ENHANCED PHOTOPROTECTION PATHWAYS IN SYMBIOTIC DINOFLAGELLATES
OF SHALLOW-WATER CORALS AND OTHER CNIDARIANS

by

JENNIFER CARRYE MCCABE REYNOLDS

(Under the direction of Gregory W. Schmidt and William K. Fitt)

ABSTRACT

Endosymbiotic dinoflagellates of the genus *Symbiodinium* provide their coral hosts up to 100% of their energy requirements for reef building. *Symbiodinium* is a symbiont common to many cnidarians including corals, jellyfish, and anemones, as well as mollusk species including giant clams. Here, we provide evidence for the first time using the novel serial irradiation pulse (SIP) chlorophyll fluorescence technique that most members of clade A *Symbiodinium*, but not clades B–D or F, exhibit enhanced capabilities for alternative photosynthetic electron transport pathways including cyclic electron transport.

Unlike other clades, clade A *Symbiodinium* engage cyclic electron transport and regularly undergo pronounced light-induced dissociation of antenna complexes from photosystem II (PSII) reaction centers. These photoprotections promote the survival of cnidarians with clade A *Symbiodinium* at high irradiance intensities and warm ocean temperatures, conditions that commonly cause coral bleaching. Laboratory experiments using cultured *Symbiodinium* investigated the effects of long-term warm water temperatures on the ability to utilize the photoprotections unique to clade A
Symbiodinium. Clade A Symbiodinium yielded the lowest amounts of the integral D1 protein, a common site of photosynthetic damage, but showed recovery when temperatures were reduced. In addition, field studies were conducted to determine the distinct physiological responses of Symbiodinium to long-term, seasonal ocean temperature changes. During all field seasons and in each colony harboring clade A Symbiodinium, cyclic electron transport was actively engaged. As a result, these corals maintained the highest maximum quantum yield and chlorophyll-a densities even during high irradiance and warm ocean temperatures.

Cyclic electron transport and light harvest complex dissociation are unique photoprotections that confer resistance to bleaching conditions that conspicuously impacted corals in symbiosis with non-clade A Symbiodinium. Such photoprotections are now easily identified through the use of the non-invasive SIP chlorophyll fluorescence technique. The results presented here show that clade A Symbiodinium may be better suited to withstand high irradiance and warm ocean temperatures, environmental conditions that may enhance coral bleaching.

Index words: coral, Symbiodinium, zooxanthellae, dinoflagellate, photosynthesis, photoprotection, photoinhibition, light-harvesting complexes, cyclic electron transport
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DEDICATION

To Ami and Poppy –

Thank you for being the best grandparents anyone could ever have. My first memories of the ocean are with you. Thank you for always supporting me during my initial career explorations. In our travels together, you provided me some of the most formative and meaningful experiences of my life. The time we spent in the Bahamas, Florida Keys, Australia, and Belize allowed me to develop a curiosity about and great respect for marine life that led me to a Ph.D. focused on coral physiology. Thank you for being my inspiration and for always supporting me.
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CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

Coral reefs thrive in tropical nearshore oceans due to an ancient endosymbiosis between corals and photosynthetic dinoflagellates of the genus *Symbiodinium* (Freudenthal). *Symbiodinium* as a genus was originally described in the early 1960s (Freudenthal, 1962), but the dinoflagellates have existed in symbiosis with corals since the Triassic period, approximately 250 million years ago (Falkowski et al., 2004, Trench, 1993). *Symbiodinium*, commonly called zooxanthellae, are of vital importance to corals because they provide corals at least 90% and up to 100% of the essential photosynthates necessary for survival and reef-building (Muscatine et al., 1981, Porter et al., 1984) in the oligotrophic marine environments where they live. *Symbiodinium* is a diverse group of organisms that live in symbiosis not only with corals, but with other marine invertebrates including jellyfish, anemones, and giant clams (Freudenthal, 1962, LaJeunesse, 2001, Rowan, 1998, Trench, 1993).

Threats to the delicate symbiosis between corals and zooxanthellae are becoming more common and severe due to the influences of global warming. The more frequent occurrence of prolonged time periods of warm ocean temperatures with the synergistic effects of high irradiance often result in a phenomenon called coral bleaching (Douglas, 2003, Fitt et al., 2008, Fitt et al., 2000, Fitt et al., 1993, Glynn, 1996, Hoegh-Guldberg, 2004, Hoegh-Guldberg & Smith, 1989, Lesser, 2004, Manzello et al., 2009, Warner et al., 2006). Coral bleaching was first described in the early 1900s
and the documented cause was warm ocean temperatures (Edmondson, 1928, Mayer, 1914, Yonge & Nichols, 1931). Further research has shown that coral bleaching occurs when there is loss of zooxanthellae, loss of their photosynthetic pigments or a combination thereof which results in the pale or white color of coral tissue (Fitt et al., 2001, Hoegh-Guldberg & Smith, 1989). In 1997-1998, the National Oceanic and Atmospheric Administration reported that the most severe bleaching episode in modern history occurred (Aronson et al., 2002, Fitt et al., 2001, Loch et al., 2002, Lough, 2000). In addition, more recent global warming models predict increasing ocean temperatures (IPCC, 2007), which will further threaten corals worldwide.

Fitt et al. (2001) described three main categories of coral bleaching – 'physiological bleaching' or bleaching resulting from decreased coral tissue, the area in which zooxanthellae live, 'algal-stress bleaching' usually resulting from photoinhibition of zooxanthellae photosynthesis, and the more rare 'animal-stress bleaching' which occurs by the loss of zooxanthellae with the shedding of host cells. In all cases of severe coral bleaching, the photosynthetic function of the zooxanthellae and subsequent transfer of photosynthates to the host is compromised.

The survival of coral reefs and their surrounding communities is dependent upon the photosynthetic function of *Symbiodinium*. The ability to maintain photosynthesis through the use of acclimations or adaptations to environmental change is necessary for survival of the symbiont, host, and symbiosis. Mechanisms for protecting photosynthesis exist and the use thereof is driven by host and respective symbiont biogeography and ecology. As *Symbiodinium* species-level phylogenetics have developed (LaJeunesse, 2001, LaJeunesse et al., 2003, Santos et al., 2004), it has
become possible to explore the unique physiological characteristics within clades and between individual isolates to identify adaptations or the ability to acclimate to global warming conditions.

The relationship between photosynthesis and coral bleaching

All life on earth ultimately depends on solar radiation that primary producers convert, using photosynthesis, into usable forms of energy. Solar energy drives carbohydrate and oxygen production from carbon dioxide and water. Pigments, including chlorophylls, are necessary to absorb light energy. The light reactions of photosynthesis, which harness energy from the sun, take place in the thylakoid membranes of chloroplasts while the carbon fixation reactions occur in the stroma, the aqueous region surrounding the thylakoid membranes. Within the chloroplast, there are two photosystems, photosystem II (PSII) and photosystem I (PSI) which work together to absorb and convert light energy into chemical energy. Light energy is used to move electrons through the electron transport chain between PSII and PSI and to reduce \( \text{NAD(P)}^+ \) to \( \text{NAD(P)}\)H and oxidize \( \text{H}_2\text{O} \) to \( \text{O}_2 \). ATP is produced during this process from the proton gradient around the thylakoid membrane resulting from electron transport activity. The products of the light reactions are passed on to the Calvin-Benson cycle and ultimately produce carbohydrates and oxygen, products that are necessary for coral and all other organisms’ survival (Buchanan et al., 2000).

The well-tuned and complex model of photosynthesis has many points where processes and pathways can breakdown, resulting in damage to the integral photosynthetic complex if stressful environmental conditions are present.
Photoinhibition results when there is loss of photosynthetic capacity from conditions such as high temperatures, ultraviolet radiation, and high irradiance (Gorbunov et al., 2001). Such stressors can produce damaging reactive oxygen molecules cause oxidative damage to the photosynthetic machinery (Lesser, 1996, Nishiyama et al., 2006). Oxidative damage is generated at three locations in the light reactions of the photosynthetic chain – the light harvesting complex of PSII, the reaction center associated with PSII, and the acceptor side of PSI (Niyogi, 1999). All photosynthetic organisms have adaptations to protect their photosynthetic complex from photoinhibition. Sometimes, however, chronic, or irreversible, photoinhibition occurs and that results in the death of the photosynthetic organism. When this happens to Symbiodinium, a breakdown of the coral-dinoflagellate symbiosis can occur with coral bleaching as the result (Douglas, 2003, Fitt et al., 2000).

**Symbiodinium Diversity**

Symbiotic dinoflagellates of the genus Symbiodinium, the most common genus of endosymbionts found in reef-dwelling cnidarians and some mollusks (Rowan, 1998, Trench, 1993) were initially thought of as a single pandemic species (Blank & Trench, 1985). Further analyses revealed multiple species such as Symbiodinium microadriaticum (Blank, 1986, Blank & Huss, 1989, Freudenthal, 1962, Trench & Blank, 1987) and high diversity within cultured Symbiodinium (Schoenberg and Trench 1980 a,b; Trench 1993). Rowan and Powers used restriction fragment length polymorphism (RFLP) of DNA to reveal three major clades – A, B, and C (Rowan & Powers, 1991). Genetic techniques continued to develop with polymerase chain reaction denaturing
gradient gel electrophoresis (PCR-DGGE) first using internal transcribed spacer (ITS) analysis of the rDNA regions of the *Symbiodinium* genome to distinguish between clades A-H (Coffroth & Santos, 2005, LaJeunesse, 2001, LaJeunesse et al., 2003, LaJeunesse & Trench, 2000). Using the ITS technique, ecological zonation patterns of *Symbiodinium* diversity have been documented and biogeographic patterns established (Iglesias-Prieto et al., 2004, LaJeunesse, 2002, LaJeunesse et al., 2003, LaJeunesse et al., 2004). Such studies have been guided by the foundation that diversity of *Symbiodinium* is not random (Iglesias-Prieto & Trench, 1997), but it is driven by host specificity (LaJeunesse, 2002, Thornhill et al., 2005).

Physiological characteristics of *Symbiodinium* are dependent upon host biogeography and ecology. The light regime on coral reefs is fairly stable (Brown & Dunne, 2008, Iglesias-Prieto & Trench, 1994, Iglesias-Prieto & Trench, 1997), and ecological distribution of corals and their symbionts are correlated (Brown et al., 2002, Chang et al., 1983). Clades and specific isolates show differential tolerances to environmental conditions including light intensities (Enriquez et al., 2005, Hennige et al., 2009, Iglesias-Prieto & Trench, 1994, Iglesias-Prieto & Trench, 1997, Trench, 1993) and temperature (Bhagooli & Yakovleva, 2004, Bruns et al., 2004, Dove et al., 2006, Tchernov et al., 2004, Warner et al., 1996, Warner et al., 1999). Shallow-water *Symbiodinium* have been documented as bleaching resistant while deeper water species are more sensitive to the conditions that cause bleaching (Warner et al., 2002, Warner et al., 2006). Iglesias-Prieto and Trench (1997) proposed that photosynthetic adaptation is the most effective driver of niche diversification.
Photosynthetic Damage in *Symbiodinium*

Within groups of related species comes variation in their adaptation to environmental conditions (Darwin, 1859). *Symbiodinium* is a diverse group of dinoflagellates that possess a variety of photosynthetic acclimation and adaptation abilities directly linked to their ecological placement (Iglesias-Prieto et al., 2004, LaJeunesse, 2002) and that respond differently to changing environments (Brown & Dunne, 2008). Damage from stressful environmental conditions can target numerous locations within the complex series of biochemical photosynthetic pathways and ultimately cause photoinhibition (Lesser, 2004). Photoinhibition is the loss of photosynthesis due to factors that eliminate the ability of the organism to produce and use photochemical energy (Franklin & Forster, 1997, Niyogi, 1999, Osmond & Forster, 2006). The following sections highlight some environmental causes of photosynthetic damage in *Symbiodinium*.

**Prolonged high temperature combined with increased irradiances**

Sunlight and photosynthetic organisms have a delicate relationship. Photon energy is needed to drive photosynthesis, but too much light can have deadly affects. It has been a goal of cnidarian researchers to locate the points of damage in the photosynthetic complex of *Symbiodinium*. When sea surface temperatures increase just 2 to 3°C more than the normal summer averages, if warm summer water temperatures extend for an unusually long period of time, and/or if high irradiance conditions are present, corals may exhibit zooxanthellae loss (Coles & Brown, 2003, Fitt, 2000, Hoegh-Guldberg & Smith, 1989, Jokiel & Coles, 1990, Lesser, 1997).
extent of bleaching is related to the severity of the temperature and irradiance increase (Lesser, 2004) and correlated with the ability of the organism to rebuild and repair damaged photosynthetic machinery.

Photosynthetic damage often occurs from the production of reactive oxygen species (ROS) such as superoxide \( (O_2^-) \) and singlet oxygen \( (1^O_2) \) due to the high photosynthetic rates and oxygen production by *Symbiodinium* (Lesser, 2006). ROS affect *Symbiodinium* and their hosts and both organisms have mechanisms which minimize potential damage including the enzymes superoxide dismutase and catalase (Weis, 2008). ROS causes dysfunction of photosynthetic membranes, proteins, and nucleic acids (Lesser, 2006) and can inhibit necessary repair (Nishiyama et al., 2006).

The integral PSII protein, D1, is commonly the major site of damage in photosynthesis. Warner et al. (1999) discovered that the D1 protein within the PSII reaction center of the *Symbiodinium* is damaged irreversibly as a result of prolonged temperature and high irradiance. Individual species and phylotypes of *Symbiodinium* show differential responses to such conditions depending upon ecological placement (Iglesias-Prieto et al., 2004, Robison & Warner, 2006, Warner et al., 1999). Under less stressful environmental conditions, the photosynthetic complex is able to turnover more D1 protein preventing photoinhibition. More recently, it has been shown that there is variation in the temperature tolerance among phylotypes and clades of *Symbiodinium* (Tchernov et al., 2004). Tchernov et al. (2004) described isolate-specific sensitivity to warm temperatures as related to the lipid composition in the thylakoid membranes. Warm temperatures also differentially affect the ability of the organism to repair integral
proteins and can inhibit the de novo synthesis of such proteins resulting in chronic photoinhibition (Takahashi et al., 2009b).

**High temperatures and ultraviolet radiation**

Ultraviolet radiation (UVR - 290-400 nm) detrimentally effects photosynthesis and thus the growth of corals (Shick et al., 1996). Fortunately, *Symbiodinium* is able to produce UVR-absorbing compounds, mycosporine-like amino acids (MAAs) that act as sunscreens protecting the symbiont and host from ultraviolet, high energy, wavelengths of light (Banaszak et al., 2000, Banaszak et al., 2006, Brown et al., 1999). The production of MAAs decreases with depth (Banaszak et al., 1998, Lesser et al., 2000) as shallow-water species need more direct protection from UVR. However, UVR in combination with high sea surface temperatures inhibits production of MAAs (Lesser & Farrell, 2004, Shick & Dunlap, 2002), making the zooxanthellae-coral symbiosis more vulnerable to photosynthetic damage.

**Photoprotection in Symbiodinium**

All photosynthetic organisms must protect themselves from net damage to their photosynthetic apparatus. They accomplish photoprotection in many ways from moving their leaves or chlorophylls, adjusting their chlorophyll antennae size, using non-photochemical quenching or thermal dissipation, through photochemical processes, production of oxidizing molecules and antioxidant systems, to degradation, repair and synthesis of new proteins (Lesser, 2006, Niyogi, 1999, Tchernov et al., 2004, Warner et al., 1999). Initial research on zooxanthellae photosynthetic protection showed that coral
tissues can withstand high temperatures more successfully if they are in low light conditions (Coles & Jokiel, 1978, Jokiel & Coles, 1977), and they can adapt to changing conditions (Dubinsky et al., 1984, Porter et al., 1984).

Photosynthetic adaptation capabilities of *Symbiodinium* are at least partially determined by host ecology (Chang et al., 1983, Iglesias-Prieto et al., 2004). Once culturing methods for *Symbiodinium* were developed, it was discovered that zooxanthellae from different hosts employed varying methods for photoprotection, such as changing the number or size of photosynthetic unit (Falkowski & Dubinsky, 1981, Iglesias-Prieto & Trench, 1994), changing carbon dioxide fixation enzymes (Chang et al., 1983, Chang & Trench, 1984, Leggat et al., 2004), or via the electron transport system (Iglesias-Prieto & Trench, 1997). Iglesias-Prieto and Trench (1994, 1997) first showed adaptation of 3 species of *Symbiodinium* to different irradiances. They found that *S. microadriaticum*, found in symbiosis with *Cassiopeia xamachana*, has the greatest ability for acclimation, most likely a result of the constantly changing light conditions as the jellyfish moves through the water column. More recently, it has been determined that *S. microadriaticum*, a clade A *Symbiodinium*, utilizes the alternative photosynthetic pathway of cyclic electron transport (CET) in addition to engaging peridinin-chlorophyll a-protein complex (PCP)/light harvesting dissociation (Reynolds et al., 2008) as well as producing MAAs (Banaszak et al., 2000, Banaszak et al., 2006) to protect their photosynthetic capacity. Examination these and other mechanisms by which *Symbiodinium* protect themselves from excess light energy and possibly warm ocean temperatures is critical.
Nonphotochemical Quenching

Protection of the photosynthetic apparatus is ultimately beneficial, but it also comes with costs. Photoprotections minimize oxidative damage to the photosynthetic apparatus, but the resulting maximum quantum yield of photochemistry also decreases (Gorbunov et al., 2001, Long et al., 1994). As photochemistry is the amount of actual production from photosynthesis, nonphotochemical processes protect the photosynthetic apparatus from damage (Demmig-Adams & Adams III, 2006). In *Symbiodinium* and other dinoflagellates there are multiple types of nonphotochemical quenching (NPQ) (Gorbunov et al., 2001). Thermal dissipation, which is commonly referred to as NPQ of chlorophyll fluorescence of PSII reaction centers, leads to the down-regulation of photochemistry so the reaction centers slow or stop operating but are not damaged (Krause & Weis, 1991, Weis & Berry, 1987). Some shallow water zooxanthellae dissipate up to 80% of the absorbed photon flux as heat, four to five times of the light energy that is absorbed (Gorbunov et al., 2001). Zooxanthellae show predictable diurnal cycles of changing the amount of thermal dissipation needed to protect their reaction centers while utilizing other photosynthetic protection mechanisms as they respond to fluctuating solar radiation throughout the day (Brown et al., 1999, Gorbunov et al., 2001, Levy et al., 2004).

Xanthophyll Cycling

The xanthophyll de-epoxidation cycle depends on light energy, but is still considered nonphotochemical quenching because excess absorbed energy is thermally dissipated from the conversion. Dinoflagellates contain chlorophyll-\(c_2\) and chlorophyll-\(a\)
and have a reversible xanthophyll cycle that converts diadinoxanthin to diatoxanthin (Brown et al., 1999), different carotenoids from those found in green algae and higher plants. Brown et al. (1999) found decreased photosynthetic efficiency at solar noon when the photosynthetic active radiation (PAR) is highest because of increased production of xanthophylls as a photoprotection. Xanthophyll de-epoxidation protects the PSII reaction center complexes in midday sun (Brown et al., 1999), especially in shallow water corals though it is not necessarily correlated to bleaching resistance (Venn et al., 2006) or host ecology (Warner & Berry-Lowe, 2006).

**Chlororespiration**

Chloroplast respiration, or chlororespiration, thought not well understood is defined as a nonphotochemical respiratory electron transport pathway which operates around PSI in the thylakoid membrane of chloroplasts and interacts and sometimes competes with the photosynthetic electron transport carriers (Peltier & Cournac, 2002). In addition to oxidation by the cytochrome $b_6/f$ complex, chlororespiration is another pathway which oxidizes plastoquinol and reduces plastoquinone in the dark via an oxidase (Bennoun, 2002). Chlororespiration can consume reactive oxygen species, preventing damage to the photosystem. The chlororespiratory model has lacked some fundamental molecular support until recently with the discovery of a plastid-encoded NAD(P)H-dehydrogenase (Ndh) complex and a nuclear-encoded plastid terminal oxidase (PTOX) (Joet et al., 2002, Rumeau et al., 2007). The Ndh complex is, in many systems, involved in nonphotochemical reduction of plastoquinones. The discovery of the Ndh and PTOX proteins added support to the chlororespiratory model as the former
has reducing ability and the latter may oxidize the reduced electron acceptors (Peltier & Cournac, 2002).

Much of the evidence for chlororespiration has been gathered using green algae (Bennoun, 2002, Peltier & Schmidt, 1991, Schmidt et al., 1977), though there have been discoveries of the Ndh and PTOX in higher plants (Joet et al., 2002). Chlororespiration is important to the functioning of photosynthesis in Arabidopsis as it oxidizes electron acceptors that drive linear and cyclic electron transport (Munekage et al., 2002, Rumeau et al., 2007). It also becomes more predominant under certain environmentally stressful conditions. In Chlamydomonas reinhardtii grown under nitrogen limiting conditions, the chlororespiratory pathway became more pronounced (Peltier & Schmidt, 1991). Under anaerobic conditions and continuous illumination, however, Symbiodinium show a substantial, though gradual, plastoquinone reduction most likely due to the activity of a chlororespiratory pathway (Reynolds et al., 2008).

State Transitions and Light Harvesting Complex Dissociation

State transitions allow photosynthetic organisms to adapt to changing light conditions through the reversible association of light harvesting complexes (LHCs) with PSII (state 1) or PSI (state 2). The LHCs migrate between PSII and PSI due to the phosphorylation of a protein kinase. State transitions have been documented in vascular plants, green and red microalgae, and cyanobacteria (Finazzi & Forti, 2004). In a state transition from state 1 to state 2, a portion of the excitation energy is diverted away from PSII and to PSI (Finazzi & Forti, 2004).
Two hypotheses attempt to explain the phenomenon of state transitions. Conformational changes within the protein during phosphorylation might trigger LHC migration (Nilsson et al., 1997) or increased negative charges in the thylakoid cause electrostatic repulsion and LHCII disassociation with PSII (Allen, 1992). In state 1, photosynthesis is oxygenic where there is reducing power and ATP is produced. State 2 is different in that it has a bacterial-type of photosynthesis where only ATP is produced. A rationale for the transition to state 2 is the need for protection of the photosynthetic unit as a result of environmental stress where there is an inability to assimilate CO₂ by the organism (Finazzi & Forti, 2004).

The phenomenon of state transitions allows the photosynthetic organism to survive changing light conditions, and it is not a uniform occurrence. For example, in the green alga *Chlamydomonas* as much as 85% of the LHCII may dissociate (Delsome et al. 1996) while 20-25% of LHCII may migrate in vascular plants (Allen 1992). Finazzi (2004) suggested that state transitions may be used differently in plants and green algae, whether to enhance PSI activity at the expense of PSII, as in *Chlamydomonas*, or to balance the use of both photosystems, as in vascular plants.

True state transitions in dinoflagellates have never been documented previously. It has been suggested that *Symbiodinium* utilize state transitions as an important part of nonphotochemical quenching when exposed to warm temperatures and high irradiance (Jones & Hoegh-Guldberg, 2001), though sufficient data has not been presented. Different from green algae or vascular plants, dinoflagellates have unique light harvesting antennae comprised of the soluble peridinin-chlorophyll-a-protein complex (PCP) and membrane-bound chl-a-chl-c₂-peridinin-protein complexes (acPCP).
(Hennige et al., 2009, Iglesias-Prieto & Trench, 1997). These protein complexes readily
dissociate from intrinsic LHCs when exposed to high irradiance conditions but only in
shallow-water clade A Symbiodinium (Reynolds et al., 2008).

**Photochemical photoprotective processes**

There are a number of photoprotective processes that rely on photochemical energy. They are described in green algae and higher plants (Niyogi, 1999), but rarely in dinoflagellates, including Symbiodinium.

**Cyclic Electron Transport Pathway**

Cyclic photophosphorylation is a series of reduction-oxidation (redox) reactions that result in cyclic electron transport (CET) around PSI and ATP production (Munekage et al., 2002, Munekage et al., 2006). CET was first described in the 1950s by Daniel Arnon and his co-workers (Arnon, 1955). CET is an important photoprotective process which protects PSII from photodamage by creating an alternative route for electron flow around PSI that prevents photoinhibition (Bukhov & Carpentier, 2004, Heber & Walker, 1992).

Two types of CET have been described, one involving a ferredoxin-plastoquinone oxidoreductase (FQR) and the other employing an NADPH/NADH dehydrogenase (NDH) complex. Recently, Munekage et al. (2002) discovered that a mutation in the protein Proton Gradient Regulation 5 (PGR5) inhibited ferredoxin dependent cyclic electron flow. This redox poising of the electron transport chain in favor of a ferredoxin-dependent, or PGR5, cyclic electron flow most likely occurs under
conditions where electron acceptors, such as oxygen and carbon dioxide, are limiting (Munekage et al., 2002, Munekage et al., 2006).

In the more than 50 years since its discovery, documenting CET activity in vivo has been challenging. More research has taken place in recent years because of CET’s role in protection of the photosynthetic apparatus (Long et al., 2008, Munekage et al., 2004, Reynolds et al., 2008, Takahashi et al., 2009a). The cyclic pathway generates necessary ATP production in green algae, cyanobacteria, and possibly C4 plants because they are commonly exposed to conditions that favor the cyclic pathway, such as drought or CO₂ limitation. The significance of CET in C3 plants is still under investigation (Bendall & Manasse, 1995, Johnson, 2005), though the work of Munekage et al. (2002, 2004) shows that CET is essential for photoprotection in Arabadobsis.

CET has never before been described in dinoflagellates.

Different from CET, the non-cyclic photosynthetic phosphorylation pathway produces oxygen, oxidizes water and reduces an electron acceptor, usually NAD(P)⁺ (Allen, 2002). NAD(P)H continues to the Calvin-Benson cycle which consumes NAD(P)H and ATP while reducing of CO₂ to produce carbohydrates. The prevailing model of the linear electron transport pathway begins with photons activating the PSII chlorophyll reaction center (P680), exciting an electron which then travels along the electron transport pathway through the plastoquinone pool, the cytochrome b₆/f complex and plastocyanin, and then to the PSI reaction center (P700). Photosystem I then reduces NAD(P)⁺ to NAD(P)H via electron transfer by ferredoxin. Simultaneously, PSII oxidizes water to O₂ and releases H⁺ into the thylakoid lumen. NAD(P)H is then used in the Calvin-Benson or dark reactions of photosynthesis to fix carbon. Protons are also
released by the cytochrome \textit{b}$_6$/f into the lumen. The ATP synthase uses protons as it phosphorylates ADP to produce ATP and also releases protons back into the stroma (Buchanan et al., 2000).

The CET pathway differs from the linear pathway in that electrons are cycled around PSI, it does not require water oxidation or oxygen evolution, and it is commonly activated by far red light, a wavelength of light that is higher than what is required for the linear pathway (Allen, 2002). Excess light energy may promote passage of electrons from PSII to the PQ pool at a rate that exceeds oxidation of plastoquinol and causing constipation of electrons as a result of complete reduction of the PQ pool. When the PQ pool is fully reduced, the reaction center of PSII, P680, is in danger of photodamage. If the photosynthetic organism is capable of activating the CET pathway, production of damaging oxygen radicals and other potentially damaging processes can be prevented. In \textit{Chlamydomonas}, CET may be regulated by the PSII generation of oxygen because CET is inhibited under aerobic conditions (Finazzi et al. 1999). In symbiotic clade A \textit{Symbiodinium}, however, CET is active under aerobic conditions and enhanced when exposed to an anaerobic environment (Reynolds et al., 2008). CET allows electrons to cycle from PSI to the cytochrome \textit{b}$_6$/f complex and back to PSI without reducing NAD(P)$^+$. CET also produces ATP and a pH gradient that allows thermal dissipation (Clarke & Johnson, 2001, Munekage et al., 2002), which is important because nonphotochemical quenching mechanisms may be damaged under environmentally stressful conditions, such as high light or temperature.
Photorespiration is an oxygen-dependent electron transport mechanism employed by mainly by C3 plants under conditions of limited CO₂ to maintain linear electron transport activity as light energy is used (Buchanan et al., 2000, Niyogi, 1999). The process evolves CO₂ and NH₃ and consumes oxygen and ATP as a result of the oxygenase activity of ribulose-1,5-bisphosphate carboxylase/oxygenase, commonly known as RuBisCO, the enzyme that catalyzes the first major step in the Calvin-Benson cycle of carbon fixation (Wingler et al., 2000). C4 plants and green algae show low rates of photorespiration as a result of the high concentrations of CO₂ in the cells (Ogren, 1984). The photorespiratory pathway, though it has been thought of as wasteful because it consumes energy, has been found to be necessary for healthy functioning of photosynthetic organisms (Wingler et al., 2000).

Dinoflagellate photorespiration has not been well studied. Dinoflagellates have a Type II RuBisCO with a lower affinity for CO₂ than the Type I RuBisCO common to higher plants. As a result, many dinoflagellates, as well as other microalgae and cyanobacteria, have carbon concentrating mechanisms (CCM) which increase the concentration of CO₂ around RuBisCO decreasing the chance for RuBisCO to bind to O₂ instead of CO₂. The activity of the CCMs may increase the amount of inorganic carbon fixation (Leggat et al., 2002) and potentially suppress photorespiratory activity (Ögren, 1991).
Mehler-ascorbate peroxidase reaction (water-water cycle)

The Mehler reaction, another oxygen-dependent cycle, may provide alternative consumption or dissipation of electrons when metabolism is slower than electron transport (Polle, 1996). It may also help to maintain a pH gradient necessary for dissipation of excess light energy because it does not consume H⁺ nor produce ATP (Osmond & Grace, 1996, Schreiber & Neubauer, 1990).

In the Mehler reaction, water is oxidized by PSII producing O₂ and four electrons. The O₂ is reduced and O₂⁻ is produced on the acceptor side of PSI. The O₂⁻ molecule is metabolized by superoxide dismutase (SOD) and ascorbate peroxidase (APX) to generate water and monodehydroascorbate. Four electrons from the oxidation of water by PSII are then consumed by PSI reduction of O₂ to H₂O (Niyogi, 1999). The cycle begins and ends with water.

The Mehler reaction is commonly, but not unanimously, perceived as a photoprotective mechanism which may protect PSII from photodamage (Niyogi, 1999). Clarke and Johnson (2001) postulated that electron transport to O₂ in the Mehler reaction alone is not sufficient for protection of PSII especially under stressful conditions such as high temperatures combined with high irradiance, which can cause oxidative stress (Lesser, 2006). Photorespiration and a low rate of Mehler reaction working congruently may provide the necessary protection under such environmentally stressful conditions (Clarke & Johnson, 2001).
Linking Photosynthetic Protections – Cyclic Electron Transport and State Transitions

Protections of the photosynthetic apparatus are essential for all photosynthetic organisms because photosynthesis rarely operates under optimal conditions and is commonly exposed to environmental stressors, such as high irradiances, low availability of terminal electron acceptors like CO₂ or O₂, or other extreme environmental conditions (Bukhov & Carpentier, 2004). Under natural conditions, multiple active pathways protect photosynthetic capacity of the organism. Cyclic electron transport and state transitions have been examined together in *Chlamydomonas reinhardtii*, a green alga (Finazzi & Forti, 2004, Finazzi et al., 2002). The *Chlamydomonas* sp. model provides insight into how dinoflagellates and other photosynthetic organisms may employ these photoprotections.

State transitions, the reversible disassociation of a fraction of the LHCII with PSII and re-association with PSI, occur in response to changing natural light conditions (Finazzi & Forti, 2004). The LHCII kinase that affects phosphorylation is activated by reduction of the plastoquinone pool by PSII activity. In contrast, its inactivation is caused by the oxidation of the plastoquinone pool by PSI activity (Allen, 1992, Allen et al., 1981). It has also been suggested that the cytochrome *b₆/f* complex plays an integral role in state transitions by sensing the redox state of the plastoquinone pool (Wollman, 2001). There are multiple models for how the LHCII kinase is activated by activity of the cytochrome *b₆/f* complex (Finazzi & Forti, 2004), but they all involve transfer of electrons.
In *Chlamydomonas*, as stated earlier, up to 85% of LHCII (Delosme et al., 1996) and a portion of the cytochrome *b*$_6/f$ complex (Vallon et al., 1991) may become dissociated from intrinsic photosystems during a state transition. Linear electron flow would be inefficient in state 2 because of the lack of absorption ability of PSII. Therefore, CET has been proposed as a way *Chlamydomonas* can afford such a state transition (Vallon et al., 1991). Finazzi et al. (1999) showed that in state 2, the electron flow to the cytochrome *b*$_6/f$ complex was insensitive to 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), which blocks the reduction of PQ, but the process was sensitive to 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) which blocks flow of electrons from the cytochrome *b*$_6/f$ complex to PQ (Finazzi et al., 1999).

Under potentially stressful conditions, such as where electron acceptors are limited or irradiance is high, state 2 with active CET around PSI could allow for the photochemical usage of absorbed light and ATP production, vital to other metabolic processes. Linear electron flow is inhibited under conditions of high irradiance, likely a consequence of photoinhibition (Finazzi et al., 2001). A state transition in conjunction with CET activity may provide temporary, but necessary protections of the photosynthetic apparatus in such situations. Finazzi and Forti (2004) proposed that a linkage between state transitions and CET similar to what is seen in *Chlamydomonas* may be present in vascular plants as well.

**Conclusion**

Conclusions about the most important role of cyclic electron transport (Allen 2002, Johnson 2005) and state transitions are not definite, but their role in
photoprotection is actively recognized (Niyogi, 1999). Relative to other photosynthetic organisms, little is known about dinoflagellate photosynthesis. The focus of my research was to explore the photosynthetic protection role of CET in symbiotic dinoflagellates of corals and other cnidarians when exposed to stressful environmental conditions such as high irradiance and warm sea surface temperatures. I hypothesized that CET actively protects symbiotic clade A *Symbiodinium*, the only species which has retained the ability to engage CET. In addition, I hypothesized that the dissociation of intrinsic LHCs and the soluble PCPs, a form of state transition, is active in symbiotic clade A *Symbiodinium* and works together with CET to maintain photosynthesis when the dinoflagellate is exposed to high irradiance. The data presented here are from experiments utilizing cultured and *in hospite* *Symbiodinium* and a variety of methods, including a novel fluorescence technique, and the resulting data support our hypotheses.
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CHAPTER 2
ENHANCED PHOTOPROTECTION PATHWAYS IN SYMBIOTIC DINOFLAGELLATES OF SHALLOW-WATER CORALS AND OTHER CNIDARIANS

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ABSTRACT

Photoinhibition, exacerbated by elevated temperatures, underlies coral bleaching, but sensitivity to photosynthetic loss differs among various phylotypes of *Symbiodinium*, their dinoflagellate symbionts. *Symbiodinium* is a common symbiont in many cnidarian species including corals, jellyfish, anemones, and giant clams. Here, we provide evidence that most members of clade A *Symbiodinium*, but not clades B–D or F, exhibit enhanced capabilities for alternative photosynthetic electron-transport pathways including cyclic electron transport (CET). Unlike other clades, clade A *Symbiodinium* also undergo pronounced light-induced dissociation of antenna complexes from photosystem II (PSII) reaction centers. We propose these attributes promote survival of most cnidarians with clade A symbionts at high light intensities and confer resistance to bleaching conditions that conspicuously impact deeper dwelling corals that harbor non-clade A *Symbiodinium*.

Keywords: coral bleaching, light-harvesting complexes, photoinhibition, *Symbiodinium*, cyclic electron transport
INTRODUCTION

Corals and other cnidarians thrive in tropical, near-shore oceans due to intracellular symbioses with dinoflagellates of the genus *Symbiodinium*, which provide their hosts essential photosynthates (1, 2). Coral species distribution largely correlates with prevailing light and temperature gradients and likely is influenced by the differing photosynthetic and light tolerance properties of their symbionts coupled with host-symbiont specificity and biogeographical distribution (3-6). Hence, *Symbiodinium* phylotypes, predominantly clades A, B, and C and sometimes clade D and their respective hosts are found at characteristic depth ranges (4, 7). Clade A *Symbiodinium* are especially prevalent in shallow-water cnidarians including corals, jellyfish, and anemones in the Caribbean (4). Clade A isolates abundantly produce UV-protective mycosporine-like amino acids (MAAs) in culture whereas all clades, with a depth dependent correlation, synthesize MAAs *in hospice*, especially mycosporine-glycine, which effectively absorbs harmful UV-B wavelengths (8). Little else is known about distinct physiological properties of *Symbiodinium* species and their susceptibilities to temperature and high light stress, although corals harboring clade D have been observed to be high-temperature tolerant (9).

*Symbiodinium*, like other photosynthetic organisms, are susceptible to photodamage of PSII (10). The resulting photoinhibition can lead to breakdown of symbioses and is an initial event in the onset of coral bleaching during episodes of elevated ocean temperatures (11). Differences in temperature sensitivity to photodamage (12) and rates of replenishment of the PSII D1 reaction center protein are documented in a few representatives of *Symbiodinium* in culture and *in hospice* (10,
Low degrees of fatty acid desaturation in thylakoid lipid membranes of cultured dinoflagellates have been correlated with elevated temperature tolerance and reduced production of reactive oxygen species under high irradiance, but there is not a strict correlation with heat sensitivity along *Symbiodinium* cladal lines (12).

Some corals bleach seasonally and others rarely (13), suggesting differential survival capabilities of the symbionts and their hosts when subject to environmental extremes. In the Caribbean, deeper water corals that commonly harbor clades B and C *Symbiodinium* are generally more susceptible to bleaching at elevated temperatures than are shallow-water corals in symbiosis with clade A (10), indicating that thermal and high irradiance tolerance can be interrelated. Photoprotection mechanisms identified in *Symbiodinium* include engagement of xanthophyll deepoxidation associated with nonphotochemical quenching (NPQ) within intrinsic light-harvesting complexes (LHCs) (14, 15) and longer-term photoacclimation processes that modulate the abundance and size of photosynthetic units and peripheral peridinin–chlorophyll-a–protein (PCP) antenna complexes (16, 17).

We show here that members of clade A *Symbiodinium* in culture and those inhabiting shallow-water cnidarians are conspicuously capable of PSII-independent CET. Evidence is also presented for the occurrence of chlororespiration, wherein oxygen serves as an alternative photosynthetic electron transport acceptor and is supplemental to CET. Both chlororespiration and CET can sustain ATP synthesis when PSII is inactivated (18). These studies employ a modified pulse amplitude modulated (PAM) fluorometry method that is easily applied in field studies. Clade A *Symbiodinium* are also found to readily undergo high light-induced dissociation of antenna complexes.
from PSII, particularly the “soluble” PCPs found only in dinoflagellates. Conventional PAM fluorometry uses dark-adapted photosynthetic samples to assess PSII photosynthetic efficiency from minimal and maximal chlorophyll fluorescence levels. Emission from a weak modulated measuring light (ML) \((F_0)\) and maximal fluorescence \((F_m)\) induced by a saturating light pulse estimates maximum quantum yield as \(F_{v/m} = (F_m - F_0)/F_m\). Typically, continuous actinic irradiation is subsequently imposed along with intermittent saturating flashes to monitor decreases of maximal fluorescence \((F_m)\) due to NPQ \([(F_m - F_m)/F_m]\). We show a simple method to detect nonlinear photosynthetic pathways by measuring chlorophyll-a (chl-a) fluorescence in response to serial irradiation pulses (SIP) of 1-s duration without concurrent actinic irradiation.

RESULTS AND DISCUSSION

Evidence for CET in clade A Symbiodinium by Using SIP Protocol. When using the SIP method with dark-adapted symbiotic clade A Symbiodinium cells in culture and in hospice, incremental increases in the levels of \(F_0\) chl-a fluorescence are initially detected, reflecting progressive reduction of the electron acceptor pool for PSII, plastoquinone (PQ) (Fig. 2.1 A and C). SIP treatment clearly does not lead to reduction of PQ in clade B Symbiodinium (Fig. 2.1 B and D) indicating sustained linear electron transport activities between PSII and PSI in contrast to clade A cells. The lengthy duration of flashes and limited sensitivity of the instruments and detectors used enable only an approximation of \(F_m\) and \(F_m\)' levels (19). However, the multi-turnover excitation of PSII to drive water oxidation is beneficial to initiating and poising PQ in a reduced state as is apparent from clade A \(F_0\)' increases. As shown below, dissociation of PSII
LHCs, particularly PCPs, lead to preferential PSI excitation in later phases of the clade A SIP response.

When the PQ pool becomes significantly reduced in clade A samples at approximately the fifth flash and thereafter, abrupt $F'_0$ decreases are then induced by each subsequent flash (Fig. 2.1A). These signals are attributed to transient PSI oxidation of the electron transport interchain, including PQ. During following dark phases, PQ reduction ($F'_0$) then returns to levels above that of the prepulse signal with approximate half-times of $\approx 10$ s in culture (Fig. 2.1A Inset) and 5 s in hospice (Fig. 2.1C Inset). A similar phenomenon has been described in a few other algae, but under conditions of limited electron acceptors and/or anaerobiosis (20). Such postillumination PQ reduction signals have also been observed after prolonged illumination of isolated spinach chloroplasts (21) and in leaves of C4 plants (22). The SIP response is significantly enhanced when clade A *Symbiodinium* are subjected to anaerobic conditions [supporting information (SI) Fig. S2.1]. Overall, the clade A fluorescence responses are ascribed to PSI-dependent plastoquinol oxidation followed by PQ rereduction by CET and/or chlororespiration (23, 24). The postillumination PQ reduction signal is likely to reflect only a small portion of CET activity that occurs during constant illumination.

Further evidence that PQ reduction/oxidation is PSI and CET-dependent in SIP-subjected clade A cells is that irradiation with far-red light (FR) and then administration of methyl viologen (MV), an artificial PSI acceptor, leads to decline of $F'_0$ to the initial dark-adapted $F_0$ level (Fig. 2.1A). Because MV interacts directly with PSI (17), it intercepts PSI electron transfer and downstream ferredoxin and NADPH-mediated
processes, including ferredoxin-mediated CET through cytochrome $b_6/f$ and/or NAD(P)H-driven chlororespiration. The MV effect also indicates that PSII photodamage is not responsible for the SIP-induced $F_0^\prime$ increase.

Negative results of SIP treatments with clade B *Symbiodinium* certify the fluorescence phenomena in clade A are not an experimental artifact (Fig. 2.1 B and D). Also, measurements with clade A yield identical SIP responses when PAM fluorometry is used with settled cells in culture flasks (data not shown). Hence, the postillumination decrease and then increase of $F_0^\prime$ is not due to sample stirring and detector array configuration (25).

**SIP Induced CET in Hospice.** The cultured clade A representative in Fig. 2.1A is of western Pacific origin (from the giant clam *Tridacna gigas*). Fig. 2.1C shows that clade A *Symbiodinium* from a Caribbean coral also exhibits diagnostic clade A SIP responses *in hospice*. *Porites astreoides* specimen were collected from shallow water (2 m), and their symbionts show an identical response to cultured clade A cells (Fig. 2.1A) when SIP-assayed with the submergible Diving-PAM (Fig. 2.1C). Subsequent genotyping by using PCR-denaturing gradient gel electrophoresis (DGGE) analysis of the rDNA ITS2 region (5) confirmed the predominance of subclade A4a symbionts. Symbionts of deeper dwelling *P. astreoides* consistently show no SIP-induced $F_0$ fluctuations and harbor C1 *Symbiodinium* (4). Similarly, corals like *Montastraea faveolata* hosting subclade B1 *Symbiodinium* exhibit flat line $F_0^\prime$ and $F_m^\prime$ when SIP-probed (Fig. 2.1D). We additionally surveyed many other species of cnidarians known to host clade A *Symbiodinium* in the Caribbean including corals *Acropora cervicornis* and *Acropora*.
palmata, anemones *Aiptasia pallida* and *Bartholomea annulata*, and the jellyfish *Cassiopeia xamachana*, as well as others known to host clades B and C. Employing SIP, only cnidarians harboring clade A *Symbiodinium* consistently display CET signals and flash-induced NPQ *in hospice*. Among cultured clade A, only subclade A2 isolates from a sea fan and a clam do not yield a CET SIP response (Table S2.1).

*Porites furcata* harbors multiple phylotypes of *Symbiodinium* in the Caribbean (4). A blind test was conducted by using SIP with *P. furcata* collected from a 1-m depth off Key Largo, Florida, revealing fluorescence patterns indicative of clade A, but not in this species when found at a 1.5-m depth (Fig. 2.2 A and B). Presence of the dominant phylotype A4a (in combination with B1) in the shallowest samples was confirmed by PCR-DGGE analysis of the rDNA ITS2 region. Thus, the SIP technique provides a noninvasive means for detecting the probable presence of clade A *Symbiodinium* and is potentially applicable to tracking symbiont genotypic and/or phenotypic fluctuations in the course of seasonal and long-term environmental change.

SIP induces a marked decrease in $F_{m^\prime}$ levels in clade A but not clade B *Symbiodinium* (Fig. 2.1 A and B) reflecting strong induction of NPQ and decreased light energy transfer to PSII. Analogous to model systems subjected to continuous illumination, this $F_{m^\prime}$ decrease can follow from reduced cytochrome $bc_6/f$-dependent activation of chloroplast protein kinases to phosphorylate/dissociate PSII LHCs, conformational changes of LHCs by means of a large decrease of thylakoid lumen pH, activation of xanthophyll deepoxidase on lumen pH decline, or a combination of such excitation energy dissipation mechanisms (18). Dinoflagellates uniquely possess peripheral antennae PCP complexes, presumed to reside in the thylakoid lumen, that
function to maximize green light energy transfer to PSII (26, 27). Shown below,
dissociation of PCPs from PSII and possibly integral antennae complexes are
conspicuous features of NPQ in clade A *Symbiodinium*.

**Anaerobic Enhancement of Dark PQ Reduction and Engagement of NPQ in clade A *Symbiodinium***. Clade A cells do not exhibit a transient PQ reduction/$F_0$' increase after termination of prolonged irradiation with moderate actinic light (250 $\mu$mol quanta $m^{-2} \cdot s^{-1}$) and after NPQ approaches steady-state (Fig. 2.3A), likely due to activation of processes that divert PSI electron acceptors from CET such as carbon fixation, nitrate/sulfate reduction, the Mehler/ascorbate peroxidase pathways, or photorespiration. PSI-dependent oxygen evolution would promote the two latter pathways. We found that anaerobic conditions double the rate of SIP-induced PQ reduction (Fig. S2.1). Imposing anaerobic conditions (Fig. 2.3C), light-adapted clade A cells exhibit a postillumination $F_0$' increase with an overall half-time of 30 s. A similar signal, although weaker, is observed in clade B *Symbiodinium* but under both aerobic and anaerobic conditions (Fig. 2.3 B and D). Clearly, clade A *Symbiodinium* is capable of more complete engagement of NPQ as indicated by the fluorescence decrease in response to continuous illumination as compared with clade B cells. Additionally, substantial but gradual dark PQ reduction, likely due to partial engagement of a chlororespiration pathway, is discernable when the oxygen electron acceptor for photorespiration, chlororespiration, and/or pathways related to the Mehler reaction is diminished. Collapse of a transthylakoid pH gradient at the onset of darkness in clade B
*Symbiodinium* and, partially in clade A cells, could also contribute to the $F_0'$ postillumination increase.

**Light-Induced Dissociation of PSII Antennae in clade A Symbiodinium.** High light induction of NPQ is fairly well documented in *Symbiodinium* in culture and *in hospice* (11, 14, 15, 28). Only the initiation of xanthophyll deepoxidation to facilitate a NPQ thermal decay of excitation energy within integral LHC chl-$a/c_2$ complexes has been documented (29). Other photoprotective processes akin to state transitions, in which LHCs dissociate from PSII, also have been theoretically deduced (30, 31). However, no definitive evidence for state transition-like mechanisms in *Symbiodinium* or other dinoflagellates have been documented. Fluorescence spectra at 77 K of dark-acclimated clade A *Symbiodinium* show that both intrinsic LHCs and extrinsic PCPs are fully connected to PSII as is apparent in the major fluorescence emission peak at 686 nm, presumably from the CP43 core antennae (Fig. 2.4A) (32). Excitation spectra of 686 nm show a strong contribution of PCP with novel peridinin absorption in the 530-nm region, along with the $\approx 400$-nm Soret bands of chl (Fig. 2.4B). That PCPs are fully PSII-associated in dark-adapted cells is evidenced by minimal emission at 673 nm where a fluorescence shoulder from unassociated PCP chl-$a$ would be selectively detected (Fig. 2.4B) (26, 33).

After exposure to actinic white light (250 $\mu$mol quanta m$^{-2}$·s$^{-1}$) for 100 min, fluorescence emission spectra of clade A cells exhibit dual peaks at 675 nm and 686 nm on preferential excitation of either chl (440 nm) or peridinin (530 nm) (Fig. 2.4C). The 675-nm peak is close to the PCP chl-$a$ emission band of purified complexes (26,
Peridinin, as a major contributor to the 675-nm emission peak, is further evidenced by an elevated 530 nm-induced excitation signal as compared with that obtained by the 440-nm excitation spectrum (Fig. 2.4D). We estimate from light/dark fluorescence spectral differences that almost half of the PCP population, perhaps together with integral LHCs dissociates from PSII as a major component of NPQ on prolonged light exposure in clade A *Symbiodinium*. No modifications of fluorescence emission spectra are detected in clade B and C *Symbiodinium* in response to these illumination conditions (Fig. S2.2).

Enhancement of fluorescence peaks at wavelengths longer than 700 nm would reflect intrinsic antenna translocation from PSII to PSI, the classical state transition earmark in green algal systems (34). In *Symbiodinium*, such a State 2 antenna association with PSI is not substantial (Fig. S2.2). When exposed to prolonged FR, the classical algal state transition response is antenna reversion from PSI to PSII connectivity (State 1) (35). Surprisingly, clade A cells respond to far-red irradiation differently, displaying dual fluorescence peaks at 675 nm and 683 nm, whereas 686-nm emission from PSII is minimized (Fig. 2.4C). The emergent 683-nm peak is best ascribed to disconnected intrinsic chl-a/c LHCs (33) antenna as evidenced by a chl-c contribution near 458 nm (27). The loss of 686-nm fluorescence could be due to diminished PSII integrity resulting from dissociation of core antenna CP43 in combination with the PSII disconnection of LHCs (Fig. 2.4 A and C). We attribute pronounced CET and cytochrome *b*/*f* reduction as an underlying basis for the distinctive response of clade A *Symbiodinium* to far-red and high light illumination.
Clades B and C exhibit no fluorescence changes as a consequence of far-red (Fig. 2.4C) or prolonged high light acclimation (Fig. S2.3).

**CONCLUSIONS**

Clade A is the most ancestral *Symbiodinium* phylotype (36). Most closely related subphylotypes found in shallow water appear to have evolutionarily retained constitutive CET/chlororespiration and PCP/LHC dissociation mechanisms. Moreover, when thermal perturbation of PSII photodamage and repair occurs, CET conceivably would sustain symbiont/host survival together with likely synergisms of xanthophyll deepoxidation contribution to NPQ and production of UV-protective MAAs. Clades B, C, and D symbionts are analogous to shade plants because they are usually found in symbioses in deeper waters than clade A *Symbiodinium* and would benefit from enhanced light harvesting capability. Some subclade B *Symbiodinium* are found in symbiosis with corals in shallow water but they likely employ photoprotection mechanisms other than antenna translocation under such circumstances. *Symbiodinium* clades B and C of deeper-dwelling corals, normally more susceptible to bleaching, can engage NPQ and varying degrees of chlororespiration (data not shown). However, kinetics and magnitude of their SIP-induced fluorescence patterns indicate they primarily sustain photosynthesis through linear photosynthetic electron transport and not CET.

Understanding *Symbiodinium* photoprotection and thermal tolerance mechanisms is crucial to predicting how coral-dinoflagellate symbioses will respond to increased ocean temperatures, irradiance conditions, and perhaps disease pressures.
From the studies presented here, corals with clade A *Symbiodinium* are likely more resistant to combinations of high light and high temperature, conditions common at the end of summer (13), because of their enhanced and constitutive alternative photosynthetic electron pathways and photoprotection processes. CET and chlororespiration (37) may also serve to minimize oxygen evolution thereby decreasing the probability of the formation of and damage by reactive oxygen species. We propose that CET can sustain ATP synthesis and underlies clade A symbiont survival during warm conditions that can exacerbate PSII loss. Analogous to the case of PSII-less tobacco mutants (38), CET and/or chlororespiration could become dominant means for photosynthetic energy transduction during bleaching episodes. However, whether some non-clade A symbionts might conditionally develop CET and significant antenna dissociation capacity in response to elevated temperature deserves further study.

**MATERIALS AND METHODS**

**Symbiodinium Cultures and in Hospice.** Cultures of *Symbiodinium* were grown in ASP-8A medium (39) and maintained at 50 µmol quanta m⁻²·s⁻¹ under cool white fluorescent lamps and at a constant temperature of 26°C under a 14:10-h light/dark photoperiod (16). Cells were harvested during exponential growth. Coral samples gathered from Admiral Reef, Alligator Reef, or Little Grecian Reef in the northern Florida Keys included *P. astreoides*, *M. faveolata*, and *P. furcata*. Other samples were collected from Florida Bay directly offshore such as coral *Siderastrea radians*, anemones *A. pallida* and *B. annulata* and jellyfish *C. xamachana* (see Table 2.1 for a list of cnidarians analyzed). Specimen were immediately placed in fresh seawater and
dark adapted for at least 60 min before SIP assays. *Symbiodinium* were genotyped by using PCR-DGGE analysis of the rDNA ITS2 region as described by LaJeunesse and Trench (40) and LaJeunesse (5).

**SIP Fluorescence Analyses.** Chlorophyll fluorescence of cultured *Symbiodinium* was measured by using a 101/103 PAM fluorometer (Walz) with the ED-101US/MD optical unit and HPL-L470 blue light-emitting diode (LED) source for saturating flashes. A Hansatech LS2 halogen light source was used for actinic white light (250 µmol quanta m\(^{-2}\)∙s\(^{-1}\)) and when filtered, far-red irradiation (> 690 nm). Culture samples were concentrated 10-fold by centrifugation, resuspended in fresh media, and dark-adapted for at least 20 min. Except for the comparison of anaerobic and aerobic experiments described below, cells were constantly stirred by using a cuvette mixer. Cnidarian samples and cultured cells settled at the bottoms of culture flasks were assayed after dark-adaptation for at least 1-h (cnidarians) and 20 minutes (culture cells) with the submergible Diving-PAM (Walz), configured with a blue LED ML source. FR was not used for these samples but the fluorescence responses among clades were similar in cultures and *in hospice* specimens. Baseline fluorescence (*F\(_0\) and *F\(_0\)´*) was monitored by a modulated ML (<1 µmol quanta m\(^{-2}\)∙s\(^{-1}\)) to assess minimal fluorescence from PSII in dark periods. For SIP, maximal fluorescence (*F\(_m\) and *F\(_m\)´*) was measured by administering a 1-s saturating flash of 470 nm of light (∼ 5000 µmol quanta m\(^{-2}\)∙s\(^{-1}\)) every 20 s beginning at 30 s into the sampling period. When used, MV (1,1´-dimethyl-4,4´-bipyridium-dichloride) was added at a final concentration of 50 µM.
**Anaerobic Measurements.** Cultures were incubated in the dark for 10 min in the presence of 10 mM bicarbonate to maximize carbon fixation rates during subsequent light treatments. The samples also were bubbled with air or argon without stirring to impose aerobic and anaerobic conditions, respectively, during the dark adaptation and fluorescence measurement periods.

**Fluorescence Spectra.** Whole cell low-temperature (77 K) emission and excitation spectra were measured by using a SLM-Aminco SPF-500 spectrofluorometer. Cells were harvested and maintained for 100 min in darkness or 250 µmol quanta m⁻² s⁻¹ actinic light. Immediately after treatment, samples were quickly suspended in 40% glycerol and frozen in liquid nitrogen in a custom glass Dewar. Emission spectra were measured with 440-nm or 530-nm excitation. Excitation spectra were measured at 673 nm or 683 nm and corrected for instrument response.
REFERENCES


Fig. 2.1. SIP detection of postillumination PQ reduction in clade A but not B

_Symbiodinium in culture and in hospice_. Dark-adapted cultured cells and corals were subjected to saturating light pulses every 20 s after applying low-intensity ML. A progressive increase in $F_{0'}$ levels reflect substantial reduction of the PQ pool in clade A3 cultured cells (A) but not in clade B1 cultured cells (B). (Insets) Light-induced PQ oxidation and dark rereduction between the 13th and 15th saturating pulses in clade A but not clade B phylotypes. (A and B) After clade A3 SIP treatment, 50 µM MV was used in combination with FR. (C and D) A Diving-PAM was used for SIP with dark-adapted corals. (C) _Symbiodinium_ phylotype A4a in shallow-dwelling _P. astreoides_
exhibits progressive PQ reduction and postillumination PQ reduction. (D) Fluorescence responses of *M. faveolata* harboring B1 *Symbiodinium* show no change with SIP.
Fig. 2.2. Detection of clade A *Symbiodinium* in coral hosts. SIP of dark-adapted *P. furcata*, from a 1-m depth, reveals a fluorescence pattern typical of clade A. (A) Genetic analysis by using DGGE revealed the presence of a combination of A4a and some B1 phylotypes of *Symbiodinium*. (B) Dark-adapted *P. furcata* from a 1.5-m depth, and found to harbor only B1 *Symbiodinium*, does not display a clade A specific fluorescence pattern SIP response.
Fig. 2.3. Enhanced dark PQ reduction in anaerobic clade A *Symbiodinium*. After dark incubation for 10 min and supplementation with 10mM bicarbonate to maximize carbon fixation, cultures of subclades A3 (A and C) and B2 (B and D) were bubbled with air (A and B) or argon (C and D) to impose aerobic and anaerobic conditions, respectively. Fluorescence decreases after onset of continuous actinic illumination reflect NPQ processes, which are almost complete after 5-min exposure to 250 $\mu$mol quanta m$^{-2}$·s$^{-1}$. During subsequent darkness, anaerobic clade A (C) and, to a lesser extent, both aerobic and anaerobic clade B exhibit PQ reduction (B and D).
Fig. 2.4. Light-Induced PCP and Intrinsic LHC Dissociation from PSII in clade A3 *Symbiodinium*.  

(A) The 77 K fluorescence emission spectra at excitation wavelengths 440 nm (solid line) and 530 nm (dashed line).  

(B) Excitation spectra of the same clade A3 cells detected at 673 nm (solid line) and 683 nm (dashed line) after 60-min dark adaptation. The dark-acclimated excitation spectrum, specifically the peridinin excitation shoulder at 530 nm, is higher at 683 nm than 673 nm as a result of the greater PSII connectivity of the PCP antenna complexes and resulting fluorescence.  

(C) Fluorescence emission spectra at excitation wavelengths of 440 nm (solid line) and 530 nm (dashed line) after exposure to 100 min of white light. The two emission peaks (675 and 686 nm) in both excitation spectra from white light-adapted clade A3 cells
reflect partial dissociation of LHCs from PSII. Prolonged FR illumination (> 690 nm) results in further loss of PSII 686 nm fluorescence, whereas 683-nm emission from PCPs and intrinsic LHCs is enhanced (solid line). No far-red-induced fluorescence shifts occur in clade B (dotted-dashed line). (D) Fluorescence excitation spectra of the same clade A3 cells as in C at detection wavelengths of 673 nm (solid line) and 683 nm (dashed line) after 100 min of actinic light exposure, showing a pronounced peridinin contribution to 675-nm emission by 530-nm excitation in comparison with that of dark-adapted cells in B.
**SUPPLEMENTAL INFORMATION**

**Fig. S2.1.** Augmented non-linear electron transport under anaerobic conditions.

Aerobic cells of culture subclade A4 (A) exhibit an 18% increase in the level of $F_0^-$ in serial irradiation pulses (SIP) progression through the sixth flash whereas an anaerobic sample of the same culture (B) shows a 39% $F_0^-$ increase. Oxygen as a downstream photosystem I (PSI) and chlororespiratory electron acceptor promotes both dark and flash-induced PQ oxidation and diminishes $F_0^-$ increases by SIP.
Fig. S2.2. Long wavelength emission spectra in clade A3 *Symbiodinium*. (A) The 77 K fluorescence emission spectra with excitation at 440 nm of cultured phylotype A3 *Symbiodinium* cells following a 60 min dark adaptation (solid line) and 100 min light acclimation (dashed line). (B) Emission spectra at excitation wavelengths of 530 nm following the same treatments. Dual peaks in both A and B under light acclimated conditions result from partial dissociation of peridinin–chlorophyll-a–proteins (PCPs) and intrinsic light-harvesting complexes (LHCs) from PSII. It is unclear to what extent PCPs and/or LHCs become associated with PSI because of the absence of a pronounced long-wavelength fluorescence peak. Both A and B were normalized to the 686-nm emission peak.
Fig. S2.3. Clades B and C LHC remain connected to PSII under high light conditions. The 77 K fluorescence emission spectra with a 440-nm excitation of phylotypes B2.1 (A) and C1 (B) from cultured Symbiodinium cells are identical after 60 min of dark (solid line) or high light (dashed line) acclimation. As compared with clade A Symbiodinium (Fig. 4), clades B and C do not exhibit photoprotective antenna dissociation from PSII.
Table S2.1. SIP detection of CET in cultured and in hospice *Symbiodinium*

<table>
<thead>
<tr>
<th>Clade/subclade †</th>
<th>Isolate Number</th>
<th>Symbiodinium species</th>
<th>Geographical Origin</th>
<th>Host Origin</th>
<th>CET present or absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>61</td>
<td><em>Symbiodinium microadriaticum</em></td>
<td>Gulf of Aqaba</td>
<td>Stylophora pistillata</td>
<td>present</td>
</tr>
<tr>
<td>A1</td>
<td>370</td>
<td><em>Symbiodinium microadriaticum</em></td>
<td>Florida</td>
<td>Cassiopeia xamachana</td>
<td>present</td>
</tr>
<tr>
<td>A2</td>
<td>97</td>
<td><em>Symbiodinium sp.</em></td>
<td>Puerto Rico</td>
<td>Gorgonia ventalina</td>
<td>absent‡</td>
</tr>
<tr>
<td>A2</td>
<td>185</td>
<td><em>Symbiodinium pilosum</em></td>
<td>Jamaica</td>
<td>Zoanthus sociatus</td>
<td>present</td>
</tr>
<tr>
<td>A2</td>
<td>350</td>
<td><em>Symbiodinium corculorum</em></td>
<td>Palau</td>
<td>Corculum cardissa</td>
<td>absent</td>
</tr>
<tr>
<td>A3</td>
<td>292</td>
<td><em>Symbiodinium sp.</em></td>
<td>Palau</td>
<td>Tridacna maxima</td>
<td>present</td>
</tr>
<tr>
<td>A3a§</td>
<td>PHMS TDle 3.4¶</td>
<td><em>Symbiodinium sp.</em></td>
<td>Philippines</td>
<td>Tridacna derasa</td>
<td>present</td>
</tr>
<tr>
<td>A4</td>
<td>379</td>
<td><em>Symbiodinium sp.</em></td>
<td>Bahamas</td>
<td>Plexaura homamalla</td>
<td>present</td>
</tr>
<tr>
<td>B1</td>
<td>2</td>
<td><em>Symbiodinium sp.</em></td>
<td>Florida</td>
<td>Aiptasia pallida</td>
<td>absent</td>
</tr>
<tr>
<td>B1</td>
<td>13</td>
<td><em>Symbiodinium bermudense</em></td>
<td>Bermuda</td>
<td>Aiptasia tagetes</td>
<td>absent</td>
</tr>
<tr>
<td>B1</td>
<td>64</td>
<td><em>Symbiodinium sp.</em></td>
<td>Jamaica</td>
<td>Cassiopeia xamachana</td>
<td>absent</td>
</tr>
<tr>
<td>B1</td>
<td>74</td>
<td><em>Symbiodinium sp.</em></td>
<td>Jamaica</td>
<td>Cassiopeia xamachana</td>
<td>absent</td>
</tr>
<tr>
<td>B1</td>
<td>146</td>
<td><em>Symbiodinium sp.</em></td>
<td>Jamaica</td>
<td>Oculina diffusa</td>
<td>absent</td>
</tr>
<tr>
<td>B2</td>
<td>141</td>
<td><em>Symbiodinium sp.</em></td>
<td>Bermuda</td>
<td>Oculina diffusa</td>
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<tr>
<td>C1</td>
<td>152</td>
<td><em>Symbiodinium goreaui</em></td>
<td>Jamaica</td>
<td>Discsoma sanctithomae</td>
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<tr>
<td>C2</td>
<td>203</td>
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<td>Palau</td>
<td>Hippopus hippopus</td>
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<tr>
<td>D1a</td>
<td>A001</td>
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<td>Okinawa, Japan</td>
<td>Acropora sp.</td>
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<tr>
<td>D1a</td>
<td>CCMP2556</td>
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<td>Florida Keys</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Symbiodinium sp.</td>
<td>Soritid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>-----------------</td>
<td>---------</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>D2</td>
<td>401</td>
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<td>Meandrina meandrites absent</td>
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</tr>
<tr>
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<td>133</td>
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<td>Jamaica</td>
<td>Meandrina meandrites absent</td>
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</tr>
<tr>
<td>A3</td>
<td>in hospice</td>
<td>Symbiodinium sp.</td>
<td>Bahamas</td>
<td>Acropora cervicornis present</td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>in hospice</td>
<td>Symbiodinium sp.</td>
<td>Key Largo, FL</td>
<td>Acropora cervicornis present</td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>in hospice</td>
<td>Symbiodinium sp.</td>
<td>Bahamas</td>
<td>Acropora palmata present</td>
<td></td>
</tr>
<tr>
<td>A3</td>
<td>in hospice</td>
<td>Symbiodinium sp.</td>
<td>Key Largo, FL</td>
<td>Acropora palmata present</td>
<td></td>
</tr>
<tr>
<td>A4a</td>
<td>in hospice</td>
<td>Symbiodinium sp.</td>
<td>Bahamas</td>
<td>Porites astreoides present</td>
<td></td>
</tr>
<tr>
<td>A4a + B1</td>
<td>in hospice</td>
<td>Symbiodinium sp.</td>
<td>Key Largo, FL</td>
<td>Porites astreoides present</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>in hospice</td>
<td>Symbiodinium sp.</td>
<td>Bahamas - deep</td>
<td>Porites astreoides absent</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>in hospice</td>
<td>Symbiodinium sp.</td>
<td>Key Largo, FL</td>
<td>Montastraea faveolata absent</td>
<td></td>
</tr>
<tr>
<td>B1 shallow</td>
<td>in hospice</td>
<td>Symbiodinium sp.</td>
<td>Bahamas</td>
<td>Montastraea faveolata absent</td>
<td></td>
</tr>
<tr>
<td>C12 deep</td>
<td>in hospice</td>
<td>Symbiodinium sp.</td>
<td>Bahamas</td>
<td>Montastraea faveolata absent</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>in hospice</td>
<td>Symbiodinium sp.</td>
<td>Key Largo, FL</td>
<td>Montastraea annularis absent</td>
<td></td>
</tr>
<tr>
<td>B1</td>
<td>in hospice</td>
<td>Symbiodinium sp.</td>
<td>Bahamas</td>
<td>Montastraea annularis absent</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>in hospice</td>
<td>Symbiodinium sp.</td>
<td>Key Largo, FL</td>
<td>Montastraea cavernosa absent</td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>in hospice</td>
<td>Symbiodinium sp.</td>
<td>Bahamas</td>
<td>Montastraea cavernosa absent</td>
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<tr>
<td>B1 or C3</td>
<td>in hospice</td>
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<td>Key Largo, FL</td>
<td>Montastraea franski absent</td>
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<td>B1 or C12</td>
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<td>Bahamas</td>
<td>Montastraea franski absent</td>
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<tr>
<td>C3</td>
<td>in hospice</td>
<td>Symbiodinium sp.</td>
<td>Bahamas</td>
<td>Siderastrea siderea absent</td>
<td></td>
</tr>
<tr>
<td>B5a or C3</td>
<td>in hospice</td>
<td>Symbiodinium sp.</td>
<td>Key Largo, FL</td>
<td>Siderastrea siderea absent</td>
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<tr>
<td>B5</td>
<td>in hospice</td>
<td>Symbiodinium sp.</td>
<td>Florida Bay</td>
<td>Mancinia aerolata absent</td>
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</tr>
</tbody>
</table>
ND, Not determined.

* Certain conditions such as increased high light exposure or high temperatures may change the response.

† Unless otherwise noted, all numbered culture clade/subclades are referenced in LaJeunesse (1).

‡ G. ventalina has been documented as hosting a B1 Symbiodinium in the Florida Keys (2, 3). Culture 97 likely arose as a nonsymbiotic epiphyte.

§ Referenced in Baillie et al. (4).

¶ Referenced in LaJeunesse et al. (5).


CHAPTER 3

THERMAL TOLERANCE AND USE OF ALTERNATIVE PHOTOSYNTHETIC PATHWAYS IN CULTURED SYMBIODINIAM²

ABSTRACT

The physiological effects of prolonged warm temperatures on ten cultured isolates of *Symbiodinium*, clades A-D and F were explored. High sea surface temperatures usually have a detrimental effect on the photosynthetic apparatus of *Symbiodinium*. However, the use of alternative electron transport pathways such as cyclic electron transport may act as a protection to the photosynthetic apparatus during times of heat stress. The integral photosystem II (PSII) protein, D1, is the most common site of damage when exposed to high temperatures and a resulting loss of photochemistry is often seen. Cyclic electron transport around photosystem I would utilize excess light energy and maintain ATP production when many PSIIIs have been damaged. Using the serial irradiation pulse (SIP) chlorophyll-a fluorescence technique, cyclic electron transport was detected in symbiotic members of the clade A *Symbiodinium* only, though all isolates were monitored for the duration of the treatment period. D1 protein contents were also examined at to determine if any relationships exist between amount of D1 and use of specific photoprotections. Differential damage as well as recovery was observed among phylotypes when exposed to 9 days of 32°C after gradually increasing the temperature 1°C per day from 26°C to 32°C. The cultures were examined again after a 25 day period at 26°C. Symbiotic A1, A3, and A4a *Symbiodinium* and isolates B1 and B2.1 were the most sensitive to prolonged high temperatures. Cyclic electron transport and nonphotochemical quenching mechanisms were always active in A1, A3, and A4a when SIP-probed. The clade B representatives and phylotype A1 showed the greatest amount of recovery of photochemistry when temperatures were decreased following the high temperature treatment. Phylotype C2 was moderately sensitive and
yielded a distinct chlorophyll-a fluorescence pattern when SIP-probed. The non-symbiotic A2 and isolates C1, D1a, and F2 were most resilient to hot temperatures.
**INTRODUCTION**

*Symbiodinium* (Freudenthal) is a genetically diverse group of photosynthetic dinoflagellates, characterized as clades (A-H) (Rowan and Powers 1991; Coffroth and Santos 2005; Pochon et al. 2006) that live most commonly as obligate endosymbionts of cnidarians and as free-living marine species. The high diversity of *Symbiodinium* evolved during their long existence and symbiosis with invertebrates which has lasted over 200 million years (Veron 1995; Stanley 2003; LaJeunesse 2005). As endosymbionts of corals, they provide their host over 90% of their carbon requirements from photosynthesis (Muscatine et al. 1981; Falkowski et al. 1984) in the tropical, shallow, oligotrophic marine environments where they live (Muscatine and Porter 1977).

*Symbiodinium* have developed niche specializations that correlate to host biogeography and ecology. In the Caribbean, clade A phylotypes are only found in symbiosis with shallow-water (1-3-m) cnidarians, while clades B – D associate with hosts in both shallow and deep water (LaJeunesse 2002). Under normal conditions, *Symbiodinium*-cnidarian symbioses are exposed to predictable light regimes and temperature fluctuations of the reef ecosystem. Every summer, most nearshore corals are exposed to warm sea surface temperatures, though not all *Symbiodinium* species respond in the same way to these seasonal changes and predictable stresses (Fitt et al. 2001; Brown and Dunne 2008). Shallow-water *Symbiodinium*-coral symbioses tend to be more bleaching resistant while deeper-water symbioses are more susceptible to bleaching in the Caribbean (Warner et al. 1996; Fitt et al. 2001). During an El Niño Southern Oscillation (ENSO) event, extra stress is placed on the coral-dinoflagellate symbiosis because of the unusually long period of warm summer sea surface
temperatures (Hughes 2003). Under such conditions, the photosynthetic apparatus of *Symbiodinium* may become severely damaged by the synergistic effects of warm temperatures and high irradiance (Iglesias-Prieto et al. 1992; Warner et al. 1996, 1999; Fitt et al. 2001; Jones and Hoegh-Guldberg 2001; Brown and Dunne 2008).

Prevention of damage to the core photosynthetic complex is integral to the survival of *Symbiodinium* and its cnidarian host. *Symbiodinium* have differential and in most cases limited abilities to acclimatize to dramatic changes in temperatures (Warner et al. 1996; Takahashi et al. 2004; Tchernov et al. 2004; Venn et al. 2006; Takahashi et al. 2008), thus are confined to narrow thermal regimes (Middlebrook et al. 2008). The inability to modify thylakoid lipid composition and its susceptibility to heat-induced damage (Tchernov et al. 2004; Takahashi et al. 2009) greatly influences the ability to acclimate to prolonged warm sea surface temperatures. Repair or synthesis of certain thylakoid membrane proteins, including the D1 protein and the peridinin-chlorophyll-a-protein complex (PCP), may become compromised due to temperature stress and result in loss of photosystem II (PSII) activity, eventually resulting in bleached corals (Warner et al. 1999; Tchernov et al. 2004; Takahashi et al. 2008).

Functional PSIIIs are essential for photosynthesis and are prone to damage and as a result, engagement of active protections of the photosynthetic apparatus is crucial for survival under all conditions. *Symbiodinium* can engage photoprotections such as the alternative photosynthetic pathway cyclic electron transport (CET), which re-routes excess light energy away from PSII (Reynolds et al. 2008). Nonphotochemical quenching (NPQ) mechanisms dissipate excess energy as heat, including xanthophyll de-epoxidation (Warner et al. 1996; Brown et al. 1999; Robison and Warner 2006) and
PCP dissociation from intrinsic light harvesting complexes (LHC) (Iglesias-Prieto and Trench 1997; Reynolds et al. 2008). Also, it has been shown that LHC phosphorylation stimulates D1 protein degradation (Georgakopoulos and Argyroudi-Akoyunoglou 1997) allowing for the synthesis of new proteins. If these and additional photoprotections are not sufficient to protect core the photosynthetic apparatus, severe damage can occur due to the inability of *Symbiodinium* to turnover PSII proteins, specifically D1 (Warner et al. 1999). However, a clear clade-level response to thermal stress does not exist as the rates of recovery and actual amounts of photosynthetic proteins vary within and among clades (Tchernov et al. 2004; Takahashi et al. 2009).

Symbiotic members of clade A *Symbiodinium* show enhanced capabilities to perform CET under normal temperature conditions, while clades B-D and F do not. The objectives of this study were to explore whether a clade-level correlation exists between cultured *Symbiodinium* and their ability to engage PSII-independent CET under prolonged high temperature conditions. Some clade A *Symbiodinium* phylotypes have lower D1 protein levels resulting in a greater sensitivity to thermal stress (Robison and Warner 2006) and the need for pathways that move electrons away from PSII. CET can maintain ATP production when PSII is not fully functional and may allow the species to recover after extended periods of high temperatures if their integral photosynthetic complex is not damaged too severely.
MATERIALS AND METHODS

Dinoflagellate Cultures

Ten *Symbiodinium* isolates (Table 3.1) in 5 clades, A-D and F, were monitored for their use of CET when probed with the serial irradiation pulse (SIP) method. The cultured isolates were genetically characterized previously (LaJeunesse 2001) using analysis of the rDNA ITS2 sequence. (See Table 1 for a full description of geographic and host origin of the isolates.) Cultures of *Symbiodinium* were grown in ASP-8A medium (Blank 1987) and maintained at 50 µmol quanta m⁻² s⁻¹ under cool white fluorescent lamps and at a 14:10 hour light:dark photoperiod (Iglesias-Prieto and Trench 1994).

Temperature shift

Cultures were grown at 26°C prior to experimentation in a temperature controlled growth chamber. The temperature was increased 1°C per day for 6 days from the control of 26°C until 32°C was reached. The temperature remained at 32°C for 9 days and then was decreased to 26°C for a 25-day period. SIP measurements were performed each day of the temperature shift, day 5 at 32°C, day 9 at 32°C, and on the last day of the 25-day period at 26°C. Cultures were refreshed with new growth media 2 days before the temperature shift began, after measurements were completed on the 9th day at 32°C, and 24 days into the recovery.

Serial Irradiation Pulses (SIP) Fluorescence Analysis.

Chlorophyll-a (chl-a) fluorescence of cultured *Symbiodinium* was measured using a Diving PAM (Walz, Germany) configured with a blue LED measuring light source. SIP
measurements were performed on cells settled on the bottom of the flasks to detect presence of CET and NPQ activity at each temperature time point in every isolate.

Baseline fluorescence ($F_0$ and $F_0^\prime$) was monitored by a modulated measuring light (<1 μmol quanta m$^{-2}$ s$^{-1}$) to assess fluorescence emission of PSII in the dark. Multiple 1-second saturating flashes of 470 nm of light (approximately 18,000 μmol quanta m$^{-2}$ s$^{-1}$) were administered to measure the maximum ($F_m$ and $F_m^\prime$) fluorescence over 6.5 minutes and to monitor fluorescence changes. For SIP, the samples were subjected to a saturating pulse of light 30-seconds into the sampling period and every 20 seconds thereafter. SIP measurements were completed within one hour of ‘dawn’ in the cultures’ light/dark cycle.

Interpretations of SIP-induced fluorescence were derived from $F_0$, $F_0^\prime$, $F_m$, and $F_m^\prime$ levels. The post-saturating pulse dip and immediate rise of $F_0^\prime$ was indicator of plastoquinol (PQH$_2$) oxidation and fast plastoquinone (PQ) re-reduction. $F_0^\prime$ rise together with either decreasing $F_m^\prime$, indicative of active NPQ mechanisms, or stable $F_m^\prime$ was the fluorescence fingerprint of CET. If total fluorescence (both $F_0^\prime$ and $F_m^\prime$) increased with exposure to the SIP method, damage to PSII had occurred. Initial $F_0$ levels were used as a determinant of amount of active PSII.

**Protein Content Analysis**

Samples were removed from cultures on the first day of the experiment at 26°C, the first day at 32°C, the fifth day at 32°C, the ninth day at 32°C, and on the twenty fifth and final day at 26°C. Cells were concentrated by centrifuging at 2000 x g for two minutes. The pellet was resuspended in the remaining media, transferred to a 2-mL Eppendorf tube and spun in for 15-seconds at top speed. The pellet was resuspended
in 300-μL of gel loading buffer (* see below). Glass beads (Ceroglass; Glass Beads, 0.4-0.6 mm, SLG-5) were then added and bead beat (Mini-Beadbeater, Biospec Products) for 2 minutes at top speed. The sample was cooled on ice for 5 minutes and supernatant transferred into a new Eppendorf tube via a spin column-like assembly. The concentration of protein in 10-μL of each sample was determined using a modified Bradford Assay (Bradford 1976) and samples were subsequently adjusted to a concentration of 1-μg total protein μL⁻¹.

*Symbiodinium* total protein extracts were adjusted (20-μg per lane) loaded evenly into an 15% acrylamide gel (Laemmli) to separate proteins through gel electrophoresis. Western blotting was then employed using polyclonal antibodies raised against a synthetic peptide, equivalent to a membrane spanning portion of the natural D1 protein to quantify relative D1 protein amounts, as described previously (Warner et al. 1999).

*Breaking and Loading Buffer:*

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</tr>
<tr>
<td>DTT</td>
<td>60 mM</td>
</tr>
<tr>
<td>Sucrose</td>
<td>15%</td>
</tr>
<tr>
<td>ACA</td>
<td>5 mM</td>
</tr>
<tr>
<td>BAM</td>
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</tbody>
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RESULTS

Chlorophyll Fluorescence Analysis

Each isolate of *Symbiodinium* revealed unique physiological responses to long-term exposure at 32°C as measured by chl-a fluorescence through SIP-probing, however similarities were found between specific phylotypes (Figures 3.1 – 3.5, Table 3.2). Generally, some isolates including A2, C1, D1a, and F2 were resilient when exposed to 32°C for 9 days (Figure 3.1D, 3.3A, 3.4, and 3.5), while A1, A3a, and A4a appeared to have enhanced PSII sensitivity at 30°C (Figure 3.1A – C). When severe damage to PSII occurred in A1, A3, A4a, B1, B2.1, and D1a phylotypes, a lack of chl-a fluorescence resulted and interpretations of results could not be made.

The maximum (*F_\text{m}/F_\text{m}^\prime*) and minimum (*F_\text{0}/F_\text{0}^\prime*) chl-a fluorescence were used to determine presence of alternative photosynthetic pathways (Table 3.2). Nonphotochemical quenching (NPQ) mechanisms were active and interpreted from the decreases of *F_\text{m}^\prime* and, where present, diminishing *F_\text{0}^\prime* after each saturating pulse. Changes in *F_\text{0}^\prime* between each saturating pulse were closely analyzed to determine oxidation and reduction of the electron acceptor pool for PSII, PQ. Increases in *F_\text{0}^\prime* reflect PSI-dependent PQH$_2$ oxidation followed by progressive reduction of PQ indicative of CET and seen in A1, A3, and A4a (Figures 3.1A, B, C) at all temperatures where *F_\text{m}/F_\text{m}^\prime* peaks were either stable or decreased. No other clades showed use of CET. Subclade A2 (Figure 3.1D) is unique among the other *Symbiodinium* members of clade A in that they are not symbiotic (Coffroth et al. 2006) and possibly a cryptic species which becomes opportunistic when placed in a culture environment.
(LaJeunesse 2001; Coffroth et al. 2006). As a result, A2 did not show the SIP-probed CET chl-a fluorescence pattern characteristic of symbiotic members of clade A.

Other isolates showed fluorescence signatures indicative of slow PQH₂ oxidation only where F₀' decreased slowly to baseline levels in the dark following a saturating pulse, possibly due chlororespiration (Bennoun 2002). B1 and B2.1 (Figure 3.2A, B) showed slow PQH₂ oxidation after every saturating flash at each temperature except after prolonged exposure to 32°C when PSIIIs were damaged and Fₘ' was not detected. C1 revealed slow PQH₂ oxidation until 31°C where the F₀' instead returned to baseline levels after each saturating pulse (Figure 3.3A). Throughout the entire treatment period F2 (Figure 3.4) was responsive to the SIP method as Fₘ' was detected and slow PQH₂ oxidation was clear. Isolates A2 (Figure 3.1D) and D1a (Figure 3.5) showed slow PQH₂ oxidation after only the first 6 to 8 saturating pulses.

Phylotype C2 revealed a unique signature of chl-a fluorescence that was consistent under all temperatures (Figure 3.3B). The only changes were the total amount of fluorescence which diminished gradually with increased temperatures and prolonged exposure to 32°C. After the first 5 saturating pulses, Fₘ' remained stable while F₀' increased between each pulse due to slow PQH₂ oxidation possibly a result of chlororespiration or slow enzyme activity. Inactive ferredoxin-NADP⁺-reductase (FNR) or ribulose-1,5-biphosphosphate-carboxylase/oxygenase (RuBisCO) could prevent passage of electrons from ferredoxin to NADP⁺ or NADP(H) to carbon fixation reactions, respectively. These potential points of slowed electron transport caused F₀' to initially increase due to electron blockage and subsequently, F₀' decreased while Fₘ' remained stable due to the resulting damage to PSII which was enhanced by high temperatures.
The maximum dark-acclimated quantum yield \([F_v/F_m = (F_m-F_0)/F_m]\) was used as a quantitative measurement to interpret changes in the efficiency of photochemistry as the isolates were exposed to high temperatures. All isolates had an inverse relationship between \(F_v/F_m\) and increased temperatures (Figure 3.6A). F2, D1a, C1, and A2 were the most resilient phylotypes to the long-term 32°C conditions while A3, B1, and B2.1 were hypersensitive to high temperatures. The moderately sensitive C2, A1, and A4a showed a greater decrease in \(F_v/F_m\) relative to the first day at 26°C than the most resilient isolates and less than the moderately sensitive types. When the recovery time point was included (Figure 3.6B), the slopes of the percent change in \(F_v/F_m\) and resulting sensitivity categories changed slightly. Most phylotypes recovered at least partially when temperatures were abruptly returned to 26°C with the exception of cultures D1a and A3 which did not survive the recovery period. Phylotypes varied in the amount of recovery of \(F_v/F_m\), for example both isolates of clade B had the most \(F_v/F_m\) increase during the recovery time period while subclades A3, A4, and D1a showed the least. F2, C1, and A2 remained the most resilient isolates as these cultures showed the least sensitivity to high temperatures and yielded pre-treatment levels of \(F_v/F_m\) at the end of the recovery period. Isolate A1 showed sensitivity to the high temperature treatment, but was able to recover as indicated by the \(F_v/F_m\) after 25 days at 26°C. In addition, D1a, A4a, B1, C2 were classified as moderately sensitive. D1a did not survive the recovery period, so the slope of the change in \(F_v/F_m\) was steeper with the recovery point included. B1 was sensitive to high temperatures, but was able to recover. Isolates A3 showed no recovery and B2.1 only slight recovery of photochemistry but
there was fluorescence indicating presence of live cells, thus their consistent appearance in the hypersensitive category.

**D1 Protein Analysis**

With increased temperatures, the SIP measurements revealed decreased overall chl-a fluorescence in all clades likely due to damage to the D1 reaction center protein. In A1, A3, and A4a, activity of photosynthetic protection pathways that divert energy from PSII to PSI could diminish the amount of fluorescence. From 26°C to the first day at 32°C, phylotypes A1, A3, A4a, B1, B2.1, and C1 had notable decreases in D1 protein (Figure 3.7). However, they began with lower amounts of protein when compared with the other phylotypes. A2, C2, F2, and D1a maintained high amounts of D1 protein during the treatment period. Most phylotypes recovered their amounts of D1 protein during the 25 day period where the cultures were maintained at 26°C, though recovery amounts were specific to each isolate. Cultures D1a and A3 did not survive the 25 day recovery period so we were not able to show a distinct band for this time point (Figure 3.7).

**DISCUSSION**

Prolonged exposure to high ocean temperatures has been demonstrated as a major cause of photosynthetic damage in *Symbiodinium* (Iglesias-Prieto et al. 1992; Hoegh-Guldberg 1999; Warner et al. 2002). However, clade-specific responses to elevated temperatures do not usually exist (Tchernov et al. 2004; Takahashi et al. 2009). The distinct physiological characteristics of each *Symbiodinium* phylotype
affects their individual ability to respond to seasonal temperature fluctuations and especially during periods of prolonged high sea surface temperatures. Some isolates have been described previously as high temperature tolerant, like D1a (Baker et al. 2004) and others as low temperature tolerant such as B2.1 (Thornhill et al. 2008).

Exposure to extremely warm sea surface temperatures often with the added effects of high irradiances causes the loss of PSII function (Warner et al. 1996; Hill et al. 2004; Middlebrook et al. 2008) by damaging the integral D1 protein (Warner et al. 1999). If the damage is severe enough, death of the symbiont can occur. Protections of the photosynthetic apparatus exist to increase the potential for survival of the symbiont and as a result, the host. For example, it has been shown that PSI is not as sensitive as PSII to high temperatures (Havaux 1996). Therefore, CET around PSI likely remains active when PSII becomes damaged in order to generate additional ATP to fuel CO₂ fixation (Rumeau et al. 2007) and to minimize electron pressure on PSII. Not all Symbiodinium show active CET around PSI (Reynolds et al. 2008), which indicates other photosynthetic protection mechanisms must be used in all clades when damage to PSII occurs. Excess light energy, which becomes more detrimental with fewer functioning PSII, can be dissipated through nonphotochemical processes like PCP/LHC dissociation, a state transition-like event (Iglesias-Prieto and Trench 1994; Reynolds et al. 2008), which may stimulate CET (Rumeau et al. 2007). Xanthophyll de-epoxidation also uses light energy that may otherwise over-reduce PSII and its electron acceptors, thus protecting them from photodamage. Though these and other photoprotections exist to perpetuate survival of the organisms when they are exposed to high irradiance conditions, not all have been examined in detail in a broad spectrum of
Symbiodinium isolates to determine their activity under high temperature stress. Here, the presence of CET and NPQ while temperatures were slowly increased from 26°C to 32°C and maintained at 32°C for 9 days was monitored using the SIP method at each day. However the development of techniques independent of chl-a fluorescence to monitor alternative electron pathways are needed to determine if PSI-dependent CET is active during times of severe PSII dysfunction caused by warm temperatures.

Isolates could not be grouped according to clade by their thermal tolerance (Tchernov et al. 2004), but common cladal characteristics did exist. Symbiotic clade A Symbiodinium, A1, A3 and A4a, isolated from a Caribbean scleractinian coral, western Pacific giant clam, and a Caribbean gorgonian (LaJeunesse 2001), respectively, revealed clear SIP-induced CET at all temperatures where chl-a fluorescence could be detected. At 30°C, they showed decreases in total fluorescence levels, the earliest noticeable effect from the increased temperatures in all isolates. They also did not have as much D1 protein, and likely, had fewer functioning PSIIIs than the other 7 isolates. CET may have protected the remaining functional PSIIIs when isolates A1, A3, and A4a were exposed to high temperature conditions that cause degradation of their core protein complexes. Phylotype A3’s dramatic $F_m$ decreases in response to each saturating pulse indicated the presence of NPQ, likely partially due to LHC/PCP dissociation as was seen previously (Reynolds et al. 2008). However, even at the lowest levels of chl-a fluorescence where $F_m$ were detectable, CET activity was clear. CET may have enhanced NPQ mechanisms (Hennige et al. 2008) which would have caused additional decreases in the level of chl-a fluorescence. However, it is unknown how long CET around PSI can maintain ATP production for survival of the organism.
under a period of high temperature stress. Also, it is unclear to what extent CET may drive NPQ mechanisms following a prolonged temperature stress event. In these isolates of Symbiodinium, a 25-day period may not have been long enough to complete the protein recovery process due to the sensitivity to warm temperatures and likely some of the damage incurred was irreversible. It is unclear how long these cultures may take to recover completely.

Phylotypes A2, C1, and F2 maintained high total fluorescence during the entire treatment period indicating consistent PSII activity. A2 and C1 were isolated a Caribbean gorgonian and corallimorph, respectively, while F2 was isolated from a Caribbean scleractinian coral. Isolates F2 and A2 likely were cryptic free-living Symbiodinium species when originally cultured (LaJeunesse 2001) and appear resilient when exposed to warm temperature conditions that present severe stress and damage in other Symbiodinium isolates. The total fluorescence of the A2, C1, and F2 isolates decreased as temperature increased, but photosynthetic activity remained high, assessed by the maximum quantum yield and the SIP-probing. Phylotype F2 has been shown to be thermally tolerant when exposed to warm temperatures even under high light stress (Robison and Warner 2006). Total fluorescence from isolates A2, C1, and F2 recovered fully after the cultures completed a 25-day recovery period at 26°C. Each of these isolates also had only small decreases D1 protein amounts at each time point, including the recovery.

Clade D has been documented as a “heat tolerant” species that sometimes appears in hospite after severe bleaching episodes (Baker 2001; Baker et al. 2004), though documentation of existence of clade D in the Caribbean is not common even
after a period of thermal stress (Thornhill et al. 2006). Here, phylotype D1a showed high tolerance when exposed to 32°C for 9 days, and when SIP-probed, D1a showed little change in fluorescence levels or patterns throughout the treatment period. Isolate D1a actively engaged NPQ and had similar D1 protein content levels to A2, C1, and F2, the other heat-tolerant phylotypes. However, D1a did not survive the recovery period where fluorescence and D1 protein levels were negligible, though it is unclear at which point the isolate is dead as compared with having inactive PSIIIs that are in the process of repair. Lack of recovery of D1a may have been due to culture contamination, nutrient deficiency, or to the inability of this culture to survive after a period of high temperature stress.

Isolated from the same species of scleractinian coral from the western Atlantic, phylotypes B1 and B2.1 had similar patterns of fluorescence in response to the SIP treatment, though B1 is known as a “cold tolerant” species (Thornhill et al. 2008). B1 and B2.1 revealed slow post-pulse PQ oxidation when compared with other isolates except C2 and F2. The speed of post-pulse PQ oxidation increased with higher temperatures, consistent with other experiments (Rumeau et al. 2007). B1 and B2.1 both experienced a gradual decrease in total fluorescence as temperatures increased until day 5 at 32°C when the cultures appeared dead. However, by the end of the recovery period both cultures showed fluorescence levels indicative of relatively normal photosynthetic activity. Phylotype B2 had a higher $F_v/F_m$ after 25 days at 26°C than at the initial 26°C time point. Both phylotypes were isolated from the same species of coral in the same location and show similar tolerance to increased temperatures, but they also have slightly different abilities to recover.
The C2 isolate consistently revealed a fluorescence pattern different from all the others including C1. The levels of total fluorescence decreased gradually throughout the entire experiment and though the culture was still alive, the total fluorescence after 25 days at 26°C remained at the low. The distinct SIP-induced fluorescence pattern with slow post-illumination PQH₂ oxidation caused an increase in \( F_0^- \) until the 5th or 6th saturating pulse while \( F_m^- \) decreased. After the 6th saturating pulse, \( F_0^- \) decreased while \( F_m^- \) increased. Slow activation of RuBisCO and the passage of electrons from NAD(P)H to the process of carbon fixation likely caused the \( F_0^- \) increases during the middle and end of the SIP treatment (Jones et al. 1998). C2 may also be deficient in PSI which would result in \( F_0^- \) increases after exposure to multiple saturating pulses.

Long-term warm temperature exposure differentially influences the photosynthetic function of *Symbiodinium* isolates. Protections to the integral photosynthetic apparatus exist, but may be compromised when severe heat stress is present. Tolerance of prolonged 32°C temperatures here was isolate-specific with some cultures showing more resilience to the warm temperatures and others with a greater ability to recover when temperatures were lowered. Different *Symbiodinium* species have varying methods for surviving elevated temperatures. For example, symbiotic members of clade A utilize CET to maintain photosynthesis when PSII is severely damaged. Other phylotypes have a relatively high amount of D1 protein and are able to maintain active photosynthesis even when some PSIIIs are damaged. This study provides isolate-specific information about resilience to warm water temperatures, including the ability to recover, and the protections to the photosynthetic apparatus that continue to be utilized during such a stress event.
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Fluorescence (relative values)

Time (seconds)

30  390  720

A

B

$F_{m}'$

$F_{0}'$

ML

$F_{m}$

25°C

30°C

32°C

Day 9 at 32°C

Recovery

87
Figure 3.1. SIP detection of post-illumination plastoquinone reduction in symbiotic clade A *Symbiodinium* and not free-living clade A during exposure to prolonged warm temperatures. Phylotypes A1 (A), A3 (B), and A4a (C) *Symbiodinium* showed enhanced capacity for CET at temperatures up to 30°C. After exposure to a weak measuring light (ML), progressive increases in minimum fluorescence (*F₀´*) levels reflect substantial post-illumination plastoquinone (PQ) pool reduction. After the 5th or 6th saturating pulse, light-induced plastoquinol oxidation and PQ dark re-reduction occur. Decreases in maximum fluorescence (*Fₘ´*) were indicative of NPQ mechanisms likely light harvesting complex/peridinin-chlorophyll-a-protein complex dissociation in conjunction with xanthophyll de-epoxidation. After exposure to 30°C, noticeable decreases in total fluorescence were clear due to temperature-induced damage of PSII reaction centers. We were unable to detect any post-illumination *F₀´* changes using the SIP method during the days with the highest temperature stress because of the lack of PSII fluorescence. CET may have been active during these times to maintain ATP production. Phylotypes A1 (A) and A4a (C) showed recovery in the form of higher chl-a fluorescence after 25 days at 26°C following 9 days at 32°C. A3 (B) showed no recovery as measured by the SIP method. The free-living A2 *Symbiodinium* had stable fluorescence in response to the SIP treatment under increased temperatures (D) with no post-illumination PQ oxidation and re-reduction. As temperature increased, the chl-a fluorescence decreased due to photodamage. After 9 days at 32°C, both the *Fₘ´* and *F₀´* decreased, indicative of damage to the photosystem, but after 25 days at 26°C, fluorescence levels recovered completely.
Figure 3.2. CET is not active in phylotypes B1 or B2.1 *Symbiodinium* under control or prolonged high temperatures. Even in the same clade, *Symbiodinium* phylotypes have different responses to high temperatures. Both B1 (A) and B2.1 (B) showed similar sensitivity to daily temperature increases of 1°C as the levels of total fluorescence decreased due to thermal sensitivity of PSII. They had similar slow post-illumination PQ oxidation at the control temperature of 26°C. After a 9 day exposure to 32°C, neither culture responded to the SIP treatment as a result of the damage to PSII that caused flat-line fluorescence. However, differential recovery was observed as culture B1 showed much less chl-a fluorescence than culture B2.1 after 25 days at 26°C.
Figure 3.3. Individual members of clade C *Symbiodinium* yield phylotype-specific fluorescence patterns when SIP-probed. C1 *Symbiodinium* (A) consistently showed stable fluorescence in response to the SIP treatment independent of temperature stress. Total fluorescence decreased as temperature was increased to 32°C and remained at 32°C for 9 days. The C1 culture showed a complete recovery when temperatures were returned to 26°C. Saturating pulses initiated slow PQH₂ oxidation until 31°C where the $F₀¹$ levels returned to baseline after each saturating pulse. C2 *Symbiodinium*, in contrast to C1, showed a chl-a fluorescence pattern when SIP-probed that is completely unique (B). Similar to other cultures, total fluorescence levels decreased with increasing temperature. Damage to the PSII reaction center and its proteins increased during the treatment period. The SIP method revealed very slow plastoquinol oxidation after the first 3-4 saturating pulses causing an increase in $F₀¹$. After the 5th pulse, the speed of plastoquinol oxidation increased and revealed a decrease in $F₀¹$ between saturating pulses. A congruent decrease in $Fₘ¹$ with decreased in $F₀¹$ indicates a block in the electron transport chain due to slow enzyme activity. Inactive ferredoxin-NADP⁺-reductase (FNR) or RuBisCO could prevent passage of electrons from ferredoxin to NADP⁺ or NADP(H) to carbon fixation reactions, respectively. These potential points of slowed electron transport caused initial $F₀¹$ increases from electron blockage and then $F₀¹$ decreases due to the resulting damage enhanced by high temperatures.
Figure 3.4. Phylotype D1a reveals strong nonphotochemical quenching at all temperatures. D1a showed large $F_{m'}$ decreases when SIP-probed indicating strong activity of NPQ mechanisms. $F_{0'}$ increased at all temperature time points but initially from slow PQ oxidation after the first 3-4 saturating pulses. $F_{0'}$ after the 4th or 5th saturating pulse may have been due to slow enzymatic activity which blocked the passage of electrons to carbon fixation mechanisms. The fluorescence of D1a decreased gradually as temperature increased 1°C per day and under prolonged warm temperatures, though not to severely low levels. Following the temperature treatment and after 25 days at 26°C, D1a unexpectedly showed no response to SIP-probing and had very low levels of fluorescence indicating a dead culture.
Figure 3.5. F2 *Symbiodinium* maintains photosynthetic ability under high temperatures. Phylotype F2 showed little PSII damage when exposed to prolonged high temperatures. It also showed a complete recovery when temperature was returned to the control level of 26°C. F2 had a stable pattern of chl-a fluorescence including slow
post-pulse plastoquinol oxidation after all saturating pulses. When the temperature was maintained at 32°C for 9 days, however, the post-pulse plastoquinol oxidation was slow after the first 5-6 saturating pulses only. The slow plastoquinol oxidation during the first part of the SIP treatment caused the initial $F_0'$ increase, while the continued increase of $F_0'$ resulted from light inputs from the saturating flashes incrementally oxidizing more plastoquinol. Interestingly, the recovery time point revealed a slightly different fluorescence pattern than seen previously. Similar to the 9th day at 32°C, slow plastoquinol oxidation caused increases in initial $F_0'$ levels. However, after the 8th saturating pulse, the speed of plastoquinol oxidation between each saturating pulse was slow and then rapidly increased causing the fluorescence to reach a point lower than the initial $F_0'$ immediately following the next saturating flash. The increased speed of plastoquinol oxidation induced by the saturating pulses from the SIP method reflected in chl-a fluorescence was likely due to the rate at which $Q_B$ can pass electrons to PQ coupled with slow enzymatic activity of FNR and/or RuBisCO.
Slope of linear regression from % of control of $F_1/F_n$

Symbiodinium phylotype

Hypersensitive

Moderately Sensitive

Resilient
Figure 3.6. High temperature stress caused differential damage of photosynthetic capacity of 10 *Symbiodinium* phylotypes. Maximum quantum yield \( \frac{F_v}{F_m} = \frac{F_0 - F_m}{F_0} \) was used as an initial indicator of the photosynthetic capacity of each *Symbiodinium* phylotype during every day of treatment from 26°C to 32°C, 9 days at 32°C, and then a return to 26°C for 25 days. Each point represents the slope of the linear regression from a scatter plot of the percent change of \( \frac{F_v}{F_m} \) from the initial time point throughout the course of the warm temperature treatment period for an individual culture. Points without the recovery time point (A) and with the recovery time point (B) were plotted to emphasize the importance of examining \( \frac{F_v}{F_m} \) not only during a stress event, but also during a period of recovery following the stress event. Most phylotypes have different damage and recovery rates as measured by SIP fluorescence.
techniques. The most resilient phylotypes were F2, C1 and A2. Phylotypes with the most dramatic recoveries include A1, A4a, and B1 showing damage at the highest temperatures but also the most substantial recovery in $F_v/F_m$ when the temperature was decreased to 26°C. Phylotype D1a had high levels of $F_v/F_m$ throughout the entire experiment, but died during the recovery from unknown reasons. Thus, this is the only culture whose slope of $F_v/F_m$ was higher without the recovery point included. B2.1 showed the lowest $F_v/F_m$ values, and inclusion of the recovery time point did not matter as the slopes of $F_v/F_m$ were almost identical.
Figure 3.7. Change in *Symbiodinium* D1 protein amounts from 26°C, after prolonged exposure to high temperatures at 32°C and followed by 25 days of 26°C. Ten isolates were sampled at 4 time points for D1 protein content and are labeled accordingly. The bands indicate relative amounts of D1. The clade D isolate was only sampled after 5 days at 32°C because the samples were lost for the other time points. Other blank spaces indicate lost sample for that time point.
Table 3.1. Host and geographic origin of cultured isolates from LaJeunesse (2001).

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<td>370</td>
<td>Symbiodinium</td>
<td>A1</td>
<td>Red Sea, Gulf of Aqaba</td>
<td>Stylophora pistillata (Scleractinaria)</td>
</tr>
<tr>
<td>97</td>
<td>Symbiodinium sp.</td>
<td>A2</td>
<td>Caribbean, Puerto Rico</td>
<td>Gorgonia ventallina (Gorgonacea)</td>
</tr>
<tr>
<td>350</td>
<td>Symbiodinium corculorum</td>
<td>A2</td>
<td>Western Pacific, Palau</td>
<td>Corculum cardissa (Bivalvia)</td>
</tr>
<tr>
<td>292</td>
<td>Symbiodinium sp.</td>
<td>A3</td>
<td>Western Pacific, Palau</td>
<td>Tridacna maxima (Bivalvia)</td>
</tr>
<tr>
<td>PHMS TDle 3.4</td>
<td>Symbiodinium sp.</td>
<td>A3a</td>
<td>North Pacific, Philippines</td>
<td>Tridacna derasa (Philippines)</td>
</tr>
<tr>
<td>379</td>
<td>Symbiodinium sp.</td>
<td>A4a</td>
<td>Caribbean, Bahamas</td>
<td>Plexaura homamalla (Gorgonacea)</td>
</tr>
<tr>
<td>146</td>
<td>Symbiodinium sp.</td>
<td>B1</td>
<td>Western Atlantic, Bermuda</td>
<td>Oculina diffusa (Scleractinaria)</td>
</tr>
<tr>
<td>141</td>
<td>Symbiodinium sp.</td>
<td>B2.1</td>
<td>Western Atlantic, Bermuda</td>
<td>Oculina diffusa (Scleractinaria)</td>
</tr>
<tr>
<td>152</td>
<td>Symbiodinium goreau</td>
<td>C1</td>
<td>Caribbean, Jamaica</td>
<td>Discosoma sancti-thomae (Corallimorph.)</td>
</tr>
<tr>
<td>203</td>
<td>Symbiodinium sp.</td>
<td>C2</td>
<td>Western Pacific, Palau</td>
<td>Hippopus hippopus (Bivalvia)</td>
</tr>
<tr>
<td>A001</td>
<td>Symbiodinium sp.</td>
<td>D1a</td>
<td>Northern Pacific, Japan</td>
<td>Acropora sp.</td>
</tr>
<tr>
<td>133</td>
<td>Symbiodinium sp.</td>
<td>F2</td>
<td>Caribbean, Jamaica</td>
<td>Meandrina meandrites (Scleractinaria)</td>
</tr>
</tbody>
</table>
Table 3.2. Isolate-specific fluorescence responses at low, high, prolonged high and recovery temperatures.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>26°C</th>
<th>32°C</th>
<th>Day 9 at 32°C</th>
<th>Recovery at 26°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CET</td>
<td>NPQ</td>
<td>Low fluorescence; CET</td>
<td>Low Fluorescence</td>
</tr>
<tr>
<td>A1</td>
<td></td>
<td></td>
<td>Low fluorescence; CET</td>
<td>Low Fluorescence</td>
</tr>
<tr>
<td></td>
<td>Slow PQH₂ oxidation</td>
<td>Stable F₀⁻ and Fₘ⁻</td>
<td>Slow PQH₂ oxidation between first 5 pulses; NPQ</td>
<td>Full recovery; Slow PQH₂ oxidation between first 5 pulses; F₀⁻ increase</td>
</tr>
<tr>
<td>A2</td>
<td>CET</td>
<td>NPQ</td>
<td>Low fluorescence</td>
<td>Low Fluorescence</td>
</tr>
<tr>
<td>A3</td>
<td>CET</td>
<td>NPQ</td>
<td>Low Fluorescence</td>
<td>Low Fluorescence</td>
</tr>
<tr>
<td>A4a</td>
<td>CET</td>
<td>NPQ</td>
<td>Low fluorescence; Fₘ⁻ peaks visible</td>
<td>Low Fluorescence</td>
</tr>
<tr>
<td>B1</td>
<td>Slow PQH₂ oxidation</td>
<td>Slow PQH₂ oxidation with possible NPQ</td>
<td>Stable F₀⁻ and Fₘ⁻; Full Recovery</td>
<td></td>
</tr>
<tr>
<td>B2.1</td>
<td>Slow PQH₂ oxidation</td>
<td>Stable F₀⁻ and Fₘ⁻; Decrease in total fluorescence</td>
<td>Low Fluorescence</td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>Stable F₀⁻ and Fₘ⁻; Decrease in total fluorescence</td>
<td>Stable F₀⁻ and Fₘ⁻; Decrease in total fluorescence</td>
<td>Stable F₀⁻ and Fₘ⁻ with a full recovery</td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>Very slow PQH₂ oxidation; Increase in F₀⁻ after first 6 pulses with stable Fₘ⁻ then decrease in F₀⁻ with decrease in Fₘ⁻</td>
<td>Very slow PQH₂ oxidation; Decrease in total fluorescence but same pattern as 26°C</td>
<td>Low total fluorescence with same pattern as 26°C</td>
<td>Low total fluorescence but Fₘ⁻ peaks present</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1a</td>
<td>Slow PQH₂ oxidation after 1st 4 pulses; NPQ</td>
<td>NPQ</td>
<td>NPQ</td>
<td>Low fluorescence</td>
</tr>
<tr>
<td>-----</td>
<td>-------------------------------------------</td>
<td>-----</td>
<td>-----</td>
<td>-----------------</td>
</tr>
<tr>
<td>F2</td>
<td>Stable $F_0^\prime$ and $F_m^\prime$; Slow PQH₂ oxidation between pulses</td>
<td>Slow PQH₂ oxidation between pulses resulting in slight $F_0^\prime$ increase; NPQ</td>
<td>Slow PQH₂ oxidation between first 5 pulses; NPQ</td>
<td>Recovery; Slow PQH₂ oxidation between first 5 pulses; NPQ and $F_0^\prime$ increase</td>
</tr>
</tbody>
</table>
CHAPTER 4

RESILIENCE OF SHALLOW-WATER CORAL-DINOFLAGELLATE SYMBIOSES: THE EFFECTS OF SEASONAL OCEAN TEMPERATURE AND HIGH IRRADIANCE ON THE PHOTOBIOLOGY OF *SYMBIODINIUM IN HOSPITE*.\(^3\)

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ABSTRACT
Seasonal influence on photosynthetic physiology of dinoflagellate endosymbionts of the genus *Symbiodinium* was investigated for three common Caribbean coral species, *Porites astreoides, Montastraea faveolata, and Siderastrea siderea* for 17 months in the northern Florida Keys. Use of photosynthetic protection mechanisms, maximum photosynthetic efficiency, genetic identity, and chlorophyll and cell densities were monitored. Additionally, coral fragments were exposed to increased irradiance to encourage physiological stress responses. Seasonal fluctuation in sea surface temperature promoted corresponding changes in all variables monitored. However, only the clade A symbionts of *P. astreoides* was the alternative photosynthetic pathway cyclic electron transport documented. With increased irradiance, photosynthetic efficiency measured by the maximum quantum yield ($F_v/F_m$) decreased in *M. faveolata* and *S. siderea*, and sometimes in *P. astreoides*. In October 2006, the end of summer, symbionts of *P. astreoides* showed no difference in $F_v/F_m$ between ambient and prolonged high light treatments. When exposed to the warm temperatures of summer and high light conditions, *P. astreoides* maintained constant densities of chlorophyll while *M. faveolata* revealed an increase and *S. siderea* showed a decrease in chlorophyll density. These physiological responses are consistent with ecological specialization of coral species. *P. astreoides* and *M. faveolata* are able to maintain photosynthesis in shallow-water high irradiance conditions while *S. siderea* lives in more shaded areas of the reef and was negatively impacted by light stress. Distinct physiological responses of *Symbiodinium* to long-term, seasonal ocean temperature changes may provide a predictive measure of how some corals will survive when
exposed to stressful environmental conditions such as high light or prolonged warm sea surface temperatures.
INTRODUCTION

Coral reefs are common to tropical, nearshore oceans around the world. Scleractinian, or reef building, corals are host to many symbionts, but the photosynthetic endosymbiotic dinoflagellates in the genus *Symbiodinium* (Freudenthal) are especially important as they supply corals with over 90% of their energy requirements for reef building (1, 2). *Symbiodinium* are a diverse group, categorized in clades A – H (3, 4), which have developed distinct physiological adaptations for survival in specific irradiance and temperature regimes (5-11). Like all photosynthetic organisms, *Symbiodinium* change the way they utilize and dissipate sunlight throughout the year and during each day. Diurnally, short-term photoacclimation mechanisms actively adjust to changing light conditions (6, 7, 12-14). Over the long-term, coral symbionts developed adaptations to their habitat which correspond to host biogeography (15-17), including ocean depth (10, 17, 18) and seasonal environmental fluctuations (19). Host-specificity also influences the ability to acclimate and most Caribbean corals maintain very stable symbioses over many years (20-22). As a result, the symbionts have developed mechanisms to survive typical environmental changes. Daily and seasonal temperature and irradiance regimes as well as symbiont composition within a coral colony are important factors when determining how acclimations are utilized effectively and may potentially provide resistance to some corals when stressful conditions are presented (17, 23, 24).

Photosynthetic acclimation processes utilized by *Symbiodinium* are altered according changing irradiance and sea surface temperatures, but not all *Symbiodinium* have the same capacity for surviving potentially stressful conditions (25). For example,
clade A *Symbiodinium* are only found on shallow-water reefs (1–3 meters in depth) most commonly in the Caribbean (15). As a result, they have retained unique photosynthetic protection mechanisms encouraging their survival (13, 26) including the alternative electron transport pathway cyclic electron transport (CET) (27). Some clade A *Symbiodinium* have shown resilience of photosynthetic membranes and protein synthesis (25, 28, 29) when exposed to warm water temperatures. Other *Symbiodinium*, specifically members of clade D, also appear stress-tolerant when exposed to very warm ocean temperatures (24, 30, 31). The use of photosynthetic protection mechanisms such as nonphotochemical quenching (NPQ) (28, 32-34), including xanthophyll de-epoxidation within intrinsic light harvesting complexes (12, 13), are integral to the ability of *Symbiodinium* to maintain active function. Over the long-term, photoacclimation processes including the adjustment of size and number of photosynthetic units (6, 35) and extrinsic peridinin-chlorophyll-a-protein complexes (PCP) (7) are utilized. *Symbiodinium* also produce mycosporine-like amino acids (36) which protect the coral and symbiont from ultraviolet light (37, 38), and they have the ability to engage antioxidant systems (39).

In order to understand the relationship between photosynthetic capacity, use of active alternative photoprotection pathways, chlorophyll (chl) and dinoflagellate cell content, 3 coral species from Admiral Reef in the northern Florida Keys were monitored every 3 to 4 months from March 2006 to August 2007. Activity of photosynthetic protection mechanisms in *Porites astreoides* (Lamarck), *Montastraea faveolata* (Ellis and Solander), and *Siderastrea siderea* (Ellis and Solander) was monitored seasonally and for four days during the application of increased irradiance. In addition,
Symbiodinium cell and chlorophyll densities were also quantified seasonally and after light treatments in March and August 2007 with the coolest and warmest sea surface temperatures to mimic an event that could cause stress to the symbiosis.

Host survival is dependent not only on its own life history strategy, but also the ability of its symbionts to acclimate and continue photosynthesis during times of stress. While function of the photosynthetic apparatus is critical, the seasonal change in structure can predict vulnerability of corals if a stress event occurs. Corals exhibit lower tissue and pigment densities in the warmer summer months (9, 19) while Symbiodinium chl and cell densities negatively correlate to increased sea surface temperatures (SST) and solar radiation (17, 40, 41). During the summer, Symbiodinium often does not show visible signs of bleaching, but reveals lower cell and photosynthetic pigment densities which correspond to lower coral host tissue levels (9). If warm summer temperatures are prolonged, visual paling or bleaching may occur. Usually during the following winter, as sea surface temperatures decrease, the density of Symbiodinium cells and pigments increase allowing for recovery of the symbiosis (40, 42).

Seasonal changes in photosynthetic physiology occur (9), but it is not yet clear how all species of corals and their symbionts are affected. Throughout each year, corals experience fluctuations in the abundance of their symbionts and the amount of photosynthetic pigments in Symbiodinium which increase or decrease according to seasonal sea surface temperatures (40, 42). Under such conditions, visual bleaching may or may not occur (19). Some damage to the photosynthetic apparatus is almost always present during each day (23) but usually recovers overnight. Damage may become chronic if high sea surface temperatures are prolonged and combined with high
irradiance to which many corals are exposed (32, 33). This study addresses the seasonal influence on physiological photosynthetic processes, including photoacclimation and photoprotection mechanisms *in hospite* to determine how and which corals may survive in a time of global change. We propose that CET is an integral photoprotection and may afford the clade A *Symbiodinium* of *P. astreoides* greater resistance to the damaging synergistic effects of high light and temperature.

**MATERIALS AND METHODS**

*In hospite* seasonal chlorophyll fluorescence – ambient sea surface temperatures combined with daily increases in irradiance.

Corals were collected from Admiral Reef (1-3 m) off Key Largo, Florida in approximately three to four month intervals March 2006 to August 2007. Coral fragments (6-10 cm²) of *Porites astreoides*, *Montastraea faveolata*, and *Siderastrea siderea* were collected from the unshaded tops of six distinct colonies between 0800 and 1030 each morning. Coral fragments were collected with a rock pick and hammer and then placed in a labeled plastic bag and transported to the laboratory in a cooler filled with seawater. Each coral fragment was placed in an individually marked glass beaker and put into a circulating water bath that was set at ambient seawater temperature. Beakers were randomly relocated at least 4 times per day. The water in each beaker was changed every 4 hours throughout the experiment. The seawater was collected daily from Admiral Reef.

Sea surface temperatures were collected hourly by the National Oceanic and Atmospheric Administration National Data Buoy Center, C-MAN Station on Molasses
Reef (25°0'36" N 80°22'48" W), which is near Admiral Reef in the upper reef tract of the Florida Keys.

Corals were maintained at the control irradiance intensity that was approximately equal to that on Admiral Reef during the collection day using shade cloth. The following 2 days, light intensity was increased and then the corals were maintained at the highest intensity for 1 more day. Light intensity fluctuations were seasonally dependent and measured with a LiCor light sensor. The midday light intensity was approximately 250 $\mu$mol quanta m$^{-2}$·s$^{-1}$ on day 1, 750 $\mu$mol quanta m$^{-2}$·s$^{-1}$ on day 2, and between 1250 $\mu$mol quanta m$^{-2}$·s$^{-1}$ and 1650 $\mu$mol quanta m$^{-2}$·s$^{-1}$ on days 3 and 4.

Serial irradiation pulse (SIP) measurements (27) were collected each day one hour after dark using a Diving PAM fluorometer (Walz, Germany) configured with a blue LED measuring light (ML) source in March 2006, June 2006, August 2006, October 2006, January 2007, March 2007 and May 2007. Baseline PSII fluorescence ($F_0$ and $F_0'$) was monitored by a modulated ML (<1 $\mu$mol quanta m$^{-2}$·s$^{-1}$). Maximum fluorescence ($F_m$ and $F_m'$) was measured while administration of a 1-s pulse of saturating light (470nm, $\approx$5000 $\mu$mol quanta m$^{-2}$·s$^{-1}$). The maximum dark acclimated maximum quantum yield of PSII ($F_v/F_m$, where $F_v$ = $F_m-F_0$) measurements were also recorded along with $F_v'/F_m'$ values.

**Chlorophyll from whole *Symbiodinium* cells isolated from coral tissue**

All coral tissue in a 16 to 20 cm$^2$ area was removed from the coral skeletons using a recirculating WaterPik™ with filtered seawater (45-µm). The saltwater “blastate” was homogenized and mucopolysacharides dispersed using a Tissue Tearor (Biospec Products Inc.). *Symbiodinium* cells for genetic analysis were separated by
centrifugation at 1000-g and preserved in DMSO buffer (20% dimethyl sulfoxide and 0.25 M EDTA in NACl-saturated water) (43). *Symbiodinium* genotype was identified using PCR-DGGE of the internal transcribed spacer region 2 (ITS2) of the ribosomal RNA (44, 45).

Homogenate for *Symbiodinium* cells for chl-a content analysis was subsampled twice (15-mL), cells separated by centrifugation (1,500-g for 5 minutes), and frozen overnight to break open the cells. After extraction in 90% acetone, chl-a contents were measured using the equations of (46). For *Symbiodinium* cell counts, a small aliquot (1-mL) of homogenate was preserved with formalin. Number of cells was determined by replicate counts using a haemocytometer (n=9). Waterpiked coral surface areas were calculated by the aluminum foil method (47) and used to determine densities of *Symbiodinium* cells cm⁻².

In March 2006, June 2006, August 2006, January 2007, and May 2007 cells were extracted under control conditions and chlorophylls were isolated to create a baseline for possible seasonal changes in fluorescence. In March and August 2007 cells were collected and chlorophylls isolated both before and after increased irradiance treatments to determine effect of light intensity on physiological characteristics at a cool and warm time of year.

**Data Analysis**

All statistical analyses were conducted using analysis of variance followed by a means comparison of all pairs using Tukey-Kramer HSD at α = 0.05. When necessary, multivariate analysis was completed by calculating Pearson correlation coefficients.
RESULTS

Seasonal SIP and $F_v/F_m$ response and correlation to phylotype identity.

Seasonal *Symbiodinium* phylotype identification revealed stable symbioses with *P. astreoides*, *M. faveolata*, and *S. siderea* from Admiral Reef, Florida Keys, consistent with previous long-term data sets (Thornhill 2006) and was independent of sea surface temperature (Figure 4.1). *P. astreoides* maintained a symbiosis with specialist A4a and *M. faveolata* with generalist B1. *S. siderea* was the only coral with slight variation in phylotype composition, but the corals always maintained symbioses with specialists B5a and/or C3. However, June 2006 was the only date where B5a and C3 occurred together in some colonies. All other dates, 2 of the colonies were in symbiosis with B5a and 4 of the colonies with C3.

The SIP technique was used to probe for alternative photosynthetic pathways in *Symbiodinium* species in symbiosis with *P. astreoides*, *M. faveolata*, and *S. siderea* (Figure 4.2). *P. astreoides*, harboring phylotype A4a *Symbiodinium*, during all seasons and under both ambient and high irradiance revealed the use of CET and/or chlororespiration. NPQ was also commonly utilized, likely a result of light harvesting complex (LHC) and PCP dissociation (Reynolds et al. 2008) as well as xanthophyll de-epoxidation (12, 13, 26). High irradiance slightly lowered overall fluorescence and encouraged NPQ as confirmed by the decrease in $F_{m^*}$, but it did not affect the use of CET in *P. astreoides* (Figure 4.2A). *M. faveolata* initiated NPQ when exposed to high irradiance especially during the warmest months. The amount of NPQ increased after multiple days of high irradiance and was accompanied by slightly lower overall fluorescence during seasons with the warmest sea surface temperatures with
accompanying decreases seen in the $F_m^*$ and $F_0^*$ (Figure 4.2B). *S. siderea* engaged NPQ during ambient and high irradiance at all temperatures (Figure 4.2C). During August and October 2006, the summer months with the warmest sea surface temperatures, photodamage was also observed in some colonies of *M. faveolata* and *S. siderea*. Any seasonal photodamage was not chronic because low irradiance intensity from thunderstorms on the fourth day of the high light treatment in August 2006 allowed for a day of recovery after which all corals showed increased fluorescence (data not shown).

Under ambient irradiance conditions of approximately 250 $\mu$mol quanta m$^{-2}$s$^{-1}$, all *Symbiodinium* species in symbiosis with the 3 coral species showed similar responses of $F_v/F_m$ to seasonal temperature regimes (Figure 3.3). A significant effect of season on $F_v/F_m$ occurred in March 2006 where *P. astreoides* and *M. faveolata* had higher $F_v/F_m$ than *S. siderea* ($P = 0.0002$) and where *P. astreoides* had a higher $F_v/F_m$ than *S. siderea* in August 2006 ($P = 0.0468$) and March 2007 ($P = 0.0441$). When irradiance was gradually increased over 4 days, most species showed a very significant negative response in $F_v/F_m$ (Figure 4.3). However, in October 2006, the warmest time during the 17 month monitoring period, *Symbiodinium* A4a in symbiosis with *P. astreoides* revealed no affect of irradiance on $F_v/F_m$ (Figure 4.3) likely due to its ability to perform alternative electron transport pathways which can protect PSII from excess light energy. In August 2006, though there was a significant effect of irradiance intensity on $F_v/F_m$ in *P. astreoides* ($P < 0.0001$), both $F_v/F_m$ values were the highest during the 17 months of sampling. The *Symbiodinium* of *S. siderea* revealed greater sensitivity to photosynthetic loss at all time points. *M. faveolata* *Symbiodinium* yielded the lowest
quantum efficiency of PSII when exposed to high irradiance (≈ 1500 µmol quanta m⁻²·s⁻¹) during seasons with the warmest temperatures.

**Seasonal Effect on Chlorophyll Density**

Sea surface temperature significantly affected the amount of chl-a per unit area (µg chl-a cm⁻², P < 0.0001). The highest amount of chl-a cm⁻² was seen in the cooler winter months while the lowest amounts were observed in the warmer summer months (Figure 4.4A), consistent with previous observations (19). *P. astreoides* and *M. faveolata* revealed the highest levels of chl-a cm⁻² while *S. siderea* had the lowest levels. From January 2007 to August 2007, *M. faveolata* presented a downward trend of chl-a cm⁻² that was lower than the previous year, potentially signaling problems if dramatic winter recovery does not take place.

*M. faveolata* showed slight decreases in average chl-a cell⁻¹ from March 2006 to August 2007 (Figure 4.4B) and the lowest overall amounts compared to the other coral species. *S. siderea* has a similar pattern of decreasing chl-a cell⁻¹, but began significantly higher than *M. faveolata* and *P. astreoides* in March 2006 (P = 0.0009) and June 2006 (P < 0.0001). For the remainder of the time points, average chl-a cell⁻¹ did not differ between *S. siderea* and *P. astreoides*. *P. astreoides* maintained significantly greater amounts of chl a cell⁻¹ from March to August 2007 than *M. faveolata* (Fig. 4.4B). Both *M. faveolata* (P = 0.033) and *S. siderea* (P = 0.0002) had lower chl-a cell⁻¹ in March 2007 compared with March 2006. *P. astreoides* showed no difference in chl-a cell⁻¹ level between March 2006 and March 2007. Similar to chl-a cm⁻², 2007 levels of chl-a cell⁻¹ were lower than 2006 levels.
M. faveolata revealed higher Symbiodinium cell densities than P. astreoides and S. siderea for almost all seasons ($P < 0.0001$) except March 2006 where cell densities of both M. faveolata and P. astreoides were higher than S. siderea ($P = 0.0243$) (Figure 4.2C). P. astreoides cell density decreased between March and June 2006 and maintained lower levels through August 2007, though not always as low as S. siderea who showed the lowest cell densities from March 2006 to August 2007.

In March 2006, June 2006 and January 2007, with some of the coolest SSTs, chl-a cm$^{-2}$ was positively correlated with cell density. In addition, the amount of chl-a cell$^{-1}$ was negatively correlated with cell density in June 2006. There were no other correlations.

When irradiance was added as a variable in addition to sea surface temperature, seasonal variation between March (24°C) and August (30.5°C) in chl-a cm$^{-2}$, chl-a cell$^{-1}$, and Symbiodinium cell densities in each species of coral were recorded. The chl-a cm$^{-2}$ in all 3 coral species in March 2007 was not affected by irradiance level (Figure 4.5A). However, in August 2007 with warmer ocean temperatures, a significant increase of in response to high irradiance was seen in M. faveolata ($P = 0.0073$) and a significant decrease in chl-a cm$^{-2}$ in S. siderea ($P = 0.0366$), and P. astreoides did not change (Figure 4.5B). While irradiance had no effect on all coral species in March 2007 (Figure 4.5C), P. astreoides was the only coral to exhibit an increase of chl-a cell$^{-1}$ ($P = 0.0153$) in August 2007 (Figure 5D). Increased light intensity had a negative effect on cell density in P. astreoides ($P = 0.0095$) in March (Figure 4.5E) and August 2007 ($P = 0.0153$) (Figure 4.5F) and had a slight effect on M. faveolata ($P = 0.0659$) in March.
High irradiance had no effect on cell density of *M. faveolata* in August 2007 and never significantly influenced *S. siderea* (Figure 4.5E, F).

**DISCUSSION**

The ability of *Symbiodinium* to survive seasonal environmental changes is complex. In order to most clearly understand adaptability of *Symbiodinium* to environmental stresses, it is necessary to consider specific *in hospite* symbioses and where possible, investigate long-term trends to capture physiological change over time. *Symbiodinium* and their cnidarian hosts respond to seasonal fluctuation of sea surface temperature and irradiance by acclimating and activating appropriate photoprotections (6, 7, 12, 26, 27, 48). The present study examined seasonal changes in the photosynthetic physiology of distinct *Symbiodinium* phylotypes *in hospite*. In addition, this study encouraged physiological stress, especially in times of warm ocean temperatures, by experimentally increasing irradiance to mimic conditions which could cause bleaching (19).

The identity of *Symbiodinium* phylotype *in hospite* is necessary when probing for physiological trends in coral symbionts. Symbioses between corals and *Symbiodinium* in the Florida Keys and Caribbean have been documented as extremely stable and predictable over many years (20, 21). Here, *Symbiodinium* genotypic identity was closely linked to host species and was very predictable and stable in *P. astreoides*, *M. faveolata*, and *S. siderea*. *P. astreoides* and *S. siderea* maintained symbioses with less common specialists, phylotypes A4a and C3 and/or B5a respectively, while *M. faveolata* with phylotype B1, a more common generalist species of *Symbiodinium* (15). Each
genetically distinct *Symbiodinium* showed unique physiological mechanisms for survival in their individual habitats on the same reef.

Seasonal utilization of alternative photosynthetic pathways was documented and compared among *Symbiodinium* species using the SIP method. Though all corals were collected from the same reef and approximate depth, species-specific physiological responses to light stress differed. CET and NPQ were detected under both low and high irradiance conditions during each season in the A4a *Symbiodinium of P. astreoides*. The observed CET fluorescence pattern yielded decreased or stable \( F_{m}^- \) and increased \( F_0^- \) in conjunction with post saturating pulse dips which were indicative of dark plastoquinol oxidation and fast re-reduction of the plastoquinone pool (27). \( F_{m}^- \) decreases observed in *P. astreoides* were most likely a result of NPQ from PCP and/or LHC dissociation (27) and activation of xanthophyll de-epoxidation (12, 13, 26). *P. astreoides* rarely showed signs of paling in color between seasons and light treatments, unlike the other species. CET can maintain ATP production and likely preserve host-symbiont survival during times of irradiance and/or temperature stress which damage PSII (28). Photodamage was most common in *S. siderea* when exposed to high irradiance, and was more severe in colonies with C3 *Symbiodinium*. On the fourth day of the high irradiance treatment in August 2006, recovery during a day of thunderstorms occurred, though fluorescence levels remained lower than the control indicating that photodamage existed and was at least partially reversible. Symbionts of *S. siderea* were adapted to lower irradiance levels from shading by sea fans or other corals on Admiral Reef, thus were more vulnerable to damage by high irradiance.
Under ambient irradiance levels, the maximum quantum yield of PSII showed predictable seasonal variation and was highest during the winter and early spring and lowest during the end of summer, consistent with prior studies (40, 49). The conditions during which the $F_v/F_m$ measurements were recorded did not cause coral bleaching, as supported by the seasonally stable Symbiodinium cell and chl-a density, nor did they cause irreversible damage to PSII. In addition, the symbionts of all three coral species maintained similar photosynthetic capacities between seasons (23, 48) suggesting that any variation observed would be present during years with typical temperature regimes in the Florida Keys.

When high light conditions ($> 1250 \mu \text{mol m}^{-2}\cdot\text{s}^{-1}$) were imposed and combined with ambient sea surface temperature, photochemical efficiency of Symbiodinium decreased at almost every seasonal time point as a result of photodamage and photoacclimation processes (6, 7, 10, 12, 50). However, in October 2006 when sea surface temperatures maintained summertime levels of approximately 31°C, maximum photosynthetic efficiency of P. astreoides was not different between low light and high light conditions. Similarly, Cassiopeia xamachana did not show late summer photoinhibition, common in symbiotic cnidarians, possibly due to a decrease in light penetration through jellyfish tissues (40, 51), but also because C. xamachana is known to harbor phylotype A1 Symbiodinium. Clade A Symbiodinium has been previously documented as bleaching resistant Symbiodinium clade (26, 48, 52), and P. astreoides as a bleaching resistant coral (48, 53). The clade A Symbiodinium of P. astreoides consistently maintained a significantly higher $F_v/F_m$ and PSII activity compared to the clade B and C symbionts of S. siderea after high irradiance exposure, suggesting
photoprotective ability and strong survivorship when exposed to the combined effects of high irradiance and warm sea surface temperatures (26, 48).

Seasonal changes in sea surface temperatures encouraged predictable patterns of *Symbiodinium* cell density and pigment content (9, 40). At no point during the sampling period, however, did chl-a densities decrease to levels that would indicate the inability to recover. Season had a significant effect on pigment content where *P. astreoides* maintained a higher amount of chl-a cm⁻² compared to *S. siderea* during each season. *M. faveolata* did not always differ in chl-a cm⁻² from *P. astreoides* and *S. siderea*, but always had the highest *Symbiodinium* cell density and usually the lowest amount of chl-a cell⁻¹ suggesting that the B1 *Symbiodinium* are adapted to shallow-water, high light conditions that encourage lower chl densities. Instead, *M. faveolata* colonies have thick coral tissue which can accommodate higher dinoflagellate cell densities (9). *S. siderea*, which grew in more shaded areas on the reef, revealed chl-a amounts and cell densities consistent with a shade-adapted species (54) since C3 *Symbiodinium* is commonly found in deep water or shaded areas on the reef (32). However, the seasonal downward trend of chl-a cell⁻¹ and upward trend of *Symbiodinium* cells cm⁻² during the sampling period suggests acclimation to higher irradiance in *S. siderea* colonies since the photosynthetic capacity remained stable.

Warm ocean temperatures of summer combined with high irradiance can cause seasonal coral paling or severe bleaching due to decreased *Symbiodinium* cell and/or chl densities (9). Under the influence of warm ocean temperatures in August 2007, *M. faveolata* showed a significant increase in chl-a cm⁻² after high light exposure suggesting the ability of B1 *Symbiodinium* to acclimate to increased irradiance likely to
meet the coral host’s energy demands (55). In contrast, *Symbiodinium* chl density within *S. siderea* colonies decreased in response to high irradiance indicating an inability of the shade-acclimated species to withstand high light conditions when combined with the warm ocean temperatures of August. Under the same conditions, amounts of chl-a cell\(^{-1}\) in *P. astreoides* increased while cell density decreased and chl-a cm\(^{-2}\) remained relatively unchanged between high and low irradiance. *P. astreoides* was able to acclimate to increased irradiance and host demands by maintaining stable chl densities independent of sea surface temperature.

Describing the complexity and unique photosynthetic physiology and activity of distinct phylotypes of *Symbiodinium* leads to a greater understanding of how some cnidarian species survive normal seasonal environmental variation and potentially stressful temperature and irradiance conditions. We have shown that phylotype A4a symbionts of *P. astereoides*, with the capability to perform CET, chlororespiration, and PCP/LHC dissociation, likely afford protection against chronic photoinhibition as their chl densities remain unchanged when exposed to high irradiance and sea surface temperatures. *M. faveolata* has an unwavering symbiosis with B1 *Symbiodinium* in the Florida Keys (21) which also remain acclimated to high irradiance with the ability to avoid severe bleaching under normal ocean temperature fluctuations likely a result of long-term ecological adaptations (6, 10, 17, 56). However, during the summer with the warmest sea surface temperatures of the year, decreased photochemical efficiency and some mortality occurred when exposed to high irradiances. *S. siderea* with either C3 and/or B5a *Symbiodinium* was the most susceptible to conditions that may cause bleaching. It is clear that even on the same reef, differences exist between
photosynthetic capacity, use of photoprotections and photoacclimation during each season.

Examining long-term seasonal patterns of physiological responses of 3 coral species in symbiosis with distinct phylotypes of *Symbiodinium* to environmental fluctuations provides more information about how some coral-*Symbiodinium* symbioses survive in the face of global climate change and increased environmental pressures (57-61). Though *M. faveolata* and *S. siderea* may be adapted to survival on a shallow, high-light reef ecosystem, *P. astreoides* continues to thrive when exposed to the synergistic effects of high irradiance and warm sea surface temperatures because of their unique ability to protect photosynthetic processes and to maintain a balanced chlorophyll density which provides the host necessary nutrients for reproduction and reef building. *P. astreoides* has been documented as a coral species whose populations are increasing across the Caribbean while other species show decline (53). Likely this is partially due to the brooding life history strategy of *P. astreoides*, but the present study provides symbiont-specific physiological evidence that supports phylotype A4a in symbiosis with *P. astreoides* may be more resistant to conditions which may cause coral bleaching.
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Figure 4.1. Sea surface temperature and *Symbiodinium* genotype for marked colonies of *P. astreoides*, *M. faveolata*, and *S. siderea* from March 2006 to August 2007. Sea surface temperatures (SST) were recorded hourly by the National Oceanic and Atmospheric Administration’s National Buoy Data Center from Molasses Reef in the Florida Keys. *Symbiodinium* genotype was identified using denaturing gradient gel electrophoresis of the ITS2 ribosomal RNA site. Each pie chart represents 6 distinct colonies of 1 coral species. From left to right, the pie charts represent the corals *P. astreoides* (PA), *M. faveolata* (MF), and *S. siderea* (SS). Each *Symbiodinium* phylotype is represented by a color in the legend in the bottom left corner of the figure.
Figure 4.2. Saturating pulse irradiation (SIP) detection of alternative photosynthetic pathways in symbionts of *P. astreoides*, *M. faveolata*, and *S. siderea* during representative seasons. All 3 coral species harbored distinct *Symbiodinium* phylotypes, *P. astreoides* in symbiosis with phylotype A4a (A), *M. faveolata* with B1 (B) and *S. siderea* with C3 and/or B5a (C). Saturating pulses of light were administered every 20 seconds after applying a low-intensity measuring light (ML). Post-illumination plastoquinone reduction, interpreted from the progressive increase in \( F_0^- \) is indicative of cyclic electron transport (CET) and/or chlororespiration. The A4a *Symbiodinium* of *P. astreoides* (A) revealed consistent use of CET/chlororespiration independent of high irradiance treatment, while the clades B and C *Symbiodinium* found
in *M. faveolata* and *S. siderea* did not. After the fourth day of exposure to increased irradiance (or third day in August 2006), NPQ activity was more active in *P. astreoides* as compared with the first day. Decreases in maximum fluorescence (*F*$_{m}^\prime$), sometimes in conjunction with decreased minimum fluorescence levels (*F*$_{0}^\prime$) indicate the use of nonphotochemical quenching (NPQ) mechanisms. NPQ was regularly seen in *M. faveolata* (B) and *S. siderea* (C) after high irradiance exposure, though *S. siderea* also used NPQ when under ambient irradiance conditions.
Figure 4.3. Seasonal photosynthetic efficiency of \textit{P. astreoides}, \textit{M. faveolata}, and \textit{S. siderea} from March 2006 to August 2007. Dark-acclimated maximum quantum yield was measured one hour after sunset to allow for late-afternoon recovery of the photosynthetic apparatus. When exposed to ambient irradiance, \textit{P. astreoides} and \textit{M. faveolata} were significantly higher than \textit{S. siderea} in March 2006. In August 2006, \textit{P. astreoides} was significantly higher than \textit{S. siderea}, but \textit{M. faveolata} did not differ from any other species. In March 2007, \textit{P. astreoides} was significantly higher than the other species. Significant effects of high irradiance were seen at all seasons in \textit{M. faveolata} and \textit{S. siderea}. However, at the end of the summer, in October 2006, \textit{P. astreoides} did not show a significant effect light on photosynthetic efficiency suggesting this species
may be well acclimated for high irradiance, warm sea surface temperature conditions.

For all points, n=6 and $\alpha=0.05$. 
Figure 4.4. Seasonal *Symbiodinium* chl-a density, cellular chl-a content, and cell densities of symbionts in *P. astreoides*, *M. faveolata*, and *S. siderea* from March 2006 to August 2007. Seasonal patterns in amount of chl-a cm⁻² (a), average chl-a cell⁻¹ (b), and million cells cm⁻² (c). Significant effects of species were seen for every variable measured at all seasons by analysis of variance and then between species differences were confirmed by Tukey/Kramer HSD. The significant effects between species are notated by a different letter next to each point (a, ab, b, or c) at α=0.05. In general, during the cooler months, the corals had higher concentrations of algal cells and higher densities of chl-a cm⁻². Error bars represent standard error, n=6.
Figure 4.5. Comparison of the effects of high (black bar) and low (grey bar) irradiance on *Symbiodinium* chl-a density, cellular chl-a content and cell density during March and August 2007. The corals examined here reveal a high tolerance to maximum irradiance levels when acclimated to both cool (March – A, C, E) and warm...
(August – B, D, F) sea surface temperatures. Significant effects of irradiance level are indicated by * above the bars in the graph at $\alpha=0.05$. Both *M. faveolata* and *S. siderea* yielded decreased chl-a cm$^{-2}$ when exposed to high irradiance in August. *P. astreoides* chlorophyll density did not change when exposed to high irradiance because of the significant increase in chl-a cell$^{-1}$ even though a decrease in cell density occurred. Chl-a density cell$^{-1}$ of *P. astreoides* was highest while *M. faveolata* revealed the highest *Symbiodinium* cell density suggesting implementation of different acclimation strategies by these two species. Error bars represent standard error, n=6.
CHAPTER 5
CONCLUSION

Coral reef ecosystems are ancient and dynamic, but in recent decades they have been under a constant threat (Brown, 1997, Hoegh-Guldberg, 1999, Lesser, 2004). Global warming has altered sea surface temperature dynamics with warm temperatures for long periods of time becoming more common. Understanding how the coral reef ecosystem may survive stressful environmental conditions such as warm ocean temperatures especially combined with the synergistic effects of high irradiance is crucial to conservation and scientific studies of coral reefs and their surrounding ecosystems.

Cnidarians such as corals and their dinoflagellate endosymbionts of the genus Symbiodinium have unique relationships and depend on their combined tolerance to thermal and irradiance pressures. The ability of Symbiodinium to photosynthesize drives the maintenance of the symbiosis with their coral or other cnidarian host. The breakdown of Symbiodinium photosynthesis generally results in coral bleaching and if severe, the possible death of the host (Fitt et al., 2001).

Shallow-water clade A Symbiodinium is the most ancestral phylotype (LaJeunesse, 2001) and retained constitutive photoprotections that offer resistance to environmental stress such as high irradiance and warm ocean temperatures. Cyclic electron transport (CET) in conjunction with peridinin-chlorophyll a-complex (PCP) and light harvesting complex (LHC) dissociation from photosystem II (PSII) confers the
ability to cycle and deflect excess light energy, protecting the photosynthetic complex and maintaining photosynthesis (Reynolds et al., 2008). When thermal perturbations disrupt the processes of photodamage and repair of PSII, CET likely sustains the symbiosis between the cnidarian host and dinoflagellate symbiont. In addition, xanthophyll de-epoxidation contribution to nonphotochemical quenching and the production of mycosporine-like amino acids (MAAs) compliment CET and PCP/LHC dissociation to protect photosynthetic function.

The discovery of CET in clade A *Symbiodinium* relied upon the development of a novel fluorescence technique which provides a non-invasive technique for clade-level *Symbiodinium* identification *in hospite*. Typically, PSII photosynthetic efficiency is monitored by continuous actinic irradiation imposed with intermittent saturating flashes to measure the amount of nonphotochemical quenching, interpreted from decreases in maximum fluorescence ($F_{m}'$). The serial irradiation pulses (SIP) method measures chlorophyll-a fluorescence recorded by a modulated measuring light (<1 µmol m$^{-2}$·s$^{-1}$) while administering multiple flashes of saturating light ($\approx$ 5,000 µmol m$^{-2}$·s$^{-1}$) in the dark. The saturating pulses of the SIP method encourage the progressive reduction of the plastoquinone (PQ) pool, eventually resulting in increases in the levels of minimum fluorescence ($F_{0}'$) in phylotypes which are able to engage CET. When exposed to SIP, symbiotic clade A *Symbiodinium* show a unique fluorescence “fingerprint” that can be used as identification *in hospite*. In addition, though clades B – D and F do not show the typical CET fluorescence pattern, they too reveal “fingerprints” typical to their individual clades. Exceptions to clade-level SIP fluorescence “fingerprints” do exist, so future work is needed to identify unique clade or phylotype fluorescence patterns.
These phylotype-specific “fingerprints” could possibly lessen the damage to corals incurred by the destructive techniques that are currently used to identify *Symbiodinium* genotypes.

CET was detected in clade A *Symbiodinium* in studies presented here using *in hospite* corals and cultured isolates with the SIP method. During the course of one year, one study showed that CET was utilized by corals hosting clade A *Symbiodinium*, and not by corals harboring clades B or C. The SIP method revealed unique patterns of nonphotochemical quenching and photodamage in non-clade A *Symbiodinium*. In addition, through the use of studies using cultured isolates when exposed to prolonged warm temperatures, similar unique SIP-induced fluorescence patterns were revealed and give information about how different isolates survive high temperature stress. Some clade A *Symbiodinium*, which actively used CET as a photoprotection yielded almost flat-line $F_{0}^{′}$ and $F_{m}^{′}$ fluorescence at temperatures exceeding 31°C. As a result, the SIP method was unable to detect CET during the warmest conditions because of the lack of chlorophyll-a fluorescence. *In hospite* clade A *Symbiodinium*, when exposed to seasonal warm temperatures, revealed normal levels of fluorescence and active CET, though the sea surface temperature did not exceed 31°C during the study period. Further studies using cultured *Symbiodinium* should focus on detection of CET when it is likely a dominant means for electron flow such as under high temperature conditions. Such environments encourage CET around photosystem I because of the lack of active PSIIIs which were damaged by the heat. CET would maintain ATP production and photosynthetic processes while PSIIIs are repaired and rebuilt. Long-term *in hospite* coral-*Symbiodinium* symbioses should also be monitored with the SIP-technique to
capture the effects of prolonged warm temperature conditions during an El Nino Southern Oscillation (ENSO) event which have become more common in past decades and resulted in widespread coral bleaching.

With the use of CET and LHC/PCP dissociation, corals hosting clade A Symbiodinium have a photosynthetic advantage when exposed to stressful environmental conditions. Porites astreoides is an example of a resilient coral-Symbiodinium symbiosis that is rarely threatened in the Caribbean (Green et al., 2008). P. astreoides colonies have grown and spread following ENSO bleaching events which negatively affected other corals, likely due to their life history strategy combined with photoprotective capabilities among other physical characteristics of both host and symbiont. Acropora palmata, a coral whose populations have been decimated by disease in the Caribbean since the 1970s, has reversed the trend and is showing population increases in the Florida Keys (Porter, pers. comm.). If A. palmata populations can withstand disease pressures, the use of CET by their clade A Symbiodinium will enhance their ability to survive the high light and potentially damaging warm temperatures in the shallow waters they inhabit. P. astreoides and A. palmata would be two corals to compare in long-term studies of Caribbean reef ecosystem change over time. Increasing populations of P. astreoides may signify a temporary or permanent phase-shift of coral populations while higher coverage of A. palmata represents reef recovery.

The discovery of cyclic electron transport in conjunction with CET in symbiotic clade A Symbiodinium is important to understanding how the coral-dinoflagellate symbiosis and coral reefs as we know them today may survive in response to our
rapidly changing climate. The biochemical pathways of photosynthesis, providing most of the corals’ energy demands for reef building are the basis for the many services provided by coral reef ecosystems worldwide. Future studies will further interpret how CET is used *in hospite* and whether it assists corals hosting clade A *Symbiodinium* in survival and recovery after the next significant bleaching episode in the Caribbean.
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