Ocean Acidification Refugia of the Florida Reef Tract

Derek P. Manzello[1,2]*, Ian C. Enochs[1,2], Nelson Melo[1,2], Dwight K. Gledhill[3], Elizabeth M. Johns[2]

1 Cooperative Institute for Marine and Atmospheric Studies, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, Florida, United States of America, 2 Atlantic Oceanographic and Meteorological Laboratories, National Oceanic and Atmospheric Administration, Miami, Florida, United States of America, 3 Ocean Acidification Program, National Oceanic and Atmospheric Administration, Silver Spring, Maryland, United States of America

Abstract

Ocean acidification (OA) is expected to reduce the calcification rates of marine organisms, yet we have little understanding of how OA will manifest within dynamic, real-world systems. Natural CO_2, alkalinity, and salinity gradients can significantly alter local carbonate chemistry, and thereby create a range of susceptibility for different ecosystems to OA. As such, there is a need to characterize this natural variability of seawater carbonate chemistry, especially within coastal ecosystems. Since 2009, carbonate chemistry data have been collected on the Florida Reef Tract (FRT). During periods of heightened productivity, there is a net uptake of total CO_2 (TCO_2) which increases aragonite saturation state (Ω_{arag}) values on inshore patch reefs of the upper FRT. These waters can exhibit greater Ω_{arag} than what has been modeled for the tropical surface ocean during preindustrial times, with mean (± std. error) Ω_{arag}-values in spring = 4.69 (±0.101). Conversely, Ω_{arag}-values on offshore reefs generally represent oceanic carbonate chemistries consistent with present day tropical surface ocean conditions. This gradient is opposite from what has been reported for other reef environments. We hypothesize this pattern is caused by the photosynthetic uptake of TCO_2 mainly by seagrasses and, to a lesser extent, macroalgae in the inshore waters of the FRT. These inshore reef habitats are therefore potential acidification refugia that are defined not only in a spatial sense, but also in time; coinciding with seasonal productivity dynamics. Coral reefs located within or immediately downstream of seagrass beds may find refuge from OA.

Introduction

Ocean acidification (OA) is the global decline in seawater pH due to the uptake of carbon dioxide (CO_2) by the surface ocean [1]. This uptake of CO_2 reduces the concentration of carbonate ions $[CO_3^{2-}]$ and leads to a decline in the carbonate mineral saturation state (Ω, where $\Omega = [CO_3^{2-}][Ca^{2+}]/K'_{sp}$, and K'_{sp} is the apparent solubility product of a carbonate mineral). The saturation state of a given carbonate mineral is a key driver of inorganic carbonate mineral kinetics [2] and thought to be an important controlling factor on the biogenic calcification of that mineral (e.g., corals and their aragonite skeletons) [3]. Coral reef ecosystems are especially vulnerable as their continued persistence is dependent on the deposition of $CaCO_3$ exoskeleton by scleractinian corals [4].

Despite these concerns, we still have only a rudimentary understanding of the spatial and temporal variability of carbonate chemistry within reef environments. There is a pressing need to ascertain which locations, habitats or regions may be relatively susceptible or even resilient to OA. This is a challenging undertaking which will take years, if not decades to unravel, as a given areas risk to OA will be a function of localized biogeochemical feedbacks that may locally alter the rates of OA [5], differing species-specific susceptibilities, and interactions with other stressors. The first step towards addressing this issue is to document the present day, baseline conditions of particular reef systems. Regions that are naturally high in CO_2 and have low aragonite saturation states (Ω_{arag}), such as upwelling areas in the eastern tropical Pacific, are OA "hotspots" in that rising CO_2 will force the already low Ω_{arag} to potentially critical levels [6], [7]. However, the larger natural variability in Ω_{arag} found in upwelling areas could also make organisms there more tolerant to future OA [8]. Regardless, areas that act as natural CO_2 sinks may serve as OA refugia because calcareous organisms will experience higher Ω_{arag} relative to the open ocean.

Seagrass beds are often located near coral reefs and the Florida Reef Tract (FRT) is no exception [9]. A non-continuous offshore barrier reef parallels the Florida Keys [10]. Between the Florida Keys islands and offshore barrier reef lies Hawk Channel that contains high abundances of seagrass habitat [9], [11]. Patch reefs are also found within Hawk Channel, and they often exist as isolated islands surrounded by seagrass beds [12]. Recent work has shown that calcification of calcareous macroalgae can be stimulated by CO_2 uptake of seagrasses [13]. To determine if photosynthetic CO_2 uptake associated with seagrass beds has the potential to create OA refugia, carbonate chemistry was repeatedly sampled across an inshore-to-offshore gradient in the upper, middle, and lower FRT over two years. During periods of

heightened productivity, there is a net uptake of total CO_2 (TCO_2) which significantly increases aragonite saturation state (Ω_{arag}) values on inshore patch reefs of the upper FRT.

Materials and Methods

Discrete seawater sampling was started in April 2009 from paired inshore and offshore sites in the upper Keys (UK) (Fig. 1). No specific permits were required for the described field studies. Paired inshore and offshore sites were carefully selected so that they had the same depth of ~4–5 m, as water depth is known to exert a strong influence on the impact that benthic biota have on seawater carbonate chemistry [7]. Samples were most often collected from the surface at 1 m depth. The timing and location of all discrete sampling is indicated in Table S1, whereas sample sizes are listed in Table 1. Additionally, seawater samples were taken from the surface (~1 m depth) during repeat biophysical oceanographic cruises of the South Florida Program aboard the R/V F. G. Walton Smith (Table S1). Carbonate chemistry sample collection and analysis were performed as previously described [6], [7]. Briefly, total CO_2 (TCO_2) was measured coulometrically and total alkalinity (TA) was determined using gran titration. The remaining carbonate parameters were calculated from these values and *in situ* temperature with CO2SYS [14] using the dissociation constants of Mehrbach et al. [15] for carbonic acid as refit by Dickson and Millero [16] and Dickson [17] for boric acid.

Statistical comparisons were made between inshore and offshore sites in the UK, middle (MK), and lower (LK) Florida Reef Tract (FRT) after data were divided into seasons. Student's t-tests ($\alpha = 0.05$ significance level) were used when data were normally distributed, whereas Mann-Whitney U-tests were used when they were not. One-way ANOVAs (or Kruskal-Wallis tests when data were not normal or homoscedastic) were used to ascertain significant effects of region (UK, MK, LK) and season within the inshore and offshore grouping. When significant differences were indicated, post-hoc t-tests were used to determine the relationship of regions/seasons.

Inshore values of TCO_2, TA, pCO_2, and Ω_{arag} were subtracted from their paired offshore values to determine the magnitude (Δ) of the gradient between inshore and offshore sites. A positive Δ indicates that inshore values are higher than offshore, whereas a negative Δ shows the opposite. TCO_2 and TA values were normalized to salinity ($nTCO_2 = TCO_2 \times 35/S$) prior to calculating Δ. Mean [$\pm$ std. error of the mean (SE)] values by season, as well as the sum of the mean values across all seasons (Σ) are presented. Means represent the average gradient encountered by season, whereas the summed values indicate the annual net direction and magnitude of the gradient. TA-TCO_2 plots were used to infer the dominant forcing mechanisms of the carbon cycle eliciting the inshore-to-offshore gradient in carbonate chemistry [18].

Results

Inshore waters at all sites were depleted in both TCO_2 and TA relative to offshore during spring and summer (Fig. 2) with the pattern generally reversing in autumn and winter (Fig. 3). In the spring, the significant inshore depletion of TCO_2 was sufficient to elevate the Ω_{arag}-values relative to offshore at all sites (Table 1; t-tests, p<0.05). The lack of significance between inshore and
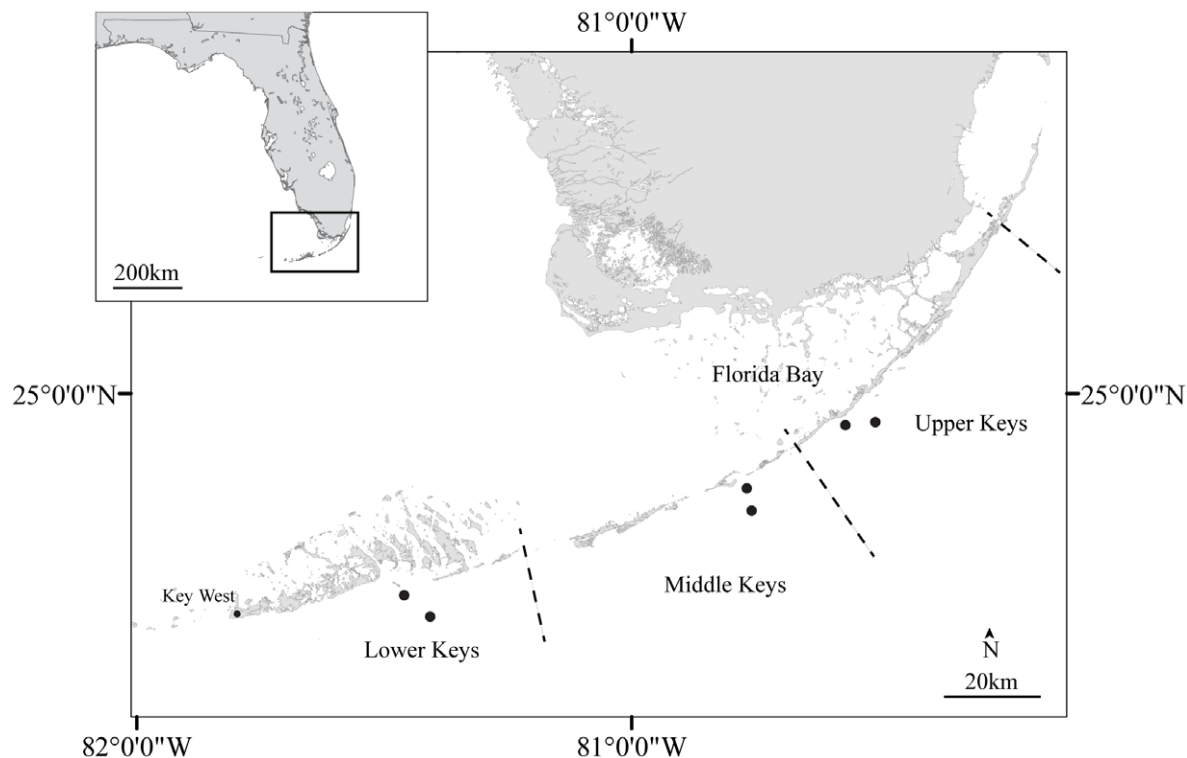
Figure 1. Map of Florida Keys portion of the Florida Reef Tract. Paired inshore and offshore sites where discrete seawater samples were obtained are indicated in the Upper, Middle, and Lower Keys. GPS coordinates are Upper Keys Inshore (24.93898N, 80.56272W), Offshore-24.9465N, 80.50207W); Middle Keys Inshore (24.81216N, 80.76075W), Offshore (24.76724N, 80.75227W); and Lower Keys Inshore (24.59723N, 81.45505W), Offshore (24.55141N, 81.40251W).
doi:10.1371/journal.pone.0041715.g001

Table 1. Mean values ($\pm$ std. error of mean) for salinity, TCO_2, $nTCO_2$, TA, nTA, pCO_2, and Ω_{arag} by season for Upper Keys (UK), Middle Keys (MK), and Lower Keys (LK) paired inshore and offshore sites.

Location	Season	N	N	Salinity (psu)	TCO_2 (μmol kg^{-1})	$nTCO_2$ (μmol kg^{-1})	TA (μEq kg^{-1})	nTA (μEq kg^{-1})	pCO_2 (μatm)	Ω_{arag}
UK										
Inshore	Spring	12	5	36.66 (0.141)	1868.0 (41.33)*	1784.1 (44.14)*	2298.5 (32.07)	2195.1 (37.12)*	257 (19.2)**	4.69 (0.101)***
	Summer	6	3	36.62 (0.226)	1925.5 (44.80)	1840.8 (46.43)	2309.2 (20.62)	2207.5 (24.46)	379 (35.2)	4.34 (0.253)
	Autumn	6	4	36.07 (0.310)	2085.3 (32.70)	2023.8 (38.53)	2386.4 (25.24)	2316.1 (36.94)	452 (38.5)	3.42 (0.157)
	Winter	4	3	35.82 (0.386)	2103.0 (84.40)	2054.0 (69.10)	2457.4 (72.22)	2400.7 (58.87)	299 (11.8)	3.91 (0.197)
Offshore	Spring	7	4	36.28 (0.062)	2036.7 (8.08)*	1964.8 (5.91)*	2386.1 (6.47)	2301.8 (5.41)*	377 (6.6)**	3.92 (0.067)***
	Summer	4	4	36.13 (0.157)	2027.0 (5.72)	1960.3 (13.97)	2381.3 (9.38)	2302.9 (15.37)	425 (18.2)	4.07 (0.148)
	Autumn	4	4	35.78 (0.324)	2053.0 (9.18)	2008.6 (16.69)	2374.8 (7.27)	2323.5 (16.61)	403 (13.3)	3.64 (0.069)
	Winter	4	4	36.22 (0.135)	2061.9 (19.78)	1992.6 (12.59)	2400.0 (21.09)	2319.3 (13.31)	340 (12.8)	3.73 (0.029)
MK										
Inshore	Spring	4	3	36.84 (0.071)*	1951.7 (15.03)*	1854.1 (12.91)*	2348.7 (12.08)	2231.4 (12.10)*	316 (8.7)*	4.39 (0.081)*
	Summer	4	4	37.29 (0.060)	1830.2 (46.18)*	1769.5 (85.47)	2223.3 (30.36)**	2123.6 (59.25)	338 (35.2)	4.32 (0.147)
	Autumn	4	3	35.93 (0.074)	2078.8 (43.36)	1956.2 (87.62)	2388.0 (35.01)	2277.2 (71.20)	404 (47.9)	3.47 (0.172)
	Winter	3	3	36.03 (0.448)	2112.5 (100.02)	2053.7 (112.63)	2479.1 (90.54)	2410.1 (108.99)	323 (23.4)	4.08 (0.150)
Offshore	Spring	3	3	36.23 (0.114)*	2030.3 (5.38)*	1961.4 (1.16)*	2346.9 (32.04)	2297.8 (7.49)*	384 (22.0)*	3.92 (0.055)*
	Summer	5	4	36.28 (0.329)	2014.7 (28.44)*	1944.6 (40.78)	2373.8 (22.32)**	2291.0 (40.12)	417 (19.5)	4.11 (0.123)
	Autumn	5	4	35.68 (0.447)	2023.4 (12.91)	1985.6 (19.54)	2367.5 (13.56)	2323.1 (18.96)	366 (9.62)	3.88 (0.093)
	Winter	3	3	36.24 (0.137)	2057.6 (22.51)	1987.3 (14.39)	2377.1 (12.45)	2296.0 (4.30)	366 (9.2)	3.54 (0.147)
LK										
Inshore	Spring	5	4	36.95 (0.291)	1839.2 (47.19)*	1743.2 (55.16)*	2216.7 (41.25)*	2100.8 (53.05)*	301 (28.1)	4.07 (0.097)
	Summer	5	4	36.75 (0.262)	1808.0 (70.04)	1720.9 (55.62)	2162.7 (50.59)	2059.2 (34.78)	367 (44.4)	3.90 (0.191)
	Autumn	5	3	36.22 (0.240)	2056.3 (29.07)	1987.7 (38.92)	2367.4 (28.71)	2288.2 (37.03)	395 (39.4)	3.47 (0.103)
	Winter	3	3	36.05 (0.196)	2103.8 (37.31)	2042.5 (26.67)	2455.8 (29.49)	2384.4 (16.09)	330 (30.0)	3.91 (0.130)
Offshore	Spring	5	4	36.33 (0.219)	1986.8 (20.54)*	1914.7 (30.76)*	2330.1 (21.53)*	2245.5 (33.77)*	375 (18.7)	3.83 (0.058)
	Summer	4	4	36.75 (0.533)	1949.0 (69.08)	1856.4 (61.34)	2324.6 (56.45)	2214.4 (49.48)	380 (36.4)	4.22 (0.137)
	Autumn	5	4	35.40 (0.478)	2023.7 (16.43)	2001.4 (15.06)	2360.9 (26.66)	2334.5 (12.46)	374 (13.0)	3.81 (0.165)
	Winter	3	3	36.28 (0.138)	2078.9 (32.30)	2005.5 (23.59)	2404.0 (27.36)	2319.2 (17.68)	359 (6.0)	3.60 (0.070)

Means are mean values for each sampling excursion by season. Significant differences between inshore and offshore as indicated with t-tests marked by symbol (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). n, number of samples. N, number of trips.
doi:10.1371/journal.pone.0041715.t001

offshore pCO_2 and Ω_{arag} measured in the LK during the spring was because of the large magnitude of TA decline inshore (Fig. 2B, 4; Table 1). The increase of inshore TCO_2 in autumn depressed Ω_{arag} relative to offshore. By winter, the inshore TA increase compensated for increased TCO_2 and caused an increase in Ω_{arag} relative to offshore once again (Fig. 4).

### Inshore-to-Offshore Gradient: Seasonal and Site Comparisons

The UK had the greatest magnitude of $\Delta\Omega_{arag}$ (one-way ANOVAs, $p < 0.05$, Fig. 4), due primarily to very high inshore Ω_{arag} values in spring (mean $\Omega_{arag} \pm$ SE = 4.69$\pm$0.101, Table 1). The increase of inshore Ω_{arag} during the spring and summer is greater than its depression during autumn for both the UK and MK (Table 2), which is also evident in time-series data (Fig. 5). $\Sigma\Delta pCO_2$ was negative for all sites (Table 2). A large negative $\Sigma\Delta nTA$ value in the LK, which was greater than the large and negative $\Sigma\Delta nTCO_2$ value, resulted in a negative $\Sigma\Delta\Omega_{arag}$. Also unlike the other sites, LK had a negative $\Delta\Omega_{arag}$ during the summer (Fig. 4).

The TA-TCO_2 plots confirm the predominance of photosynthesis and calcification during the spring and summer at all sites (Fig. 6). The slopes of the UK and LK spring trendlines were no different (Fig. 6, Table S2). However, during the summer, the UK slope declined, indicating an increase in photosynthesis relative to calcification, whereas the LK slope increased, indicating an increase in calcification versus photosynthesis. The MK had large amplitude during summer because of the influence of Florida Bay waters that are depleted in TCO_2 and TA during this time (Fig. 6C) [19]. The opposite occurred during the winter. During the winter months, net respiration and net dissolution were documented for all sites. In autumn, there was a scatter between the dominant spring/summer and winter processes for all sites.

Paired inshore and offshore time-series

There was a large seasonal amplitude for both the inshore TCO_2 and TA that was not apparent offshore (Fig. 5). Seasonally fluctuating temperatures result in corresponding seasonality in Ω_{arag} for both inshore and offshore waters, but the amplitude is enhanced for the inshore waters (Fig. 5). The inshore UK site had

Spring 2011
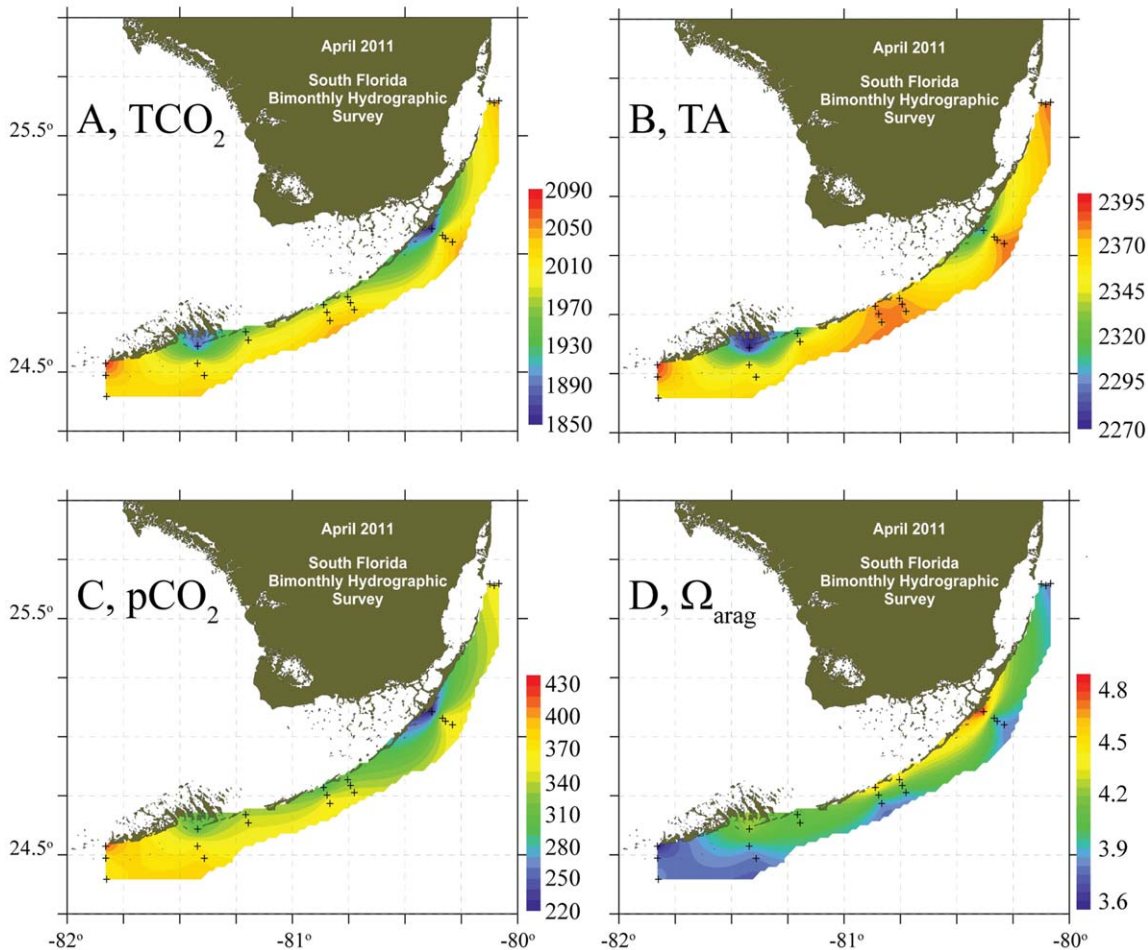
Figure 2. Carbonate Chemistry of Florida Reef Tract, Spring 2011. (A) Total CO_2 (TCO_2, µmol kg^{-1}), (B) Total Alkalinity (TA, µEq kg^{-1}), (C) partial pressure of CO_2 (pCO_2, µatm), and (D) aragonite saturation state (Ω_{arag}) from April 2011 for the Florida Reef Tract.
doi:10.1371/journal.pone.0041715.g002

lower pCO_2 most of the time except during or after anomalous events.

Three anomalous events impacted carbonate chemistry. First, there was an increase of inshore TCO_2 and TA in the UK after the cold-water mass mortality of reef-building corals that occurred at inshore patch reefs in January 2010 (Fig. 5) [20–22]. This particular inshore site was seriously affected, as coral mortality in four species of corals was 83–100% (Manzello, unpub data). During August 2010, there were persistent winds at 5 to 7.5 m s^{-1} and overcast skies during sampling. As a result of lower than normal nTA, there was a spike in pCO_2 and depression in Ω_{arag} at both sites, erasing any inshore-to-offshore gradient for these two parameters (Fig. 5). Interestingly, at this time the TA-TCO_2 plot indicated net calcification in the absence of photosynthesis (Fig. 6B). Lastly, during October 2011, there was unseasonably high rainfall in south Florida and low salinity water was observed at all sites, with salinities inshore and offshore ranging from 32.04–32.685 and 34.035–34.835, respectively. At the inshore sites, low salinity was coincident with a spike in pCO_2 and depression in Ω_{arag} (Fig. 5). All sites showed net respiration during this time (Fig. 6).

Site and Seasonal comparisons

The carbonate chemistry was similar across the UK, MK, and LK when grouped by season and inshore/offshore (Figs. S1 and S2). A single exception was that UK inshore Ω_{arag} which, in addition to $\Delta\Omega_{arag}$, was significantly higher than the LK in the spring (Fig. S1, t-test, $p < 0.01$).

Seasonal effects were pronounced at the inshore sites, while the offshore sites were relatively stable (Table S3, Figs S1 and S2). The two exceptions were 1) pCO_2 at the UK offshore site, which was highest in summer (t-tests, $p < 0.05$), and 2) nTA at the LK offshore site, where there was a significant depletion in TA during summer (Fig. S2). The only other site where pCO_2 varied significantly by season was the UK inshore site (Table S3). Ω_{arag} values were significantly higher in the spring versus autumn at all the inshore sites ($p < 0.05$). Spring and summer TCO_2 and TA values were significantly lower than the winter values at all inshore sites ($p < 0.05$).
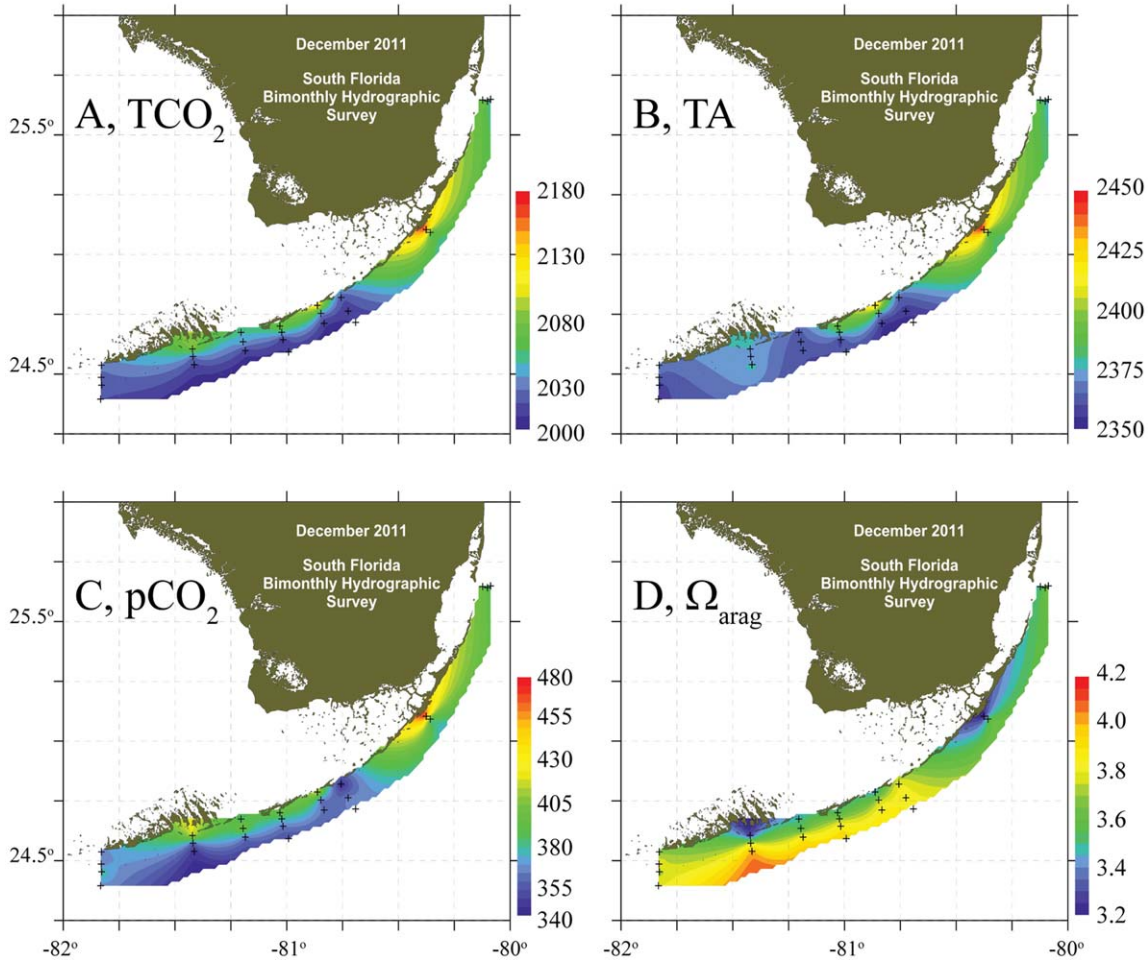
Figure 3. Carbonate Chemistry of Florida Reef Tract, Late Autumn 2011. (A) TCO_2, (B) TA, (C) pCO_2, and (D) Ω_{arag} from December 2011 for the Florida Reef Tract.
doi:10.1371/journal.pone.0041715.g003

Discussion

The inshore-to-offshore variability in carbonate chemistry on the FRT is primarily driven by large changes in TCO_2, indicating that net primary productivity is the dominant mechanism forcing this gradient. The magnitude of the TCO_2 depletion in the spring and summer is sufficiently large to elevate Ω_{arag}-values despite a coincident decline in TA. The standing crop, abundance, and productivity of the dominant species of seagrass in the Florida Keys, *Thalassia testudinum*, follow a sinusoidal pattern, peaking from June to July, and reaching minimum values in January [11]. Macroalgae in the Florida Keys follow a similar seasonal pattern, whereby they increase in abundance from the spring to summer, then decline and reach a minimum in the winter [23], [24]. The temporal variation of these benthic primary producers coincide with the seasonal trend in TCO_2 at the inshore sites.

Seagrass beds covered 46.2–55.6% of 3,141 km^2 in the Florida Keys during a recent mapping study that included terrestrial and marine habitats [9]. The dominance of seagrass habitats within the Florida Keys lends support to the hypothesis that their productivity is responsible for the patterns in TCO_2 reported herein.

Seagrasses are usually net autotrophic ecosystems [25], which would explain the net uptake of TCO_2 at all inshore sites (Table 2, Fig. 4). Net TCO_2 uptake was also indicated by the persistence of the inshore-to-offshore gradient during nighttime sampling in May 2009, June 2010, April 2011, June 2011, and February 2012. Net autotrophy within seagrass meadows is due, in part, to low levels of herbivory, as <20% of seagrass productivity is consumed by herbivores [26]. Furthermore, seagrasses bury up to 50% of their production within their roots and rhizomes, which act as a CO_2 sink [27], [28]. Reef areas with high macroalgal cover may also exhibit net CO_2 uptake [29–31], though this carbon may be less permanently sequestered than in seagrasses [32]. A large fraction of the macroalgal production on reefs is directly respired, resulting in P/R ratios very close to 1, even for macroalgal dominated systems [33]. We hypothesize that the carbon sequestered by macroalgae is less persistent than that fixed by seagrasses. Macroalgae are either consumed by herbivores, or degraded microbially when the algae senesce on a seasonal basis. Either route represents a relatively short temporal fate whereby CO_2 is ultimately respired back into the water column. There is no obvious route whereby the CO_2 sequestered would be preserved
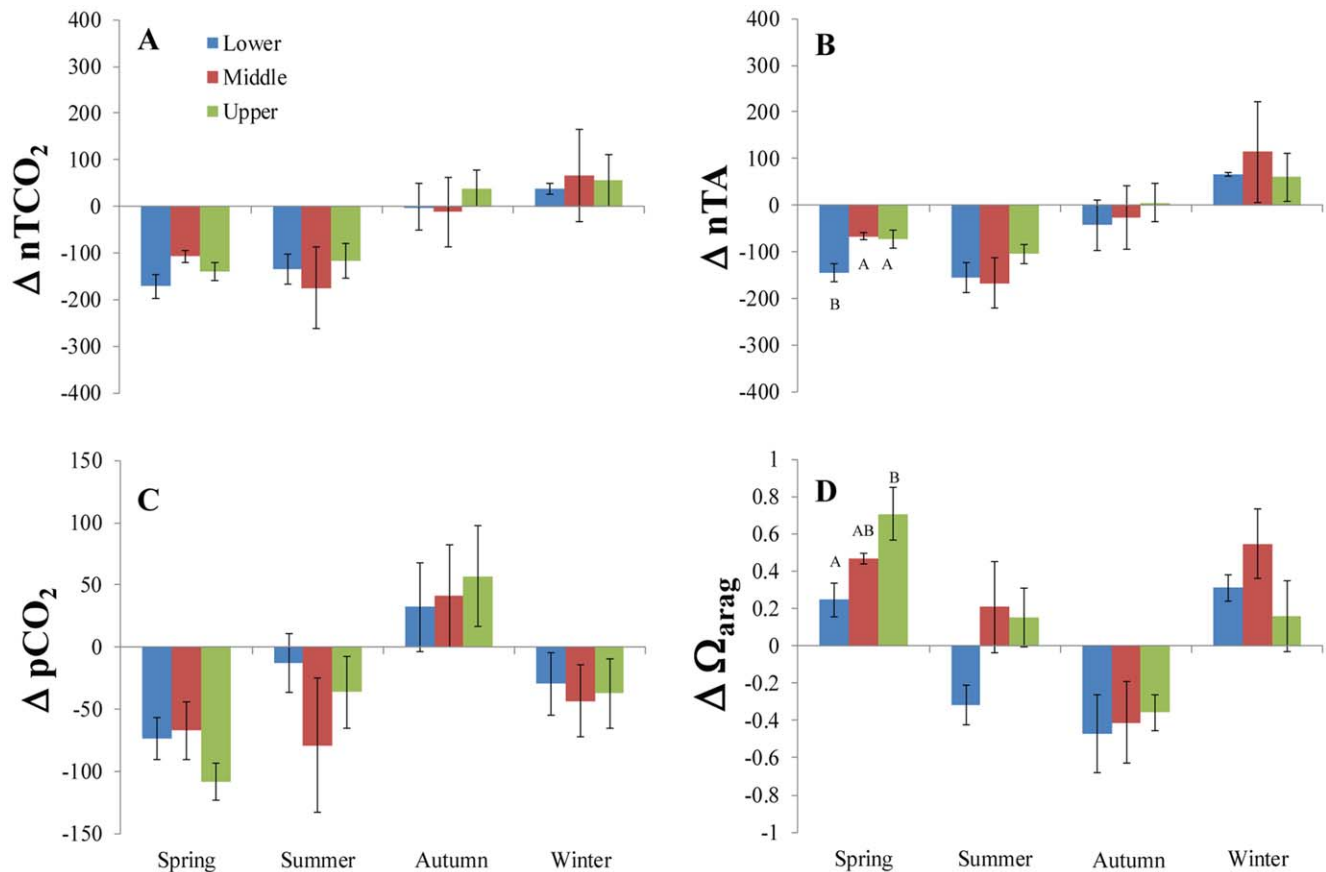
Figure 4. Seasonal Gradient in Carbonate Chemistry between Inshore and Offshore reef sites on the Florida Reef Tract. The difference (Δ) between inshore and offshore values of (A) $nTCO_2$, (B) nTA, (C) pCO_2, and (D) Ω_{arag} for the upper, middle, and lower Keys plotted by season. Values are means and error bars are standard error of the mean. Non-matching letters indicate significant differences (t-tests, $p < 0.05$).
doi:10.1371/journal.pone.0041715.g004

like that of seagrass rhizomes and roots that are buried in sediments.

While reductions in CO_2 associated with seagrass photosynthesis may ameliorate OA-related stress, several aspects of seagrass habitats are not conducive to coral growth and reef development. First, the soft substrate of seagrass beds is a poor habitat for both corals and other sessile reef-associated organisms [34]. While corals can survive as free-living motile colonies in areas of unstable substrate, these "coralliths" are usually small in size and have stunted growth rates [35]. Corals thrive on stable substrata, thus areas of antecedent hard bottom downstream or within seagrass areas may be important areas for coral persistence with accelerating OA.

Second, temperature fluctuations inshore on the FRT are high and potentially harmful to thermally sensitive coral species. Mean temperature values at our six sites from June 2010 to May 2011 were similar, ranging from 26.0 to 26.4°C. The variance in temperature was much greater at the inshore sites, as minimum and maximum temperatures were more extreme (Fig. S3). The greatest temperature fluctuations occurred in the MK, likely a result of the net outflow of Florida Bay waters through the wide channels in the middle Keys [36]. Florida Bay is shallow (average depth <3 m) and responds rapidly to changes in atmospheric temperatures [20]. At the inshore MK site, the minimum temperature was 14°C in Dec 2010 and the maximum temperature was >34°C in the summer of 2010 (Fig. S3). These temperatures are well outside the thermal tolerances of most coral

species (18–30°C) [37]. Corals can tolerate conditions outside of this range if the duration and magnitude of temperature fluctuations is not too long or great, respectively. This 20°C range observed at the inshore MK site effectively restricts the proliferation of the majority of coral species. There are, however, a few especially hardy species, such as *Solenastrea bournoni*, and *Siderastrea radians*, but they are present in low abundances. Thus, while the $\Sigma\Delta\Omega_{arag}$ was highest in the MK, thermal extremes preclude the viability of this area as a refuge for corals from OA. The range in temperature was less at the inshore sites in the UK and LK. These sites do experience extreme temperatures, but they are not as great in magnitude or as long in duration. This helps explain why isolated patch reefs are able to persist in these locations and not in the MK [38].

Overall, thermal conditions are more favorable at the offshore sites (Fig. S3). This is because the Florida Current effectively buffers these reef sites from the thermal extremes that occur close to shore [38]. Again, the MK offshore site, just like for the inshore sites, had the greatest range in temperatures (17.1°C), likely a result of the exchange of Florida Bay waters in the middle Keys [36]. This large temperature variability, by itself, is likely a primary factor of why the MK reefs are the most poorly developed of FRT [10]. The range in temperatures at the UK and LK offshore sites was less. However, it is important to realize that the FRT experiences some of the greatest temperature fluctuations documented for coral reefs. There are only a few locations, such as the Persian Gulf, where corals that form reef frameworks

Table 2. Sum of differences between inshore and offshore sites across all seasons.

| Site	$\Sigma\Delta TCO_2$	$\Sigma\Delta nTCO_2$	$\Sigma\Delta TA$	$\Sigma\Delta nTA$	$\Sigma\Delta pCO_2$	$\Sigma\Delta\Omega_{arag}$
Upper	−144.4	−162.5	−71.0	−111.2	−125	0.66
Middle	−160.9	−228.0	−37.3	−147.3	−149	0.81
Lower	−244.9	−271.5	−243.3	−277.0	−84	−0.23

doi:10.1371/journal.pone.0041715.t002

experience a wider range in temperatures (25°C) [39] . The FRT is at the upper and lower thermal limits for coral survival and reef development in the North Atlantic, due to cold water in the winter and warm water in the summer.

The FRT has experienced at least five warm-water bleaching events since 1987 that have encompassed the entire reef tract [40].

Inshore patch reefs experienced catastrophic coral mortality due to extreme cold weather in early 2010 that was an order of magnitude greater than any of the mortality associated with warm-water bleaching events to date [20–22]. Cold water stress, while rare, has repeatedly been identified as a primary limiting factor for the FRT and inshore sites are more prone to thermal extremes [38]. Although these sites may provide refuge from chronic OA stress, they will likely continue to be susceptible to acute cold weather events.

Intriguingly, the inshore patch reef environments of the upper FRT, which were found to have higher Ω_{arag} values than what has been modeled for the tropics prior to the industrial revolution [4], are known to have higher coral cover and faster coral growth rates compared to reefs offshore [41]. One hypothesis for the better condition of these nearshore patch reefs is that these areas may be more resistant to recurrent warm-water bleaching because of increased turbidity and chromophoric dissolved organic matter (CDOM) shading corals, partially reducing the photo-oxidative
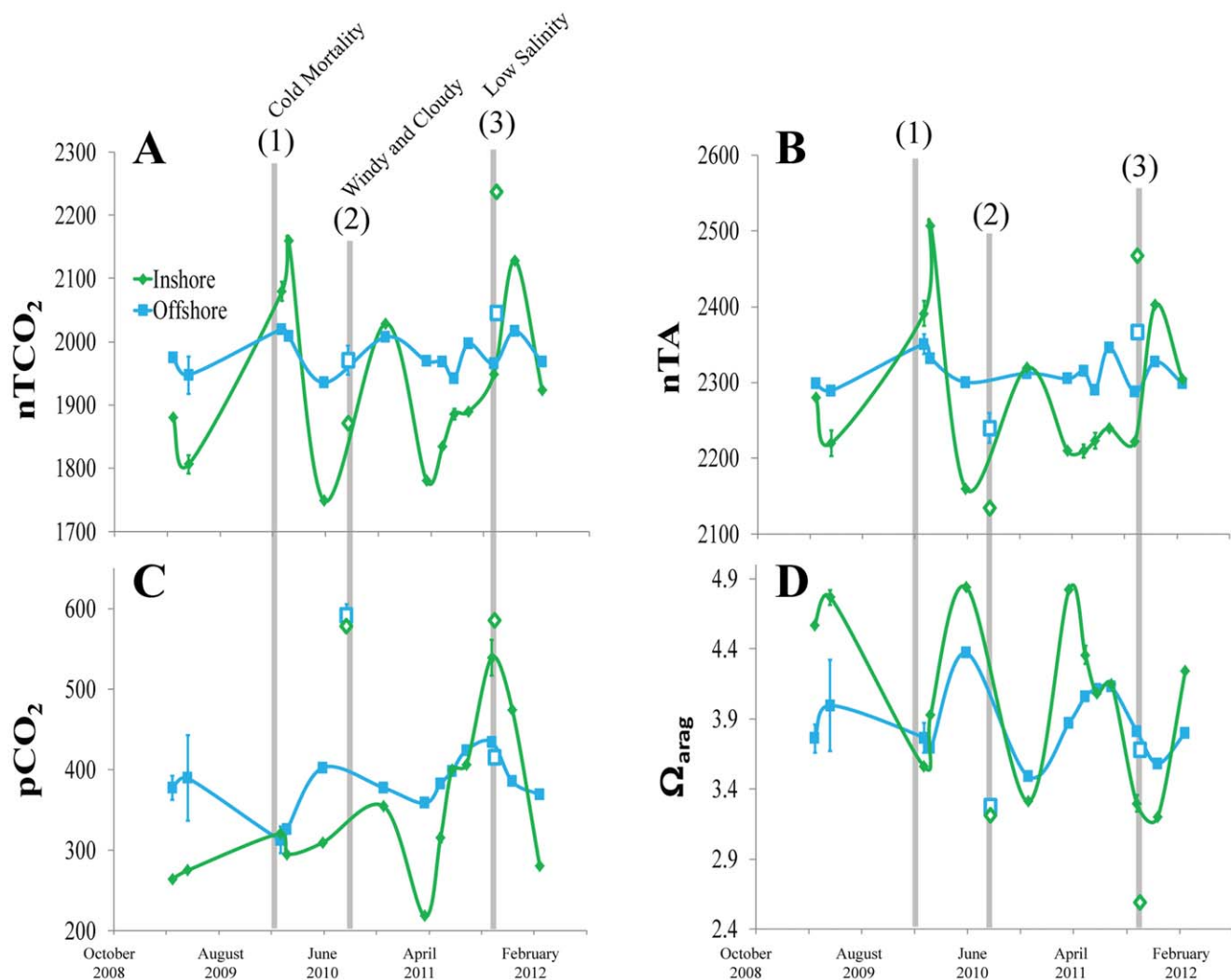
Figure 5. Time-series of Carbonate Chemistry at paired Inshore and Offshore reef sites in upper Florida Keys. Time-series of (A) nTCO$_2$, (B) nTA, (C) pCO$_2$, and (D) Ω_{arag} for paired inshore (green diamonds) and offshore (blue squares) reef sites in the Upper Florida Keys from April 2009 to February 2012. Anomalous events are noted for (1) sampling after cold-water mass mortality of inshore patch reefs in early 2010; elevated pCO$_2$ and depressed Ω_{arag} associated with (2) high winds and overcast conditions in August 2010, and (3) low salinity in October 2011. Events (2) and (3) are represented by open diamonds (inshore) and squares (offshore) and are not linked to line as they deviate greatly from the seasonal pattern. The data points immediately after the cold-water mass mortality (1) are included because they represent important winter endpoints for 2009–2010.
doi:10.1371/journal.pone.0041715.g005
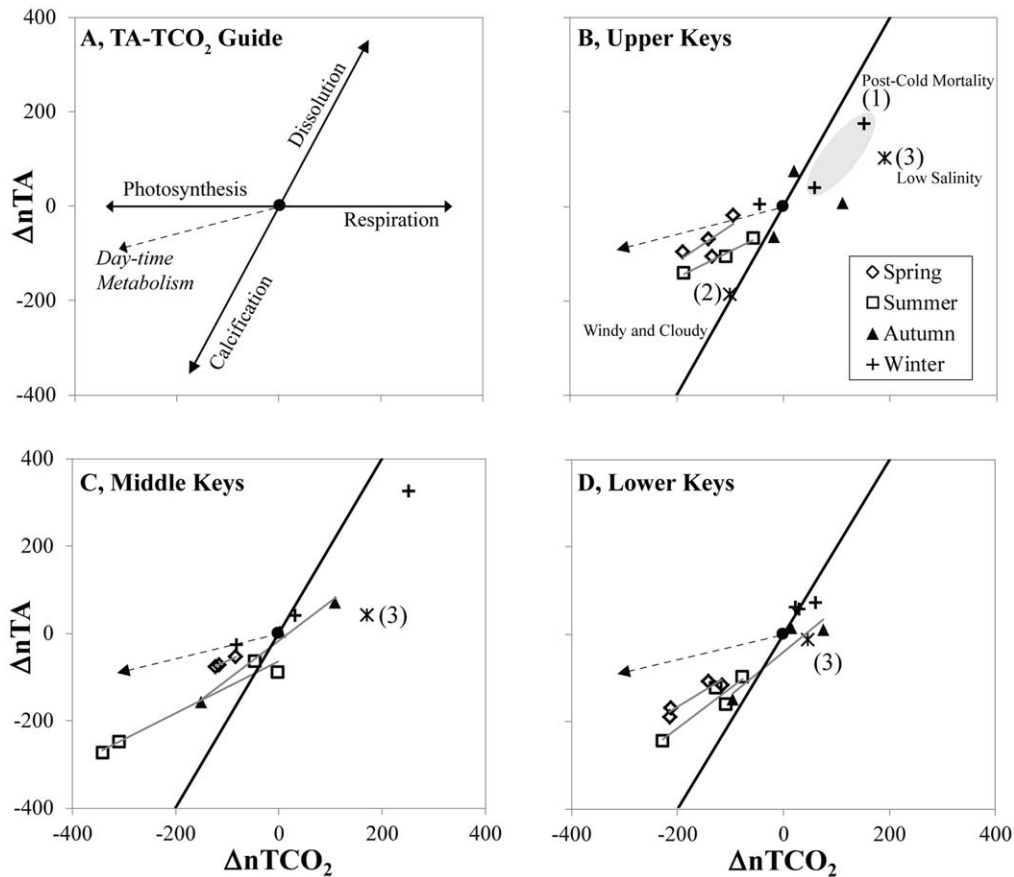
Figure 6. Total alkalinity (TA) vs. Total CO_2 (TCO_2) plots for upper, middle, and lower Florida Keys. (A) Guide to TA-TCO_2 plots illustrating how net photosynthesis, respiration, calcification and dissolution affect location of points. TA and TCO_2 are salinity normalized (S = 35) to allow comparison across seasons and sites. The reference offshore water mass is illustrated by the solid circle in the center of the plot. Vector addition of day-time metabolism assumes a photosynthesis/calcification molar ratio of 6 [17]. (B) Upper Keys, (C) Middle Keys, and (D) Lower Keys. The same three events listed in Fig. 5 are highlighted. (1) The samples obtained one and two months after the cold mortality are within the gray ellipse. The data point closer to the origin is from 16–17 February 2010, whereas the one farther removed is from 8 March 2010. Asterisks denote events (2) and (3).

doi:10.1371/journal.pone.0041715.g006

stress that occurs within the algal symbionts of corals during thermal stress [42], [43]. We suggest that the favorable Ω_{arag} conditions for these sites should also be considered as a contributing reason for their increased resilience.

Inshore patch reefs of the upper FRT may be OA refugia. The thermal extremes of the MK eliminate this area as a potential refuge. The $\Sigma\Delta\Omega_{arag}$ of the LK is negative, indicating that Ω_{arag}-values inshore are more often lower than those offshore. Low TA values were measured during the summer for both the inshore and offshore LK sites (Figs. S1 and S2). Inshore TA values were sufficiently low to decrease Ω_{arag}-values relative to those offshore (Table 1). It is not clear what is causing this apparent increase in calcification relative to photosynthesis. Tidally-driven flushing of the shallow waters between the numerous islands of the LK (see Fig. 1) may be resulting in the increased depletion of TA relative to TCO_2, favoring $CaCO_3$ precipitation that is not directly linked to photosynthesis. The bedrock of the LK islands is an oolitic deposit, whereas the Key Largo Limestone that makes up the UK and MK islands is coral reef derived [44]. Both facies were formed about 125,000 years ago, during the Pleistocene, when sea level was 4–6 m higher than the present day [45]. The same factors that favored the inorganic precipitation of $CaCO_3$ (i.e., ooid shoals) in

the LK, versus the biogenic coral reef deposits of the UK and MK, may be similar to what is occurring today on the FRT.

Seagrasses are also known to stimulate the dissolution of the calcareous sediments where their roots and rhizomes are buried [46–48]. This is because seagrasses pump a fraction of the oxygen produced during photosynthesis into their roots, which drives aerobic respiration and carbonate dissolution via CO_2 production [46]. The addition of TA to seawater via this process has been suggested as a potential negative feedback to OA, as TA addition lowers pCO_2 [47], [48]. Our data from the water column showed that TA often declined in concert with TCO_2, indicative of net calcification (Fig. 6). Burdige and Zimmerman [46] showed that dissolution rate was a function of seagrass density and photosynthesitic productivity. Yet, TA was seasonally elevated at all sites (inshore and offshore) during the winter months (Table 1), and even more so at the inshore sites (Fig. S1, 4). This is when productivity of seagrasses on the FRT is at its minimum [11]. It is not clear if the TCO_2 enrichment during autumn and winter is directly causing this elevated TA by driving dissolution. Whatever the mechanism, the elevation of TA during the winter offsets the TCO_2 enrichment that occurs at this time, resulting in lower pCO_2 and higher Ω_{arag} at the inshore sites (Fig. 4).

Seagrasses are carbon limited, whereas macroalgae are able to effectively utilize the abundant HCO_3^- in seawater for photosynthesis [49]. Consequently, seagrass productivity is expected to be stimulated with OA [49–52], whereas macroalgae, already carbon-saturated, should not exhibit any increases in photosynthesis or growth [49]. For example, seagrasses exposed to high-CO_2 conditions for 1 yr had increased reproduction, rhizome biomass, and vegetative growth of new shoots, which could represent a potential positive feedback to their ability to serve as OA refugia [51]. These results have implications for other calcifying organisms other than corals. Seagrasses, unlike shallow coral reefs, are an ecosystem that is not limited to the warm tropical latitudes. As such, the potential for seagrasses to locally buffer OA within coastal zones may be much greater than what is considered here and could even increase over time.

In summary, coral reefs in close proximity to seagrass beds may find refuge from OA. In the upper Florida Keys, inshore patch reefs have exhibited greater resilience relative to the offshore barrier reefs to a range of environmental stresses [41]. These inshore reefs are located where the uptake of TCO_2 drives seasonal enhancement of Ω_{arag} (Fig. 4D). The natural sequestration of CO_2 on the FRT may facilitate the high resilience of these sites.

Supporting Information

Figure S1 Carbonate Chemistry at Inshore Sites by Season. Mean values for (A) $nTCO_2$, (B) nTA, (C) pCO_2, and (D) Ω_{arag} plotted by season for inshore sites from upper, middle, and lower keys. Error bars represent standard error of the mean. Means represent the average of mean values for each sampling excursion grouped by season. Non-matching letters indicate significant differences (t-tests, p<0.05).
(TIF)

Figure S2 Carbonate Chemistry at Offshore Sites by Season. Mean values for (A) $nTCO_2$, (B) nTA, (C) pCO_2, and (D) Ω_{arag} plotted by season for offshore sites from upper, middle, and lower keys. Error bars represent standard error of the mean.

Means represent the average of mean values for each sampling excursion grouped by season.
(TIF)

Figure S3 Seawater temperature at paired inshore and offshore sites. *In situ* temperature data, collected every 30 min from paired inshore (green lines) and offshore (blue lines) sites for (A) upper, (B) middle, and (C) lower Keys. Depths of temperature values are 5 m.
(TIF)

Table S1 Timing of discrete sampling at paired inshore and offshore sites in the upper (UK), middle (MK), and lower (LK) Florida Keys. WS indicates sample taken aboard the R/V Walton Smith in coordination with the South Florida Program's repeat biophysical oceanographic cruises rather than small boat sampling (X).
(DOC)

Table S2 nTA-nTCO$_2$ trendline equations and R^2 by site and season.
(DOC)

Table S3 Kruskal-Wallis results to identify seasonal differences within each of the six sites.
(DOCX)

Author Contributions

Conceived and designed the experiments: DPM ICE EJ. Performed the experiments: DPM ICE NM. Analyzed the data: DPM ICE DKG. Wrote the paper: DPM ICE DKG.

References

1. Caldeira K, Wickett ME (2003) Anthropogenic carbon and ocean pH. Nature 425: 365.
2. Morse JW, Andersson AJ, Mackenzie FT (2006) Initial responses of carbonate-rich shelf sediments to rising atmospheric pCO$_2$ and "ocean acidification": Role of high Mg-calcites. Geochim Cosmochim Acta 70: 5814–5830.
3. Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009) Ocean acidification: The other CO$_2$ problem. Ann Rev Mar Sci 1: 169–192.
4. Kleypas JA, Buddemeier RW, Archer D, Gattuso J-P, Langdon C, et al. (1999) Geochemical consequences of increased atmospheric carbon dioxide on coral reefs. Science 284: 118–120.
5. Bates NR, Amat A, Andersson AJ (2009) The interaction of ocean acidification and carbonate chemistry on coral reef calcification: evaluating the carbonate chemistry Coral Reef Ecosystem Feedback (CREF) hypothesis on the Bermuda coral reef. Biogeosci Discuss 6: 7627–7672.
6. Manzello DP, Kleypas JA, Budd DA, Eakin CM, Glynn PW, et al. (2008) Poorly cemented coral reefs of the eastern tropical pacific: Possible insights into reef development in a high-CO$_2$ world. Proc Natl Acad Sci U S A 105: 10450–10455.
7. Manzello DP (2010) Ocean Acidification Hotspots: Spatiotemporal dynamics of the seawater CO$_2$ system of eastern Pacific coral reefs. Limnol Oceanogr 55: 239–248.
8. Friedrich T, Timmermann A, Abe-Ouchi A, Bates NR, Chikamoto MO, et al. (2012) Detecting regional anthropogenic trends in ocean acidification against natural variability. Nat Clim Change 2: 167–171.
9. Lidz BH, Reich CD, Shinn EA (2005) Systematic mapping of bedrock and habitats along the Florida reef tract: Central Key Largo to Halfmoon Shoal (Gulf of Mexico). US Geol Survey Prof Paper 1751, Available: http://pubs.usgs.gov/pp/2007/1751. Accessed 2012 March 19.
10. Ginsburg RN, Shinn EA (1964) Preferential distribution of the reef-building community in Florida and the Bahamas. Am Assoc Pet Geol Bull 48: 527.
11. Fourqurean JW, Willsie A, Rose CD, Rutten LM (2001) Spatial and temporal pattern in seagrass community composition and productivity in south Florida. Mar Biol 138: 341–354.
12. Jones JA (1977) Morphology and development of southeastern Florida patch reefs. Proc 3rd Int Coral Reef Symp 2: 231–235.
13. Semesi IS, Beer S, Bjork M (2009) Seagrass photosynthesis controls rates of calcification and photosynthesis of calcareous macroalgae within a seagrass meadow. Mar Ecol Prog Ser 382: 41–47.
14. Lewis E, Wallace DWR (1998) Basic program for CO$_2$ system in seawater. ORNL/CDIAC-105, Oak Ridge National Laboratory.
15. Mehrbach C, Cilberson CA, Hawley JE, Pytokwicz RM (1973) Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnol Oceanogr 18: 897–907.
16. Dickson AG, Millero FJ (1987) A comparison of the nequilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Res 34: 1733–1743.
17. Dickson AG (1990) Thermodynamics of the dissociation of boric acid in synthetic seawater from 273.15 to 318.15°K. Deep-Sea Res 37: 755–766.
18. Suzuki A, Kawahata H (2003) Carbon budget of coral reef systems: An overview of observations in fringing reefs, barrier reefs and atolls in the Indo-Pacific regions. Tellus B 55: 428–444.
19. Millero FJ, Hiscock WT, Huang F, Roche M, Zhang JZ (2001) Seasonal variation of the carbonate system in Florida Bay. Bull Mar Sci 68: 101–123.
20. Kemp DW, Oakley CA, Thornhill DJ, Newcomb LA, Schmidt GW, et al. (2011) Catastrophic mortality on inshore coral reefs of the Florida Keys due to severe low-temperature stress. Glob Change Biol 17: 3468–3477.
21. Lirman D, Schopmeyer S, Manzello D, Gramer LJ, Precht WF, et al. (2011) Severe 2010 cold-water event caused unprecedented mortality to corals of the Florida Reef Tract and reversed previous survivorship patterns. PLoS ONE 6(8): e23047.

22. Collela MA, Ruzicka RR, Kidney JA, Morrison JM, Brinkhuis(2012) Cold-water event of January 2010 results in catastrophic benthic mortality on patch reefs in the Florida Keys. Coral Reefs doi: 10.1007/s00338-012-0880-5

23. Lirman D, Biber P (2000) Seasonal dynamics of macroalgal communities of the Northern Florida Reef Tract. Bot Mar 43: 305–314.

24. Collado-Vides L, Rutten LM, Fourqurean JW (2005) Spatiotemporal variation of the abundance of calcareous green macroalgae in the Florida Keys: A study of synchrony within a macroalgal functional-form group. J Phycol 41: 742–752.

25. Duarte CM, Marba N, Gacia E, Fourqurean JW, Beggins J, et al. (2010) Seagrass community metabolism: Assessing the carbon sink capacity of seagrass meadows. Glob Biogeochem Cycl 24, GB4032.

26. Duarte CM, Cebrian J (1996) The fate of marine autotrophic production. Limnol Oceangr 41: 1758–1766.

27. Duarte CM, Merino M, Agawin NSF, Uri J, Fortes MD, et al. (1998) Root production and belowground seagrass biomass. Mar Ecol Prog Ser 171: 97–108.

28. Duarte CM, Kennedy H, Marba H, Hendriks I (2011) Assessing the capacity of seagrass meadows for carbon burial: current limitations and future strategies. Ocean Coast Mgt doi: 10.1016/j.ocecoaman.2011.09.001.

29. Gattuso J-P, Payri CE, Pichon M, Delesalle B, Frankignoulle M (1997) Primary production, calcification, and air-sea CO2 fluxes of a macroalgal-dominated coral reef community. J Phycol 33: 729–738.

30. Anthony KRN, Kleypas JA, Gattuos J-P (2011) Coral reefs modify their seawater carbon chemistry –implications for impacts of ocean acidification. Glob Change Biol 17: 3655–3666.

31. Kleypas JA, Anthony KRN, Gattuso J-P (2011) Coral reefs modify their seawater carbon chemistry – case study from a barrier reef (Moorea, French Polynesia). Glob Change Biol 17: 3667–3678.

32. Gattuso J-P, Frankignoulle M, Smith SV (1999) Measurement of community metabolism and significance in the coral reef CO_2 source-sink debate. Proc Natl Acad Sci U S A 96: 13017–13022.

33. Falter JL, Atkinson MJ, Schar DW, Lowe RJ, Monismith SG (2011) Short-term coherency between gross primary production and community respiration in an algal-dominated reef flat. Coral Reefs 30: 53–58.

34. Jackson JBC (1985) Distribution and ecology of clonal and aclonal benthic invertebrates. In: Jackson JBC, Buss LW, Cook, RE (eds.), Population Biology and Evolution of Clonal Organisms. Yale Univ Press. New Haven, CT. pp 297–356.

35. Glynn PW (1974) Rolling stones among the Scleractinia: Mobile coralliths in the Gulf of Panama. Proc 2nd Int Coral Reef Symp 2: 183–198.

36. Smith NP (1994) Long-term Gulf-to-Atlantic transport through tidal channels in the Florida Keys. Bull Mar Sci 54: 602–609.

37. Kleypas JA, McManus JW, Lambert LAB (1999) Environmental limits to coral reef development: where do we draw the line? Am Zool 39: 146–159.

38. Marszalek DS, Babashoff G, Noel MR, Worley DR (1977) Reef distribution in South Florida. Proc 3rd Int Coral Reef Symp 2: 223–229.

39. Coles SL, Fadlallah YH (1991) Reef coral survival and mortality at low temperatures in the Arabian Gulf: new species-specific lower temperature limits. Coral Reefs 9: 231–237.

40. Manzello DP, Berkelmans RC, Hendee JC (2007) Coral bleaching indices and thresholds for the Florida Reef Tract, Bahamas, and St. Croix, U.S. Virgin Islands. Mar Poll Bull 54: 1923–1931.

41. Lirman D, Fong P (2007) Is proximity to land-based sources of coral stressors an appropriate measure of risk to coral reefs? An example from the Florida Reef Tract. Mar Poll Bull 54: 79–791.

42. Zepp RG, Shank GC, Stabenau E, Patterson KW, Cyterski M, et al. (2008) Spatial and temporal variability of solar ultraviolet exposure of coral assemblages in the Florida Keys: importance of colored dissolved organic matter. Limnol Oceanogr 53: 109–1922.

43. Ayoub L, Hallock P, Coble P (2009) Colored dissolved organic material increases resiliency of Coral Reefs by controlling exposure to UVR. Proc 11th Int Coral Reef Symp 16: 572–576.

44. Hoffmeister JE, Multer HG (1968) Geology and origin of the Florida Keys. Geol Soc Am Bull 79: 1487–1502.

45. Overpeck JT, Otto-Bleisner BL, Miller GH, Muhs DR, Alley RB, et al. (2006) Paleoclimatic evidence for future ice-sheet instability and rapid sea-level rise. Science 311: 1747–1750.

46. Burdige DJ, Zimmerman RC (2002) Impact of seagrass density on carbonate dissolution in Bahamian sediments. Limnol Oceanogr 47: 1751–1763.

47. Burdige DJ, Zimmerman RC, Hu X (2008) Rates of carbonate dissolution in permeable sediments estimates from pore-water profiles: The role of seagrasses. Limnol Oceanogr 53: 549–565.

48. Burdige DJ, Hu X, Zimmerman RC (2010) The widespread occurrence of coupled carbonate dissolution/reprecipitation in surface sediments on the Bahamas Bank. Am J Sci 310: 492–521.

49. Beer S, Kock E (1996) Photosynthesis of marine macroalgae and seagrasses in globally changing CO_2 environments. Mar Ecol Prog Ser 141: 199–204.

50. Zimmerman RC, Kohrs DG, Stellar DL, Alberte RS (1997) Impacts of CO_2-enrichment on productivity and light requirements of eelgrass. Plant Physiol 115: 599–607.

51. Palacios S, Zimmerman RC (2007) Response of eelgrass (*Zostera marina* L.) to CO_2 enrichment: Possible impacts of climate change and potential for remediation of coastal habitats. Mar Ecol Prog Ser 344: 1–13.

52. Jiang ZJ, Huang X-P, Zhang J-P (2010) Effects of CO_2 Enrichment on Photosynthesis, Growth, and Biochemical Composition of Seagrass *Thalassia hemprichii* (Ehrenb.) Aschers. Int J Plant Biol 52: 904–913.