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ORIGINAL PAPER

Effect of hyposalinity on the photophysiology of *Siderastrea radians*

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Abstract Tolerance to hyposalinity of the scleractinian coral *S. radians* was examined in a mesocosm study. Colonies of *S. radians* were collected from five basins in Florida Bay, USA, which occur along a northeast-to-southwest salinity gradient. Salinity treatments were based on historical salinity records for these basins. Photophysiology of the endosymbiont *Symbiodinium* spp. (maximum quantum yield; F_v/F_m) was measured as an indicator of holobiont stress to hyposalinity. Colonies from each basin were assigned four salinity treatments [The Practical Salinity Scale (PSS) was used to determine salinity. Units are not assigned to salinity values because it is a ratio and has no unit as defined by UNESCO (UNESCO Technical papers no. 45, IAPSO Pub. Sci. No. 32, Paris, France, 1985)] (30, 20, 15, and 10) and salinities were reduced 2 per day from ambient (30) to simulate a natural salinity decrease. Colonies treated with salinities of 20 and 15 showed no decrease in F_v/F_m versus controls (i.e. 30), up to 5 days after reaching their 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. Within 1 day after salinity of 10 was reached, there was a significant reduction in F_v/F_m, indicating a critical threshold for hyposaline tolerance. At the lowest treatment salinity (10), F_v/F_m for the more estuarine, northeast-basin colonies were significantly higher than the most marine southwest-basin colonies (Twin Key Basin). Our results suggest that historical salinity ranges within basins determine coral population salinity tolerances.

Introduction

Siderastrea radians (Pallas), order Scleractinia family *Siderastreidae*, is distributed throughout the tropical Atlantic and Caribbean region (Veron 2000). These ahermatypic corals form small encrusting-to-hemispherical mounds that may be attached to, or free from, the substrate and reach a maximum diameter of 10 cm (Lewis 1989; Veron 2000). They often occur in shallow lagoonal backreef areas and so-called marginal habitats where physical parameters can vary widely (Lirman 2002). A marginal habitat is defined here on the basis of proximity to an environmental condition that places 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 seasonal salinity and temperature ranges of 15–40 and 16–31°C and consistently high sedimentation rates (Lirman et al. 2003). *Siderastrea radians* has also shown high 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

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demonstrating its ability to withstand and thrive in marginal environments (Lirman 2002).

Siderastrea radians harbors symbiotic dinoflagellates of the genus *Symbiodinium* spp. The symbiont translocates photosynthate to its host supporting metabolic processes including calcification, reproduction, and growth for the host colony (Muscatine 1990; Rinkevich 1989; Anthony et al. 2002). The symbionts 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). Because of this functional importance, much work has focused on the photophysiological characteristics of *Symbiodinium* in response to stress conditions since they may determine whether the host–symbiont relationship is maintained or terminated (i.e. coral beaching; Iglesias-Prieto et al. 1992; Jones et al. 1998). A variety of stressors disrupt autotrophic functioning of the symbiont causing critical damage to host function (Jones et al. 1999; Philipp and Fabricius 2003; Kerswell and Jones 2003; Saxby et al. 2003; Ralph et al. 2005). Large-scale bleaching due to increased temperature and/or high solar radiation has been a major area of coral research as such events have become more frequent and are correlated with large-scale coral mortality (Hoegh-Guldberg 1999). However, understanding the effects of localized stressors, such as reduced salinity from increased runoff, that may impair symbiont function and thus holobiont condition are also critical for protection of corals growing in marginal habitats (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-twentieth Century (Light and Dineen 1994). As a result, Florida Bay began receiving 90% of its freshwater inputs from seasonal rainfall instead of constant flows via the Everglades (Light and Dineen 1994; Nuttle et al. 2000). The northeast region of the bay, already physically restricted, exhibited increased and highly 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 (Fig. 1). The one notable exception to this trend is Blackwater Sound (BLK). Although BLK is located in the extreme northeast region of Florida Bay, it is connected with more marine waters via cuts through Key Largo which connect it directly with the Atlantic. Thus, conditions within Blackwater Sound have been described as typical of more south-central regions of Florida Bay (Hackney and Durako 2004).

Corals have been observed scattered within hardbottom regions along the northeast to southwest physical–environmental gradient discussed above during annual large-scale

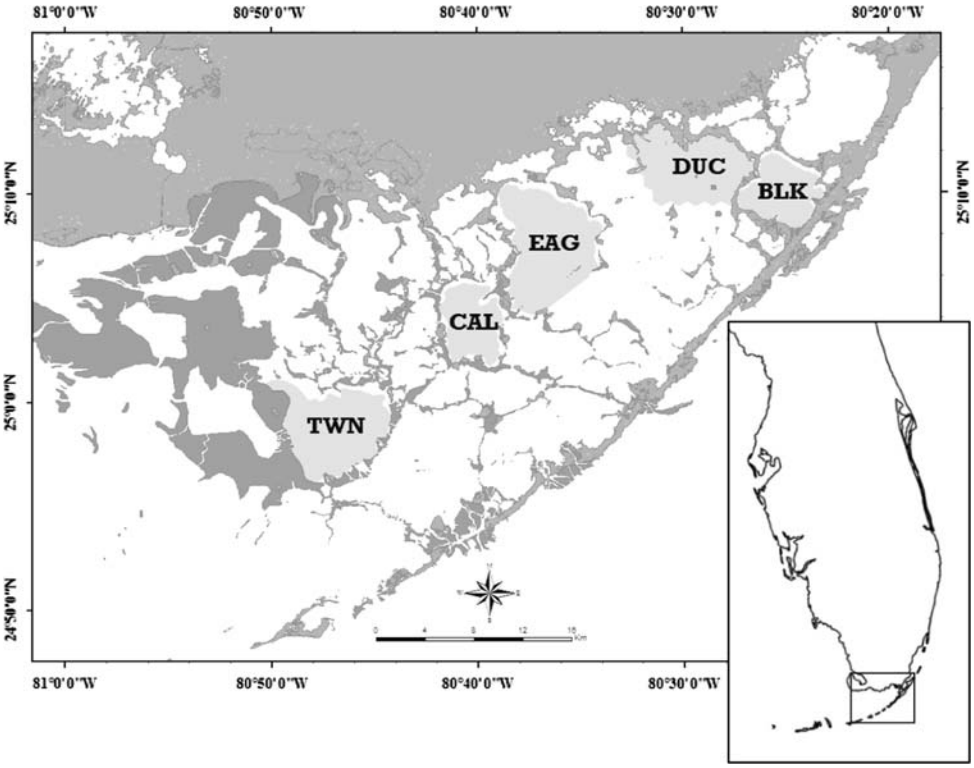

Fig. 1 Florida Bay, Florida, USA: Five basins (BLK, DUC, CAL, EAG, TWN) where *S. radians* colonies were collected in this study. See "Materials and methods" for basin abbreviation names

macrophyte monitoring in Florida Bay (Durako unpublished data). The distribution of the most common coral species in the bay, *S. radians,* is limited to five of the eleven monitored basins, in areas with little sediment and low seagrass abundance (Chartrand and Durako 2009). It is 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 *S. radians* as increased freshwater may lower salinities across Florida Bay (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 1987; Manzello and Lirman 2003; Kerswell and Jones 2003). Muthiga and Szmant (1987) investigated the effects of hyposalinity on *Siderastrea siderea* from the Florida panhandle where large river systems empty into the Gulf of Mexico. They observed that *S. siderea* placed in treatments with a change in salinity greater than 10 from controls exhibited decreased rates of gross photosynthesis. Manzello and Lirman (2003) 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. However, salinity records of the sample sites were not given in these studies. It is unclear how these corals 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 corals can thrive in coastal, brackish habitats. More recently, Kerswell and Jones (2003) employed variable chlorophyll fluorescence 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* is an important Indo-Pacific reef builder known to be intolerant of environmental fluctuations such as high temperatures and low salinity (Loya et al. 2001; Kerswell and Jones 2003; van Oppen et al. 2005). The objective of the experimental study described herein was to provide a more realistic understanding of how future water management in Florida Bay may affect the photophysiology of *Symbiodinium in hospite* of *S. radians*, 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 reductions in salinities will likely stress the corals in the bay, an effect not taken into account in the restoration plan.

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, 2005). PAM fluorometry measures the efficiency with which light energy is converted into chemical energy at photosystem II (PSII). Any decrease in efficiency and transport of photons will affect all photosystem electron transport processes downstream of PSII. Thus, PSII is considered to be the most stress-sensitive part of the photosynthetic pathway (Becker et al. 1990). The ability to redirect light energy into alternative pathways, such as photosynthesis, heat or fluorescence, will strongly determine how well an organism will handle stressful and potentially damaging light energies in parallel with other physical variables. Therefore, evaluating the chlorophyll fluorescence pathway can indicate an organism's photosynthetic efficiency in using these different pathways under changing or stressful conditions (e.g. varying salinity regimes).

Our objective was to assess the effects of hyposalinity, which may occur in Florida Bay under the proposed freshwater diversion plans associated with the CERP, on *S. radians*. We also evaluated whether the responses among different basin populations reflect acclimation to differences in basin physical conditions. Photophysiological characteristics of *Symbiodinium* spp. were used to indicate stress on the holobiont from decreased salinity, as previously applied in temperature and light stress studies (e.g. Iglesias-Prieto et al. 1992; Warner et al. 1996; Warner et al. 1999; Rodríguez-Román et al. 2006).

Materials and methods

Study site and experimental 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

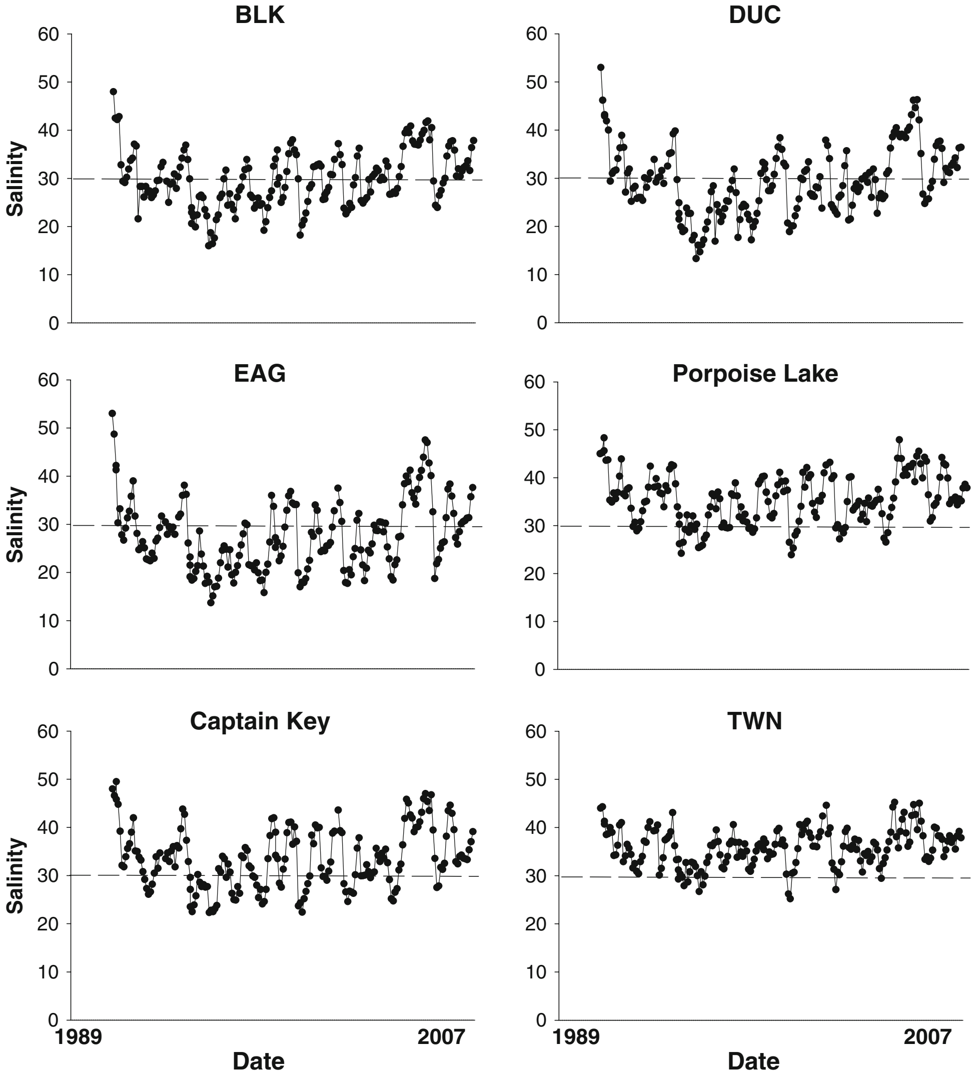

Fig. 2 Florida Bay, Florida, USA: historical salinity record for five basins where *S. radians* colonies were collected for this study. Porpoise Lake and Captain Key provide an approximation of salinity in CAL where direct records were not available. See "Materials and methods" for basin abbreviation names

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 <2 m) 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 (Fig. 2). 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 (Chartrand and Durako 2009). The five basins in a northeast to southwest orientation (Fig. 1) are Blackwater Sound (BLK), Duck Key Basin (DUC), Eagle Key Basin (EAG), Calusa Key Basin (CAL), and Twin Key Basin (TWN).

During October 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 and transported in insulated seawater-filled coolers to the Center for Marine Science (CMS), Wilmington, NC overnight. Care was taken to ensure colonies were maintained under ambient field conditions (i.e., temperature and salinity were monitored every 3–4 h). Colonies were placed in 40-l aquaria filled with filtered (1 μm), UV sterilized seawater and containing gravel-bed filters, which were used as aeration systems. Aquaria were held in fiberglass vaults (125 × 60 × 30 cm, four aquaria per vault), which were used as water baths for temperature control. One colony per basin was randomly chosen and placed in each of 16 aquaria such that each aquarium contained a colony from each of the five basins sampled. Location within aquaria was randomly assigned to one of four corners or the center, yet placement ensured that a colony from each basin was in each aquarium position at least twice. Corals were held at a salinity of 30 (the average salinity ±1 at collection sites) for 1 week to allow colonies to acclimate to greenhouse conditions. Salinities were checked every 12 h using a YSI conductivity probe (accuracy ±0.5; Yellow Springs, OH). Evaporation in aquaria was offset by the addition of deionized water. Calcium, pH, and alkalinity were assessed weekly to ensure stable water chemistry. Temperatures in the greenhouse varied from 24 to 28°C between daytime and nighttime; a 12:12 light:dark cycle was maintained using supplemental light provided by two 500 W metal-halide lamps (which compensated for latitudinal effects and light reductions due to the greenhouse walls).

Target treatment salinities of 10, 15, 20, and 30 (controls) were assigned to each of the 16 aquaria. Each complete treatment series of four aquaria were randomly located in one of the four water-filled fiberglass vaults (level of replication is the aquarium, $n = 4$). Salinities were then decreased daily following pre-dawn PAM fluorescence measurements by adding distilled water to experimental aquaria to drop salinity at a rate of 2 per day. 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 aquaria reached their assigned target salinities, they were maintained at this state for the remainder of the experiment.

Photobiology

Chlorophyll *a* fluorescence measurements were taken on all colonies at pre-dawn and post-dusk with a Diving-PAM fluorometer (Walz, Germany). Both pre-dawn and post-dusk measurements were initially taken to ensure there were no significant differences in F_v/F_m resulting from residual dynamic photoinhibition during the day, generating lower F_v/F_m at dusk. Post-dusk measurements (data not shown) ceased when no significant differences between pre-dawn and post-dusk measurements were detected (*t* tests) during the first week, indicating alternative pathways for directing excess light energy had been essentially switched off by dusk. For all measurements, the fiber optic tip of the instrument was set at a standard geometry (30° from perpendicular) and distance (5 mm) to the upper surface of sampled corals. Minimum fluorescence was first measured (F_0). The coral tissue was then subjected to a pulse of saturating light during which a second fluorescence reading is taken (F_m). F_m is the state in which all reaction centers present are considered reduced or closed. Maximum (i.e., dark-acclimated) quantum yields, which measure photosynthetic capacity of the endosymbiotic dinoflagellate, were then calculated ($F_v/F_m = F_m - F_0/F_m$). The experiment was terminated 3 days after colonies in treatment salinity of 10 had reached their target salinity, although pre-dawn quantum yields were still measured for the following 7 days.

Statistical analyses

To assess whether quantum yields (dependent variable) were significantly different among basin populations (BLK, CAL, DUC, EAG, TWN) and salinity treatments (10, 15, 20 and 30) at their respective target dates (all fixed factors), a repeated measures factorial ANOVA was applied using the MIXED procedure of SAS. Models were constructed based on various covariance structures, with each being evaluated by the Bayesian Information Criterion and the Akaike Information Criterion. The best covariance structure, which was utilized for all analyses, was a compound symmetric structure across replicate aquaria within a basin population, with a first-order autoregressive structure across respective target dates for each aquarium. 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 tests were performed to assess differences among basin populations at each target salinity date. All statistical models were calculated using SAS v.9.1 (SAS Institute, Cary, NC).

Results

A general pattern of decreasing F_v/F_m over time was apparent as treatments reached their target salinities (Fig. 3). F_v/F_m values were compared among basins 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. 3a), thus salinity treatments were compared to controls on their corresponding target dates. In salinity treatment 15, only colonies from TWN had significantly lower F_v/F_m compared to their controls 3 days after reaching the target salinity ($p < 0.05$; Fig. 3a, c). Colonies from TWN also consistently exhibited the lowest F_v/F_m over the course of the experiment in all treatments, including controls (Fig. 3). At salinity of 10, TWN exhibited the only significant drop in F_v/F_m from its controls (Figs. 3d, 4e; $t = 2.91$, $p < 0.01$). 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. 4e; $t = 3.33$ 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. 4d; $t = 2.84$, $p < 0.01$). 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. 4a–c).

After 1 day at the target salinity, all basin populations exhibited significantly lower F_v/F_m in salinity of 10 versus

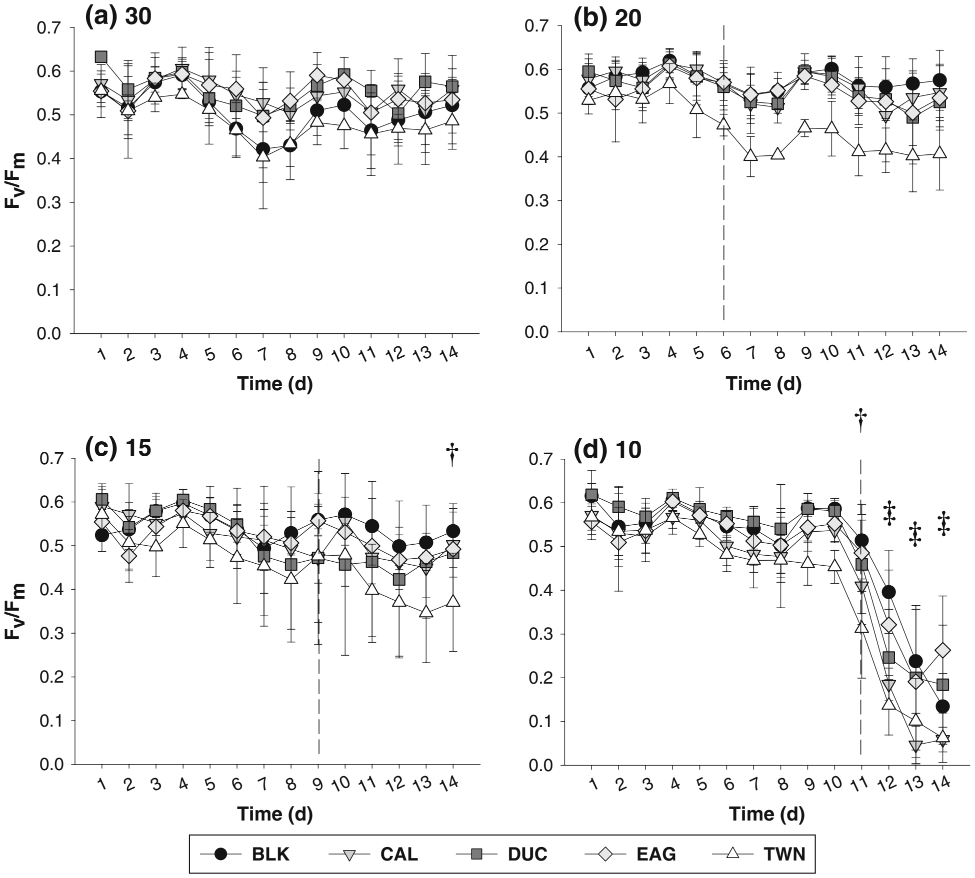

Fig. 3 *Siderastrea radians*. Mean dark-acclimated yields ($F_{\mathrm{v}}/F_{\mathrm{m}}$) ± SD by basin population over time. *Dashed gray line* indicates day target salinity was reached. †Indicates a significant difference in TWN salinity treatment versus TWN controls. ‡Indicates a significant difference in all basins versus respective basin controls. **a** 30, **b** 20, **c** 15, **d** 10

treatment salinities 15, 20 and 30 (Fig. 5; $p < 0.01$). These differences were consistent for the following three days after salinity treatment of 10 was reached. The experiment was terminated on day 14 with evidence of death and uniformly low $F_{\mathrm{v}}/F_{\mathrm{m}}$ values. Visual observations and increases in yields in the days following the significant drop in $F_{\mathrm{v}}/F_{\mathrm{m}}$ 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 $F_{\mathrm{v}}/F_{\mathrm{m}}$ 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. 6; $F = 15.88$, $p < 0.001$). $F_{\mathrm{v}}/F_{\mathrm{m}}$ values for colonies from the northeast basins were higher on average than those in the southwest (Fig. 6), with the exception of pooled BLK controls (see below). The pattern in $F_{\mathrm{v}}/F_{\mathrm{m}}$ 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 reaching target salinity until 2 days after the target salinity was reached with TWN colony $F_{\mathrm{v}}/F_{\mathrm{m}}$ values being significantly lower than all other basins 1 and 2 days after reaching the target salinity of 10 ($p < 0.01$) The TWN population also had significantly lower $F_{\mathrm{v}}/F_{\mathrm{m}}$ compared to all other basins when the target salinity of 20 was reached and thereafter (Fig. 6; $F = 4.45$, $p < 0.05$). The observed patterns in $F_{\mathrm{v}}/F_{\mathrm{m}}$ correlate with basin population location (Fig. 1). Moreover, this shift in $F_{\mathrm{v}}/F_{\mathrm{m}}$ parallels mean bottom salinity and variance recorded for each basin over the last 18 years (Fig. 2; Table 1). EAG and DUC have the highest recorded variability and lowest mean salinity of all basins while observed $F_{\mathrm{v}}/F_{\mathrm{m}}$ in these populations were consistently the highest among basins (Fig. 6). Colonies in the target salinity of 15 aquaria exhibited no significant difference in $F_{\mathrm{v}}/F_{\mathrm{m}}$ among basins throughout the experiment due to highly variable responses but they exhibited similar trends as yields decreased in parallel with the northeast to southwest physical-salinity gradient.

Discussion

Symbiotic dinoflagellates of *S. radians* demonstrated high tolerance to hyposaline stress *in hospite* as measured by maximum quantum yield of PSII ($F_{\mathrm{v}}/F_{\mathrm{m}}$), in this study. $F_{\mathrm{v}}/F_{\mathrm{m}}$ indicated that 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

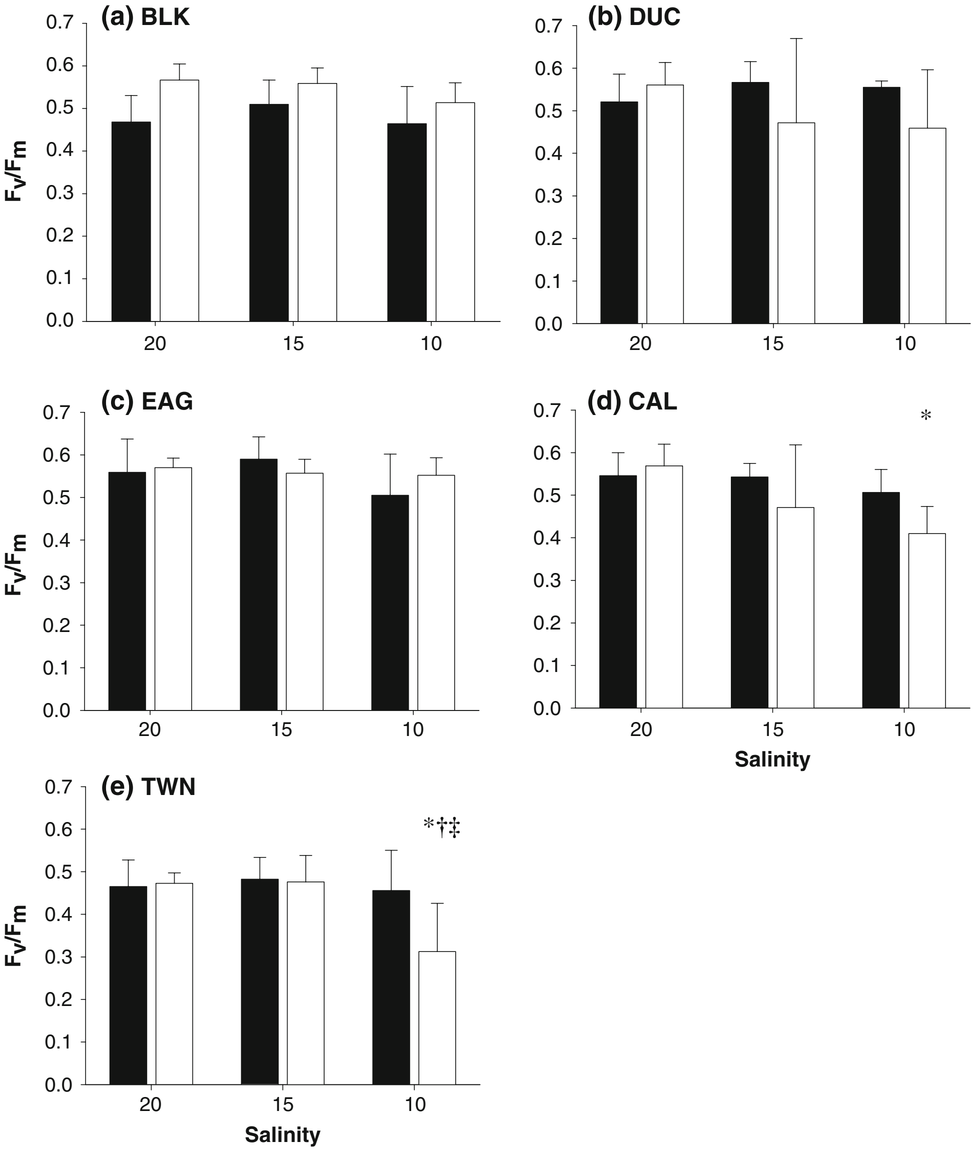

Fig. 4 *Siderastrea radians*. Dark-acclimated quantum yields (F_v/F_m) at treatment salinity (*white bars*) and for controls (*black bars*) on the target date. *Bars* represent mean yields for given treatment ± SD. *Significant difference from F_v/F_m in treatment salinity 20; †significant difference from treatment salinity 15; ‡significant difference from the treatment controls. **a** BLK, **b** DUC, **c** EAG, **d** CAL, **e** TWN

20 from ambient. Coles and Jokiel (1992) state that salinities below 15 sustained for more than 2 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 tissue sloughing for a minimum of three days while in a hyposaline 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 at least 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. 2) indicate that salinities have often dropped below 20 and occasionally down to 10 in some of the northern basins where *S. radians* occurs, reflecting the ecological relevance of the salinity treatments chosen in this study. Chlorophyll *a* and symbiont densities were not measured; however no bleaching was evident as indicated by the presence of a narrow chlorophyll absorption feature near 675 nm in colony reflectance spectra (Durako and Chartrand 2009). This may again indicate higher salinity tolerance in *S. radians* compared to other scleractinian species where substantial loss of pigmentation and/or symbionts has been documented while under minimal hyposaline stress (Kerswell and Jones 2003).

Little attention has been given to understanding osmoregulation in corals and how osmotic shifts under hypo-osmotic conditions can affect both the host and symbiont physiology. The threshold response observed in this study is indicative of the coral actively maintaining and successfully

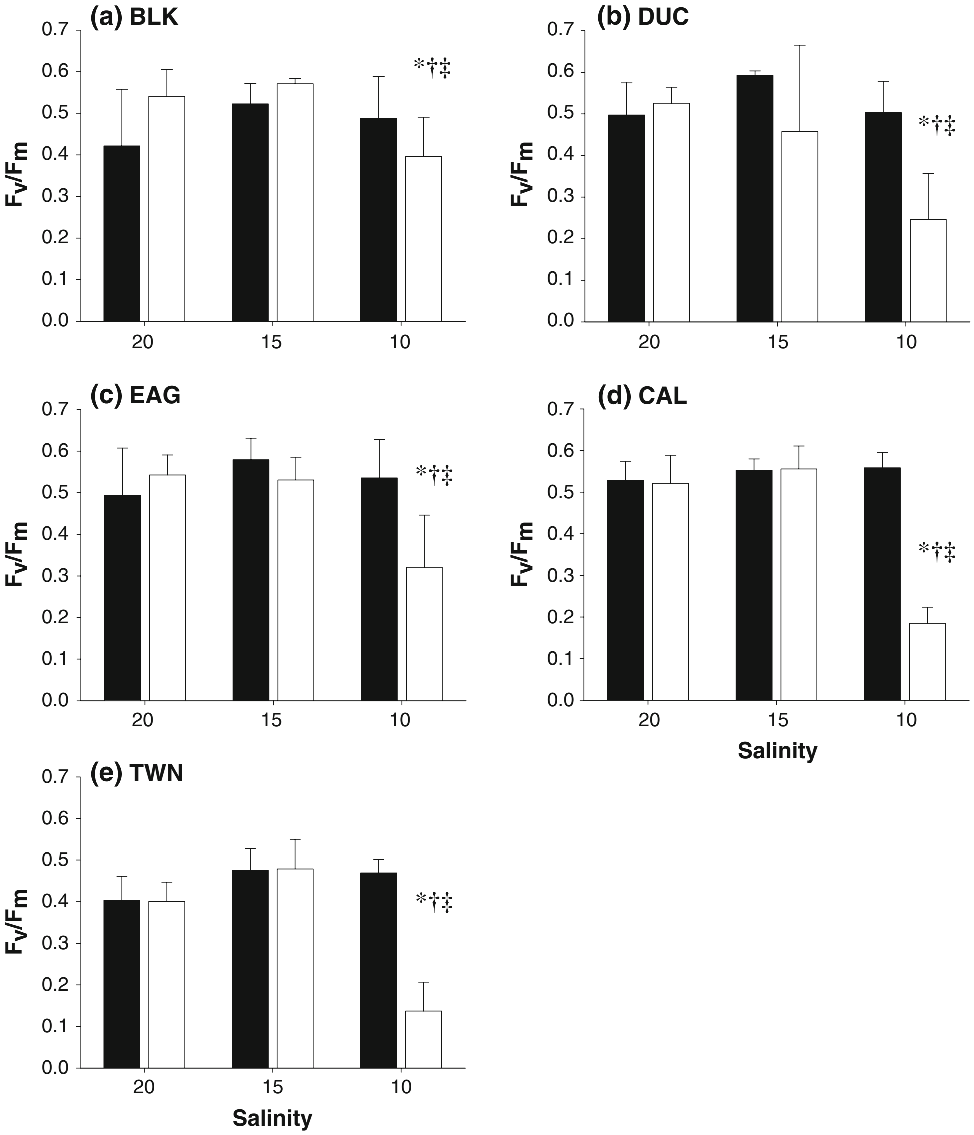

Fig. 5 *Siderastrea radians.* F_v/F_m 1 day after reaching treatment salinities (*white bars*) and comparative controls (*black bars*). Bars represent mean yield ± SD. See Fig. 4 for explanation of symbols

regulating its internal osmotic balance until a critical (and lethal) level is reached between salinities of 10 to 12. Mayfield and Gates (2007) review possible mechanisms by which symbiotic corals maintain osmotic balance with their external environment. Glycerol produced by the symbiont and transferred to the host may be rapidly respired as a major energy source or stored in cellular pools (Gates and Edmunds 1999; Mayfield and Gates 2007). The cellular 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; Schick 1991; Mayfield and Gates 2007). In *Dunaliella* (Chlorophyta) glycerol levels are reduced to eliminate its osmotic potential under hyposaline conditions (Ben-Amotz 1973; Chitlaru and Pick 1991). The effect of glycerol fluctuations within the endosymbiont could have a negative effect on the host if the levels of glycerol available for transfer are reduced below those required 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.

The significant decrease in F_v/F_m in the most extreme hyposaline 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; Philipp and

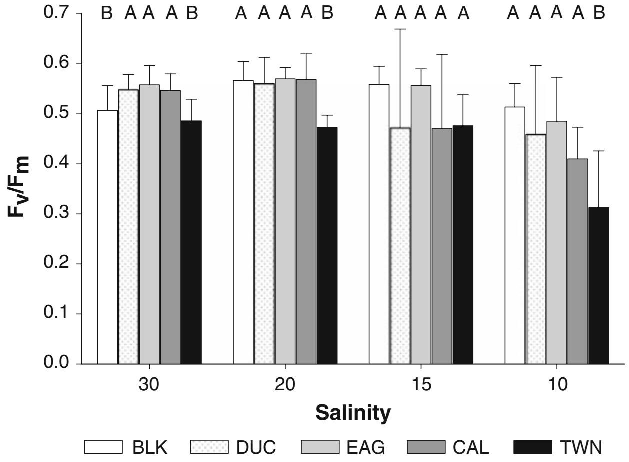

Fig. 6 *Siderastrea radians*. Among basin differences in mean F_v/F_m ± SD within salinity treatments on date treatment was reached. Differing *letters* within salinity treatments indicate a significant difference among basins

Table 1 Average basin salinity from 1989 to 2007. Monthly salinity records obtained from SERC-FIU

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[a]	220	33.27 (6.6)	33.46 (6.3)
TWN	220	36.20 (4.3)	35.85 (4.0)

Salinity was recorded near adjacent Captain Key

The numbers given in parentheses represent SD

[a] Record is an estimate

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 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 chlorophyll *a* 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 chlorophyll *a* fluorescence of an organism, which cannot be discerned using only PAM 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 hypo-osmotic conditions in *Symbiodinium* spp. is required to determine if impairments similar to those demonstrated in other marine species occur to its photosynthetic machinery. Analyzing anti-oxidant levels specific to the thylakoid membrane and measuring cell volume may provide the first indication of damage along the photosynthetic pathway in *Symbiodinium* spp. (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 study. 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 and they had the highest spectral reflectances (Durako and Chartrand 2009). Persistent algal blooms and poor water quality from November 2005 to October 2006 (Rudnick 2006; Chartrand personal observation) 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. 2). 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 more 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 3 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 the coral–dinoflagellate association may be impaired when under various environmental stressors like salinity. In recent years, an increasing focus on the role of the symbiont has determined that the genus *Symbiodinium* is morphologically, physiologically and genetically diverse (Trench 1993, 1997; Rowan 1998; LaJeunnesse 2001). At least eight known clades have been described. The discovery of distinct types or subclades within the genus *Symbiodinium* has raised concerns regarding how intra- versus inter-clade variability may influence the holobiont (Blank and Trench 1985). Large-scale sampling efforts have identified geographic and ecologically relevant patterns based on subclade classification (LaJeunnesse 2001; Iglesias-Prieto et al. 2004; Sampayo et al. 2007). Response patterns related to relevant stressors and subclade variability may help explain holobiont success or failure under increasing pressure at the local (i.e. salinity and runoff) and global (i.e. temperature and ocean acidity) scale. 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.

Management implications

The distribution of corals in Florida Bay has only recently been documented by landscape-scale monitoring surveys (Chartrand and Durako 2009). 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 the northeastern basins of Florida Bay. If freshwater reaches more southern basins such as Twin Key Basin, our results indicate that coral colonies will likely fail to survive an osmotic stress below a salinity of 15. If corals have only recently recruited to this region as a result of freshwater diversion, their reduction or loss as freshwater inflow is restored to produce more historical salinity conditions may reflect a more natural state for the bay.

The tolerance of *S. radians* to reduced salinities documented in this study is much higher than previously documented for scleractinian corals. Population-based responses to reduced salinities correlated with historical salinity patterns across a physical–environmental gradient from the northeast to southwest basins in Florida Bay. Acclimation to local salinity regimes within basins was most apparent in the lowest tolerance to hyposaline conditions by colonies from the most marine Twin Key Basin, compared to all other basins. However, F_v/F_m was reduced in all basin populations at the lowest salinities. Plans to alter the watershed of South Florida will lead to reduced salinities throughout large portions of Florida Bay with the potential to reach the Florida Keys Reef Tract. Understanding how reduced salinities will affect local coral communities will allow better models for the prediction of ecosystem shifts that will occur under watershed management plans. Furthermore, identifying corals such as *S. radians* that may have greater tolerance 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 climate conditions.

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