{max}}$ (Chl $a$ basis) from deep (~375 μmol quanta m$^{-2}$ s$^{-1}$, peak photosynthetic photon flux density (PPFD)) to shallow subtidal (~825 μmol quanta m$^{-2}$ s$^{-1}$, peak PPFD) sites was observed. Upper intertidal populations of Colpomenia peregrina (Phaeophyta) had a 166 and 134% increase in daily $P_{\text{max}}$ compared to lower elevation populations during summer and winter, respectively. Contrary to this, A. concinna had a 15% reduction in $P_{\text{max}}$ (Chl $a$ basis) in canopy relative to understory tissues. In Porphyra perforata, a similar reduction (44%) in $P_{\text{max}}$ (surface area basis) was observed between high vs. low intertidal populations. This similarity is not unexpected as both occupy the most elevated (above MLLW) niche in their respective localities and at this elevation both experience a severe combination of stresses caused by prolonged emersion. $P_{\text{max}}$ in marine macrophytes increases with increased PPFD caused by higher vertical position in the water column or decreased intra- or inter-individual shading. With further increases in exposure to emersion related stresses, decreases in $P_{\text{max}}$ are observed.

Terrestrial forests present similar changes in PPFD as are observed seen in contrasting rhodophyte turf microsites. Forest understories may receive only 0.5-2% of incident canopy PPFD compared to <0.3-10% in our model rhodophyte turfs. In general, terrestrial forest canopies have saturation irradiances 100-1000% higher than shade-tolerant understory plants. Canopy tissues of L. mcdermidiae and A. concinna exhibit higher values of saturation irradiance ($I_k$), 108% and 173%, respectively, relative to understory tissues. The above comparisons emphasize the responsiveness of A. concinna and L. mcdermidiae to alterations in PPFD. These diminutive turfs undergo similar a similar range
photoacclimative changes as are seen over much greater scales in other primary producers systems. Physiological adjustments at this fine scale appear unparalleled in eukaryotic systems and may represent an adaptation to intertidal tropical PPFD.

In addition to only irradiance stress, A. concinna's vertical position exposes this alga to extreme combinations of emersion related stresses including irradiance (280-800 nm), temperature (thallus temperatures up to 35°C), and desiccation (relative water content as low as 18%). Unlike other intertidal macroalgae in which the impacts of emersion stresses have been examined, A. concinna does not exhibit increased temperature tolerance with desiccation. Temperature and osmolarity response curves both demonstrate a broad range of tolerance, indicative of adaptation to high tidal elevations. Although canopy tissues of A. concinna are chronically photoinhibited, they have the physiological capacity to recover photosynthetic activity from emersion stresses more efficiently than do understory tissues from the same thallus. Microscale variability in stress tolerance capacity of the tropical turfs A. concinna parallels large scale variation in the temperate rhodophyte P. perforata.

The scale of acclimation adjustments in pigmentation, photosynthetic performance and emersion stress tolerance in tropical macroalgae strongly suggests that the primary scale of acclimation in temperate to tropical areas changes from vertical position (m) to intra-thallus position (cm), respectively. In general, temperate coastal zone have larger tidal amplitudes than areas at lower latitudes. Additionally, irradiance (both UV and PAR) levels increase with decreases in latitude. Broad gently sloping intertidal benches, characteristic of the Hawaiian Islands, expose macroalgae to these irradiances and tropical temperatures with the ebb of the tide. Although, this exposure is brief compared to some temperate high intertidal elevations irradiance and temperature are considerably raised at tropical latitudes. This dichotomy in physical environments has led to similar conspicuous adaptations to emersion and irradiance stresses over two different scales.
The adaptations to the stresses indicative of tropical marine habitats whether in the form of inter-life cycle phase photoadaptations or the capacity to precisely acclimate aids in the ecological success of ruderal and stress tolerant macroalgae, alike. Intertidal and planktonic niches have distinct physiological demands that select for particular traits to maximize overall success. Photoadaptations of Ahnfeltiopsis concinna, Laurencia medermidae, Ulva fasciata, and Enteromorpha flexuosa represent a small portion of the possible stress tolerance mechanisms selected for by irradiance stresses at tropical latitudes. Clearly, without the examination of numerous model systems from various latitudes and tidal elevations we will be left with a narrow perspective as how macroalgae thrive at different tidal elevations. Only with a comprehensive examination can we begin to gain an understanding of the physiological and biochemical diversity present in this important group of coastal primary producers.