DISTRIBUTION, PHOTOBIOLOGY, SALINITY TOLERANCE AND POPULATION STRUCTURE OF *SIDERASTREA RADIANS* AND ITS SYMBIONTS IN FLORIDA BAY, FLORIDA USA

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ABSTRACT

The distribution of *Siderastrea radians* (Pallas) Blainville and photophysiology of its symbiont in Florida Bay were determined during annual macrophyte surveys in spring 2006 and 2007. *Siderastrea radians* was present in five of the eleven sampled basins in areas with little sediment and low abundance of the seagrass *Thalassia testudinum*. The five basins are located along a northeast-to-southwest transect that also represents a salinity gradient from inshore, predominantly estuarine conditions adjacent to the Everglades, to offshore near-marine salinities adjacent to the Gulf of Mexico. Colony abundance was highest in the two basins at the extremes of this physical range. Effective quantum yields, measured *in situ* by PAM fluorometry, were significantly correlated between *S. radians* and the dominant seagrass *T. testudinum* among all five basins where the two species co-occurred, albeit with a 40% reduction in mean yields of *S. radians.* These findings indicate *S. radians* may function as an alternative eco-indicator species for regions in which *T. testudinum* is absent and/or act as an indicator of stress for other benthic invertebrate taxa.

The tolerance of *S. radians* to hyposalinity was examined in a mesocosm study using salinity treatment levels based on historical salinity records for the basins where this species occurs. Colonies of *S. radians* collected from the five basins in Florida Bay (i.e., along the northeast-to-southwest salinity gradient) were used to assess effects of hyposalinity on the photophysiology of the symbiont, as an indicator of holobiont stress. Colonies from each basin were assigned four salinity treatments (30, 20, 15, and 10) and salinities were reduced 2 d^{-1} from ambient (30) to simulate a natural salinity decrease. Maximum (F_v/F_m) and effective ($\Delta F/F_m'$) quantum yields were measured using PAM-fluorometry at dawn and noon, respectively. Because of high variability in $\Delta F/F_{m'}$ in response to varying mid-day light conditions only F_v/F_m

measurements were used to assess salinity treatment effects. There was generally no decrease in F_v/F_m for 20 and 15 treatment colonies versus controls (i.e. 30) up to five days post-target salinity. This indicates a greater ability to withstand reduced salinity for relatively extended periods of time in *S. radians* compared to other reef species. When salinity of 10 was reached, there was a significant reduction in F_v/F_m versus 20 and 30, indicating a critical threshold for hypo-saline tolerance. At salinity of 10, colony yields from the more salinity-variable northeast basins versus the most marine southwest Twin Key basin (TWN) were significantly different, suggesting populational acclimation or adaptation in salinity tolerance corresponding with historical basin salinity ranges.

Genetic tools were used to determine whether differences among basin populations in photophysiological responses to hypo-saline stress were due to unique symbiont type among basin populations (i.e. adaptation) or to acclimation. Subcladal identification of *Symbiodinium* for each basin was completed using PCR-DGGE analysis of the ITS2 region of the rDNA. TWN colonies possessed an ITS2 variant distinct from all other basins, correlating with differences in photobiology measured during hyposalinity experiments. These findings suggest long-term differences among basin salinities lead to differential responses in the holobiont, which are related to a shift in symbiont photophysiology and symbiont adaptation.

Despite low overall cover and frequency, *S. radians* in Florida Bay is a valuable marginal population for investigating tolerance to physical stressors across a natural physical gradient. Coral populations residing in marginal coastal habitats may provide insights as to how these communities will respond to higher environmental variability under predicted future climate patterns. The coral *S. radians* occurs along a physical-environmental gradient in Florida Bay that has been variably influenced by anthropogenic impacts to its watershed that have primarily affected salinity variability across the Bay. It is unclear what effects projected increased freshwater inflow under the Comprehensive Everglades Restoration Plan (CERP) will have on this community, but the results presented here indicate a greater tolerance to hyposaline conditions than generally reported for corals.

ACKNOWLEDGEMENTS

I thank my outstanding advisor, Dr. Michael J. Durako. I could not have asked for a better mentor to complete my master's degree. He allowed me to pursue my interests in "stony plants" while introducing me to the wonderful world of Florida Bay. His enthusiasm, direction, and dedication to my work is what every student hopes for in an advisor. I also would like to thank the rest of my advisory committee, Dr. Joe Pawlik, Dr. Craig Bailey, and Dr. Diego Lirman. You have given me valuable guidance and I am forever grateful for your expertise in completing this work. I would also like to thank the very dedicated team in Florida, the Seagrass Rangers. Most importantly, Manuel Merello for dedicating his time and energy to my project when he had none to spare. I am also grateful to Jen Kunzelman, Fay Belshe, and Donna Berns for field support; Margaret Hall for logistical support; and Brooke Landry for her technical skills in ArcGIS, which I could never have figured out alone.

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DEDICATION

I would like to dedicate this work to my father, Stephen A. Chartrand. May I strive to have your “boundless energy, fastidious work ethic, unpretentious intellect, ready wit and undying enthusiasm for teaching others”.

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CHAPTER.1

DISTRIBUTION AND PHOTOBIOLOGY OF *SIDERASTREA RADIANS* AND *THALASSIA TESTUDINUM* IN FLORIDA BAY, FL USA

INTRODUCTION

Florida Bay is a shallow lagoonal system located at the land-sea interface between the southern tip of mainland Florida and the Florida Keys. The Bay is part of the larger South Florida hydroscape that extends from north of Lake Okeechobee in central Florida to the ridges of the Florida Reef Tract (Fig 1). Historically, Florida Bay acted as the receiving body for freshwater from Lake Okeechobee that moved southward through the Everglades and mixed with the marine waters flowing in through Florida Keys channels and the open western boundary of the Gulf of Mexico, creating a rich, productive transition zone. Soft carbonate-sediments in the bay support large seagrass beds dominated by *Thalassia testudinum* Banks ex König. Its dense canopy comprises approximately 92 percent of the habitat structure in the Bay sustaining a fish and invertebrate community critical to ecosystem function as well as the local economy (Chester and Thayer 1990, Herrnkind et al. 1994, Butler et al. 1995, Matheson et al. 1999, Major and Dutton 2002). The annual economic return of the *T. testudinum* habitat in Florida Bay to both fisheries and tourism has been estimated in the millions of dollars for the South Florida region (Robblee and DiDomenico 1991, Nance 1994, English et al. 1996).

Over the last century, human development and water management projects have perturbed the natural watershed for Florida Bay via construction of canals, levees, and intensified land use. Diversion of fresh waters towards the Atlantic and Gulf of Mexico, intensified farming and development, and exponential population growth in the latter half of the 20th century exacerbated problems by all but eliminating freshwater inputs to the Bay and leaving only 50%

of the original Everglades system intact (Light and Dineen 1994). Residual drainage from developed lands continue to enter the Bay at low levels in pulses from the canal and levee system, frequently with concentrated nutrient and contaminant loads (Lapointe and Clark 1992, Brand 2002). Irregular and patchily distributed rainfall events now contribute up to 90 percent of the freshwater supply to Florida Bay (Nuttle et al. 2000). Higher average salinities and poor water quality were correlated with a large die-off of *T. testudinum* in western basins beginning in the late 1980s (Robblee 1991). Re-suspension of sediments that increased turbidity and released trapped nutrients were linked to these losses, as were recurrent algal blooms and anoxic conditions that persisted in the Bay during the early 1990s (Fourqurean and Robblee 1999). Concern over the long-term health of Florida Bay and the adjacent Everglades led to the Comprehensive Everglades Restoration Plan (CERP). The primary goal of CERP is to restore South Florida's watershed and ecosystems via habitat and water quality modifications aimed at the re-establishment of natural drainage (CERP evergladesplan.org).

As part of CERP monitoring efforts, the Fish Habitat Assessment Program (FHAP), instituted in 1995 following the original seagrass die-off, was expanded from 10 to 22 basins in 2005 (now termed FHAP-SF). FHAP-SF performs annual surveys to monitor trends in macrophyte abundance, distribution, and photobiology along South Florida's (SF) coastal waters, including Florida Bay, as historical drainage pathways are restored (Durako et al. 2002). The dominant macrophyte in this region, *T. testudinum*, is perceived as the best indicator of the health of the Bay and its distribution, morphology and photobiology has been extensively studied since FHAP monitoring began in 1994 (Durako et al. 2002, Durako and Kunzelman 2002, Hackney and Durako 2004, Landry 2005, Belshe et al. 2007).

Although most of Florida Bay has soft carbonate sediments, exposed Pleistocene calcium carbonate deposits constitute large areas of hardbottom in the central portions of many basins (Merriam et al. 1989). In these areas the lack of sediment limits rhizome development in *T. testudinum*. Thus, *T. testudinum* surveys provide limited information on community dynamics in these areas and other species that rely on such hardbottom regions may be valuable for understanding wider system function. One taxonomic group largely overlooked in the Bay is hard corals. Anecdotal observations during annual FHAP surveys indicated the occurrence of *Siderastrea radians* within five of 10 surveyed basins (Durako pers. observ.). However, no records or publications exist, to the best of our knowledge, describing the abundance or spatial distribution of this coral population in Florida Bay. A small number of peer-reviewed papers have focused on corals, including *S. radians*, in this area; however, these studies focused on paleoclimatic records and photophysiological experiments of temperature-induced stress near the Florida Keys channel systems (Swart 1996; Warner et al 1996; Thornhill et al. 2006).

Siderastrea radians is one of three species of siderastreids found in the Caribbean region. *S. siderea* and *S. stellata* have been described as 'sibling species' to *S. radians* due to overlapping morphologies and life history traits (Laborel 1974, Werner 1996). These similarities make it difficult to distinguish these species in the field based on colony size, distribution, and corallite structure alone. *S. stellata* and *S. radians* are particularly difficult to distinguish due to their physical similarities and identical reproductive strategies despite new genetic patterns confirming *S. radians* and *S. stellata* as two species (Neves et al. 2008). *S. stellata* is described as endemic to Brazilian reefs (Neves and da Silveira 2003) while *S. siderea* is more commonly found at deeper depths on the reef, however these distribution patterns may not be as restrictive as previously assumed. Based on personal field observations, measured corallite morphologies, colony size, described distributions of these three species and the ecological relevance of this Florida Bay population as a whole, all siderastreids are described here as *S. radians* which agrees with previous descriptions of these local, inshore populations. Further systematic analysis is needed to resolve any taxonomic overlap within this genus in the South Florida region as no clarification has been made among these species outside of the Brazilian region (Neves et al. 2008).

The significance of the *S. radians* population in Florida Bay is twofold: 1) Their presence in hardbottom areas of basins may allow *S. radians* to act as a valuable alternate eco-indicator in Florida Bay in areas where *T. testudinum* is typically absent or sparse and 2) The photophysiology of *T. testudinum* can be compared to that of the coral endosymbiont, *Symbiodinium spp.*, within *S. radians* as an additional physiological indicator of ecosystem health.

Since 2000, pulse amplitude modulated (PAM) fluorometry has been used to assess the photobiology of *T. testudinum* in a non-invasive manner during annual FHAP surveys (Durako and Kunzelman 2002; Belshe et al. 2007). PAM fluorometry is a measure of chlorophyll fluorescence at PSII to indirectly assess the efficiency with which light energy is converted into chemical energy (Schreiber et al. 1986). PSII is considered to be the most sensitive part of the photosynthetic pathway to stress (Becker et al. 1990). This tool may provide early indications that changing environmental conditions—as may occur with restoration efforts in Florida Bay—are beneficial or detrimental to overall ecosystem health (Warner et al. 1996, Hoegh-Guldberg et al. 1999, Jones et al. 2000, Jones et al. 2001, Warner et al. 2002, Hill et al. 2004, Hill et al. 2005, Ralph et al. 2007).

To be used as an eco-indicator, baseline information is needed on population structure including spatial and seasonal dynamics as well as physiological response to shifts in environmental conditions (Carignan and Villard 2002, Biber et al. 2004). This study describes the distribution and chlorophyll fluorescence characteristics of *S. radians* and *T. testudinum* in Florida Bay during 2006 and 2007 FHAP surveys. The objectives of this study are: 1) To provide baseline distributional data on *S. radians* populations in Florida Bay and 2) To determine whether the photobiology of this coral population can provide relevant information on ecosystem health in Florida Bay to supplement *T. testudinum* data.

METHODS

Study Site

Florida Bay (ca. 25°05'N, 81°45'W) is located at the southern tip of mainland Florida, bordered by the Florida Keys to the south and east, and by the Gulf of Mexico to the west (Fig. 1). The Bay is divided into a suite of shallow (ca. <2m) basins by a reticulated network of carbonate mud banks and mangrove islands (Schomer and Drew 1982; Smith 2002). Water quality follows an environmental gradient as runoff and flow constraints in the northeast create highly fluctuating and extreme conditions, progressing to more stable marine conditions in the south-central portions of the Bay as mixing with Gulf and Atlantic waters increases. Water quality among adjacent basins can be strikingly different due to the isolation of waters by mudbanks and mangroves; low tidal flux; and the large role wind can play in directional water movement. Sediments are carbonate-based and in the west are deep and nutrient-rich while eastern regions tend to have shallower sediments and more hardbottom (Prager and Halley 1997, Zieman et al. 1989)

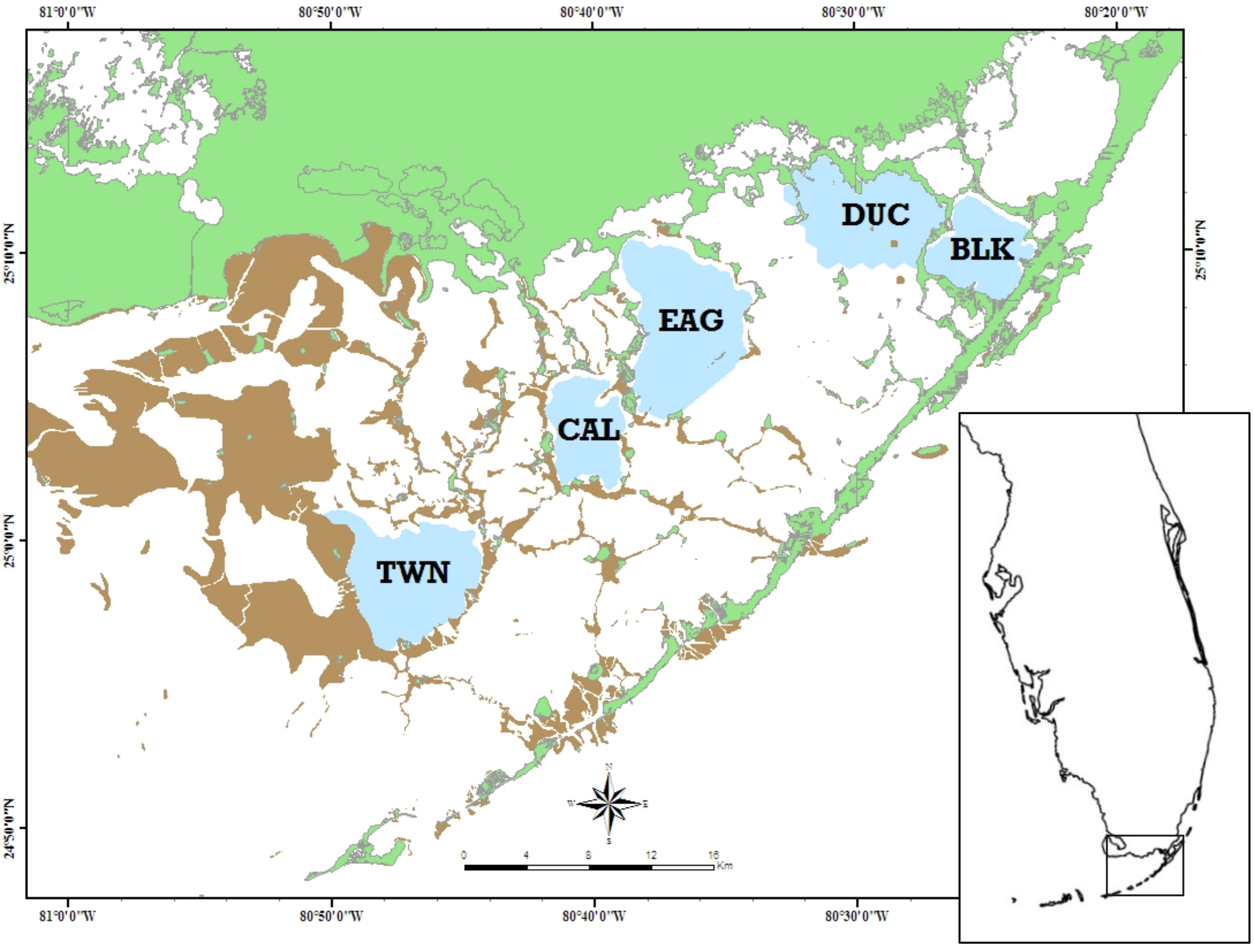


Fig. 1 Florida Bay, FL USA and basins surveyed in the current study.

FHAP-SF monitors eleven of approximately 50 basins in Florida Bay which represent the full range in physical-environmental conditions in the Bay. *Siderastrea radians* has been observed within five of the basins (see Table I for abbreviations used throughout this text) and consequently are the only basins discussed in the context of this study. These basins are located along a northeast-southwest transect that follow the environmental gradients described above (Fig. 1). Each sampled basin was subdivided into 28-30 tessellated hexagonal grids and one station randomly assigned within each polygon, to produce 28-30 stations sampled per basin. This design results in systematic random sampling which scales the sampling effort to basin size (Durako et al. 2002). Stations were located using GPS.

During May 2006 and 2007, eight replicate 0.25m^2 quadrats were haphazardly placed to score the presence and abundance of *T. testudinum* and *S. radians* at each station. A modified Braun-Blanquet abundance/density scale was used to assess frequency and density within quadrats (Table II; Braun-Blanquet 1972; Fourqurean et al. 2002). Frequency and density information for each species were calculated using the following formulas:

Frequency = # stations where observed/ total # of stations

Density = sum of Braun Blanquet scores/ # of quads

Transect surveys of *S. radians* were conducted in October 2006 at sites where colonies were recorded the previous May. Briefly, line transects were used in place of the Braun-Blanquet method to quantify local densities. This approach is similar to the quadrat method that was found to be the most accurate method available when determining species cover in coral mapping surveys (Jokiel et al. 2005, Leujak and Ormond 2007). A 50 m transect was established in an east-to-west orientation. Colonies were counted when present within 0.5m of each transect, equating to 25 m^2 of area covered per station. Data were compared with Braun-Blanquet scores collected in spring surveys.

Spatial Assessment

Distribution and density maps of both *T. testudinum* and *S. radians* were created in ArcGIS ArcMap v.9.2 according to Landry (2005). Individual sampling events for both species were transformed into shapefiles. For *T. testudinum*, grid surfaces were interpolated from Braun-Blanquet point values using the Inverse Distance Weighted function in the Spatial Analyst extension. This technique is better suited than krigging when dealing with patchy environments. It also is not smooth, meaning it does not ignore true values and it does not interpolate beyond maximum or minimum values observed. The Spatial Analyst extension applied a power of two to determine how much weight to assign distant data points. It also automatically uses a 12-point variable search radius to interpolate areas without data and an output cell size of 25 was assigned to increase boundary smoothness between areas. Basin outlines were applied as an analysis mask to keep interpolated surfaces within basin boundaries, and surfaces were classified according to Braun-Blanquet values and color-coded to represent the potential range from zero to 100% cover. Distribution of *S. radians* was overlayed on *T. testudinum* interpolated surfaces and classified by Braun-Blanquet values using graduated color-coded symbols.

Table I. Basin abbreviations used throughout the text.

ABBREVIATION	BASIN
BLK	Blackwater Sound
CAL	Calusa Key Basin
DUC	Duck Key Basin
EAG	Eagle Key Basin
TWN	Twin Key Basin

Table II. Modified Braun-Blanquet scale and corresponding percent of benthic substrate covered.

COVER CLASS	**DESCRIPTION**
0	Absent
0.1	Solitary Individual
0.5	Sparse and <5%
1	Numerous, but <5%
2	5%-25%
3	25%-50%
4	50%-75%
5	75%-100%

Photobiology

Chlorophyll fluorescence was recorded *in situ* using a diving-PAM fluorometer (Walz, Germany). Prior to the application of saturating pulse, minimum fluorescence for the ambient light state was measured (F). This steady-state fluorescence, F, represents the number of reaction centers open in the sample. The sample was then subjected to a pulse of saturating light during which a second fluorescence reading was taken (F_m'). F_m' is the state in which all reaction centers present are considered reduced or closed. Effective quantum yield of PSII ($\Delta F/F_m'$), a measure of photosynthetic capacity of light-acclimated tissues, was then calculated ($\Delta F/F_m' = F_m' - F/ F_m'$).

Healthy-appearing *S. radians* colonies and *T. testudinum* short-shoots separated by > 2m were haphazardly chosen by the diver at each station. The instrument's fiber optic end was set at a standard geometry (30^o from perpendicular) and distance (5-10 mm) to the dorsal surface of sampled corals and to the middle of the second youngest blade of seagrass short-shoots (n = 5 and n = 6 per station respectively) using a clear acrylic distance clip. Gain and measuring intensity were minimally adjusted to maintain an F of approximately 150-200 throughout the day. Bottom photosynthetic active radiation (PAR) was recorded using a 4π quantum sensor held just above the substrate.

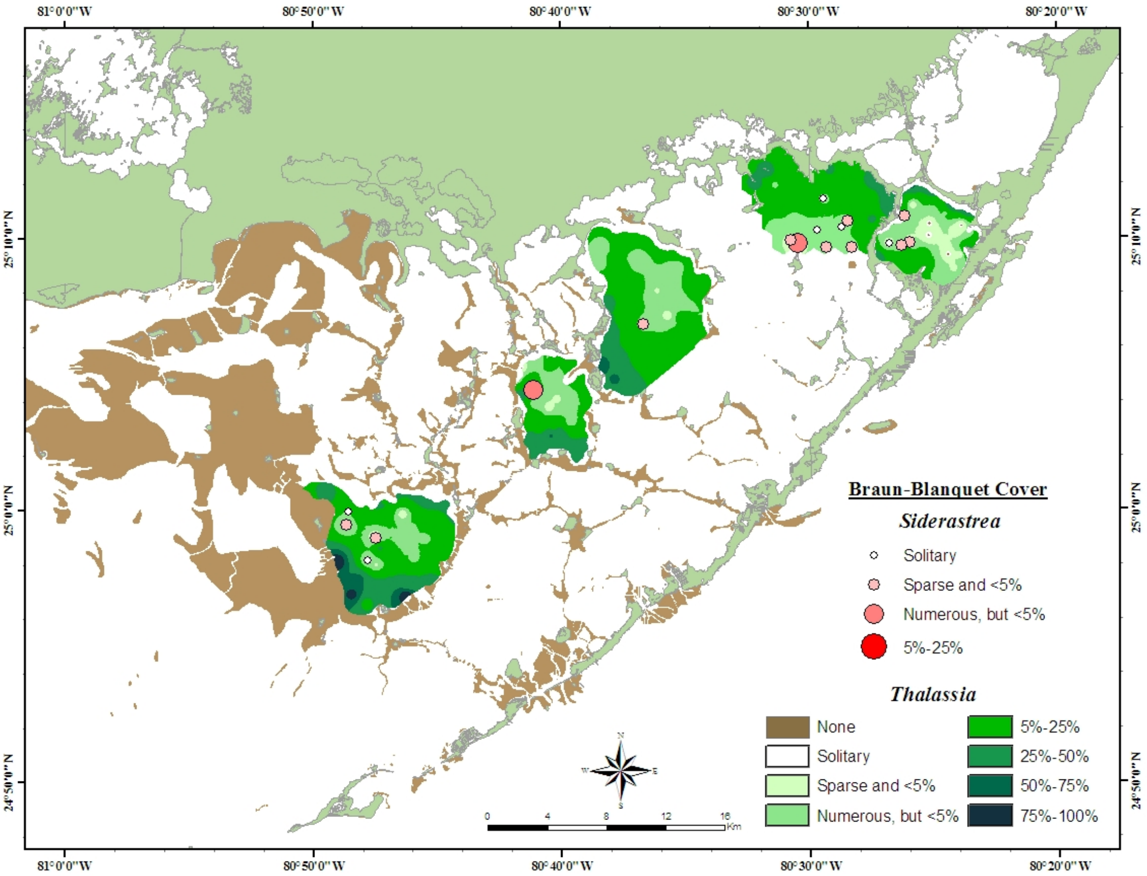
RESULTS

Distribution

Thalassia testudinum was observed at 97% of stations sampled while *S. radians* was observed at 10 and 15% of the stations in the 2006 and 2007 surveys, respectively. *Thalassia testudinum* did not appear to have any major shifts in cover between the two years except for small decreases in mean cover in BLK and TWN (Fig. 2, Table III). Coral colonies were present only where *T. testudinum* was less than 25% of benthic cover and mostly when *T. testudinum* cover was less than 5% (Fig 2). When present, *S. radians* never covered greater than 5% of the substratum; densities and cover were greatest in the northeast basins of BLK and DUC and southwest in TWN; lowest abundance occurred in EAG and CAL basins (Fig. 1). There was no significant change in cover between years, however, cover as determined by the Braun-Blanquet method, was in part low overall due to the small size of individual spherical-to-hemispherical colonies, which ranged in maximum diameter from 2.0 cm to 10.0 cm with an average of 4.5 cm. Thus, even though colony frequency may be high in a given sampling area, observed percent benthic cover within a quadrat was usually quite low due to small colony size. Line transects used to re-assess station densities of *S. radians* in October 2006 indicated cover in each basin to be comparable to values measured via the Braun-Blanquet method (Table III).

Frequency of *S. radians* was also greater in BLK, DUC, and TWN compared to CAL and EAG. DUC had the highest frequency of occurrence at stations surveyed in both years at 27% and 20% respectively followed by TWN at 13% and 16% (Table III). Colonies were rarely observed or absent among stations in both EAG and CAL at 3% and 0% for consecutive years. It is important to note that poor visibility during 2007 surveys due to rough windy conditions caused sediment re-suspension in addition to persistent algal blooms in some areas. This may have hampered visual assessments and thus lowered overall population estimates for this year. An increase in frequency of *S. radians* among basins positively correlated with shallower sediment depths while *T. testudinum* exhibited the opposite trend (Table III). There was no apparent trend in maximum colony diameter of *S. radians* colonies among basins (Table III).

a)


81°0'0"W
80°50'0"W
80°40'0"W
80°30'0"W
80°20'0"W
25°10'0"N
25°0'0"N
24°50'0"N
Braun-Blanquet Cover
Siderastrea
Solitary
Sparse and <5%
Numerous, but <5%
5%-25%
Thalassia
None
Solitary
Sparse and <5%
Numerous, but <5%
5%-25%
25%-50%
50%-75%
75%-100%
N
W
E
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0
4
8
12
16
Km

b)

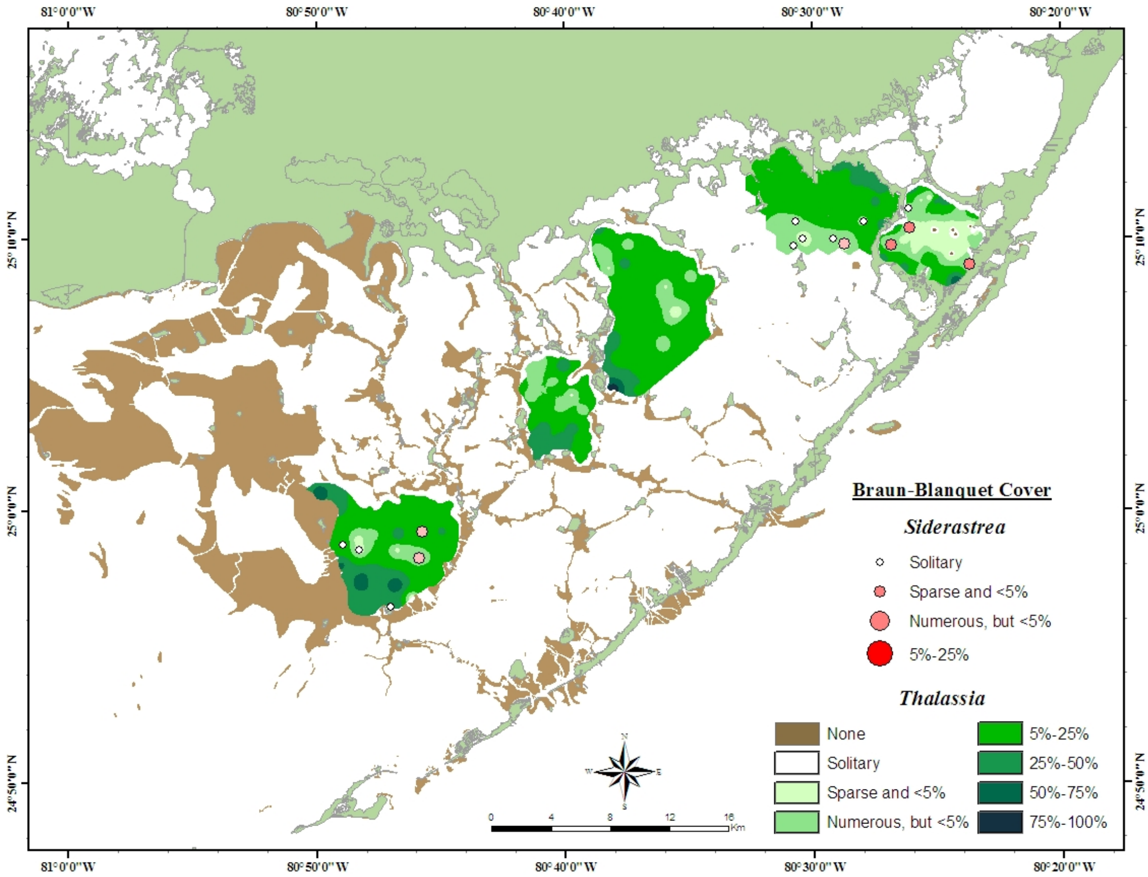


Fig. 2 Distribution and cover of *T. testudinum* and *S. radians* in Florida Bay among basins where they mutually occurred during a) 2006 and b) 2007 surveys.

Table III. Benthic surveys of *T. testudinum* and *S. radians* in 2006 and 2007 using Braun-Blanquet method. October 2006 transect surveys examine percent cover, and the mean of maximum diameter for colonies of *S. radians* within one station from each basin. SED represents mean sediment depth for stations where *S. radians* was present. Because colonies were not detected in CAL and EAG during 2007 surveys, sediment data were not available (indicated by —). Values are presented as mean (± S. D.).

BASIN	**SPRING FHAP**							**OCTOBER TRANSECTS**	
	TT-BB		**SIDR-BB**		**SIDR % FREQ**				**SIDR MAX DIAM.(cm)**
	06	**07**	**06**	**07**	**06**	**07**	**SED (cm)**	**%COVER**	
BLK	1.34 (1.25)	1.13 (1.23)	0.02 (.07)	0.02 (.06)	14	14	1.75 (2.06)	0.64	4.78
DUC	1.37 (.71)	1.49 (.64)	0.09 (.23)	0.01 (.03)	27	20	3.00 (1.89)	0.30	3.50
EAG	1.46 (.86)	1.55 (.68)	0.02 (.09)	0.0	3	0	—	0.02	4.35
CAL	1.43 (.79)	1.55 (.88)	0.02 (.09)	0.0	3	0	—	0.18	7.75
TWN	2.16 (1.6)	1.84 (1.10)	0.02 (.05)	0.02 (.04)	13	16	8.75 (4.34)	0.31	4.42

Photobiology

Effective quantum yields ($\Delta F/F_m'$) for both *S. radians* and *T. testudinum* exhibited significant among-basin variability. In 2006, these results are largely driven by the variability in ambient light among basins over the sampling period. $\Delta F/F_m'$ in both species negatively correlated with increasing bottom PAR (Fig. 3). These trends are not surprising as dynamic photoinhibition (or downregulation) is a typical photosynthetic response to high light whereby increasing fluorescence yields indicate higher photochemical efficiencies at PSII under reduced light (Hoegh-Guldberg and Jones 1999, Belshe et al. 2007). $\Delta F/F_m'$ values for *S. radians* were approximately 40% of those for *T. testudinum* (Fig. 3), but they were highly correlated ($p<0.05$, $r^2 = 0.72$) at stations where they co-occurred (Fig. 4).

In 2007, *S. radians* was not observed in CAL and EAG during photophysiological assessments of *T. testudinum*. Comparisons of mean $\Delta F/F_m'$ were thus limited to only BLK, DUC and TWN. *S. radians* in BLK and TWN exhibited similar patterns to those in 2006 in which mean $\Delta F/F_m'$ values were 40 to 50 % of those measured in *T. testudinum* (Fig. 5). However, in DUC, the two species exhibited similar mean $\Delta F/F_m'$ values. *T. testudinum* in DUC maintained $\Delta F/F_m'$ values reflecting the same trends in effective quantum yield under varying PAR as seen in 2006. Thus, the change in pattern in 2007 was a result of higher mean $\Delta F/F_m'$ versus bottom PAR measured in *S. radians* than in the previous year.

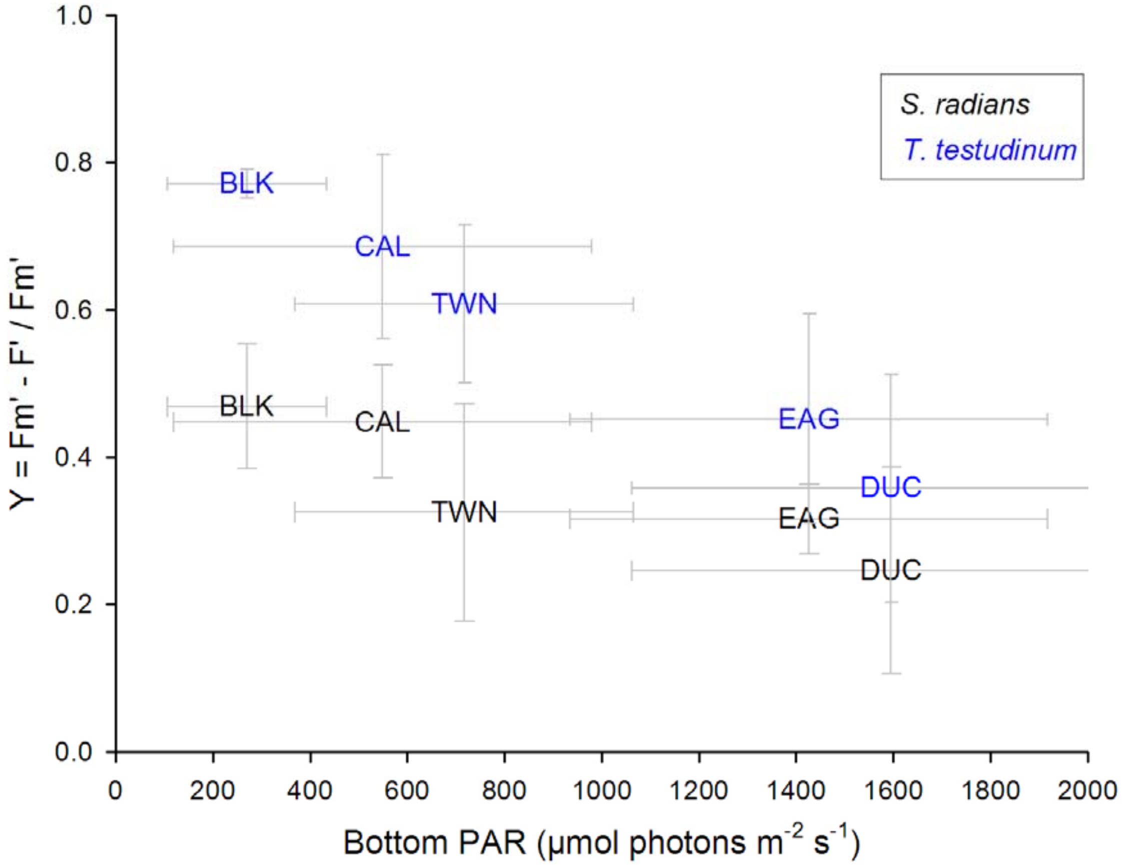


Fig. 3 2006 mean effective quantum yields ($\Delta F/F_m'$) for *S. radians* and *T. testudinum* versus mean daily bottom PAR measured among the five basins where *S. radians* and *T. testudinum* co-occurred. Error bars represent ± S.D.

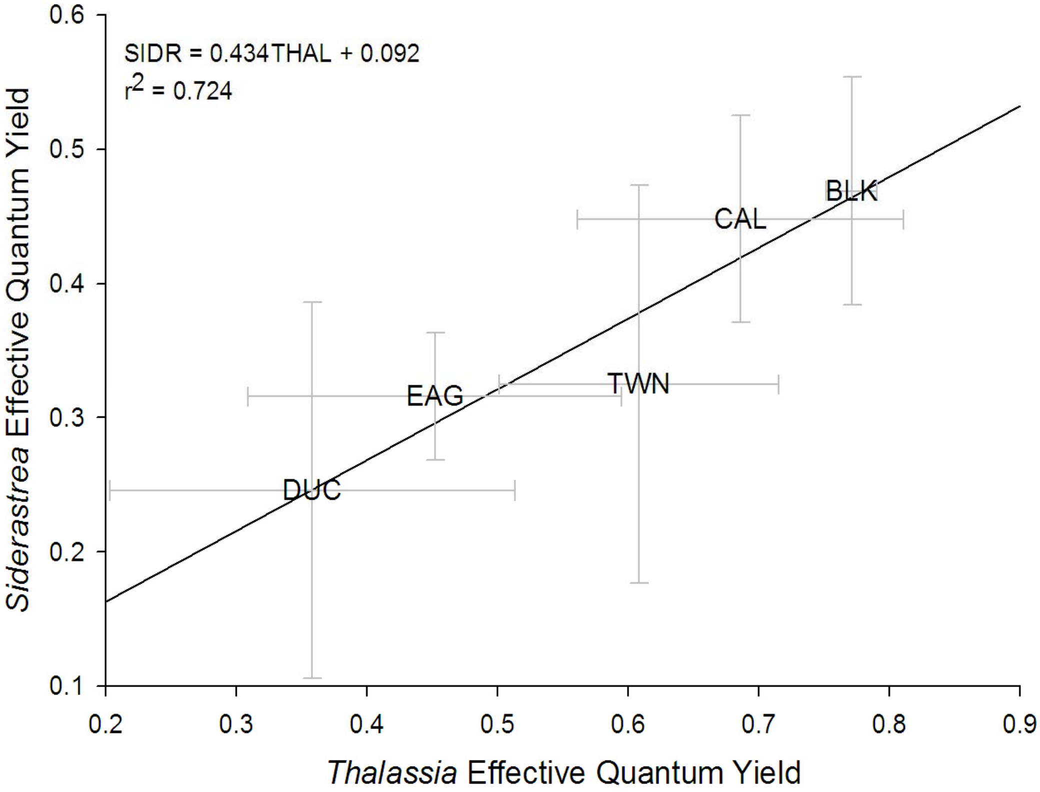


Fig. 4 Correlation of *T. testudinum* and *S. radians* mean effective quantum yields ($\Delta F/F_m'$) measured during May 2006 surveys. Error bars represent ± S.D.

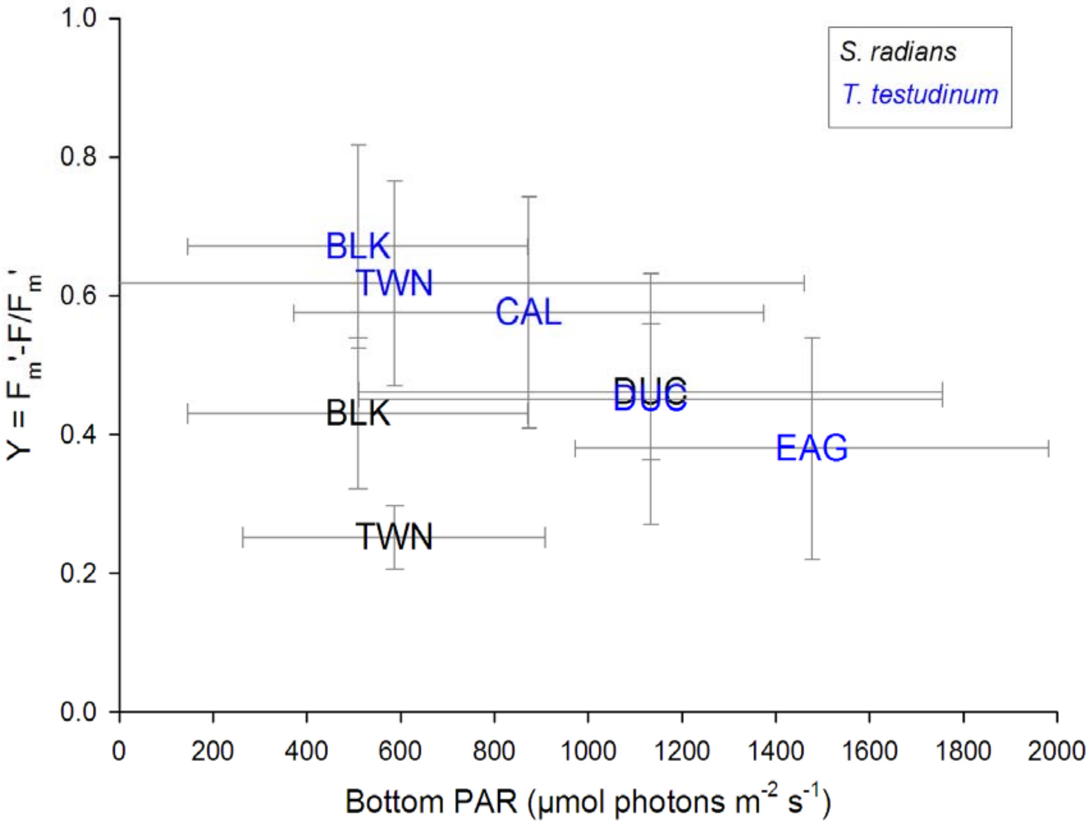


Fig. 5 2007 mean effective quantum yields ($\Delta F/F_m'$) for *S. radians* and *T. testudinum* versus mean daily bottom PAR measured among the five basins where species co-occurred. Error bars represent ± S.D.

DISCUSSION

Distribution and abundance of macrophytes in Florida Bay have been extensively characterized since the large die-off of *T. testudinum* began in 1987 (Robblee et al. 1991, Hall et al. 1999, Fourqurean et al. 2002, Durako et al. 2002, Landry 2005). These large datasets have provided management with a valuable means to assess recent macrophyte dynamics and predict how future water-management alterations under CERP may affect the macrophyte community system-wide. However, other benthic taxa have not been well documented within the bay. This work provides the first distributional assessment and physiological characterization of the dominant scleractinian coral occurring in Florida Bay, *S. radians*.

The presence of *S. radians* was minor compared to *T. testudinum* cover and frequency in the five basins where they co-occurred. However, there was an increase in coral frequency in basins at the most northeastern (BLK and DUC) and southwestern (TWN) extremes of the spatial-physical gradient. Connections between these basins and the Florida Keys Reef Tract may create a higher potential for larval recruitment from external reef source populations. Once established, local populations may proliferate creating higher densities in these regions compared to the more isolated and interior basins of CAL and EAG. *Siderastrea radians* is a brooding species and releases planulae on a lunar cycle rather than only once per year like most broadcast spawning corals (Szmant-Froelich 1984, Richmond and Hunter 1990). This strategy increases potential settlement and recruitment within the species as a trade off for smaller colony size (Szmant 1986). In addition, brooded planulae settle out relatively quickly (Babcock 1991), resulting in aggregates of colonies within a limited spatial range. Brooding corals are known to successfully recruit in habitats that are frequently disturbed due to their well-developed planulae, regular larval release throughout the year, and relatively fast settlement (Bak and Luckhurst

1980, Babcock 1991). Local densities thus increase with lower likelihood of widespread distribution. This reproductive strategy is likely advantageous for corals in Florida Bay where high environmental variability and limited hardbottom may greatly impact population distributions.

Long-term survivorship of *S. radians* in the Bay may also be affected by changes in sediment depth and re-suspension that may co-occur with altered flow regimes. Lirman et al. (2003) observed that experimental sediment burial of *S. radians* in Biscayne Bay severely impacted growth and mortality. Thus, it may be necessary in the future to assess changes in sediment depths in regions where coral colonies exist as flow regimes are altered. A lack of differing size-frequency distributions among basins suggests colony age was not affecting among-basin responses to previous salinity fluxes and that local basin salinity conditions have not influenced age survivorship. Future monitoring surveys of *S. radians* and more comprehensive assessments of hardbottom regions, outside of those surveyed during FHAP, will better establish the distribution and abundance of this species within Florida Bay.

Thalassia testudinum and the symbiont of *S. radians* exhibited significantly correlated $\Delta F/F_m'$ in 2006, but to a lesser extent in 2007. Coral colonies in DUC in 2007 exhibited higher than expected $\Delta F/F_m'$ based on previous trends which may have been due to previous days' inclement weather. Prolonged windy conditions may have led to turbid waters along with drifting algal blooms from adjacent basins leading to a decrease in the ambient light field. Under such prolonged conditions, resident symbiotic algae may exhibit a more shade-adapted response. Both species responded similarly with a decrease in efficiencies under high ambient light, a ubiquitous response in photosynthetic organisms as they downregulate as a means to avoid high excitation pressure and consequential damage to the photosynthetic apparatus (Hoegh-Guldberg and Jones

1999, Major and Dunton 2002). The significantly lower $\Delta F/F_m'$ values in *S. radians* compared to *T. testudinum* may indicate a difference in overall photochemical efficiency or capacity between these species in the field. However, photochemical efficiency of PSII does not describe the full capacity of the photosynthetic pathway nor the up-regulation of photoprotective mechanisms (Logan et al. 2007). It is therefore pre-mature to make any strong conclusions regarding overall photoefficiencies between these species. The disparity in mean quantum yields between the two species may also be affected by gross morphological differences in tissue structure, chloroplast packaging, and effects of multiple scattering (Enríquez 2005, Enriquez et al. 2005). Under ambient light conditions, the approach by which one species enhances efficiency (i.e. changing the number of light harvesting complexes or overall tissue morphology) may dramatically differ from the other, thus exhibiting overall differences in quantum yields. However, the similar responses to light availability indicate it would be valuable to continue measuring *S. radians* as a complement to *T. testudinum* surveys.

Siderastrea radians represents a sessile invertebrate population that has not been previously sampled in FHAP monitoring due to the focus on macrophytic habitat structure (Durako et al. 2002). However, this species can act as an alternative indicator of stress using the same photophysiological assessment tools and would account for a very different community of taxa, benthic invertebrates. Holobiont physiology is rather distinct from a seagrass outside of the similarities of their photosynthetic capabilities. As such, coral responses and related thresholds may be very different from *T. testudinum* for many variables including salinity, nutrient loading, and light. As the watershed of Florida Bay changes, *S. radians* may reveal impacts on the Bay's organisms that would not be readily apparent by sampling only seagrasses and macroalgae. Furthermore, higher coral abundance in hardbottom regions provides managers with an alternative physiological ecoindicator measure when *T. testudinum* is absent (Zieman et al. 1989).

LITERATURE CITED

Babcock RC (1991) Comparative Demography of Three Species of Scleractinian Corals Using Age- and Size-Dependent Classifications. Ecolog Monogr 61 (3): 225-244

Bak RPM, Luckhurst E (1980) Constancy and change in coral reef habitats along depth gradients at Curacao. Oecologia 47: 145-155

Becker G, Norman J, Moholl-Siebett M (1990) Two sites of heat-induced damage to photosystem II. In Current Research in Photosynthesis. v IV, Ed. M. Baltscheffsky 705-708

Belshe EF, Durako MJ, Blum JE (2007) Photosynthetic rapid light curves (RLC) of *Thalassia testudinum* exhibit diurnal variation. J Exp Mar Biol Ecol 342: 253–268

Biber PA, Harwell MA, Cropper WP (2004) Modeling the dynamics of three functional groups of macroalgae in tropical seagrass habitats. Ecolog Model 175: 25-54

Brand, LE (2002) The transport of terrestrial nutrients to South Florida coastal waters. 353-406, In: The Everglades, Florida Bay, and Coral Reefs of the Florida Keys, Ed. By J.W. Porter and K.G. Porter, CRC Press, Boca Raton, Florida

Braun-Blanquet J (1972) Plant Sociology: The Study of Plant Communities. Hafner Publishing, New York

Butler IV MJ, Hunt, H.J, Herrnkind WF, Childress MJ, Bertelsen R, Sharp W, Matthews T, Field JM, Marshall HG (1995) Cascading disturbances in Florida Bay, USA: Cyanobacterial blooms, sponge mortality, and implications for the juvenile spiny lobster *Panulirus argus*. Mar Ecol Prog Ser 129: 119-125

Carignan V, Villard MA (2002) Selecting Indicator Species to Monitor EcologicalIntegrity: A Review. Environmental Monitoring and Assessment. 78: 45–61

Comprehensive Everglades Restoration Plan, official website located at http://www. evergladesplan.org

Chester AJ, Thayer GW (1990) Distribution of spotted seatrout (*Cynoscion nebulosus*) and gray snapper (*Lutjanus griseus*) juveniles in seagrass habitats in western Florida Bay. Bull Mar Sci 46: 345-357

Durako MJ, Hall MO, Merello M (2002) Patterns of change in the seagrass dominated Florida Bay hydroscape. In Porter, J.W., Porter, K.G. (Eds.), The Everglades, Florida Bay, and Coral Reefs of the Florida Keys: An Ecosystem Sourcebook. CRC Press Boca Raton, FL. 479-496

Durako, Kunzelman J (2002) Photosynthetic characteristics of *Thalassia testudinum* measured in situ by pulse-amplitude modulated (PAM) fluorometry: methodological and scale-based considerations. Aq Bot 73: 173–185

English DB, Kriesel W, Leeworthy VR, Wiley PC (1996) Economic Contribution of recreating visitors to the Florida Keys/Key West. In: Linking the Economy and the Environment of Florida Keys/Florida Bay. Internal Report. National Oceanographic and Atmospheric Administration, Silver Spring MD

Enríquez S (2005) Light absorption efficiency and the package effect in the leaves of the seagrass *Thalassia testudinum*. Mar Ecol Prog Ser 289: 141-150

Enríquez S, Méndez ER, Iglesias-Prieto R (2005) Multiple scattering on coral skeletons enhances light absorption by symbiotic algae. Limnol Oceanogr 50: 1025-1032

Fourqurean J, Robblee M (1999) Florida Bay: a history of recent ecological changes. Estuaries. 22: 345-357

Fourqurean JW, Durako MJ, Hall MO, Hefty LN (2002) Seagrass distribution in south Florida: a multi-agency coordinated monitoring program. In Porter and Porter (eds), *The Everglades, Florida Bay, and Coral Reefs of the Florida Keys: An ecosystem sourcebook*. CRC Press, Boca Raton, FL. 497-522

Hackney JW, Durako MJ (2004) Size–frequency patterns in morphometric characteristics of the seagrass *Thalassia testudinum* reflect environmental variability. Ecol Indic 4: 55–71

Hall MO, Durako MJ, Fourqurean JW, Zieman JC (1999) Decadal changes in seagrass distribution and abundance in Florida Bay. Estuaries. 22 (2B): 445-459

Herrnkind WF, Jernakoff P, Butler MJ (1994) Status f the fishery for *Panulirus argus* in Florida. In Philips, B., Cobb, S., and J. Kittaka (Eds.) Spiny Lobster Management. 213-229. Blackwell Press, Oxford

Hill R, Schreiber U, Gademann R, Larkum AWD, Kuhl M, Ralph PJ (2004) Spatial heterogeneity of photosynthesis and the effect of temperature induced bleaching conditions in three species of corals. Mar Biol 144: 633-640

Hill R, Frankart C, Ralph PJ (2005) Impact of bleaching conditions on the components of non photochemical quenching in the zooxanthellae of a coral. J Exp Mar Bio Ecol 22(1): 83-92

Hoegh-Guldberg O, Jones RJ (1999) Photoinhibition and photoprotection in symbiotic dinoflagellates from reef-building corals. Mar Ecol Prog Ser 183: 73-86

Jokiel PL, Rodgers KS, Brown EK, Kenyon JC, Aeby G, Smith WR, Farrell F (2005) Comparison of Methods Used to Estimate Coral Cover in the Hawaiian Islands. Report to NOAA/NOS NWHI Coral Reef Ecosystem Reserve, Honolulu, Hawai'i

Jones RJ, Ward S, Amri AY, Hoegh-Guldberg O (2000) Changes in quantum efficiency of Photosystem II of symbiotic dinoflagellates of corals alter heat stress, and of bleached corals sampled after the 1998 Great Barrier Reef mass bleaching event. Mar Fresh Res 51: 63-71

Jones RJ, Hoegh-Guldberg O (2001) Diurnal changes in the photochemical efficiency of the symbiotic dinoflagellates (Dinophyceae) of corals: photoprotection, photoinactivation, and the relationship to coral bleaching. Plant Cell Environ 24: 89-99

Landry JB (2005) Changes in the Distribution and density of Florida Bay Macrophytes: 1995 – 2004. Thesis Submitted to University of North Carolina Wilmington, Wilmington, NC

Lapointe BE, Clark MW (1992) Nutrient inputs from the watershed and coastal eutrophication in the Florida Keys. Estuaries 15:465-476

Leujak W, Ormond RFG (2007) Comparative accuracy and efficiency of six coral community survey methods Journal of Experimental Mar Biol Ecol 351: 168–187

Lirman D, Orlando B, Macia S, Manzello D, Kaufman L, Biber P, Jones T (2003) Coral communities of Biscayne Bay, Florida and adjacent offshore areas: diversity, abundance, distribution, and environmental correlates. Aq Conserv: Mar Fres Eco 13: 121-135

Logan BA, Adams WW, Demmig-Adams B (2007) Avoiding common pitfalls of chlorophyll fluorescence analysis under field conditions. Functional Plant Biology 34: 853–859

Major KM, Dunton KH (2002) Variations in light-harvesting characteristics of theseagrass, *Thalassia testudinum*: evidence for photoacclimation. Journal of Experimental Mar Biol Ecol 275: 173-189

Matheson RE, JR, Camp DK, Sogard SM, Bjorgo KA (1999) Changes in Seagrass associated Fish and Crustacean Communities on Florida Bay Mud Banks: The Effects of Recent Ecosystem Changes? Estuaries. 22(28): 534-551

Merriam DF, Fuhr JM, Jenkins RV, Zimmerman PJ (1989) Pleistocene bedrock geology of Florida Bay, the Keys and the Everglades. Bull Mar Sci 44(1): 519-520

Nance JM (1994) A biological review of the Tortugas pink shrimp fishery through December 1993. Galveston Laboratory, Southeast Fisheries Science Center, National Marine Fisheries Service, Galveston, TX

Neves EG, da Silveira FL (2003) Release of planula larvae, settlement and development of *Siderastrea stellata* Verrill, 1868 (Anthozoa, Scleractinia) Hydrobiologia 501: 139-147

Neves EG, Andrade SC, da Silveira FL, Solferini VN (2008) Genetic variation and population structuring in two brooding coral species (*Siderastrea stellata* and *Siderastrea radians*) from Brazil. Genetica 132(3): 243-254

Nuttle WK, Fourqurean JW, Cosby BJ, Zieman JC, Robblee MB (2000) The influence of net freshwater supply on salinity in Florida Bay. Water Resour Res 36 (7): 1805-1822

Prager E, Halley RB (1997) Florida Bay Bottom Types. South Florida Information Access, U.S. Geological Survey, St. Petersburg, FL. http://sofia.usgs.gov/publications/ofr/97526/ index.html

Ralph PJ, Smith RA, Macinnis-Ng CMO, Seery CR (2007) Use of fluorescence based ecotoxicological bioassays in monitoring toxicants and pollution in aquaticsystems: Review. Toxicol Environ Chem 89(4): 589-607

Richmond RH, Hunter CL (1990) Reproduction and recruitment of corals: comparisons among the Caribbean, the Tropical Pacific, and the Red Sea. Mar Ecol Prog Ser 60: 185-203

Robblee MB, Didomenico WJ (1991) Seagrass die-off threatens ecology of Florida Bay. Park Science 11:21-22

Robblee MB, Barber TR, Carlson PR, Durako MJ, Fourqurean JW, Muehlstein LK, Porter D, Yarbro LA, Zieman RT, Zieman JC (1991) Mass mortality of the tropical seagrass *Thalassia testudinum* in Florida Bay. Mar Ecol Prog Ser 71: 297-299

Schomer NS, Drew RD (1982) An ecological characterization of the lower Everglades, Florida Bay, and the Florida Keys: U.S. Fish and Wildlife Service, Office of Biological Services, FWS/OBS-82/58

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

Smith NP (2002) Florida bay circulation studies. Recent Research Developments in Geophysics 4: 93-104

Swart PK, Healy G, Dodge R, Kramer P, Hudson H, Halley R, Robblee M (1996) The Stable Oxygen and Carbon Isotopic Record from a Coral Growing in Florida Bay: A 160 Year Record of Climatic and Anthropogenic Influence, Palaeogeo, Palaeoclim, Palaeoec 123:219-238

Szmant-Froelich A (1984) Reef coral reproduction: diversity and community patterns. In: Advances in reef science. Joint Meeting Intl. Soc. for Reef Studies and Atlantic Reef Committee. University of Miami, Miami, FL. 122-123

Szmant AM (1986) Reproductive ecology of Caribbean reef corals. Coral Reefs 5: 43-53

Thornhill DJ, Fitt WK, Schmidt GW (2006) Highly stable symbioses among western Atlantic brooding corals. Coral Reefs 25: 515-519

Warner ME, Fitt WK, Schmidt GW (1996) The effects of elevated temperature on the photosynthetic efficiency of zooxanthellae *in hospite* from four different species of reef coral: anovel approach. Plant, Cell Environ 19: 291-299

Warner ME, Chilcoat GC, McFarland FK Fitt WK (2002) Seasonal fluctuations in the photosynthetic capacity of photosystem II in symbiotic dinoflagellates in the Caribbean reef-building coral *Montastrea*. Mar Biol 141: 31-38

Zieman JC, Fourqurean JW, Iverson RL (1989) Distribution, abundance and productivity of seagrasses and macroalgae in Florida Bay. Bull Mar Sci 44: 292-311

CHAPTER 2

EFFECT OF HYPO-SALINITY ON THE PHOTOPHYSIOLOGY OF *SIDERASTREA RADIANS*

INTRODUCTION

Siderastrea radians (Pallas), order Scleractinia family *Siderastreidae*, is a hardy coral found throughout the tropical western to eastern Atlantic and Caribbean region (Veron 2000). These ahermatypic corals are small encrusting-to-hemispherical mounds that may be attached to, or free from, the substrate and reach a maximum diameter ≤ 10 cm (Lewis 1989, Veron 2000). They often occur in shallow lagoonal backreef areas where physical parameters can vary widely compared to other reef zones and are known to have greater overall tolerance to stress than many Caribbean species (Lewis 1989, Lirman 2002). As such, *S. radians* inhabits so-called marginal habitats away from characteristic reef communities. A marginal habitat is defined here on the basis of proximity to an environmental condition known or assumed, based on physiological or biogeographic evidence, to place an absolute limit on the occurrence of reef communities or key classes of reef organisms (Guinotte et al. 2003). For example, in Biscayne Bay *S. radians* thrives despite salinity and temperature ranges of 15-40 and 16 – 31ºC seasonally and consistently high sedimentation rates (Lirman et al. 2003). *Siderastrea radians* has also shown resiliency—the rate and ability of return to a pre-disturbance state—when placed directly under sedimentation and salinity stress in a controlled setting, further demonstrating its ability to withstand and thrive in atypical environments (Lirman 2002).

Siderastrea radians harbors dinoflagellates from the genus *Symbiodinium* within its endodermal tissues. Nutrient-rich photosynthate translocated from the symbiont supports metabolic processes including calcification, reproduction, and greater overall tissue thickness and quality for the colony (Muscatine 1990, Rinkevich 1989, Anthony et al. 2002). They may contribute up to 95% of the carbon and nitrogen budget to the host, making the relationship critical to host survival (Trench 1979; Muscatine 1990). As a result of their functional importance to the holobiont, focus has been placed on the photophysiological characteristics of *Symbiodinium* in response to stress conditions. The photophysiological state of the resident symbiont may determine whether the host-symbiont relationship is maintained or terminated (Iglesias-Prieto et al. 1992; Jones et al. 1998). This temporary or permanent loss of the dinoflagellate and their photosynthetic pigments from the host is known as coral bleaching (Glynn 1996, Hoegh-Guldberg 1999). A variety of stressors have been implicated in bleaching including high and low temperature, solar irradiation, and sedimentation, salinity changes, all which are thought to disrupt autotrophic functioning of the symbiont causing critical damage to host function (Jones et al. 1999, Phillip and Fabricius 2003, Kerswell and Jones 2003, Saxby et al. 2003, Ralph et al. 2005). This loss results in the host lacking a major nutrient source, thus compromising normal respiration rates and skeletal growth (Jokiel and Coles 1990). Large-scale bleaching due to increased temperature and/or high solar radiation has been a major focus of coral research in recent decades as such events have become more frequent and are correlated with large-scale coral mortality (Hoegh-Guldberg 1999). However, focus on the global concerns of temperature and light induced stress has resulted in few studies measuring effects of other localized stressors, such as reduced salinity from increased runoff, that may also negatively impact the dinoflagellate and thus the condition of the holobiont (Mayfield and Gates 2007).

Florida Bay

Over the last century, agriculture, urban development and exponential population growth in South Florida has resulted in a dramatic change to the natural watershed of the Everglades and Florida Bay. Waterflow originating from Lake Okeechobee and the Kissimmee River watershed was largely re-directed to the Gulf of Mexico and Atlantic Ocean by the Army Corps of Engineers in the mid-20th Century (Light and Dineen 1994). As a result, Florida Bay began receiving 90 percent of its freshwater inputs from seasonal rainfall instead of constant flows via the Everglades (Light and Dineen 1994, Nuttle et al. 2000). More variable and higher average salinities occurred bay-wide. The northeast region of the Bay, already physically restricted, exhibited the highest and most variable salinities whereas areas to the southwest maintained less variable and more typical marine conditions due to mixing with waters from the Gulf of Mexico. The one notable exception to this trend in Florida Bay is Blackwater Sound. Its location in the extreme northeast region connects it with more marine waters from Barnes Sound and Biscayne Bay to the north and cuts through the Key Largo connect it directly with the Atlantic. As a result, conditions within Blackwater Sound have been described as typical of more south-central regions (Hackney and Durako 2004).

Large-scale annual macrophyte monitoring in Florida Bay led to anecdotal observations of abundant coral populations within hardbottom regions along the northeast to southwest physical-environmental gradient discussed above (Durako pers. comm.). There had not been any distributional information on the most common coral species in the Bay, *S. radians,* until recently (see Chapter 1). It is still unclear how this species has responded to the widely fluctuating salinity patterns in the Bay over the last century. Colonization of hardbottom areas in the northeast may have only occurred within the last century following the rise in salinities resulting from freshwater diversion and a dramatically altered South Florida watershed. Plans to restore the watershed and increase freshwater inflow to historical levels under the Comprehensive Everglades Restoration Plan (CERP) might threaten this coral population as increased freshwater may lower salinities bay-wide (US Army Core of Engineers and SFWMD 2000).

Hyposalinity and Coral Physiology

Corals are considered stenohaline, osmoconformers yet only a few studies have investigated the quantitative effect of salinity fluctuation on coral fitness and the effect it has on the ability of the dinoflagellate to maintain its functional role within the host (Muthiga and Szmant 1989, Manzello & Lirman 2002, Kerswell & Jones 2003). Muthiga and Szmant (1989) investigated the effects of hyposalinity on *S. siderea* from the Florida panhandle where large river systems empty into the Gulf of Mexico. They found that *S. siderea* placed in treatments with a change in salinity greater than 10 from controls had significant decreases in gross photosynthetic rates. Manzello and Lirman (2002) working in Biscayne Bay also reported a significant drop in net photosynthesis of *Porites furcata* in hyposaline treatments despite the low salinities regularly observed in this coastal habitat. Both studies indicated little tolerance of low salinity *in vivo* and yet the nearshore habitats where colonies were collected were likely subjected to low salinity from rainfall and runoff events; salinity records of the sample sites were not given in these studies. It is therefore unclear how these coral populations thrive in variable-to-low saline environments. The acute nature of transferring colonies directly from controls to salinity treatments does not allow acclimatory responses to hypo-osmotic stress that may occur under more natural fluctuations. Understanding thresholds and physiological tolerances for corals exposed to more realistic salinity fluctuations may clarify how certain populations can thrive in coastal, brackish habitats. More recently, Kerswell & Jones (2003) employed fluorometric techniques to assess the effects of hyposalinity on the photosynthetic efficiency of *Symbiodinium in hospite* of *Stylophora pistillata*. However, acute changes to salinity were again applied. Furthermore, *S. pistillata*, an important Indo-Pacific reef builder, is known to be markedly intolerant of environmental fluctuations such as high temperatures and low salinity (Loya et al 2001, Kerswell & Jones 2003, van Oppen et. al 2005) and so makes a poor model for general responses by more tolerant species. The goal of the experimental study described here on the photophysiology of *Symbiodinium in hospite* of *S. radians,* a so-called resilient taxon, was to provide a more realistic understanding of how future management may affect this member of the Florida Bay community under gradually-induced hyposaline conditions. The goal of CERP to restore historical freshwater flow into Florida Bay is perceived to benefit to the overall system. However, the resulting reduced salinities will likely stress the coral population in the Bay, an effect which has not been taken into account in this restoration plan.

Photobiology

Pulse amplitude modulated (PAM) fluorometry has become a popular tool to assess the photosynthetic performance of coral dinoflagellate symbionts under natural and stressed (bleaching) conditions *in situ* (Warner et al. 1996, Hoegh-Guldberg and Jones 1999, Jones et al. 2000, Jones and Hoegh-Guldberg 2001, Warner et al. 2002, Hill et al. 2004, Hill et al. 2005). PAM fluorometry enables the study of efficiency of photochemical energy conversion by measuring chlorophyll fluorescence in a non-invasive manner (Schreiber et al. 1986). Previously, such studies of symbiont health required measurements of O_2 evolution or $^{14}CO_2$ uptake that are quite intrusive. In addition, using a respirometer to measure O_2 production by *Symbiodinium in hospite* cannot be accurately determined because some of the O_2 produced will be used by the host and the dinoflagellate in respiration (Kuhl et al. 1995).

PAM fluorometry measures the efficiency with which light energy is converted into chemical energy at photosystem II (PSII). PSII is considered a critical link in which photons are passed into the first steps of the photochemical pathway for energy conversion. Thus, at this stage, any decrease in efficiency and transport of photons will affect the entire system. It is also considered to be the most stress sensitive part of the photosynthetic pathway (Becker et al. 1990). Light energy absorbed by chlorophyll can be channeled into one of three pathways that are used in parallel: it can be used to drive photosynthesis; be dissipated as heat; or re-emitted as fluorescence to remove excess energy. When first exposed to light, PSII absorbs photons and transfers electrons downstream into the photosynthetic pathway. PSII first passes electrons to plastoquinone (Q_A), putting it in a reduced state. This is the first major limiting step, as Q_A cannot accept more electrons until it reduces the next acceptor, Q_B. As a result, reaction centers are “closed” and temporarily cannot accept more electrons. As excitation pressure builds at the reaction center, energy must be redirected into alternative pathways. The ability to redirect energy will strongly determine how well an organism will handle stressful and potentially damaging light energies in parallel with other physical variables. Therefore, evaluating the fluorescence pathway can indicate an organism’s photosynthetic efficiency in using these different pathways under changing conditions (e.g. varying salinity regimes).

The objective of this research was to assess the effects of hyposalinity conditions, such as those that might result from increased freshwater input to Florida Bay on the species *S. radians*, and to determine whether the responses among different basin populations reflect acclimation due to differences in basin physical conditions. Assessing the photophysiology of the endosymbiotic dinoflagellate *Symbiodinium* was used to indicate stress on the holobiont from decreased salinity, as has been previously examined in temperature and light stress studies (Iglesias-Prieto 1992, Warner et al. 1996, Warner et al. 1999, Rodríguez-Román et al. 2006).

METHODS

Study Site and Mesocosm Design

Florida Bay (ca. 25°05'N, 81°45'W) is located at the southern tip of mainland Florida, bordered by the Florida Keys to the south and east, and by the Gulf of Mexico to the west (Fig. 1). The Bay is divided into a complex suite of approximately 50 shallow (mean depth <2m) basins by mud banks and mangrove islands (Schomer and Drew 1982; Smith 2002). Highly-variable salinities and water quality persist in northeast basins shifting to more stable marine conditions in the south-central region (Figs. 1, 6). Spatial surveys conducted during May 2006 and May 2007 as part of annual South Florida Fish Habitat Assessment Program (FHAP-SF) monitoring indicated the presence of *S. radians* within five distinct basins along this northeast-southwest physical-environmental gradient (see Chapter 1). The five basins in a northeast to southwest orientation are Blackwater Sound (BLK), Duck Key Basin (DUC), Eagle Key Basin (EAG), Calusa Key Basin (CAL), and Twin Key Basin (TWN; Fig. 1). See Table I for basin abbreviations used throughout this text.

During Fall 2006 field surveys, *S. radians* colonies were collected for controlled- salinity experiments. Sixteen *S. radians* colonies (≤3-5 cm in diameter) were collected per basin from locations where the highest densities were previously observed (see Chapter 1). Colonies were transported to the greenhouse at the Center for Marine Science (CMS), Wilmington, NC overnight. Care was taken to ensure colonies were never removed from seawater and that temperatures and salinity were maintained under ambient field conditions. Colonies were immediately and randomly placed in 40-1 aquaria filled with high quality seawater and containing gravel-bed filter aeration systems. One colony per basin was randomly chosen and placed in each of 16 aquaria. Location within tank was randomly assigned yet placement ensured that a colony from each basin was in each tank position at least twice. Corals were held at a salinity of 30 (the average salinity ±1 at collection sites) for one week to allow colonies to acclimate to greenhouse conditions. Salinities were checked and adjusted as needed approximately every 12 hours using a YSI conductivity probe (accuracy ±0.5; Yellow Springs, OH) while calcium, pH, and alkalinity assessed weekly. Temperatures were controlled in the greenhouse (24-28ºC variation between daytime and nighttime temperatures) and a 12:12 light:dark cycle was established (additional daytime lighting was provided by 500W metal-halide lamps). A 2π LiCor quantum sensor (LiCor Omaha, NE) was placed in the middle tank to record mean PAR at 30 minute intervals throughout the course of the experiment.

After one week, target treatment salinities of 10, 15, 20, and 30 (controls) were assigned to each of 16 aquaria (level of replication is the tank). Salinities were then decreased daily following morning fluorescence measurements by adding distilled water to experimental tanks to drop salinity at a rate of 2 day^{-1}. This rate of change was chosen to represent a realistic drop in salinity in the Bay during a flushing and/or rainfall event (Kahn and Durako 2005, 2006). Similar rates have been used for assessing the response of *Thalassia testudinum* seedlings to salinity fluctuations in Florida Bay (Kahn and Durako 2006). When tanks reached their assigned target salinities, they were maintained at this state for the remainder of the experiment.

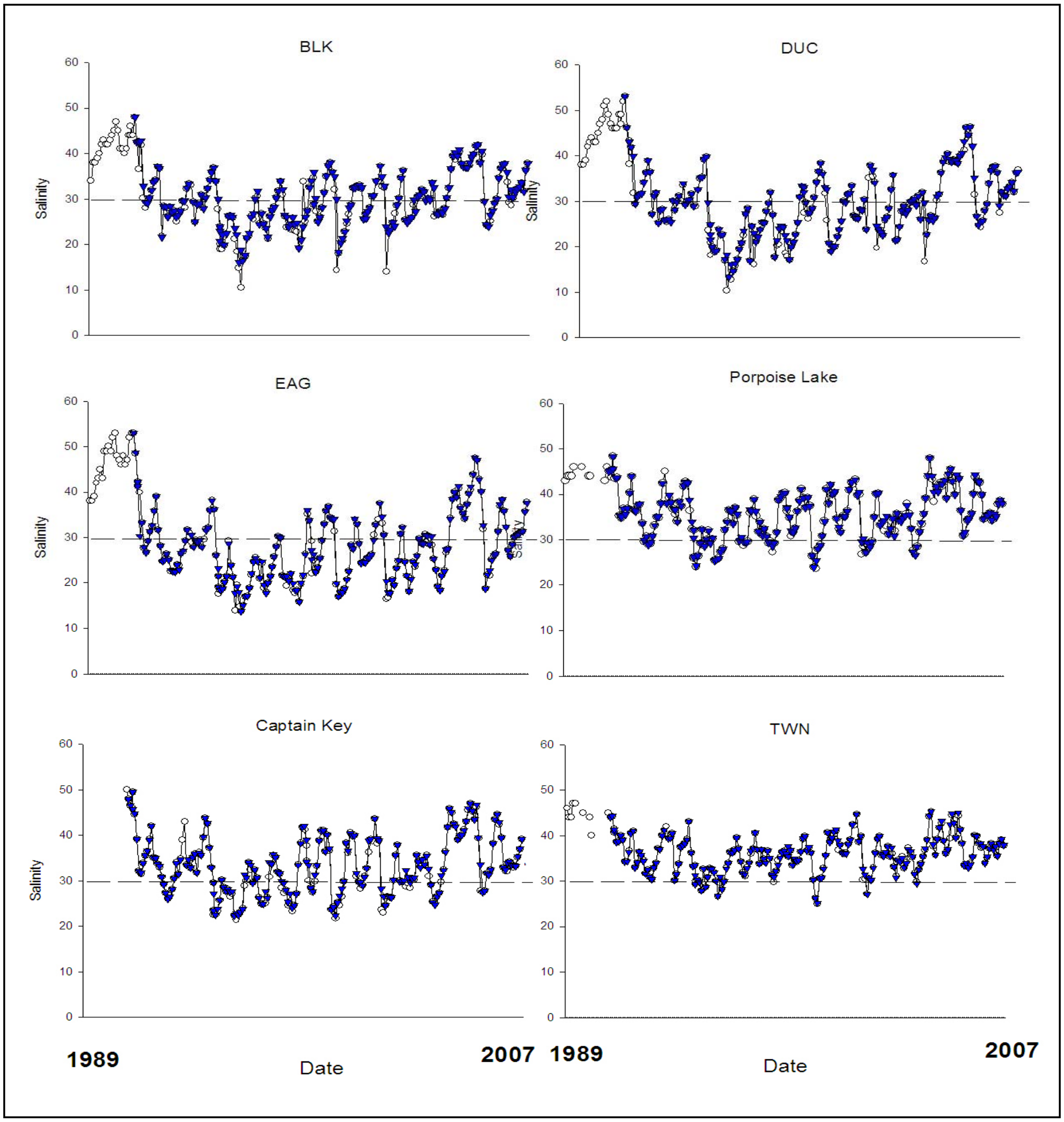


Fig. 6 Florida Bay, Florida, USA: Historical salinity record for five basins where colonies were collected for this study. Porpoise Lake and Captain Key provide an approximation of salinity in CAL where direct records were not available.

Photobiology

Fluorescence measurements were taken on all colonies at mid-day, pre-dawn, and post-dusk with a Diving-PAM fluorometer (Walz, Germany). For all measurements, the fiber optic tip of the instrument was set at a standard geometry (30^{o} from perpendicular) and distance (5mm) to the dorsal surface of sampled corals. Minimum fluorescence was first measured (F_o or F for dark- or light-acclimated states, respectively). The coral tissue was then subjected to a pulse of saturating light during which a second fluorescence reading is taken (F_m or F_m' for dark- or light-acclimated states, respectively). F_m or F_m' is the state in which all reaction centers present are considered reduced or closed. Maximum (i.e., dark-acclimated) and effective (i.e., light-acclimated) quantum yields, which measure photosynthetic capacity of the endosymbiotic dinoflagellate, were then calculated ($F_v/F_m = F_m\text{-}F_o / F_m$ and $\Delta F/F_m' = F_m'\text{-} F/ F_m'$ respectively). The dawn and dusk measurements provided F_v/F_m while $\Delta F/F_m'$ was measured at midday. F_v/F_m describes the full potential of photochemical energy conversion rather than the efficiency under a given level of ambient light ($\Delta F/F_m'$). In a dark-acclimated state the reaction centers are considered fully relaxed or open before saturation of the reaction centers with the pulse of light from the fiber optic. Measuring both $\Delta F/F_m'$ and F_v/F_m can provide information on the efficiency of photochemical processes under natural daily irradiance and on maximum efficiency in a relaxed state. Both dawn and dusk measurements were initially taken to ensure there were no significant differences in F_v/F_m resulting from residual dynamic photoinhibition during midday, generating lower F_v/F_m at dusk. Post-dusk measurements ceased when no significant difference between dawn and dusk measurements was observed during the first week, indicating alternative pathways for directing excess light energy had been essentially switched off. The experiment was terminated three days after colonies in treatment salinity of 10 had reached target although dawn and midday quantum yields were still measured for the following seven days.

Statistics

To assess whether quantum yields were significantly different among basin population (BLK, CAL, DUC, EAG, TWN) and salinity treatments (10, 15, 20 and 30) at their respective target dates, a repeated measures factorial ANOVA was applied. Significant results are reported with the associated test-statistic, hereafter represented by t, and level of significance (α). A linear model ANOVA and post hoc Student-Newman-Keul's test was performed to assess differences among basin populations at each target salinity date. All statistical models were calculated using SAS v.9.1 software and figures generated in SigmaPlot v. 9.0.

RESULTS

Maximum quantum yields (F_v/F_m) consistently exhibited smaller variance than effective quantum yields ($\Delta F/F_m'$) measured at midday, in all treatments (Fig. 7). This pattern of high variance under high and variable midday irradiance confounded the effects of salinity on quantum efficiency due to the variable pressure light puts on photosynthetic reaction centers. In effect, the signal-to-noise ratio decreases for mid-day $\Delta F/F_m'$. Thus, to increase the ability to detect effects of salinity, treatment effects were evaluated using F_v/F_m. F_v/F_m still exhibited a consistent, albeit insignificant, trend that negatively correlated with the light history from the previous 24 hours (Fig. 8). The most apparent example occurred on Nov 7 and Nov 8 when two extremely overcast days resulted in maximum PAR below 115 and 240µmol photons m^{-2} s^{-1} respectively.

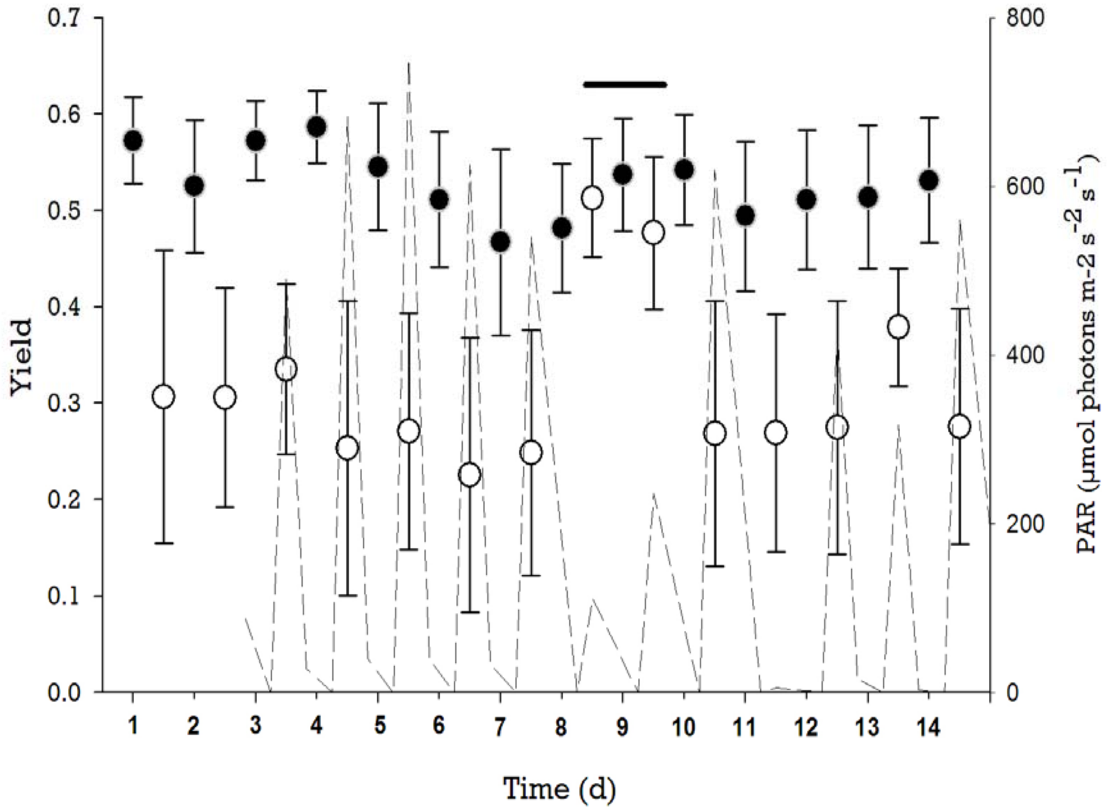


Fig. 7 Dark-acclimated dawn quantum yields (F_v/F_m; closed circles) versus light acclimated midday quantum yields ($\Delta F/F_m$'; open circles) for control colonies. Error bars represent S.D. Dashed line indicates mean PAR over 30 minute intervals throughout the experiment. ▬ denotes extreme overcast days. n=20

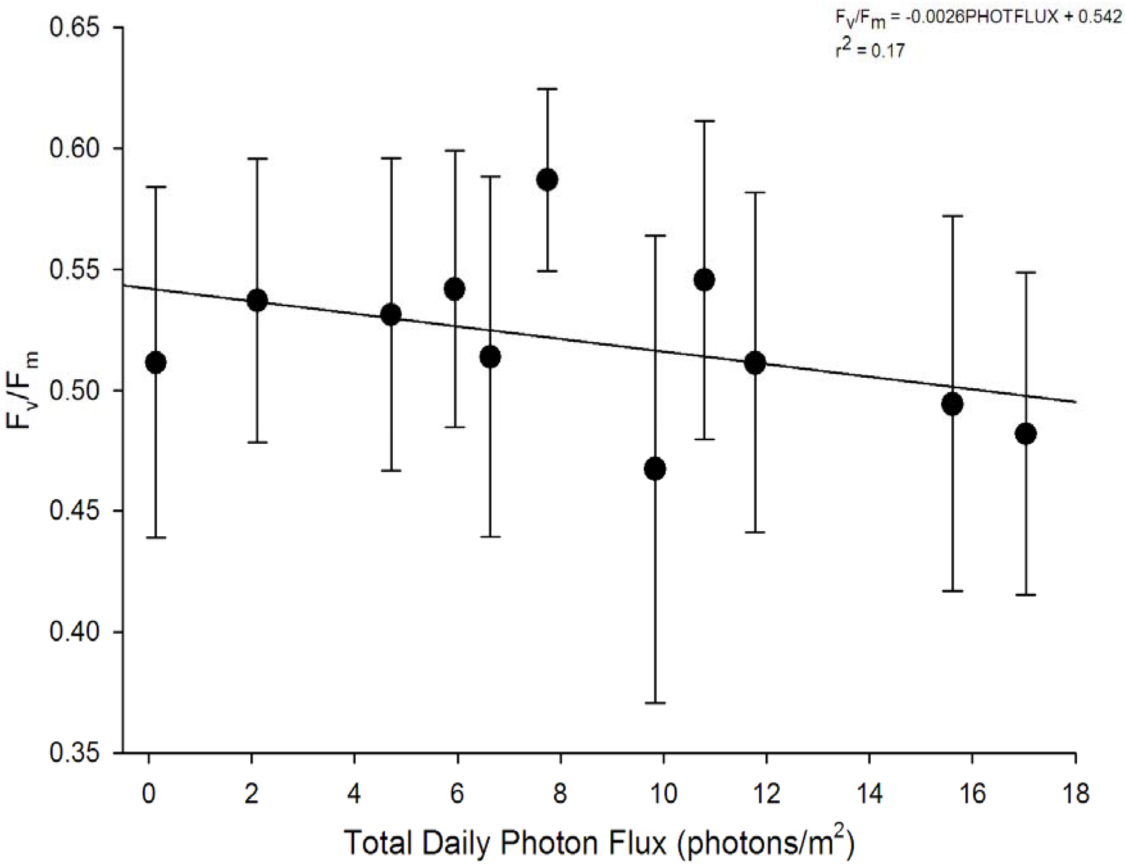


Fig. 8 Total daily photon flux versus dark-acclimated quantum yields in control colonies. Error bars represent S.D. n=20

F_v/F_m values were compared by basin population for each treatment on the day target salinity was reached (i.e. reached target salinity: 20 on Day 6, 15 on Day 9, and 10 on Day 11). No significant change in F_v/F_m occurred in control treatments (30) over the time course of the experiment ($p = 0.08$; Fig. 7 and Fig 9a), thus salinity treatments were compared to controls on their corresponding target dates.

A general pattern of decreasing F_v/F_m over time was apparent as treatments reached their target salinities and were held at target for the remainder of the experiment (Fig. 9). There was no significant decrease in F_v/F_m from controls for all basin populations in treatment salinities of 20 at target and for at least three days post-target (Fig 9a-b).

In salinity treatment 15, none of the populations were significantly below their respective basin controls when the target was reached and only colonies from TWN had significantly lower F_v/F_m three days after reaching the target salinity from the TWN controls ($p<0.05$; Fig. 9 a,c). However, there was a distinct yet insignificant trend of colonies in TWN exhibiting the lowest F_v/F_m over the course of the experiment in all treatments, including controls (Fig. 9a). At salinity of 10, TWN exhibited the only significant drop in F_v/F_m from its controls (Figs. 9d, 10e; $t = 2.91$, $p<0.01$). TWN colonies exposed to salinity of 10 also had significantly lower F_v/F_m compared to TWN colonies at target salinities of 15 and 20 (Fig 10e; $t = 3.33$ and $t = 3.25$ respectively, $p<0.01$). Colonies from CAL also exhibited significantly reduced F_v/F_m at target salinity of 10 versus CAL colonies at target 20 (Fig. 10d; $t = 2.84$, $p<0.01$). BLK, DUC, and EAG colonies did not exhibit a significant change in yields between colonies at 10 and all other salinities on their respective target dates (Fig. 10 a-c).

By one-day post-target salinity, all basin populations exhibited significantly lower dark-acclimated yields in salinity of 10 versus treatment salinities 15, 20 and 30 (Fig.

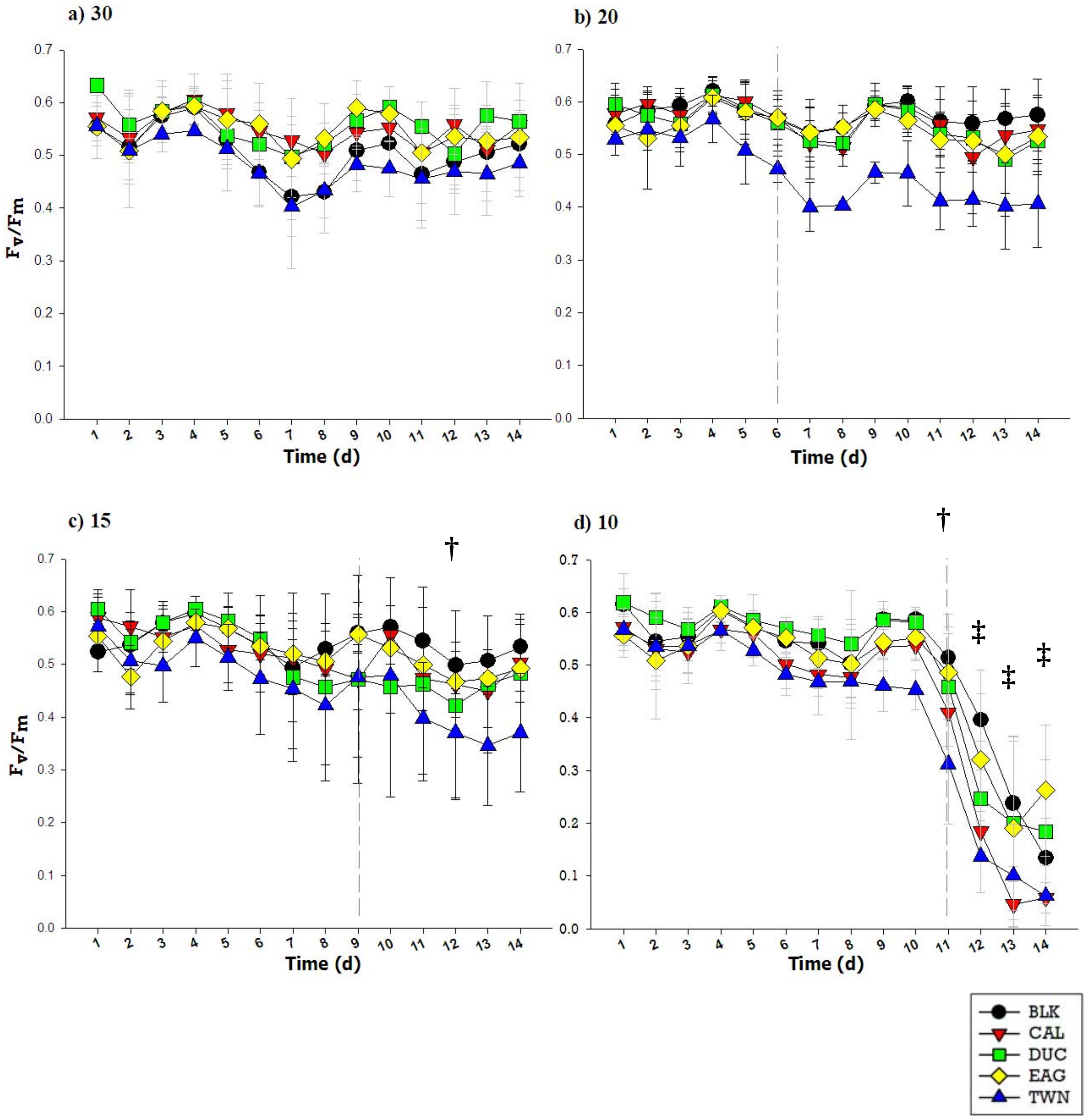


Fig. 9 Mean dark-acclimated yields (F_v/F_m) ± S.D. by basin population over time. Dashed gray line indicates day target salinity was reached. † indicates a significant difference in TWN versus TWN controls. ‡ indicates a significant difference in all basins versus respective basin controls.

11a; $p < 0.01$). These differences were consistent for the following three days after salinity treatment of 10 was reached. The experiment was terminated on day 14 when it was clear colony death had occurred in the most hyposaline treatment. Evidence of death was apparent when F_v/F_m no longer significantly differed from midday $\Delta F/F_m'$. Furthermore, visual observations and increases in yields in the days following this significant drop in F_v/F_m confirmed the overgrowth of bluegreen and green algae on colony surfaces, the proliferation of which was measured with the PAM fluorometer for seven days following the experiment (data not shown).

Among-basin differences in F_v/F_m were evident in both controls and reduced salinities. BLK and TWN exhibited significantly lower mean yields than all other basins when all dates for controls were pooled (Fig 12; $F = 15.88$, $p < 0.001$). Also, a consistent pattern from highest to lowest F_v/F_m by basin population was detected in salinity treatments, however this trend was not always significant. F_v/F_m values for colonies from the northeast basins were higher on average than those in the southwest (Fig. 12), with the exception of pooled BLK controls (see below). The pattern in F_v/F_m most commonly decreased sequentially from BLK, EAG, DUC, CAL, and TWN respectively. In treatment salinity 10, this pattern was evident from two days prior to target until 2 days post-target with TWN colonies significantly lower than all other basins one and two days after reaching 10 ($p<0.01$) The TWN population also had significantly lower yields at target salinity 20 compared to all other basins when target was reached and thereafter (Fig. 12; $F = 4.45$, $p < 0.05$). The observed patterns in F_v/F_m correlate with population location in the Bay (Fig. 1). Moreover, this shift in F_v/F_m parallels mean bottom salinity and variance recorded for each basin over the last 18 years (Fig 6, Table IV). EAG and

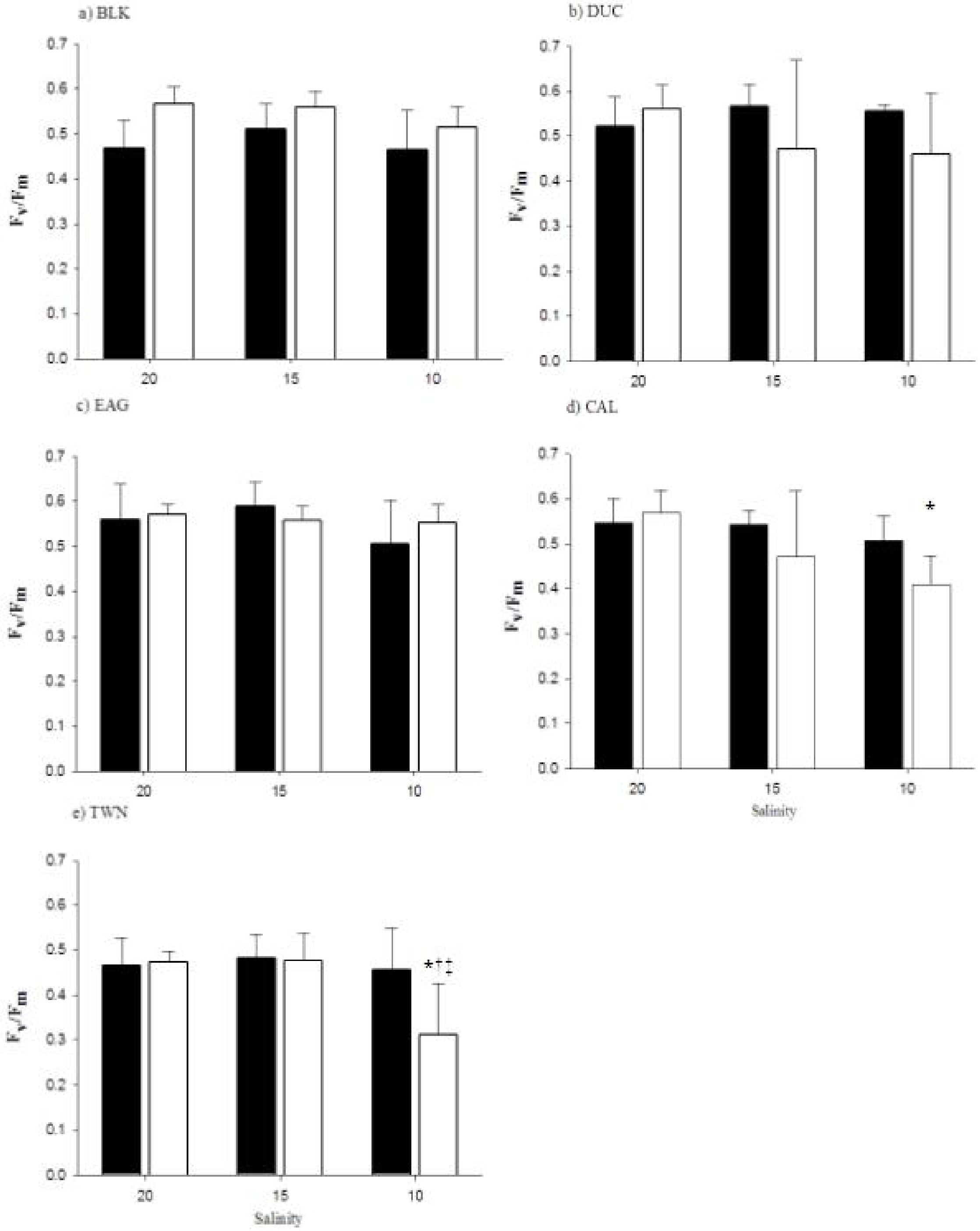


Fig. 10 a-d) Dark-acclimated quantum yields (F_v/F_m) at treatment salinity and controls on the target date. Black bars represent mean yield for controls ± S.D. White bars represent mean yields for given treatment ± S.D. Asterisks (*) indicate a significant difference from F_v/F_m in treatment salinity 20; † indicates a significant difference from treatment salinity 20; ‡ indicates a significant difference from treatment 10 controls.

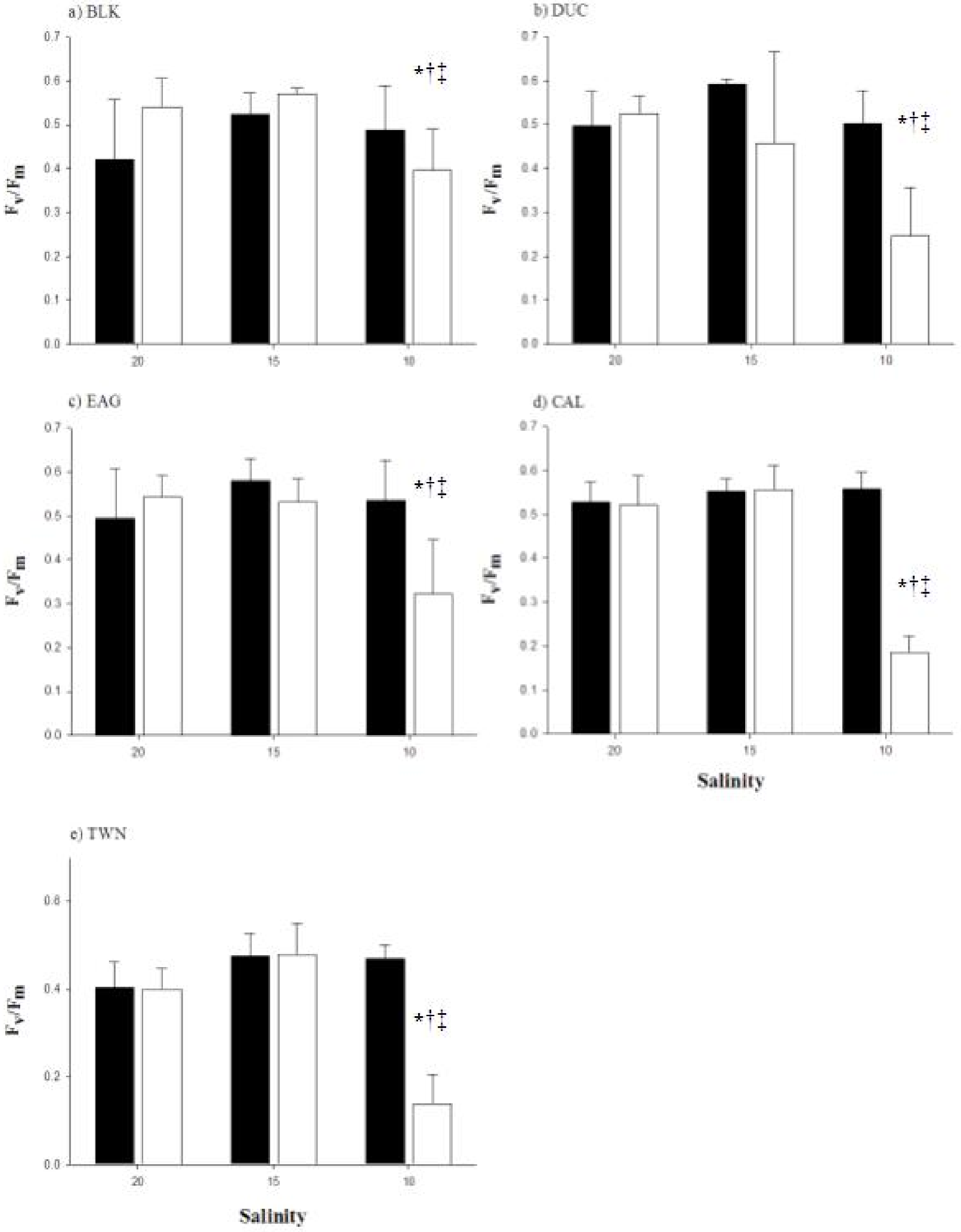


Fig. 11 a-d) One day post-target (F_v/F_m) for treatment salinities and comparative controls. Black bars represent mean yield for controls ± S.D. White bars represent mean yields for given treatment ± S.D. See Fig. 10 for explanation of symbols.

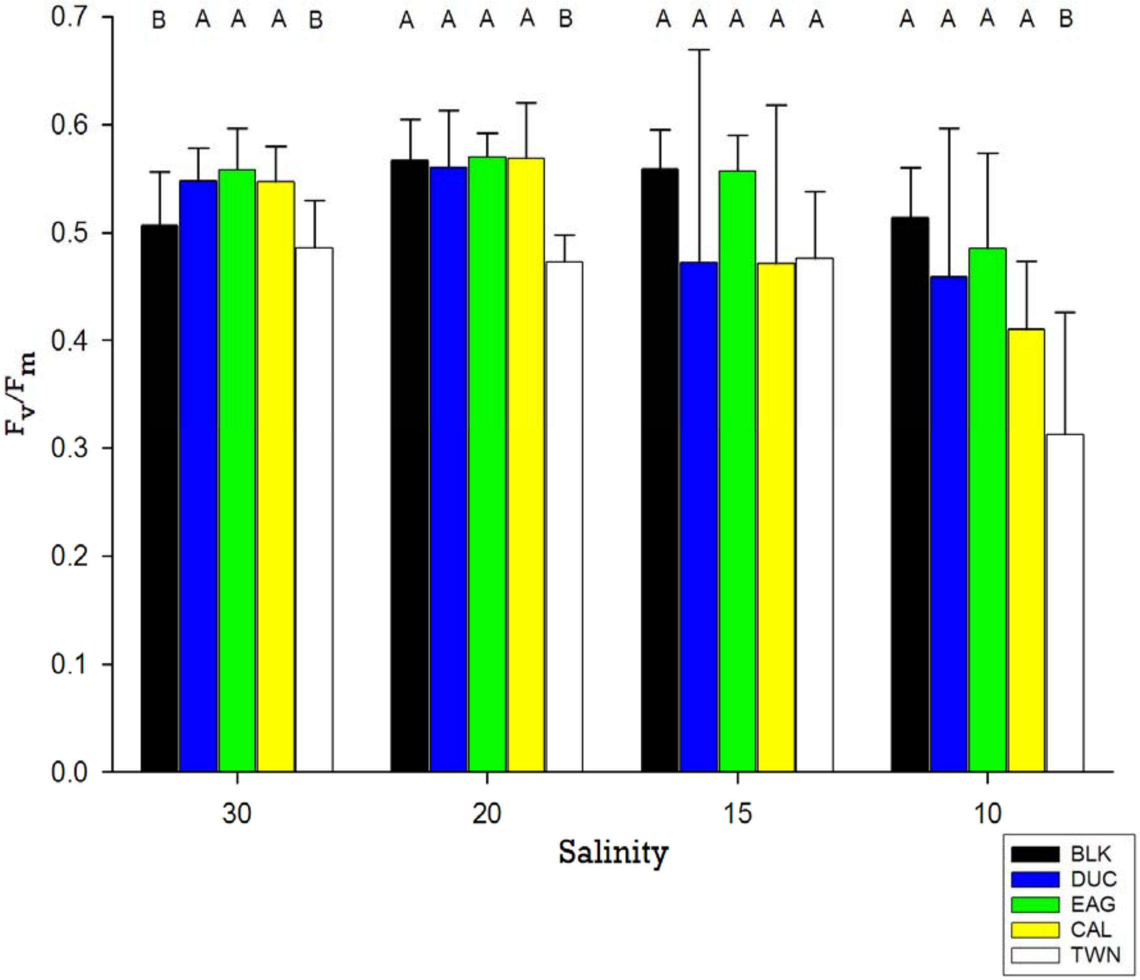


Fig. 12 Among basin differences in mean F_v/F_m ± S.D. within salinity treatments on date treatment was reached. Differing letters within salinity treatments indicate a significant difference among basins.

Table IV. Average basin salinity from 1989-2007 monthly salinity records obtained from SERC; ( ) = S.D. ; Asterisks (*) denotes record is an estimate. Salinity was recorded near adjacent Captain Key.

BASIN	**n**	**SURFACE**		**BOTTOM**	
BLK	220	30.91	(7.2)	29.91	(5.8)
DUC	220	30.83	(8.9)	29.37	(7.0)
EAG	220	29.70	(9.4)	27.64	(7.3)
CAL*	220	33.27	(6.6)	33.46	(6.3)
TWN	220	36.20	(4.3)	35.85	(4.0)

DUC have the highest recorded variability and lowest mean salinity of all basins while observed F_v/F_m in these populations were consistently the highest among basins (Fig. 12). Colonies at target of 15 exhibited no significant difference in F_v/F_m among basins throughout the experiment but exhibited similar trends as yields decreased in parallel with the northeast to southwest physical-salinity gradient.

DISCUSSION

Symbiotic dinoflagellates of *S. radians* demonstrated remarkable tolerance *in hospite* to hyposaline stress as measured by chlorophyll fluorescence in experimental aquaria. F_v/F_m measured at pre-dawn indicated symbiont health was not acutely affected by decreasing salinities until a threshold was reached between salinities of 10 and 12, a salinity drop of roughly 20 from ambient. Coles and Jokiel (1992) state that salinities below 15 sustained for more than two days will lead to coral mortality. In this study, there was no significant drop in F_v/F_m in the symbiont population and no visual pigment loss or tissue sloughing for a minimum of three days while in a hypo-saline treatment of 15. Corals in similar coastal environments with widely fluctuating salinities have also demonstrated tolerance to reduced salinities, however these studies applied acute treatments with a shift in salinity of 10 from ambient levels (Marcus and Thornhaug 1981, Muthiga and Szmant 1987, Manzello and Lirman 2003). The step-wise reduction from the mean ambient salinity, used in the current work provided a more realistic assessment of tolerance as colonies can potentially adjust to external osmotic changes by employing osmoregulatory responses (Mayfield and Gates 2007). Salinity records for Florida Bay (Fig 6) indicate that salinities have often dropped below 20 and occasionally down to 10 in some northern basins where *S. radians* occurs, reflecting the ecological relevance of the salinity treatments chosen in this study (see Chapter 1). Chl *a* and symbiont densities were not measured, however loss of color indicative of bleaching was negligible overall; only a slight color change was observed in colonies at salinity treatment 15 after three days at target. This may again indicate higher tolerance versus other scleractinian species where substantial loss of pigmentation and/or symbionts was documented while under less substantial hyposaline stress (Kerswell and Jones 2003).

In general, little attention has been given to understanding osmoregulation in this taxon, likely owing to the rarity of reduced salinity events on most reefs. However, the ability to adjust to hypo-osmotic conditions, documented in this and previous studies, highlights the need to assess how osmotic shifts can affect the host and symbiont physiology *in toto*. Furthermore, the threshold response observed in this study is indicative of the coral actively maintaining and successfully regulating its internal osmotic balance until a critical (and lethal) level is reached. Mayfield and Gates (2007) review possible mechanisms by which symbiotic corals maintain a balance with their external environment. Glycerol produced by the symbiont and transferred to the host is rapidly respired as a major energy source and/or stored in cellular pools (Gates and Edmunds 1999, Mayfield and Gates 2007). These pools may have a secondary function as essential compatible organic osmolytes (COOs), which can be abundant without affecting regular cell function (Yancey et al. 1982, Shick 1991, Mayfield and Gates 2007). In *Dunaliella* (Chlorophyta) glycerol levels are reduced to eliminate its osmotic potential (Ben-Amotz 1975, Chitlaru and Pick 1991). The effect of such fluctuations within the endosymbiont would have a downstream effect if the host is subjected to reduced glycerol levels for normal metabolic function. Free amino acids (FAAs), also abundant in the host cytoplasm and known to play a role in signaling the release of photosynthate from the symbiont, can be readily taken up or released from the host to the external medium as a means to alter internal osmolarity (Gates et al. 1995, Mayfield and Gates 2007). Further work is needed to confirm these alternative roles glycerol and FAAs may play in endosymbiotic corals. Examining interspecific differences in these pools between species tolerant to, and sensitive of, salinity change may clarify what role if any these pools may have.

The significant decrease in F_v/F_m in the most extreme hypo-saline treatment indicates photosynthetic impairment within the symbiont while residing in host tissues. Impairment of photosynthesis within coral symbionts has been studied in response to other stressors including light, temperature, herbicides, heavy metals, sedimentation, and reduced water flow (Warner et al. 1999, Hoegh-Guldberg 1999, Jones and Hoegh-Guldberg 1999, Phillipp and Fabricius 2003, Carpenter and Patterson 2007), however, the processes leading to a measurable drop in photochemical efficiency at PSII under reduced salinities are unclear in symbiotic dinoflagellates. Jahnke and White (2003) observed reductions in F_v/F_m, increased cell size and increased levels of anti-oxidants that function in the thylakoid and envelope membranes of the chloroplast in *Dunaliella* during hypo-osmotic stress. Change in cell osmolarity causes increased chloroplast volume and decreased photosynthetic electron flow as the surrounding medium of the chloroplast is altered (Gross et al. 1969). This inhibition occurs when electron transfer from plastocyanin to P700 is interrupted resulting in additional excitation pressure on the primary acceptor in PSII. The over-reduction of PSII may lead to the creation of free radicals that in turn damage the reaction center, which would be indicated by reduced F_v/F_m (Jahnke and White 2003). Alternatively, hypersaline stress, which similarly produces depressed quantum yields, impacts the photosynthetic pathway via inhibition of electron transport at the acceptor side of PSII (Xia et al. 2004). Because of these sources of variation, use of fluorometric data to assess the efficiency of PSII *in situ* must be interpreted with caution. A number of different biochemical processes may occur to bring about a change in the fluorescence of an organism, which cannot be discerned using only fluorometric data (Fitt et al. 2001). As such, ideas proposed above are only hypothetical and cannot be applied directly to *Symbiodinium* as examined here. Additional work on the impact of the osmotic conditions in *Symbiodinium spp*. is required to determine if similar impairments as demonstrated in other marine species occur to its photosynthetic machinery when under hypo-osmotic stress. Analyzing anti-oxidant levels specific to the thylakoid membrane and measuring cell volume may provide the first indication of true morphological damage along the photosynthetic pathway in *Symbiodinium spp*. as seen in other marine algae (Jahnke and White 2003).

Significant differences in F_v/F_m among basin populations were measured in the controls and during the changing treatment salinities in this experiment. Photochemical efficiencies among basin populations followed the geographic and historical water quality gradients in Florida Bay. Blackwater Sound and Twin Key Basin controls (salinity of 30) exhibited significantly lower yields pooled over the experiment compared to all other basins. Blackwater Sound is hydrologically similar to more central and western basins due in part to its close connections with marine waters from Biscayne Bay and channels directly connecting it to the Atlantic side of the Florida Keys. Colonies in Blackwater Sound were clearly paler in color compared with all other basins when collected in October 2006. Persistent algal blooms and poor water quality from November 2005 to October 2006 (Rudnick 2006, Chartrand pers. observ.) in this basin may have triggered partial bleaching in this population. It is unclear what role these factors had on this population during the experiment. However, despite lower control maximum yields, Blackwater Sound colonies generally maintained high yields within reduced-salinity treatments similar to the other northeast basin populations. Duck Key Basin and Eagle Key Basin had high F_v/F_m in both controls and at reduced salinities, suggesting acclimation to the high variance and lower mean salinities in the historical salinity record for these regions (Fig 6). Twin Key Basin diverged consistently from all other basins in both controls and in salinity treatments with a consistent trend of lower yields in all treatments over time. The overall reduced yields in TWN colonies may be an indication of a local shade-adapted population versus sun-adapted colonies in all other basins. The depth in TWN is greater than all other basins surveyed and persistent algal blooms in this region prior to and during coral sampling may have generated a shift toward greater algal accessory pigments for greater light capture while reducing the number of total reaction centers. Placing such shade-adapted colonies under higher ambient light levels would likely result in lower overall efficiencies as seen throughout the experiment. Furthermore, F_v/F_m was significantly lower in Twin Key Basin colonies once salinity treatment of 10 was reached and for the remaining three days of the experiment. The lower photochemical efficiency of PSII in this population at reduced salinities is consistent with historical salinity records, which indicate that salinities have never dropped below 20 in the last century. Colonies have likely acclimated or adapted to the more stable marine salinities of Twin Key Basin whereas widely fluctuating conditions in northeastern basins may have led to a wider tolerance range in these communities. F_v/F_m for Calusa Key Basin colonies was never significantly lower than Duck Key Basin, Eagle Key Basin, or Blackwater Sound in all salinities although yields consistently were lower. Yet, F_v/F_m for Calusa Key Basin colonies were never below the always lowest yields in the Twin Key Basin population. These results further reinforce the correlative trends between F_v/F_m in the symbiont and the physical-salinity gradient in the Bay.

Assessing the photophysiology of *Symbiodinium* with PAM fluorometry has advanced our understanding of symbiont physiology and how it may directly impair the coral-dinoflagellate association when under various environmental stressors like salinity. However, discovery of distinct types or clades within the genus *Symbiodinium* has raised concerns regarding how such variability may influence the holobiont (Blank and Trench 1985). The complexity of species identification due to indistinguishable morphological characters within the genus has fostered the use of molecular techniques to characterize distinct symbiont types or clades (Rowan and Powers 1991, Loh et al. 2001, Santos et al. 2002). Development of refined molecular markers led to resolution of symbiont types and a subclade classification scheme (Hunter et al. 1997, LaJeunesse 2001). Large-scale sampling efforts have identified geographic patterns based on subclade classification and increased our understanding of diversity in this genus. Recent work by Iglesias-Prieto et al. (2004) and Sampayo et al. (2007) identified ecologically relevant patterns in symbiont physiology as it pertains to light and depth. How such patterns relate to ecologically relevant stressors and subclade variability may provide valuable information as to holobiont success or failure under increasing stress at the local (i.e. salinity and runoff) and global scale (i.e. temperature and ocean acidity).

Practical considerations when using PAM Fluorometry

High variance in midday effective quantum yields ($\Delta F/F_m'$) obscured any apparent effect of salinity treatments on the photochemical efficiency of PSII as measured with the diving-PAM fluorometer. Dynamic light fields during midday measurements increased the inherent variability among colonies, confounding treatment effects. Measuring photochemical efficiencies at dawn allowed all colonies the same extended dark-adaptation/recovery period minimizing the effects from the previous day’s light conditions. At dawn, reaction centers should be fully relaxed and alternative pathways for energy-dissipation (i.e. non-photochemical quenching including xanthophyll cycling) no longer in use (Brown et al. 1999). By removing the “noise” of ambient light, the effect of salinity treatments on the potential photochemical efficiency of the PSII apparatus was more discernible. However, despite nearly 11 hours in full darkness, dawn F_v/F_m exhibited a consistent, albeit insignificant trend negatively correlated with the previous days’ light history in both controls and treatment colonies (Fig 7). This observation is somewhat surprising given repair and recovery from typical downregulation following high light is thought to generally occur within a few hours as non-photochemical quenching (NPQ) components are relaxed (Ruban and Horton 1995, Brown et al. 1999, Hill et al. 2005). However, only two of the three described components of NPQ, qE and qT, are thought to dissipate relatively quickly (Müller et al. 2001). The function of the third component, qI is less well described and may not have fully dissipated despite the extended period of darkness prior to dawn sampling. Alternatively, photodamage due to high light during this experiment is unlikely given irradiance levels in the greenhouse did not get above 800 μmol photons m^{-2} s^{-1} and UV is nearly totally blocked by the greenhouse glass. It is unclear what other photo-regulatory mechanisms may account for this trend following such a long dark-acclimation period. It is noteworthy to consider what other long-term processes may be active in addition to those described as part of NPQ.

Management Implications

The distribution of coral populations in Florida Bay has only recently been documented by ongoing landscape-scale monitoring surveys (see Chapter 1). Plans to restore freshwater flow through the Everglades into Florida Bay have not considered what impact reduced salinities will have on corals in this region. *S. radians* appears to have greater tolerance to reduced salinities than most symbiotic corals, as documented in the current study, however the threshold for which this species can survive may be surpassed if significant freshwater inflows reach many of the northeastern basins of Florida Bay. If freshwater reaches more southern basins such as Twin Key Basin, coral colonies will likely fail to survive an osmotic stress below a salinity of 15.

The tolerance of *S. radians* to reduced salinities documented in this study is much higher than previously documented for scleractinian corals. Effect of hyposaline conditions was measured using photochemical efficiencies of PSII of the endosymbiont *Symbiodinium* in a dark-acclimated state as an indicator of stress to the holobiont. Population-based responses to reduced salinities correlated with historical salinity patterns across a physical-environmental gradient from the northeast to southwest basins in the Bay. Acclimatization to local salinity regimes within basins was most apparent in the lowest tolerance to the hypo-saline conditions by colonies from Twin Key Basin compared to all other basins. However, consistent trends in reduced F_v/F_m by all basin populations were apparent at the most reduced salinities.

The recent focus on large-scale temperature effects on coral symbioses is understandable given future climate change predictions, however it is important to recognize other factors that can drastically impact local coral populations. Understanding the effects of reduced salinity on corals in South Florida is imperative as plans move forward to alter the watershed that flows not only to Florida Bay, but also may directly reach the Florida Keys Reef Tract. Identifying corals that appear to be more tolerant to extreme and highly-variable conditions becomes ever more important as anthropogenic impacts on coral communities increase in coastal regions and as these habitats shift landward under changing sea level and new climate conditions.

LITERATURE CITED

Anthony KR, Conolly SR, Willis BL (2002) Comparative analysis of energy allocation to tissue and skeletal growth in corals. Limnol Oceanogr 47(5): 1417–1429

Becker G, Norman J, Moholl-Siebett M (1990) Two sites of heat-induced damage to photosystem II. In Current Research in Photosynthesis. vol IV, Ed. M. Baltscheffsky. 705-708

Ben-Amotz A (1973) The Role of Glycerol in the Osmotic Regulation of the Halophilic Alga *Dunaliella parva*. J Phycol 51: 875–878

Blank RJ, Trench RK (1985) Speciation and symbiotic dinoflagellates. Science 229: 656–658

Brown BE, Ambarsari I, Warner ME, Fitt WK, Dunne RP, Gibb SW, Cummings DG (1999) Diurnal changes in photochemical efficiency and xanthophyll concentrations in shallow water reef corals: evidence for photoinhibition and photoprotection. Coral Reefs 18: 99-105

Carpenter LW, Patterson MR (2007) Water flow influences the distribution of photosynthetic efficiency within colonies of the scleractinian coral *Montastrea annularis* (Ellis and Solander, 1786); implications for coral bleaching. J Exp Mar Biol 351: 10-26

Chitlaru E, Pick U (1991) Regulation of glycerol synthesis in response to osmotic changes in *Dunaliella.* Plant Physiol 96: 50–60

Coles SL, Jokiel PL (1992) Effects of salinity on corals. In: Pollution in tropical aquatic systems. Connell DW, Hawker DW Eds. CRC Press, Boca Raton 147-166

Finelli CM, Helmuth BST, Pentcheff ND, Wethey DS (2006) Water flow influences oxygen transport and photosynthetic efficiency in corals. Coral Reefs 25 (1): 47-57

Fitt WK, Brown BE, Warner ME, Dunne RP (2001) Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. Coral Reefs 20: 51-65

Gates RD, Hoegh-Guldberg O, McFall-Ngai MJ, Bil KY, Muscatine L (1995) Free aminoacids exhibit anthozoan host factor activity: they induce the release of photosynthate from freshly isolated symbiotic dinoflagellates in vitro. Proc Natl Acad Sci U.S.A. 92: 7430-7434

Gates RD, Edmunds PJ (1999) The physiological mechanisms of acclimatization in tropical reef corals. Am Zool 39:30-43

Glynn PW (1996) Coral reef bleaching: facts, hypotheses and implications. Glob Ch Biol 2: 495-509

Goreau TF, Goreau NI (1959) The physiology of skeleton formation in corals. II. Calcium deposition by hermatypic corals under various conditions in the reef. Biol Bull 117: 239-250

Gross E, Dilley RA, San Pietro A (1969) Control of electron flow in chloroplasts by cations. Arch Biochem Biophys 134: 450–462

Guinotte JM, Buddemeier RW, Kleypas JA (2003) Future coral reef habitat marginality: temporal and spatial effects of climate change in the Pacific basin. Coral Reefs 22: 551-558

Hackney JW and Durako MJ (2004) Size–frequency patterns in morphometric characteristics of the seagrass *Thalassia testudinum* reflect environmental variability. Ecol Indic 4: 55-71

Hill R, Schreiber U, Gademann R, Larkum AWD, Kuhl M, Ralph PJ (2004) Spatial heterogeneity of photosynthesis and the effect of temperature induced bleaching conditions in three species of corals. Mar Biol 144: 633-640

Hill R, Frankart C, Ralph PJ (2005) Impact of bleaching conditions on the components of non photochemical quenching in the zooxanthellae of a coral. J Exp Mar Bio Ecol 22(1): 83-92

Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the word's coral reefs. Mar Freshwat Res 50: 839–866

Hoegh-Guldberg O, Jones RJ (1999) Photoinhibition and photoprotection in symbiotic dinoflagellates from reef-building corals. Mar Ecol Prog Ser 183:73-86

Hunter CL, Morden CW, Smith CM (1997) The utility of ITS sequences in assessing relationships among zooxanthellae and corals. Proc 8th Intl Coral Reef Symp, Panama 2: 1599–1602

Iglesias-Prieto R, Matta JL, Robins WA, Trench RK (1992) Photosynthetic Response to Elevated Temperature in the Symbiotic Dinoflagellate *Symbiodinium microadriaticum* in Culture. Proc Natl Ac Sci 89: 10302-10305

Iglesias-Prieto R, Beltran VH, LaJeunesse TC, Reyes-Bonilla H, Thome PE (2004) Different algal symbionts explain the vertical distribution of dominant reef corals in the Eastern Pacific. Proc R Soc London, Ser B, Biol Sci 271: 1757–1763

Jahnke LS, White AL (2003) Long-term hyposaline and hypersaline stresses produce distinct antioxidant responses in the marine alga *Dunaliella tertiolecta*. J Plant Physiol 160: 1193–1202

Jokiel PL, Coles SL (1990) Response of Hawaiian and other Indo-Pacific reef corals to elevated temperature. Coral Reefs. 8: 155-162

Jones RJ, Hoegh-Guldberg (1999) Effects of cyanide on coral photosynthesis: implications for identifying the cause of coral bleaching and for assessing the environmental effects of cyanide fishing. Mar Ecol Prog Ser 177: 83-91

Jones RJ, Ward S, Amri AY, Hoegh-Guldberg O (2000) Changes in quantum efficiency of Photosystem II of symbiotic dinoflagellates of corals alter heat stress, and of bleached corals sampled alter the 1998 Great Barrier Ref. mass bleaching event. Mar Fresh Res 51: 63-71

Jones RJ, Hoegh-Guldberg O (2001) Diurnal changes in the photochemical efficiency of the symbiotic dinoflagellates (Dinophyceae) of corals: photoprotection, photoinactivation, and the relationship to coral bleaching. Plant Cell Environ 24: 89-99

Jones RJ, Hoegh-Guldberg O, Larkum AWD, Schreiber U (1998) Temperature induced bleaching of corals begins with impairment of the CO2 fixation mechanism in zooxanthellae. Plant Cell Environ 21: 1219–1230

Jones RJ, Kildea T, Hoegh-Guldberg O (1999) PAM chlorophyll fluorometry: a new *in situ* technique for stress assessment in scleractinian corals, used to examine the effect of cyanide from cyanide fishing. Mar Poll Bull 38: 864-874

Kahn AE and Durako MJ (2005) The effect of salinity and ammonium on seed germination in *Ruppia maritima* from Florida Bay. Bull Mar Sci 77: 453–458

Kahn AE and Durako MJ (2006) *Thalassia testudinum* seedling responses to changes in and nitrogen. J Exp Mar Biol Ecol 335: 1-12

Kerswell AP, Jones RJ (2003) Effects of hypo-osmosis on the coral *Stylophora pistillata*: nature and cause of ‘low-salinity bleaching’. Mar Ecol Prog Ser 253: 145-154

Kuhl M, Cohen Y, Dalsgaard T, Barker Jorgensen B, Revsbech NP (1995) Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with microsensors for O2, pH and light. Mar Ecol Prog Ser 117: 159-172

LaJeunesse TC (2001) Investigating the biodiversity, ecology, and phylogeny of endosymbiotic dinoflagellates in the genus *Symbiodinium* using the ITS region: in search of a ‘species’ level marker. Journal of Phycol 37(5): 866–880

Lewis JB (1989) Spherical growth in the Caribbean coral *Siderastrea radians* (Pallas) and its survival in disturbed habitats. Coral Reefs. 7: 161-167

Light S, Dineen W (1994) Water control in the Everglades: a historical perspective, in Everglades the Ecosystem and its Restoration, S.M. Ogden and J.C. Ogden, Eds., St Lucie Press, Delray Beach, FL

Lirman D (2002) Back from the dead: the resilience of *Siderastrea radians* to severe stress. Coral Reefs 21: 291–292

Lirman D, Orlando B, Macia S, Manzello D, Kaufman L, Biber P, Jones T (2003) Coral communities of Biscayne Bay, Florida and adjacent offshore areas: diversity, abundance, distribution, and environmental correlates. Aq Conserv: Mar Fres Eco 13: 121-135

Lockau W (1979) The Inhibition of Photosynthetic Electron Transport in Spinach Chloroplasts by Low Osmolarity. Europ J Biochem 94(2): 365-373

Loh WKW, Loi T, Carter D, Hoegh-Guldberg O (2001) Genetic variability of the symbiotic dinoflagellates from the wide ranging coral species *Seriatopora hystrix* and *Acropora longicyathus* in the Indo-West Pacific. Mar Ecol Prog Ser 222: 97- 107

Loya Y, Saka K, Yamazato K, Nakano Y, Sambali H, van Woesik R (2001) Coral bleaching: the winners and the losers. Ecol Lett 4: 122–131

Manzello D, Lirman D (2003) The photosynthetic resilience of *Porites furcata* to salinity disturbance. Coral Reefs 22: 537–540

Marcus J, Thornhaug A (1981) Pacific versus Atlantic responses of the subtropical hermatypic coral *Porites spp*. to temperature and salinity effects. Proc 4th Int Coral Reef Symp, Quezon City 2: 15-20

Mayfield AB, Gates RD (2007) Osmoregulation in anthozoan-dinoflagellate symbiosis. Comp Biochem Phys Part A 147: 1-10

Muscatine L (1990) The role of symbiotic algae in carbon and energy flux in reef corals. In: Dubinsky Z (Ed.), Ecosystems of the World: Coral Reefs. Elsevier, Amsterdam, 75-87

Müller P, Li X, Niyogi KK (2001) Non-Photochemical Quenching: A response to excess light energy. Plant Physiol 125 (4): 1558-1566

Muthiga MA, Szmant AM (1987) The effects of salinity stress on the rates of aerobic and photosynthesis in the hermatypic coral *Siderastrea siderea*. Biol Bull 173: 539-551

Nuttle WK, Fourqurean JW, Cosby BJ, Zieman JC, Robblee MB (2000) The influence of net freshwater supply on salinity in Florida Bay. Water Resour Res 36 (7): 1805-1822

Philipp E, Fabricius K (2003) Photophysiological stress in scleractinian corals in response to short-term sedimentation J Exp Mar Bio Ecol 287: 57– 78

Ralph PJ, Larkum AWD, Kuhl M (2005) Temporal patterns in effective quantum yield of individual zooxanthellae expelled during bleaching. J Exp Mar Bio Ecol 316: 17- 28

Rinkevich B (1989) The contribution of photosynthetic products to coral reproduction. Mar Biol 101(2): 259-263

Robblee M (2000) Salinity Pattern in Florida Bay: A Synthesis. In: U.S. Geological Survey Program on the South Florida Ecosystem: 2000 Proceedings. Naples, Florida. 70-72

Rodríguez-Román AR, Hernández-Pech X, Thome PE, Enríquez S, Iglesias-Prieto R (2006) Photosynthesis and light utilization in the Caribbean coral *Montastraea faveolata* recovering from a bleaching event Limnol Oceanogr 51(6): 2702-2710

Rowan R, Powers DA (1991) A molecular genetic classification of zooxanthellae and the evolution of animal-algal symbioses. Science 251: 1348–1351

Ruban AV, Horton P (1995) Regulation of nonphotochemical quenching of chlorophyll fluorescence in plants. Aust J Plant Physiol 22: 221-230

Rudnick D (2006) Report on Algae Blooms in Eastern Florida Bay and Southern Biscayne Bay. South Florida Water Management District. Florida.

Sampayo EM, Franceschinis L, Hoegh-Guldberg O, Dove S (2007) Niche partitioning of closely related symbiotic Dinoflagellates. Molec Ecol 16: 3721–3733

Santos SR, Taylor DJ, Kinzie RA, Sakai K, Coffroth MA (2002) Evolution of length variation and heteroplasmy in the chloroplast rDNA of symbiotic dinoflagellates (*Symbiodinium*, Dinophyta) and a novel insertion in the universal core region of the large subunit rDNA. Phycologia. 41 (4): 311–318

Saxby T, Dennison WC, Hoegh-Guldberg O (2003) Photosynthetic responses of the coral *Montipora digitata* to cold temperature stress. Mar Ecol Prog Ser 248: 85- 97

Schick HM (1991) Functional Biology of Sea Anemones. Chapman and Hall, London.

Schomer NS, Drew RD (1982) An ecological characterization of the lower Everglades, Florida Bay, and the Florida Keys: U.S. Fish and Wildlife Service, Office of Biological Services, FWS/OBS-82/58

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

Smith NP (2002) Florida bay circulation studies. Recent Research Developments in Geophysics 4: 93-104.

Trench RK (1979) The cell biology of plant animal symbioses. Annu. Rev. Plant Physiol. 30: 485–531

US Army Corps of Engineers and South Florida Water Management District (2000) Master Program Management Plan. Vol. I. http://www.evergladesplan.org/pm/program_docs/mpmp.aspx

van Oppen MJH, Mahiny AJ, Done TJ (2005) Geographic distribution of zooxanthellae types in three coral species on the Great Barrier Reef sampled after the 2002 bleaching event. Coral Reefs 24: 482-487

Veron JEN (2000) Corals of the World. Australian Institute of Marine Science, Townsville, Australia

Warner ME, Fitt WK, Schmidt GW (1996) The effects of elevated temperature on the photosynthetic efficiency of zooxanthellae *in hospite* from four different species of reef coral: a novel approach. Plant Cell Environ 19: 291-299

Warner ME, Fitt WK, Schmidt GW (1999) Damage to photosystem II in symbiotic dinoflagellates: A determinant of coral bleaching. Proc Natl Acad Sci 96: 8007-8012

Warner ME, Chilcoat GC, McFarland FK Fitt WK (2002) Seasonal fluctuations in the photosynthetic capacity of photosystem II in symbiotic dinoflagellates in the Caribbean reef-building coral *Montastrea*. Mar Biol 141: 31-38

Xia J, Li Y, Zou D (2004) Effects of salinity stress on PSII in *Ulva lactuca* as probed by chlorophyll fluorescence measurements Aquat Bot 80: 129–137

Yancey PH, Clark ME, Hand SC, Bowlus RD, Somero CN (1982) Living with water stress: evolution of osmolyte systems. Science 217: 1214-1222

CHAPTER 3

GENOTYPIC CHARACTERIZATION OF *SYMBIODINIUM* FROM HOST TISSUES OF *SIDERASTREA RADIANS* IN FLORIDA BAY, FL USA

INTRODUCTION

The diversity of *Symbiodinium* spp. in host-symbiont relationships in corals has led to numerous studies on the change in symbiotic partnership across varying physical and spatial scales (Buddemeier and Fautin 1993; Baker 2003; LaJeunesse et al. 2004). The "shuffling" or swapping of symbiotic dinoflagellates to those better adapted or acclimated to local conditions has been proposed to provide an overall benefit to the holobiont (Rowan and Powers 1991; Buddemeier and Fautin 1993). Identifying the range of natural and long-term spatial and physical gradients in the field over which a host species thrives might provide valuable insights on how the symbiont community may be altered under different conditions. Moreover, existing patterns of variability may also demonstrate whether the host-symbiont relationship can truly "shuffle" under predicted future climate fluctuations and provide a functional benefit to the holobiont.

In recent years, an increasing focus on the role of the symbiont has determined that the genus *Symbiodinium* Freudenthal is morphologically, physiologically and genetically diverse (Trench 1993, 1997; Rowan 1998; LaJeunesse 2001). At least eight known clades have been described. Numerous biogeographical descriptions of abundance and trends among and within clades have been published (see review by Baker 2003, LaJeunesse 2001). Several studies have also described the physiologies of various symbiont types in relation to stress. For instance, observations of thermal bleaching patterns have led to the description of clade D as a stress-tolerant clade (Toller 2001, Rowan 2004). Others have described particular types as "bleaching-susceptible" or "bleaching-resistant" (LaJeunesse et al. 2003). Recent work by Sampayo et al.

(2007) demonstrated that closely-related symbionts (within clades) were ecologically distinct along a depth and light gradient. However, it is still poorly understood whether quantifiable physiological differences exist among and within clades that relate to functional differences in holobiont physiology, under variable environmental patterns in the field.

Several molecular markers have been employed to distinguish among clades of *Symbiodinium* (Rowan and Powers 1991, Rowan and Powers 1995, Wilcox 1998). However, it is apparent that these cladal-level markers do not provide the resolution needed for ecologically relevant distinctions. LaJeunesse and Trench (2000) first used the internal transcribed spacer 2 region (ITS2) for differentiating *Symbiodinium* types within clade groupings. The ITS2 region is highly conserved so small differences within the sequence can indicate taxonomically significant distinctions. Denaturing-gradient gel electrophoresis (DGGE), a molecular tool used in concert with assessing the ITS2 region, enables discrimination between sub-clade types. Individuals are differentiated by single DNA base pair deletions or insertions. DGGE is commonly used in bacterial studies but only recently has been used for other taxa such as dinoflagellates (Muyzer and Smalla 1998, LaJeunesse and Trench 2000). Specifically, DGGE has been effective in identifying subcladal differences within *Symbiodinium* (LaJeunesse 2001, 2002, Thornhill et al. 2006a). The ITS2 region of *Symbiodinium* types commonly differ by only one or a few base pairs, thus can be effectively separated by this technique and compared with DGGE libraries of *Symbiodinium* spp. Alternative molecular tools such as cloning have been suggested, however they may produce inconclusive results outside of bacterial studies unlike results of DGGE analysis (Thornhill et al. 2007).

Florida Bay

Over the last century, agriculture, urban development and exponential population growth in South Florida has resulted in a dramatic change to the natural watershed of the Everglades and Florida Bay. Further alterations were made to freshwater inputs entering via the Everglades originating from Lake Okeechobee and the Kissimmee watershed, when the Army Corps of Engineers re-directed most of the flow to the Gulf of Mexico and Atlantic Ocean by the mid-20th Century (Light and Dineen 1994). As a result, Florida Bay began receiving 90 percent of its freshwater inputs from seasonal rainfall instead of constant flows via the Everglades (Light and Dineen 1994, Nuttle et al. 2000). More variable and higher average salinities resulted bay-wide. The northeast region of the Bay, already physically restricted, has exhibited the highest and most variable salinities over the last one hundred years while areas to the southwest maintain less variable and more typical marine conditions due to mixing with waters from the Gulf of Mexico (Fig. 13).

Mesocosm studies on the photophysiological response of *S. radians* to reduced salinities indicated a populational response among basins that correlated with the historical physical-salinity gradient in the Bay (see Chapter 2). The most southwest basin, TWN (see Table I), exhibited significantly lower dark-acclimated quantum yields at both control salinities and reduced-salinity treatments compared to all other basins. This substantial reduction in photoefficiency may be associated with an acclimatory response to historical salinity patterns in the symbiont. However, it is unknown whether the symbiont population within *S. radians* in the Bay is homogenous, as expected (Thornhill et al. 2006b), or has shifted under the long-term historical changes to water quality in the Bay.

This study aimed to identify whether the symbiont assemblage in *S. radians* varies among basin populations in Florida Bay. A change in symbiont type is one mechanism by which corals may respond to changes in abiotic factors such as salinity (Buddemeier and Fautin 1993). Furthermore, the pattern in mean salinity and salinity variability is most pronounced at the extremes of the Bay's coral distribution gradient and so it would be expected that any shift in symbiont assemblage would be most notable here as was manifested in TWN (the most marine basin) during the mesocosm study (see Chapter 2).

METHODS

During August 2007, *S. radians* colonies were collected for molecular characterization of the *Symbiodinium* population. Colonies were collected from each of five basins along a northeast-to-southwest physical-environmental gradient in Florida Bay (Fig. 1). Collection locations were the same as those visited previously during distribution surveys and used in mesocosm experiments (see Chapter 1 and 2) and were re-located by GPS. Five colonies (maximum diameter ≤ 3-5 cm) were collected in each basin with hammer and chisel and returned to the lab in ambient seawater for processing. Coral tissues were extracted from individual colonies using an airbrush. The resulting slurry was homogenized briefly using a blender in order to free the symbiont from lipid-rich host tissue. The slurry was then centrifuged in 50ml sterile tubes for approximately five minutes to obtain the algal pellet while residual host tissue and seawater was discarded. The pellet was placed in 1.5ml Eppendorf tubes with 90% ethanol and stored on ice until processed further.

Subsequent processing followed the methods described in LaJeunesse et al. (2003). DNA was extracted using the Promega Wizard Kit and described briefly here. Between 20-40 mg of pelleted material was placed in a screw cap tube with 500 µl autoclaved deionized water (dH_2O) to rinse cells. After centrifuging for one minute, water was removed and 600 µl Nuclei Lysis Buffer (0.2M Tris, 2mM EDTA, 0.7% SDS, pH 7.5) and 200 µl glass beads were added. Tubes were bead beaten at high speed for 120 s in a Bio-Spec Mini-Beadbeater. The lysate was incubated with 0.1 mg ml^{-1} proteinase K for 1 h at 65ºC with vortexing every ~15 min. Samples were allowed to cool for 5 min at room temperature (RT) before adding 6 µg ml^{-1} RNAse, mixing well and keeping samples at room temp for another 10 min. 250 µl of protein precipitation buffer (Promega) was added and extracts placed on ice for 15 min. After centrifugation for 10 min at 13,000 rpm, 600 µl of supernatant was transferred to a fresh 1.5 ml tube with 700 µl 100% isopropanol (at RT) and 40 µl 3 M sodium acetate, pH 7.5. Samples were placed on ice for 20-30 min before being spun down, isopropanol vacuumed off, and 500 µl 70% ethanol added to wash the pellet. The DNA was centrifuged a final time, dried, and re-suspended in 100 µl dH_2O. Samples were run on a 0.8% agarose gel to ensure positive DNA extraction. Samples were kept at –20ºC until further processing.

PCR-DGGE analysis was used to differentiate *Symbiodinium* types at the sub-clade molecular level (LaJeunesse 2001, 2002). Each sample was diluted 1:10 and 1 µl used as a template for PCR amplification. The ITS2 was amplified using primers developed by LaJeunesse and Trench (2000) to produce a 300-360 bp sequence. The forward primer ITSinfor2 (5' - GAATTGCAGA ACTCCGTG- 3') anneals to a conserved region of the 5.8S ribosomal DNA while the reverse primer anneals to the LSU. The reverse primer, ITSCLAMP (5'- CGCCCGCCGC GCCCCGCGCC CGTCCCGCCG CCCCGCCC GGGATCCATA TGCTTAAGTT CAGCGGGT-3'), contains a GC rich tail or 'clamp' that alters melting properties of an amplified sequence and thus enables detection of single base pair changes during

DGGE that may otherwise go undetected due to the inherent properties of the sequence of interest (Sheffield et al 1989; LaJeunesse and Trench 2000; LaJeunesse et al. 2003).

A modified PCR thermal cycle profile called a “touchdown” amplification was run on all extracted samples (Don et al. 1991; LaJeunesse 2002, LaJeunesse et al. 2003). Briefly, the annealing temperature was reduced 0.5ºC after each of 20 cycles from 62ºC to 52ºC. Annealing conditions were held at 52ºC for another 20 cycles (LaJeunesse et al. 2003; Kemp pers. comm.) before loading onto an 8% polyacrylamide denaturing gradient gel (45–80% ureaformamide gradient; 100% consists of 7 M urea and 40% deionized formamide). Samples were separated by electrophoresis for 10 h at a constant 60ºC and 150 volts (LaJeunesse 2002). Samples were run with previously identified standards (Schmidt lab, Athens GA). The gel was stained with Sybr Green (Molecular Probes) according to the manufacturer's specifications and photographed. Discreet, prominent bands that did no match known profiles were excised, re-amplified and sequenced as carried out by LaJeunesse (2002).

RESULTS

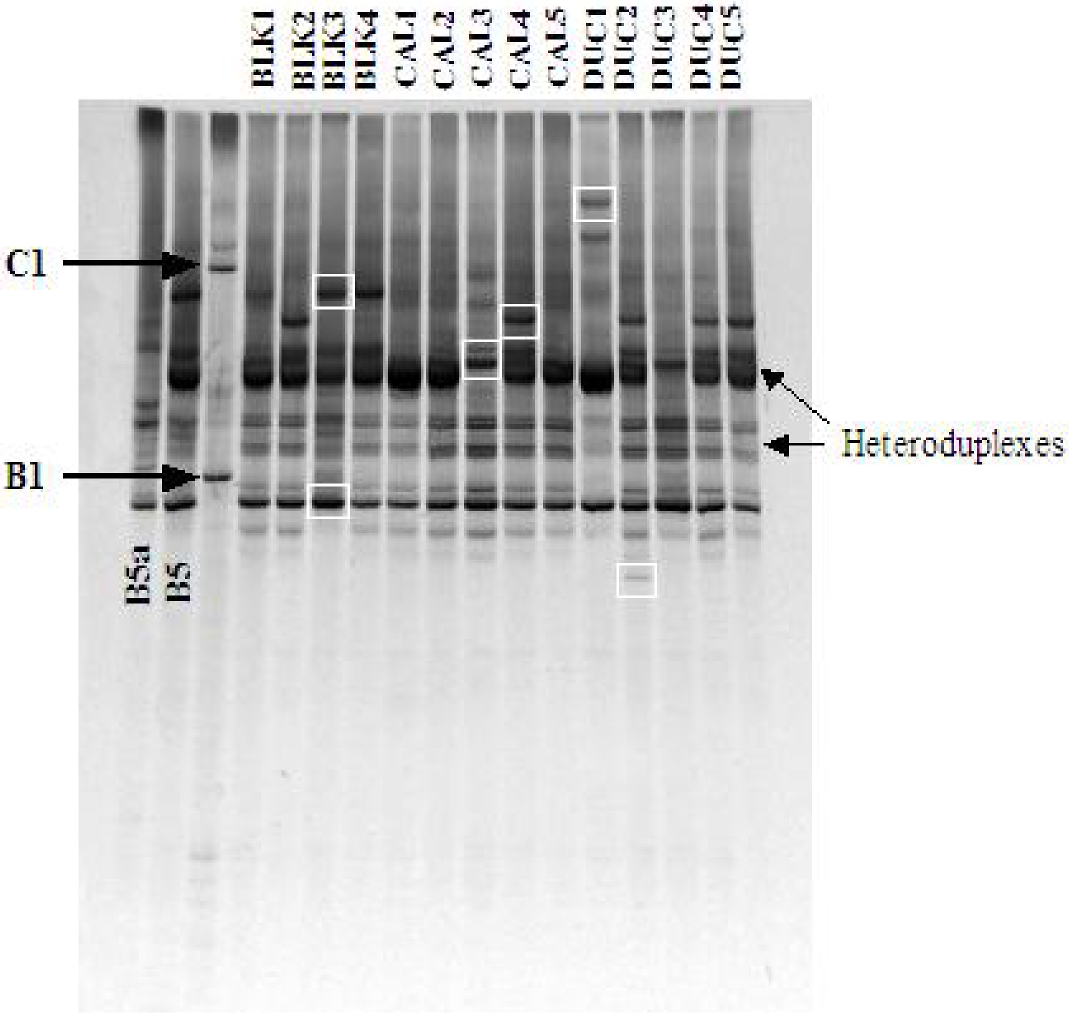
All colonies sampled from BLK, DUC, and EAG (n=5 except n=4 in BLK) displayed DGGE profiles consistent with the previously identified ITS2 type B5 (Fig. 13a-b). Residual stained bands in these DGGE profiles that had been excised and sequenced were determined to be either host ITS sequence remaining from the DNA extraction process, heteroduplexes, or lesser intragenomic variants (Thornhill pers. comm.). These additional bands are not thought to interfere with the distinct banding pattern found in samples which are comparable to ITS2 library profiles.

In contrast, all samples from TWN (n=4) showed a unique banding profile (Fig. 13b). Distinctive excised bands from TWN contained 2 base pair changes that were functional within the ITS secondary structure and therefore accepted as a true subcladal variant rather than an intragenomic variant within the B5 genome (Thornhill personal communication). The first base pair shift is consistent with B5a while the second base pair shift at 120 within Helix IIIb is a novel substitution, thus we are calling this variant 'B5b'. This base pair substitution is tolerated in folding of the secondary structure and thus is considered a functional variant (Thornhill et al. 2007). A base pair change in the ITS region is believed to become dominant within the entire genome of that variant over large evolutionary time scales (Thornhill et al. 2007). Therefore, the strong dominance of this single band within multiple samples strongly indicates a significant alternative symbiont population within this basin compared to B5 found throughout the rest of the sampled Florida Bay population.

DISCUSSION

Most colonies investigated in this study maintained a distinct association with the dominant ITS2 type of *Symbiodinium*, B5 (Fig. 14). This symbiont type was also found in *S. radians* during previous surveys in northeastern Florida Bay (Thornhill et al. 2006b). A review by Goulet (2007) found that the majority of corals (77%) examined do not change their symbiont. Furthermore, Thornhill et al. (2006b) concluded that brooding species, whose symbionts are transferred 'vertically', i.e. directly from host to offspring, maintain stable symbioses with a single discrete symbiont. Consequently, the finding of the unique *Symbiodinium* variant B5b within *S. radians* in TWN is remarkable (Fig. 14).

a)


BLK1
BLK2
BLK3
BLK4
CAL1
CAL2
CAL3
CAL4
CAL5
DUC1
DUC2
DUC3
DUC4
DUC5
C1
B1
B5a
B5
Heteroduplexes

b)

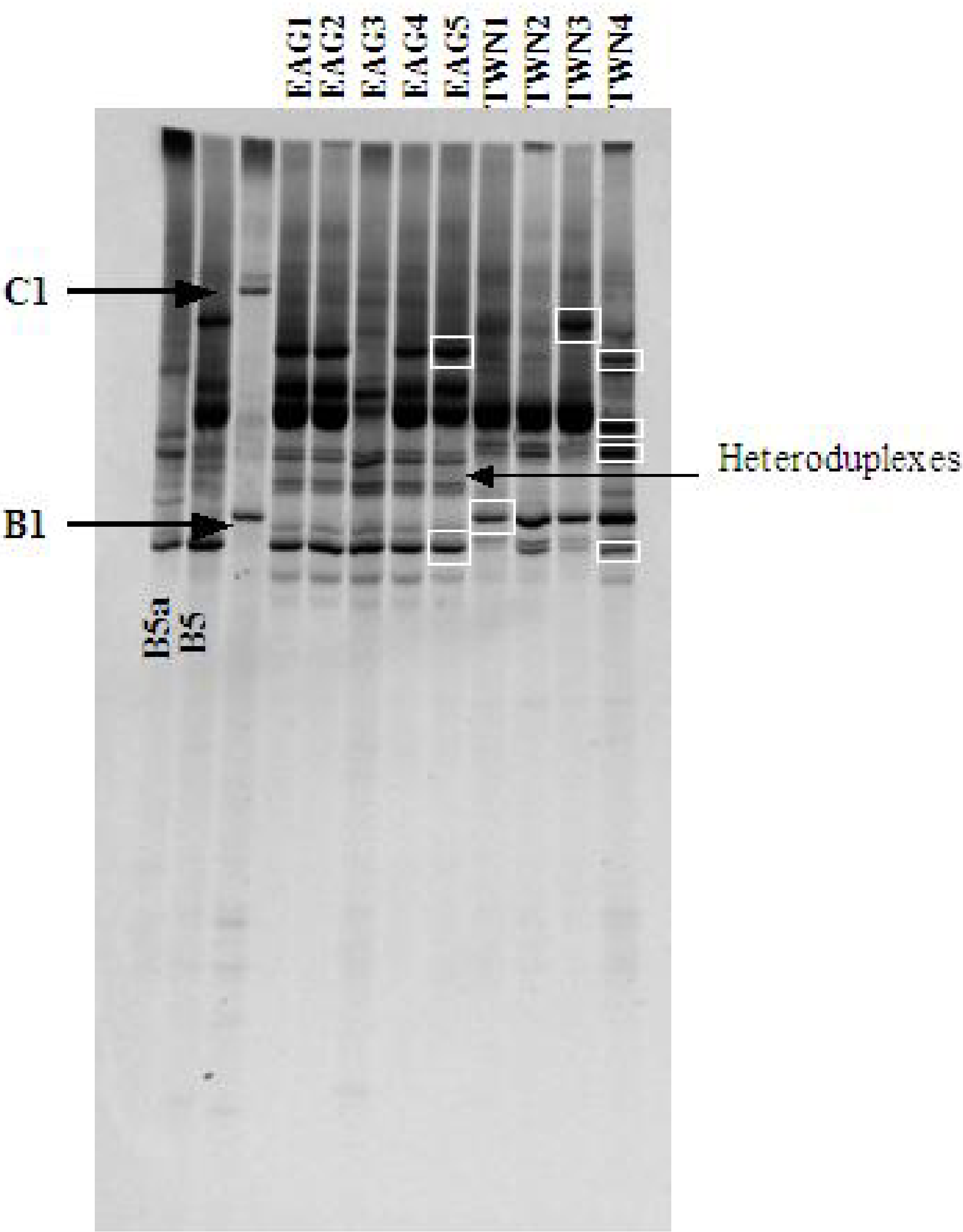


Figure 13 *Symbiodinium spp*. PCR-DGGE [polymerase chain reaction denaturing gradient gel electrophoresis (DGGE)] profile of ITS2 (internal transcribed spacer 2) from *S. radians* in Florida Bay. Samples are labeled above their respective sample column according to basin name and sample number. The first 3 columns in gels a) and b) represent purified markers used from lab stocks.

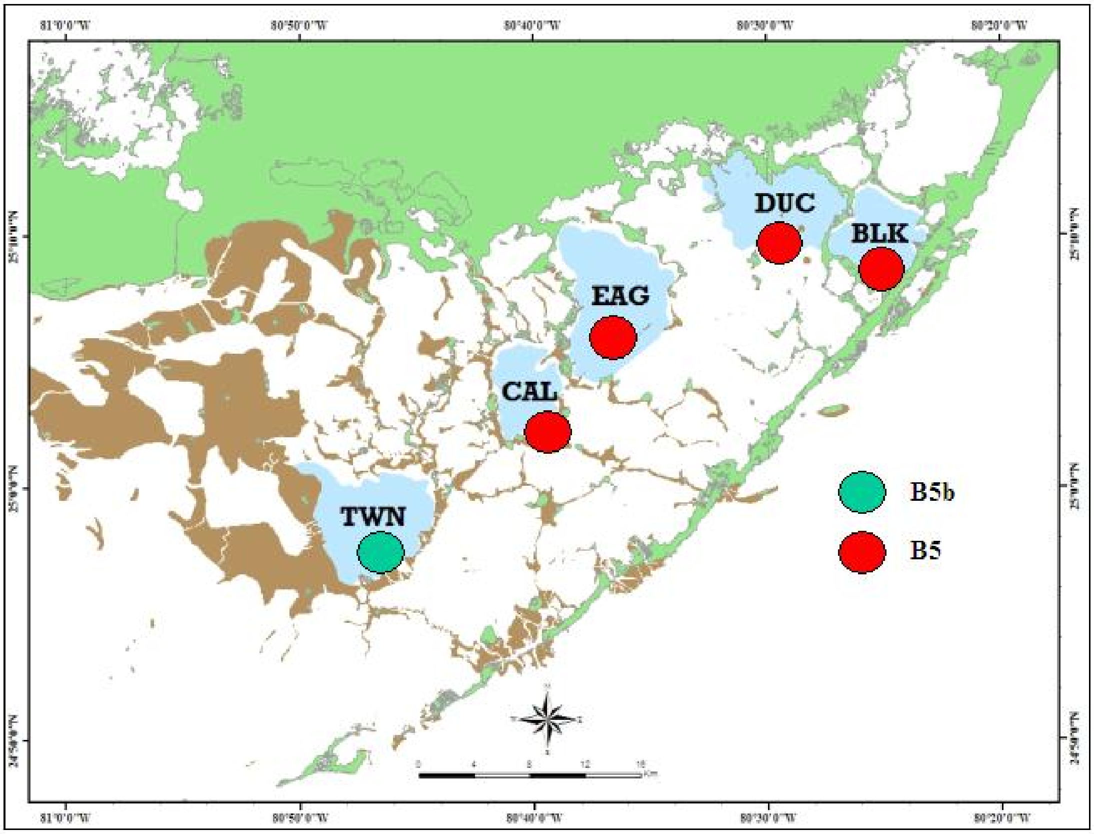


Figure 14 *Symbiodinium* sub-cladal types detected by PCR-DGGE analysis of the ITS 2 across basins of interest in Florida Bay, FL USA. n = 5 in DUC, EAG, and CAL and n =4 in BLK and TWN

TWN is the most southern and marine-influenced basin from which colonies were sampled. Bottom salinities within TWN over the past 18 years have remained within a typical marine range (mean salinity of 35.85 ± 4.0, Fig. 6) and are reported to have maintained these conditions over the last century (Robblee 2000). Conversely, large inter- and intra-annual salinity fluctuations have dominated the more northeastern basins of BLK, DUC, EAG, and to a lesser extent CAL (Fig. 6).

S. radians possesses other ITS types, B5a and C1, from Lee Stocking Island, Bahamas and Puerto Morelos, Mexico, respectively (Thornhill et al. 2006b, LaJeunesse 2002). Thornhill et al. (2006b) proposed that distinct subpopulations of brooding species may exist in which a unique symbiont type is maintained discretely from the larger species population. This hypothesis may explain the unique ITS type found in TWN. However, it was noted, that this may not account for patterns in some populations. For instance, Indo-Pacific brooding species possess a diverse symbiont community unlike trends found in Caribbean brooders (Thornhill et al. 2006b).

The new variant, B5b, is a two base pair substitution from B5 found elsewhere in the Bay and only one base pair from the variant B5a. A new ITS sequence becomes dominant within the genotype only after the turnover of numerous generations (Thornhill et al. 2007). Thus, despite the modest difference in the ITS2 sequence, these differences are considered to represent truly distinct sub-types. Previous work evaluating the photophysiology of the symbiont *in hospite* found colonies with the unique symbiont type responded differently to an environmental stressor (Chapter 2). The symbiont was measured to indirectly evaluate if the tolerance of *S. radians* to reduced salinities varied among basin populations. The results of this work indicated that there was a significant difference in photochemical efficiencies under near-ambient salinities and efficiencies under hyposalinity stress of *S. radians* from TWN versus all other sampled basins (BLK, DUC, EAG, and CAL; see Chapter 2). The genetic patterns established here correlate both with the physiological findings and historical physical-environmental gradient in the Bay, thus providing a possible explanation for the population level response.

The establishment of *S. radians* and its vertically-acquired symbiont in Florida Bay may be a result of several source populations entering the Bay during spatially or temporally separate recruitment events with shifting hydrodynamics. The population of *S. radians* in TWN may be a result of larval recruitment via large channels to the south (i.e. Long Key Channel and Seven Mile Bridge) whereas the northern population may be the consequence of recruitment from Biscayne Bay and Card Sound to the north. Alternatively, following the establishment of the coral population in TWN, water entering via the Gulf of Mexico may have provided a novel symbiont acquired ‘horizontally’, i.e. from the water column, in the most western basin. Other physical factors in the Bay may also influence the symbiont population structure. Despite a lack of significantly different mean basin temperatures where colonies were sampled (SERC data not shown), other physical factors can vary historically across the Bay (i.e. nutrient profiles and bottom PAR) and should be evaluated for their correlation with the observed symbiont population structure.

These results suggest ecological relevance of sub-cladal variation in coral symbionts and how a closely related variant may alter the overall functionality of the holobiont.

A recent study by Sampayo et al. (2007) found that symbionts within a single clade exhibited niche partitioning across a light/depth gradient. Other studies have also demonstrated that populations differing by a single base substitution exhibited distinctive environmental, geographic, and/or host taxa distributions (LaJeunesse 2001, 2002; Baker 2003; Rodriguez-

Lanetty et al. 2004). It is often stated that a wide variety of abiotic factors may influence the host-symbiont relationship. However, most research that has incorporated genetic analyses with functional and ecologically relevant trends has evaluated a limited range of such factors, namely light/depth and temperature. Certainly, these factors are at the core of coral research in light of predicted future climate patterns and the effect this will have on coral-symbiont relationships. However, other abiotic factors, like salinity, may provide valuable information on how local impacts can alter or breakdown the host-symbiont relationship. In Florida Bay, genetic identification of unique variants among local populations may correlate with the functionally distinct responses to salinity across a small-scale geographic physical-environmental gradient. This work demonstrates how these ecologically relevant physical-environmental gradients may also be linked to the unique distribution and functionally distinct physiologies of two discrete ITS2 types of *Symbiodinium*.

LITERATURE CITED

Baker AC (2003) Flexibility and specificity in coral-algal symbiosis: diversity, ecology and biogeography of *Symbiodinium*. Annu Rev Ecol Evol Syst 34:661–689

Buddemeier RW, Fautin DG (1993) Coral bleaching as an adaptive mechanism. Bioscience 43:320–326

Don RH, Cox PT, Wainwright BJ, Baker K, Mattick JS (1991) 'Touchdown' PCR to circumvent spurious priming during gene amplification. Nucl Ac Res 19(14): 4008

Goulet TL (2007) Most corals may not change their symbionts Mar Ecol Prog Ser 321: 1-7

LaJeunesse TC (2001) Investigating the biodiversity, ecology, and phylogeny of endosymbiontic dinoflagellates in the genus *Symbiodinium* using the ITS region: in search of a ‘‘species’’ level marker. J Phycol 37:866–880

LaJeunesse TC (2002) Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. Mar Biol 141:387–400

LaJeunesse TC, Trench RK (2000) The biogeography of two species of *Symbiodinium* (Freudenthal) inhabiting the intertidal anemone *Anthopleura elegantissima* (Brandt). Biol Bull 199:126–134

LaJeunesse TC, Loh WKW, van Woesik R, Hoegh-Guldberg O, Schmidt GW, Fitt WK (2003) Low symbiont diversity in southern Great Barrier Reef corals, relative to those of the Caribbean. Limnol Oceanogr 48:2046–2054

LaJeunesse TC, Bhagooli R, Hidaka M, deVantier L, Done T, Schmidt GW, Fitt WK, Hoegh Guldberg O (2004) Differences in relative dominance between closely related *Symbiodinium spp* in coral reef host communities across environmental, latitudinal, and biogeographic gradients. Mar Ecol Prog Ser 284:147–161

Light S, Dineen W (1994) Water control in the Everglades: a historical perspective, in Everglades the Ecosystem and its Restoration, S.M. Ogden and J.C. Ogden, Eds. St Lucie Press, Delray Beach, FL

Muyzer, G., and K. Smalla. (1998) Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie Leeuwenhoek 73: 127–141

Nuttle WK, Fourqurean JW, Cosby BJ, Zieman JC, Robblee MB (2000) The influence of net freshwater supply on salinity in Florida Bay. Wat Resour Res 36: (7)1805-1822

Robblee (2000) Salinity Pattern in Florida Bay: A Synthesis. In: U.S. Geological Survey Program on the South Florida Ecosystem: 2000 Proceedings. Naples, Florida. 70-72

Rodriguez-Lanetty M, Krupp D, Weis VM (2004) Distinct ITS types of *Symbiodinium* in clade C correlate to cnidarian/dinoflagellate specificity during symbiosis onset. Mar Ecol Prog Ser 275:97–102

Rowan R (1998) Diversity and ecology of zooxanthellae on coral reefs. J Phycol 344:7-17

Rowan R (2004) Thermal adaptation in reef coral symbionts. Nature 430:742

Rowan R, Powers DA (1991) A molecular genetic classification of zooxanthellae and the evolution of animal-algal symbioses. Science 251: 1348–1351

Rowan R, Knowlton N (1995) Intraspecific diversity and ecological zonation in coral algal symbiosis. Proc Nati Acad Sci USA 92: 2850–2853

Sampayo EM, Franceschinis L, Hoegh-Guldberg O, Dove S (2007) Niche partitioning of closely related symbiotic Dinoflagellates. Molec Ecol 16: 3721–3733

Sheffield VC, Coxt DR, Lerman LS, Myers RM (1989) Attachment of a 40-base-pair G+C-rich sequence (GC-clamp) to genomic DNA fragments by the polymerase chain reaction results in improved detection of single-base changes Proc Nati Acad Sci USA 86:232-236

Thornhill DJ, LaJeunesse TC, Kemp DW, Fitt WK, Schmidt GW (2006a) Multi-year, seasonal genotypic surveys of coral–algal symbioses reveal prevalent stability or post-bleaching reversion. Mar Biol 148:711–722

Thornhill DJ, Fitt WK, Schmidt GW (2006b) Highly stable symbioses among western Atlantic brooding corals Coral Reefs 25: 515-519

Thornhill DC, LaJeunesse TC, Santos SR (2007) Measuring rDNA diversity in eukaryotic microbial systems: how intragenomic variation, pseudogenes, and PCR artifacts confound biodiversity estimates Molec Ecol 1-15 doi: 10.1111/j.1365 294X.2007.03576.x

Toller W, Rowan R, Knowlton N (2001) Zooxanthellae of the *Montastrea annularis* species complex: patterns of distribution of four taxa of Symbiodinium across different reefs and across depths. Biol Bull 201:348–359

Trench RK (1993) Microalgal-invertebrate symbiosis: a review. Endo Cell Res 9:135–75

Trench RK (1997) Diversity of symbiotic dinoflagellates and the evolution of microalgal invertebrate symbioses. In: Proceedings of the 8th International Coral Reef Symposium 2:1275–1286

Wilcox TP (1998) Large subunit ribosomal RNA systematics of symbiotic dinoflagellates: morphology does not recapitulate phylogeny. Mol. Phylogenet.Evol. 10: 436–448