APPENDIX I:

Report on dissolved inorganic carbon (DIC) and total alkalinity (TA) concentrations on 14 MMP sites comparing one dry-and one wet-season

by Sven Uthicke

Australian Institute of Marine Science

SUMMARY AND RECOMMENDATIONS

- Dissolved Inorganic Carbon and Total Alkalinity in the Water near 14 inshore reefs were collected over three MMP trips
- Data were analysed statistically and $p\mathrm{CO}_2$, pH and aragonite saturation state values derived
- Largest variation was found between field trips, with generally higher values of $p\mathrm{CO}_2$ and lower values of pH detected in the wet-season
- Due to temperature effects, saturation state of aragonite was not reduced in the wet-season
- Samples obtained from the ships anchorage were not significantly different to those taken directly on the reef
- There are good indications that saturation state of aragonite on inshore reefs was lower, and $p\mathrm{CO}_2$ higher that on GBR reefs further offshore
- The latter two points may indicate that inshore GBR reefs are more vulnerable to ocean acidification and have less buffering capacity compared to reefs further offshore
- It is suggested that further samples will be collected and analysed over the next two Reef Water Quality Plan Marine Monitoring trips to complete the dataset.
- Further studies with short temporal (e.g. day vs. night) and spatial (e.g. reef flat vs. reef slope) resolution, and targeted comparisons to midshelf and outer shelf reefs would be important to test if inshore reefs (in addition to vulnerability to runoff and warming) are especially vulnerable to ocean acidification

INTRODUCTION

Present day atmospheric CO_2 concentrations are over 30% higher than the maximum observed in the previous 2 Mil yr (Hönisch et al. 2009). A large percentage of CO_2 is absorbed into the world's oceans leading to reduced carbonate ion concentrations in parallel with a reduction in seawater pH (Ocean Acidification, OA). Seawater pH has decreased by 0.1 units since pre-industrial times and is likely to be further reduced by 0.3-0.5 units in the next 100 years (Caldeira and Wickett 2005). These changes in chemistry reduce the saturations state of carbonate (both aragonite and calcite) which reduces the ability of animals using carbonate structures as skeleton (such as corals) to grow. Coral reef ecosystems are particularly vulnerable to OA and climate change induced ocean warming (Hoegh-Guldberg et al. 2007) with a range of effects on the ecosystem and associated biota evident (e.g., Anthony et al. 2008; Munday et al. 2009; Diaz-Pulido et al. 2011; Fabricius et al. 2011).

Given that many coral reefs are net-autotrophic, some reefs may have a strong buffering capacity towards ocean acidification (Anthony et al. 2011; Kleypas et al. 2011). Shallow reef flat areas are dominated by respiratory processes at night (increasing CO_2 in the water) and autotrophic processes at day (decreasing CO_2 and thus increasing pH). This can lad to extreme fluctuations of pH, aragonite saturation state and *p*CO2 (Shaw et al. 2012).

The inshore reefs of the GBR are presumed vulnerable to a variety of stressors resulting from increased agricultural runoff (Fabricius 2005; Cooper et al. 2007; Fabricius et al. 2012). For this reason the Reef Water Quality Protection Plan Marine Monitoring Programme (Reef Plan MMP) has been implemented in 2005; this program is now known as the Reef Rescue Marine Monitoring Program (MMP) and is funded by the Australian Government’s Reef Rescue initiative. This program includes an intensive benthic (e.g., coral communities, algae and foraminifera) monitoring on 14 core reefs in the main catchments of the Great Barrier Reef (GBR). In addition, three annual research trips for water quality analysis are conducted.

There is presently now information on carbon chemistry on these inshore reefs. Given that autotrophic processes are often lower on inshore reefs due to decreased light availability (Fabricius and De'ath 2008; Nobes et al. 2008; Uthicke and Nobes 2008;

Uthicke and Altenrath 2010), it is possible that these reefs are less able to absorbed some of the additional DIC from ocean acidification into plant production and thus buffer the effects of OA. Here, we analyse carbon chemistry data from 14 inshore reefs which are the core reefs of the Reef Rescue Marine Monitoring program collected during three field trips spanning one year of MMP monitoring.

MATERIALS AND METHODS

Collection and analysis

Water samples for analysis of Total Alkalinity (A_T) and Dissolved Inorganic Carbon (DIC) where collected on three water quality trips from the Reef Water Quality Marine Monitoring Program. These trips represent one late dry season (September 2011), one wet-season (February 2012) and one early dry season (June 2012). 250 ml of samples for A_T/DIC analyses were collected in duplicate from each of the stations at the 14 core reefs of this monitoring program (Fig. 1), in conjunction with the standard water quality parameters (e.g. DIN, TDP, TSS, chlorophyll, not presented here), temperature and salinity. On each reef, three types of samples were taken. At the anchor station of the research vessels (in the range of 300-2000m from the reef sites), one sample from the surface seawater and one from and intermediate depth (average: 9.4m, 1 SD = 3.1m) were obtained using Niskin bottles. Samples from the reef station (average depth 6.5m, 1 SD = 1.4m) were taken by scuba divers directly on the reef slopes close to the benthos (also using Niskin bottles). All samples were fixed with 125µl of saturated (7g in 100mL) mercuric chloride until analyses. Samples for A_T and DIC were analysed at 25°C using a VINDTA 3C titrator (Marianda, Germany) at the Australian Institute of Marine Science. Alkalinity was analysed by acid titration (Dickson et al. 2007) and DIC by acidification and coulometric detection (UIC 5105 Coulometer) of the evolved CO_2. Calibration was conducted using Certified Reference seawaters (A. G. Dickson, Scripps Institute of Oceanography, Dixon, Batch 106).

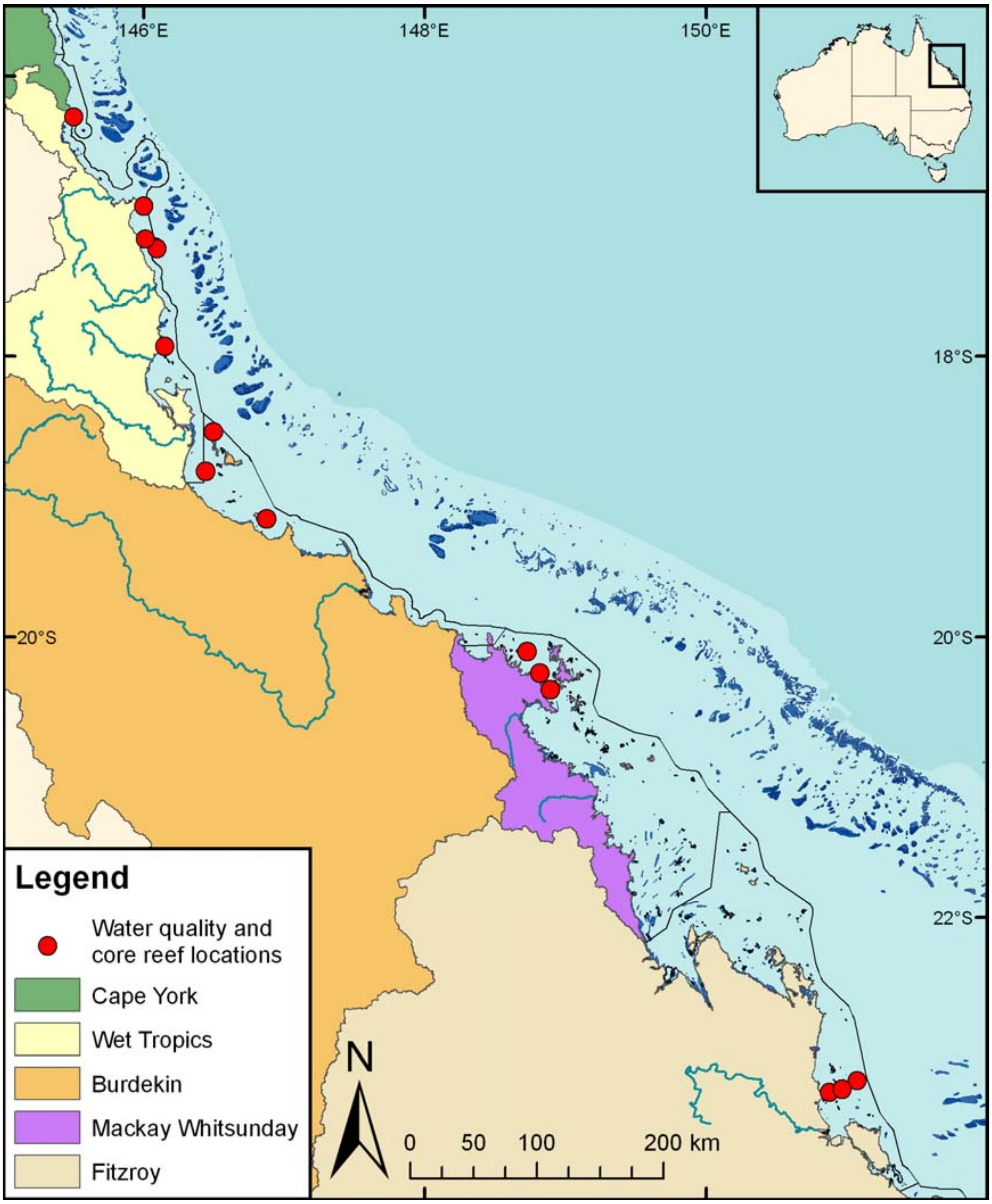

Fig. 1. Core sampling stations of the Reef Water Quality Marine Monitoring Program.

Statistical analysis

Carbon chemistry parameters (the partial pressure of CO_2 [pCO_2], pH on the total scale [pH_{Total}], and the saturation state for Aragonite [ΩAr]) were calculated using the Excel macro CO2SYS taking salinity and temperature in consideration.

We used mixed model analysis of variance (ANOVA) to analyse T_A, DIC, $p\mathrm{CO}_2$, $\mathrm{pH}_{\mathrm{Total}}$, and ΩAr. The main factor "Trip" tested for differences between the three sample periods, whereas "Region" tests for differences between 4 regions investigated (Wet-Tropics, Burdekin, Whitsunday and Fitzroy). For the purpose of this report we considered replicate "Islands" as random nested factor within Regions. To evaluate if samples taken from the reef location are different from those at the ships anchorage, "Location" was includes in the model as a third fixed factor. Differences in Temperatures and Salinity between regions and seasons were obvious and only interpreted graphically. We tested for correlations between several parameters using Pearson's product moment correlations. All statistical analysis were conducted in NCSS (Hintze 2001) or the R environment (R Development Core Team 2012).

RESULTS

Both water temperature and salinity over the 4 Regions and 3 Trips showed patterns reflecting expected seasonal trends (Fig. 2). During all three sample occasions, temperatures distinctly declined from the north to the south, and were highest during the summer wet season (average 29.7°C, 1 SD = 0.8°C) and lowest in June (21.7°C, 1 SD = 1.3°C).

Salinity was at its highest levels at the end of the dry season (September) and was lowest during the wet (February, Fig. 2). Due to the higher amount of runoff this patters is most distinct in thee two northern regions (Wet-Tropics and Burdekin). During all seasons it appears that the most southern region (Fitzroy) has slightly higher salinity values compared to other regions.

Table 1: Mixed model ANOVA for measured parameters Total Alkalinity (T_A) and Dissolved Inorganic Carbon (DIC).

		Total Alkalinity			Dissolved Inorganic Carbon		
	DF	MS	F-Ratio	p	MS	F-Ratio	p
A: Trip	2	20.94	0.01	0.9886	696.11	0.36	0.6985
B: Region	3	51784.98	28.44	**<0.0001**	48190.88	25.14	**<0.0001**
AB	6	6807.50	3.74	**0.0068**	5158.60	2.69	**0.0328**
C(AB): Island	30	1820.75	204.16	<0.0001	1917.23	126.03	<0.0001
D: Location	2	124.03	0.30	0.7414	360.21	1.23	0.3028
AD	4	1037.97	2.52	0.0563	746.22	2.55	0.0542
BD	6	624.59	1.52	0.1978	480.07	1.64	0.1617
ABD	12	300.53	0.73	0.7133	328.23	1.12	0.3707
CD(AB)	39	411.36	46.13	<0.0001	292.41	19.22	<0.0001
S	108	8.92			15.21		
Total (Adjusted)	212						
Total	213						

Total Alkalinity values showed no effects caused by the three sample months, and significant effects for geographic regions (Tab. 1). However, there was also a significant Trip x Region interaction. Alkalinity values were generally reduced during the wet season and early dry season, and during these seasons showed a clear increase from north to south (Fig. 2). Values were highest in September, and slightly elevated in the Fitzroy region compared to the other regions. In general, T_A values very closely follow changes in salinity levels (Fig. 2). Indeed, these two parameters are highly correlated ($R = 0.92$, $p < 0.0001$).

DIC showed very similar patterns to T_A, with a strong interaction between Trip and Region. Similar to T_A, highest values for DIC were observed in September, and a distinct north to south gradient was detected in the other sampling months. Also DIC was strongly correlated to salinity ($R = 0.86$, $p < 0.0001$) as well as to Alkalinity ($R = 0.95$, $p < 0.0001$). No effect of sample location (two depth at anchorage and the reef station) or interactions of this factor with other fixed factors were observed for either T_A or DIC.

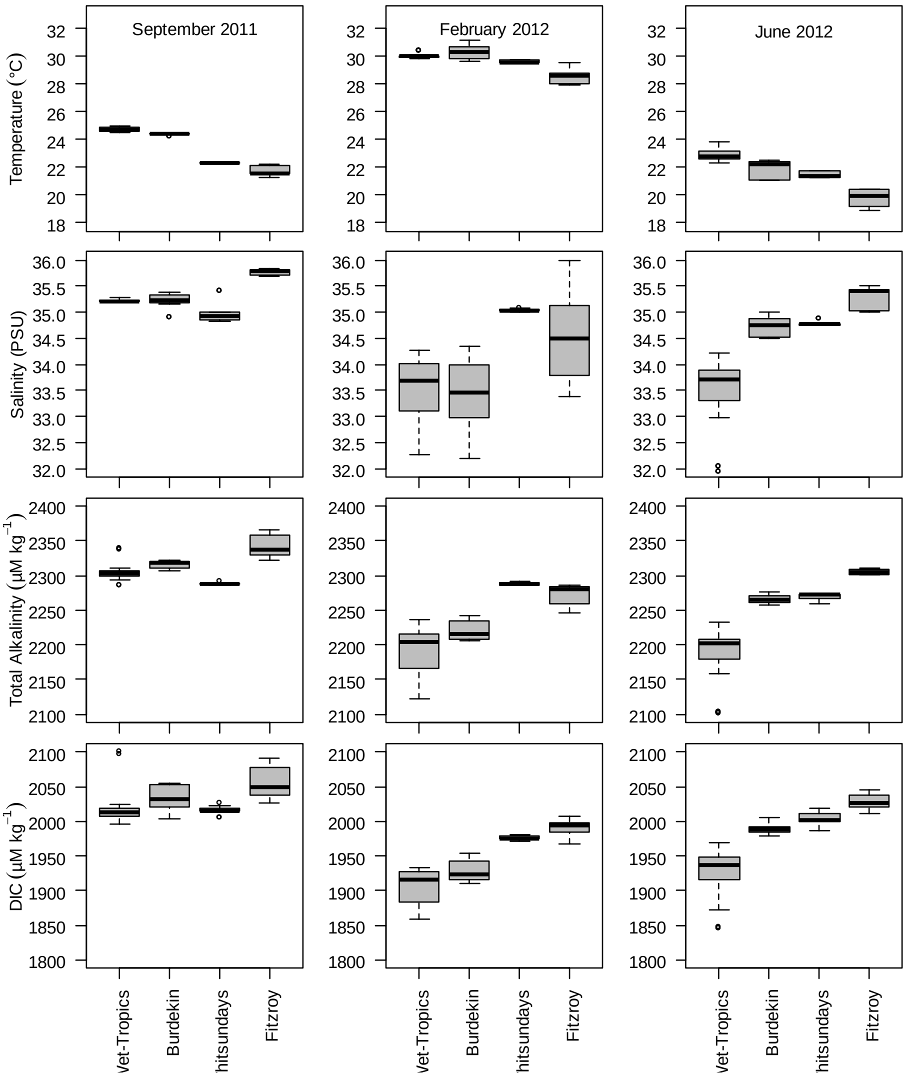

Fig. 2. Measured parameters on 14 inshore reefs during three research trips in four geographic regions along the length of the Great Barrier Reef. DIC = Dissolved Inorganic Carbon.

Sample month had a significant effect on the partial pressure of CO_2 (pCO_2), and the interaction of Trip and Region was marginally significant (Tab. 2). Variation between regions was small, and the most distinct pattern in the pCO_2 data (Fig. 3) was an elevation during the Wet-Season (total average = 460 µAtm, 1 SD = 19 µAtm), compared to June (383 µAtm, 1 SD = 21) and September (416 µAtm, 1 SD = 35 µAtm). Thus, values in the latter two months were in the range of present atmospheric values, whereas data during the wet-season were elevated.

Similar to pCO_2, the pH value varied significantly among trips and a Region x Trip interaction was significant (Tab. 2). As expected, patterns of variation were reversed compared to those of pCO_2 (Fig. 3). pH values were lowest during the wet-season

(7.97, SD = 0.01) and slightly higher in June (8.04, 1 SD = 0.02) and September (8.02, 1 SD = 0.03). The significance of the interaction term was likely caused by a slight increase in pH from north to south during dry season samples, which was not observed during the wet (Fig. 3).

Differences in aragonite saturation state were highly significant between sampling trips (Tab. 2, Fig. 3). Average values in the wet-season (3.39, 1 SD = 0.15) were higher that in June 2.99, 1 SD = 0.13) or September (3.17, 1 SD = 0.14). Thus, despite higher pCO_2 and lower pH values during the summer wet-season, aragonite saturation states were not reduced, but actually increased. This is likely to be caused by higher water temperatures in the summer wet season.

Similarly to the directly measured parameters, neither pCO_2, pH or ΩAr measurements were significantly different between sampling locations.

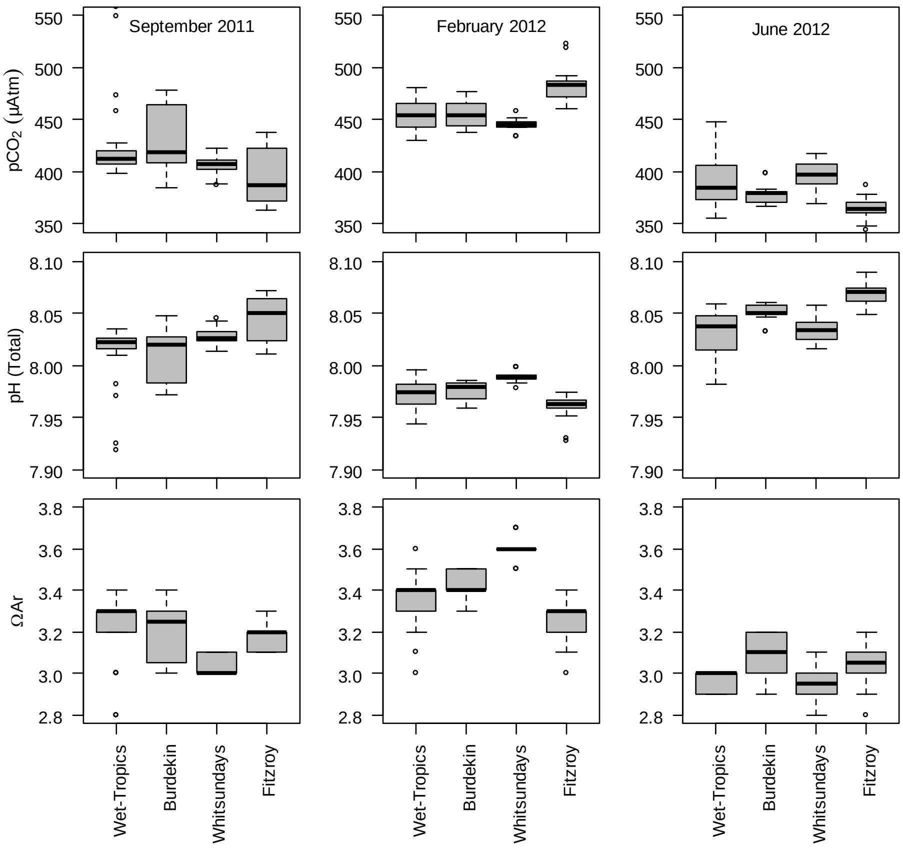

Fig. 2: Derived parameters on 14 inshore reefs during three research trips in four geographic regions along the length of the Great Barrier Reef. Ω Ar = Aragonite saturation state.

Table 2: Mixed model ANOVA for derived parameters $p\mathrm{CO}_2$, $\mathrm{pH}_{[\mathrm{Total}]}$ and aragonite saturation state (Ω Aragonite).

			$p\mathrm{CO}_2$			$\mathrm{pH}_{[\mathrm{Total}]}$			Ω Aragonite	
	DF	MS	F	p	MS	F	p	MS	F	p
A: Trip	2	8942.52	4.34	**0.0222**	7.25E-03	5.48	**0.0094**	0.288021	6.02	**0.0064**
B: Region	3	578.50	0.28	0.8390	3.06E-03	2.31	0.0961	0.04625	0.97	0.4215
AB	6	4970.90	2.41	0.0508	3.28E-03	2.48	**0.0458**	4.39E-02	0.92	0.4968
C(AB): Island	30	2062.53	46.32	**<0.0001**	1.32E-03	36.61	**<0.0001**	4.79E-02	24.92	**<0.0001**
D: Location	2	594.74	1.14	0.32971	3.75E-04	1.13	0.3343	1.22E-02	0.88	0.4239
AD	4	151.44	0.29	0.88223	7.43E-05	0.22	0.9237	3.50E-03	0.25	0.9065
BD	6	537.57	1.03	0.41950	3.39E-04	1.02	0.4273	2.12E-02	1.53	0.1951
ABD	12	628.17	1.21	0.31356	3.80E-04	1.14	0.3568	1.13E-02	0.82	0.6323
CD(AB)	39	520.92	11.7	<0.0001	3.32E-04	9.2	<0.0001	0.013854	7.21	<0.0001
S	108	44.52			3.61E-05			1.92E-03		
Total (Adjusted)	212									
Total	213									

DISCUSSION

Although some variation existed between Regions of the GBR, by far the largest amount of variation was explained by sampling season. However, whether this is a real seasonal effect needs to be established by repeated sampling in each season. Samples from a second September trip have already been collected and we suggest that the following wet season trip and the subsequent June trip will be sampled. Reasons for elevated $p\mathrm{CO}_2$ during the wet-season are not fully understood. One possible explanation is that during high runoff conditions during flooding in the wet-season reduce light availability and thus limit production (despite increased nutrient levels) to a point where heterotrophic processes in the plankton outweigh DIC uptake by phytoplankton. However, a recent plankton study showed that phytoplankton on inshore reefs of the GBR is net-autotrophic throughout the year (McKinnon et al. in review). Thus, elucidation of the reasons for higher pCO2 during the wet-season requires further investigation.

Further surprising was the finding that samples taken during the daytime directly over the coral reef benthos on inshore reefs did not vary from samples taken at the water surface or at ~ 9m depth near the ships anchorage. This casts some doubt on whether inshore reefs (or at least reef slope areas investigated) on the GBR can take up sufficient DIC during light periods to alter carbon chemistry and buffer increased DIC as was suggested for larger offshore reefs (Anthony et al. 2011; Kleypas et al. 2011;

Shaw et al. 2012). However further studies including finer scale temporal (i.e., sampling during day and night) and spatial (comparing different reef habitats) investigations are required to investigate this further.

Comparison to outer reefs

Although we did not study midshelf and outer reefs of the GBR at the same time as inshore reefs ie, there is strong evidence that inshore reefs are subjected to higher pCO_2 and somewhat reduced aragonite saturation states. Waters at Lady Elliot Island in the southern GBR usually have ΩAragonite > 3.6 (Shaw et al. 2012). The same authors showed that nighttimes values on reef flats can be far below 3, all daytime values on the reef flat were even above the value of the surrounding water, and often above 4 (NB: all values presented in this report are daytime values). Similarly, preliminary carbon chemistry analyses from samples collected in surface waters near midshelf and outer shelf reefs of the GBR showed little spatial or temporal variation (Tab. 3). pCO_2 values on these reefs were generally at or slightly below equilibrium with the atmosphere, and thus somewhat lower than on inshore reefs in summer (~20%) and the late dry season (~10%). Aragonite saturation state was in the range of 3.6-3.8, also higher than on the inshore reefs studied (~10- 20%, depending on the season).

Table 3: Carbon chemistry of water samples collected in surface waters near Midshelf and Outer shelf reefs in the Northern section and the Swains Region at three field trips (M. Furnas, unpublished data). All samples were collected in duplicate; the number of samples (N) indicates the number of reefs or locations sampled.

Time/Region	pCO_2	$pH_{[Total]}$	Ω Aragonite
November 2011 Northern Section (N=6)	389 (7)	8.04 (0.01)	3.60 (0.09)
April 2012 Swains (N = 8)	370 (6)	8.06 (0.01)	3.76 (0.06)
June 2012 Northern Section (N = 9)	372 (15)	8.06 (0.02)	3.55 (0.16)

Further investigation of the differences between inshore and offshore carbon chemistry and trophic status of the surrounding waters are crucial for our understanding of the vulnerability of inshore reefs to climate change and ocean acidification.

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