Ecological and physiological responses of hard corals to variations in seawater carbonate chemistry

E. F. Camp

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Department of Biological Sciences

University of Essex

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“People ask: Why should I care about the ocean? Because the ocean is the cornerstone of earth's life support system, it shapes climate and weather. It holds most of life on earth. 97% of earth's water is there. It's the blue heart of the planet — we should take care of our heart. It's what makes life possible for us. We still have a really good chance to make things better than they are. They won't get better unless we take the action and inspire others to do the same thing. No one is without power. Everybody has the capacity to do something.”

— Sylvia A. Earle

Summary

It is predicted that ocean acidification (OA) threatens coral reefs worldwide, by lowering seawater pH which in turn compromises essential metabolic processes such as carbonate genesis of corals. Inshore waters however, experience different spatial and temporal carbonate chemistry variability, raising questions over the future impact of OA within these habitats. It also remains unclear whether local biogeochemical conditions of some marine habitats can buffer, or provide a refuge against OA. The thesis systematically examines the response (ecological abundance, distribution, recruitment, and metabolic expenditure) of corals that have expanded their niche into variable pH habitats, to assess both the potential impact of OA and whether any habitats may act as a refuge against its effects by: (i) establishing robust methods to measure the local carbonate chemistry and the metabolic activity of corals *in situ*, (ii) characterising the natural carbonate chemistry variability over different temporal and spatial scales, and evaluating the biological versus abiotic control of non-reef habitats, (iii) quantifying the metabolic expenditure of corals living within non-reef habitats and assessing whether there are similarities in the physiological responses of corals existing in different regions to ascertain commonalities, and finally (iv) testing the impact of future predicted changes in temperature and pH on the physiological responses of corals from different variability habitats. The thesis demonstrates that across bioregion sites non-reef habitats exist that have highly variable carbonate chemistry but still house corals. These non-reef habitats have very different carbonate chemistry, influencing both their own susceptibility to future OA and their potential services (buffering versus pre-conditioning) for local coral populations. Future studies can expand on this work by assessing the molecular differences of corals found within these highly-variable habitats to explore further the potential of adaptation and/or acclimatisation of coral species to low pH.

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2013

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Abbreviations

Note: All abbreviations in equations are defined as they appear within the thesis.

AGRRA	Atlantic Gulf Rapid Reef Assessment
AIC	Akaike Information Criteria
ANOVA	Analysis of variance
ATP	Adenosine Tri-Phosphate
Ca.	Approximately
CDIAC	Carbon Dioxide Information Analysis Centre
CPCe	Coral point count with excel extensions
Cv	Coefficient of variation
DIC	Dissolved inorganic carbon
DLI	Daily light integral
G	Calcification
G_1	Skewness
G_D	Nigh-time calcification
G_{DAY}	Daily net calcification
G_L	Light enhanced calcification
H_2O	Water
HV	High-variability
LCL	Lower control limit
LME	Linear mixed effects
LV	Low-variability
LWL	Lower warning limit

NBS	National bureau of standards
NCP	Net community production
NEC	Net ecosystem calcification
NGO	Non-government organisation
OA	Ocean acidification
P	Photosynthesis
PAR	Photosynthetically active radiation
PCA	Principal component analysis
P_N	Daily net photosynthesis
PVC	Polyvinyl chloride
R	Respiration
REML	Restricted maximum likelihood
SE	Standard error
TSB	Todos os Santos Bay
UCL	Upper control limit
UNESCO	United Nations Educational, Scientific, and Cultural Organisation
UV	Ultra violet
UWL	Upper warning limit
WNP	Wakatobi National Park

Carbonate Chemistry Parameters

Ω	Saturation state
Ω_{arg}	Aragonite saturation state
CO_2	Carbon dioxide

CO_3^{2-}	Carbonate
H^+	Hydrogen proton
H_2CO_3	Carbonic acid
HCO_3^-	Bicarbonate
nA_T	Salinity normalised total alkalinity
nC_T	Salinity normalised total carbon
pCO_2	Partial pressure of carbon dioxide
pH	*potential hydrogenii*
TCO_2	Total carbon dioxide

Little Cayman Study Locations

GTB	Grape Tree Bay
MBW	Mary's Bay West
MBE	Mary's Bay East
CB	Charles Bay
SS	South Sound

Bioregion Locations

AO	Atlantic Ocean
IO	Indian Ocean
PO	Pacific Ocean

Table of Contents

List of Tables

Chapter 1| General Introduction

Chapter 2| Methodology for capturing the variance of carbonate chemistry and inherent metabolic activity of corals within reef and non-reef systems

Chapter 3| Biological versus abiotic control of coral habitats and the metabolic expenditure of corals living within them

Chapter 4| Spatial variability of carbonate chemistry and the *in situ* metabolic response of dominant coral species

Chapter 5| The impact of future predicted changes in temperature and pH on the biological performance of corals from different variability habitats

Supplementary Tables

List of Figures

Chapter 1| General Introduction

Chapter 2| Methodology for capturing the variance of carbonate chemistry and inherent metabolic activity of corals within reef and non-reef systems

Chapter 3| Biological versus abiotic control of coral habitats and the metabolic expenditure of corals living within them

Chapter 4| Spatial variability of carbonate chemistry and the *in situ* metabolic response of dominant coral species

Chapter 5| The impact of future predicted changes in temperature and pH on the biological performance of corals from different variability habitats

Chapter 1| General Introduction

1.1 | Tropical coral reefs

Coral reefs are considered a flagship ecosystem, providing life support and housing greater diversity than any other habitat on planet Earth. Coral reefs occupy less than 0.01 % of the marine environment, yield approximately 25 % of the fish catch in developing nations, and generate up to 30 % of export earnings in 100 countries that promote reef-related tourism (Burke *et al.*, 2011); together these services from coral reefs and associated connected systems (e.g. seagrass beds) have been valued between $6,075 and $22,832 US$ per hectare per year (Moberg & Folke, 1999). Reef habitats support millions of people through the economic, biological and social services they provide (Table 1.1) (Worm *et al.*, 2006; Wilkinson, 2008; Sheppard *et al.*, 2010; Barbier *et al*., 2011; Hicks, 2011).

The main skeletal structure of coral reefs is formed by scleractinian corals, an order within the phylum Cnidarian. Scleractinian corals are typically colonial and are comprised of individual polyps that secrete calcium carbonate ($CaCO_3$), which forms the basis of the reef infrastructure. The secreted $CaCO_3$ remains after a coral dies which allows reef accretion by providing a suitable framework for future growth and reef evolution (Merks *et al.*, 2004; CoRis, 2012). Scleractinian coral communities around the world's oceans are highly diverse with more than 700 species found in the Indo-Pacific, but fewer than 70 species found in the Atlantic basin (Sorokin, 1995). Corals are both auto- and hetero-trophs with many taxa reliant upon a symbiotic relationship with endosymbiotic algae (collectively termed zooxanthellae), which can transfer up to 90 % of the organic products produced by photosynthesis to the host coral (Sumich, 1996). In some instances coral species can up-regulate their heterotrophic capacity under conditions that

are not optimal for the alga symbionts (e.g. Anthony, 1999), thus, in addition to providing complex reef architecture, scleractiniain corals act as keystone primary producers at the base of reef food webs.

Corals exhibit different life histories that influence their growth rates, energy expenditure and reproductive strategies (Hughes *et al.*, 1992; Sorokin, 1995; Hall & Hughes, 1996), which in-turn can determine the survival and resilience of coral species challenged by increasing disturbances and environmental change (Garrabou & Harmelin, 2002). Massive corals tend to have a slow growth rate and expend large amounts of energy into growth and metabolic regulation (Sorokin, 1995). The low-profile of massive corals often provides greater stability to the reef infrastructure (Lirman & Fong, 1997) and consequently their presence is thought to enhance reef resilience (Kenyon *et al.,* 2006). However, ecosystems dominated by massive corals appear to support less biodiversity and productivity (Alvarez-Filip *et al.,* 2009). Other corals, such as the branching and plating corals have a faster growth rate and can repair damage quicker than the massive corals (Lirman & Fong, 1997; McClanahan *et al.*, 2002). Branching corals provide the greatest three-dimensional infrastructure and are thus considered the dominant habitat-creating corals on a healthy reef system (Bellwood *et al.,* 2004; Sheppard *et al.*, 2010).

Table 1.1| Summary of key services provided by coral reef habitats (Hoegh-Guldberg, 1999; Moberg & Folke, 1999; Barbier *et al.*, 2011)

Biological	Social	Economic
Support high-biodiversity	Heritage and culture	Support over 500 million people's livelihoods
Nutrient cycling	Recreation	Support fisheries
Biological control	Intrinsic value	Coastal protection
Water purification	Artistic inspiration	Tourism and coastal jobs
Biogeochemical services	Support of spiritual and religious values	Erosion control
Information services	Education and research	Supply many ecological goods such as: • Raw materials • Pharmaceuticals • Live fish and coral for the aquarium trade • Algae for agar • Materials for jewellery and gifts

1.2 | Coral calcium carbonate genesis

Coral reefs precipitate *ca.* half of the world's $CaCO_3$ (Smith, 1978), with rates of calcification estimated at 10 kg m^2 $year^{-1}$ (Chave *et al.*, 1975). Coral skeleton formation is thought to use carbonate ions (CO_3^{2-}) as described by equation 1, whereby CO_3^{2-} combines with calcium ions (Ca^{2+}) to form $CaCO_3$. However, bicarbonate (HCO_3^-) has also been proposed as the main source of dissolve inorganic carbon (DIC) due to the low ratio of [CO_3^{2-}]/[HCO_3^-] at the physiological pH of 7.5-9.0 (Ichikawa, 2007). In this case, calcification would occur by the reaction of $2HCO_3^-$ and Ca^{2+}producing two by-products: CO_2 and H_2O as shown in equation 2. Thus the major source of DIC for calcification remains debated. Similarly, both external DIC (Land *et al.*, 1975; Goiran *et al.*, 1996; Gattuso *et al.*, 1999) and internal metabolically-derived sources of DIC (Taylor, 1983; Furla *et al.*, 2000) have been argued as the preferred carbon source. As yet, no "one size fits all model" has been determined for the process of calcification in corals, perhaps reflecting that different taxa may ultimately have evolved different strategies to calcify.

$$Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \quad [1]$$

$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O \quad [2]$$

Within corals, the site of calcification is between the base of the calicoblastic epithelium and the skeleton surface. Seawater is broadly thought to provide the starting fluid for coral calcification, which passively diffuses to the site of calcification (Cohen *et al.*, 2001; Braun & Erez, 2004; Cohen & Holcomb, 2009), for example, seawater leakage through the tissue membranes as the calicoblastic epithelium pulsates (Tambutté *et al.*, 1996; Furla *et al.*, 2000;

Cohen & Holcomb, 2009). Coral calcification will not occur spontaneously due to kinetic barriers which include: (i) low concentrations of CO_3^{2-} (Garrels & Thompson, 1962; Lippmann, 1973), (ii) high hydration energy of Ca^{2+} (Lippmann, 1973), and (iii) high concentration of sulphate and magnesium (Kastner, 1984). Consequently, calcification occurs in compartments that can be modified and tightly regulated to the required conditions (Cohen & Holcomb, 2009; Tambutté *et al*., 2011). In 2003, Al-Horani *et al.* demonstrated a pH change at the site of calcification, which has been attributed to a plasma membrane Ca^{2+}-ATPase antiporter elevating the calcification fluid pH and saturation state (Ω). The antiporter removes two hydrogen ions (H^+) for every Ca^{2+} transported from the calicoblastic epithelial cells (Cohen & Mc Connaughey, 2003; Zoccola *et al.*, 2004; Tambutté *et al*., 2012). The described ion-transporter demonstrates the energetic cost of calcification to corals with estimates that up to 20 % of a coral's energy budget can be spent on calcification (Cohen & Holcomb, 2009).

Coral calcification does not directly require photosynthesis as corals can calcify at night (Cohen & McConnaughey, 2003). However, coral calcification appears to be elevated in the light, with average calcification rates reported to be three times greater during light-periods (Gattuso *et al*., 1999). The relationship between photosynthesis and calcification remains debated despite extensive research into this area (Allemand *et al.,* 2011). One train of thought is that photosynthesis increases the concentrations of CO_3^{2-} and the aragonite saturation state (Ω_{arg}) which may stimulate calcification (Allemand *et al*., 1998). However, it seems unlikely that the elevation of the Ω_{arg} by photosynthesis is solely responsible for light-enhanced calcification because: (i) photosynthesis lowers CO_2 levels which is self-inhibiting and consequently limits the ability to increase the Ω_{arg} (Cohen & McConnaughey, 2003), and (ii) an experimental increase in the Ω_{arg} driven by the addition of Ca^{2+} was shown to have minimal impact on

calcification rates (Gattuso *et al.,* 1998). Photosynthesis does however result in an active carbon cycle and the production of Adenosine Tri-Phosphate (ATP), which can enhance processes that require ATP, such as calcification (Al-Horani *et al*., 2003).

An alternative view is that calcification may stimulate photosynthesis by maintaining CO_2 levels via the by-product of $CaCO_3$ formation, thus ensuring that CO_2 levels are not depleted for photosynthesis (McConnaughey *et al.,* 2000). However, work by Gattuso *et al.* (2000) found that photosynthesis continued uninhibited when calcification was almost ceased. Another suggestion has been that calcification may stimulate nutrient uptake by the proton secretion of the Ca^{2+}-ATPase antiporter, aiding nutrient uptake (Cohen & McConnaughey, 2003). As summarised, review of the current literature demonstrates that the exact relationship between calcification and photosynthesis remains unclear. It seems plausible that species differences exist for the way these two processes interact, which would explain some of the differences in experimental results. The interaction of these two processes is explored further in Chapters 5 & 6.

1.3 | Ocean acidification

The ocean plays a vital role in climate regulation through its role as a 'biological pump' and through the absorption of atmospheric CO_2 (Ducklow *et al.,* 2001; IPCC, 2007). However, since the industrial revolution elevated atmospheric CO_2 has driven ocean warming and the simultaneous absorption of this CO_2 by the ocean (*ca*. 33-50 %, Sabine *et al*., 2004) is making it more acidic (ocean acidification, OA, Gattuso *et al.,* 1999; Hoegh-Guldberg, 2011). OA is the net result of lowered pH via alterations in carbonate chemistry (Gattuso & Hansson, 2011; Hoegh-Guldberg, 2011). The oceans' pH has already dropped from 8.2 to 8.1 between pre-

industrial times and may get as low as 7.8 by 2100 (Gattuso & Lavigne, 2009), with various anthropogenically-driven CO_2 emissions scenarios suggesting that by the end of the century ocean pH could be the lowest it has been in more than 50 million years (Figure 1.1, IPCC, 2015).

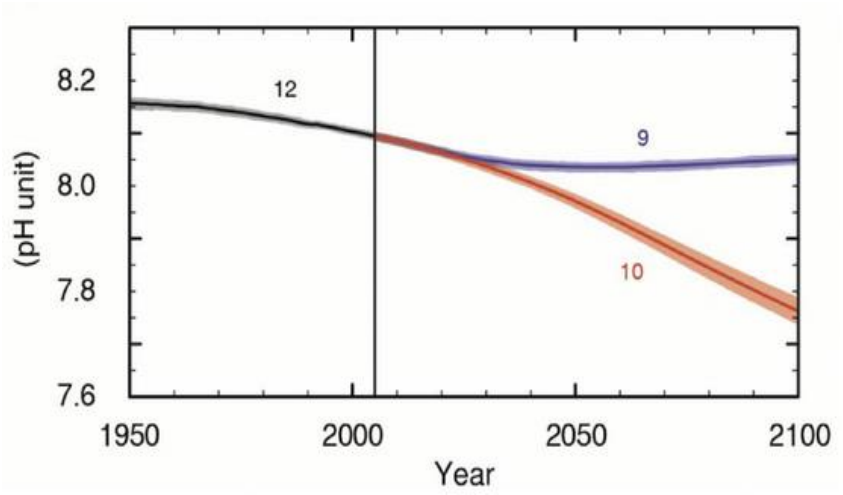


Figure 1.1| Global ocean surface pH historical record and future predicted trajectory. Taken from the Intergovernmental Panel on Climate Change (IPCC) 5th assessment report, the multi-model assessment shows time series data from 2050 to 2100 for global mean surface pH. The blue line is the RCP2.6 projection (best-case) and the red line is the RCP8.5 projection (worst-case, business-as-usual). The black line shows the historical model using reconstructed data. The shading around each line shows the levels of uncertainty. Numbers indicate the number of models that went into the projection (IPCC, 2015).

The carbonate system of seawater – The carbonate system of seawater consists of three main inorganic forms: CO_2 (aq), HCO_3^- and CO_3^{2-}. A fourth form, carbonic acid (H_2CO_3) also exists but is normally represented by CO_2 (aq) as its concentrations are minimal (~ 0.3 %) (Zeebe & Wolf-Gladrow, 2001). The carbonate system is related via a series of chemical equilibrium reactions that can shift in accordance to changes in temperature, pressure and salinity (see equations 3-5). As atmospheric levels of CO_2 increase the carbonate system of the ocean shifts to re-establishes equilibrium. Atmospheric CO_2 is absorbed by surface seawater and is in

thermodynamic equilibrium according to Henry's Law. Dissolved CO_2 forms a weak acid (H_2CO_3) which rapidly dissociates to form HCO_3^- and a proton (H^+). The HCO_3^- also dissociates to form CO_3^{2-} and another H^+; this H^+ formation lowers the pH to make the water more acidic (strictly, less alkaline for OA where even the most severe emission scenarios predict pH to remain >7.0, i.e. neutral).

$$CO_{2(aq)} + H_2O \leftrightarrow H_2CO_3 \quad [3]$$

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+ \quad [4]$$

$$HCO_3^- \leftrightarrow CO_3^{2-} + H^+ \quad [5]$$

The buffering capacity of seawater is quantified by the Revelle factor which is a measure of how the partial pressure of CO_2 in seawater changes for a given change in DIC (Sabine *et al.*, 2004). The Revelle factor means that a doubling in atmospheric CO_2 only results in a 10 % change in DIC (provided temperature and other factors remain the same). Buffering ability of seawater is due to CO_3^{2-} which reacts with CO_2 and H_2O to form $2HCO_3^-$ (see equation 6). However, despite the buffering from CO_3^{2-}, the acidity of seawater still increases slightly as some of the HCO_3^- dissociates to form $CO_3^{2-}$and H^+. Current rates of change in CO_2 (and consequently H^+ production) exceed the natural geological scales of buffering; historically (over the last 50 million years) ocean mixing over longer time scales has been able to buffer the seawater chemistry via interactions with carbonate-sediment. As the oceans absorb more CO_2 their ability to buffer changes in seawater chemistry are reduced (Zeebe & Wolf-Gladrow, 2001).

The total alkalinity (TA) of seawater also influences the buffering capacity of seawater and according to Dickson (1981) is defined as:

> *"the number of moles of hydrogen ion equivalent of excess proton acceptors with a dissociation constant $K \leq 10^{-4.5}$ over proton donors (acids $K > 10^{-4.5}$) in 1kg of sample*" (Dickson, 1981).

TA is a conservative property and relational to charge balance in seawater. In seawater, the charge balance of conservative species is not equal with slightly more cations than anions (~ 2.2 mmol kg^{-1}). Such an imbalance is equal to the TA and is compensated for by the anions of H_2CO_3. Thus, TA can be described as the difference between the sum of conservative cations minus the sum of conservative anions in seawater (see equation 7). Both TA and DIC are important to ocean chemistry and are influenced by biogeochemical processes such as precipitation and dissolution of $CaCO_3$.

$$CO_2 + CO_3^{2-} + H_2O \rightarrow 2HCO_3^- \quad [6]$$

$$\sum conservative\ cations - \sum conservative\ anions = Total\ Alkalinity \quad [7]$$

The saturation states of calcium carbonate – The saturation state (Ω) of carbonate minerals calcite and aragonite are influenced by changes in the oceans' acidity. Ω of $CaCO_3$ in seawater is dependent on the concentrations of Ca^{2+} and CO_3^{2-} and their solubility products (see equation 8). If Ω is > a value of 1.0 then the water is supersaturated with regards to aragonite, and if < 1.0 then seawater is under-saturated. Importantly, Ω for the different mineral forms of $CaCO_3$ are not the same as aragonite is more soluble than calcite due to its orthorhombic structure (Figure 1.2) (Zeebe & Wolf-Gladrow, 2001). Consequently some marine organisms, e.g. corals (aragonite skeleton), are at a greater risk from OA than others, e.g. soft corals (calcite skeleton). There are differences in Ω of $CaCO_3$ between the North Pacific and North Atlantic

Oceans; the North Pacific has a lower Ω because the water is 'older', meaning that it has been longer since it was last in contact with the atmosphere so it has taken up more CO_2 from remineralisation, resulting in lower CO_3^{2-} concentrations (Zeebe & Wolf-Gladrow, 2001). As the oceans become more acidic, there will be less CO_3^{2-} resulting in lower Ω of $CaCO_3$. The reduced Ω is extremely problematic for marine calcifers as seawater will start to become under-saturated at shallower depths, making calcification a more costly process (Gattuso *et al*., 1999; Gattuso & Hansson, 2011; Ries, 2011a).

$$\Omega = \frac{[Ca^{2+}]_{sw} \times [CO_3^{2-}]_{sw}}{Ksp} \quad [8]$$

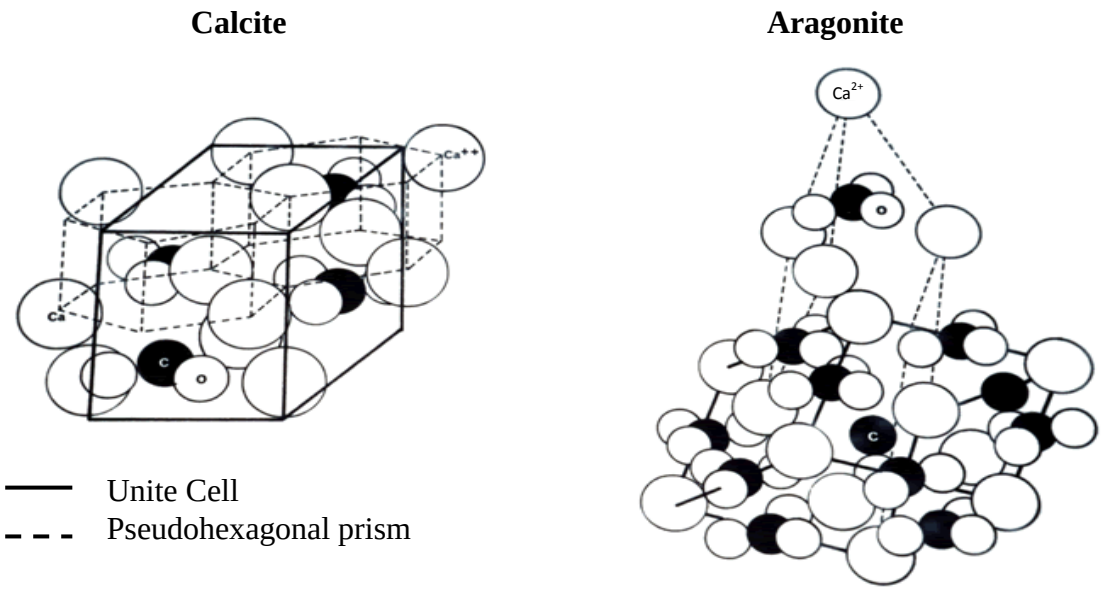


Figure 1.2| The different structures of the calcium carbonate minerals calcite and aragonite (Pengelly, 2012). Aragonite is the more soluble form of calcium carbonate due to its orthorhombic structure and is the form typically used by hard corals. The lines indicate the bonding structure.

Threats of ocean acidification on reef processes – The range of threats of OA on marine life and reef processes are still being uncovered. A decrease in pH of 0.4 units (expected by 2100) will result in an 100-150 % increase in H^+ concentrations (Orr *et al.,* 2005), a 30-50 % decrease in CO_3^{2-} (Sabine *et al.,* 2004) and a resulting decrease in the Ω of $CaCO_3$ (Figure 1.3). All of these chemical changes threaten to disrupt marine systems and their functions (Gattuso *et al.,* 1999; Zeebe & Wolf-Gladrow, 2001; Gattuso & Hansson, 2011; Hoegh-Guldberg, 2011). Some of the major threats identified include: (i) interference with extracellular and intercellular pH of organisms (Crawley *et al.,* 2010; Gattuso & Hansson, 2011; McCulloch *et al.,* 2012), (ii) reduced buffering capacity of the ocean and thus increased sensitivity to environmental change (Egleston *et al.,* 2010), (iii) disrupted and reduced rates of calcification which can alter species fitness and potentially create a phase-shift from a $CaCO_3$ dominated reef system to an organic algal dominated system (Fabry *et al.,* 2008; Hall-Spencer *et al.,* 2008; Kuffner *et al.,* 2008), (iv) altered biological processes like photosynthesis that consume inorganic carbon (Gattuso *et al.,* 1999; Gattuso & Hansson, 2011), (v) potential metabolic suppression (Portner & Reipschlager, 1996; Guppy & Withers, 1999), (vi) disruption to larval stages (Munday *et al.,* 2009), (vii) a shift in species distribution, (viii) disruption of trophic levels, and (ix) an altering of $CaCO_3$ and organic matter cycling (Fabry *et al.,* 2008). Collectively these impacts threaten marine food webs, ecosystem services and biodiversity; negative effects are predicted on the survival, growth, calcification and reproduction of many marine organisms by the end of the century (Kroeker *et al.,* 2010).

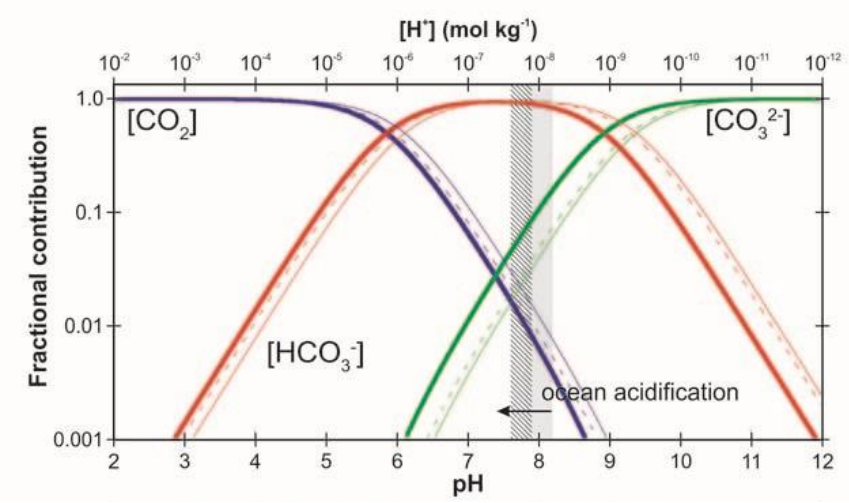


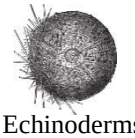
Figure 1.3| The Bjerrum Plot. Illustration of the carbonate species of seawater and their equilibrium relationships (Nature Education, 2012). Changes in the relative proportions of carbon dioxide (CO_2), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) influence seawater pH and the buffering ability of seawater.

The impact of ocean acidification on scleractinian corals – Global CO_2 emissions are tracking above worst-case scenarios from the 5th Intergovernmental Panel on Climate Change (IPCC) report, with dire consequences predicted for coral reef ecosystems (van Hooidonk *et al.*, 2014). Corals with their $CaCO_3$ skeleton are at risk as Ω decrease (Anthony *et al.*, 2011a; Pandolfi *et al.*, 2011). As the oceans become more acidic rates of dissolution will increase and the availability of CO_3^{2-} will fall making it harder to form $CaCO_3$ (Rodolfo-Metalpa *et al.*, 2011; McCulloch *et al.*, 2012). Coral reefs produce the more soluble form of $CaCO_3$, aragonite during bio-mineralisation (Cohen & Holcomb, 2009) and thus are more susceptible to dissolution. Notably dissolution of $CaCO_3$ is increasing by 0.003 to 1.2 μmol kg^{-1} $year^{-1}$ near the Ω_{arg} horizon (Feely *et al.*, 2004). When $[CO_3^{2-}]$ reach 200 μmol kg^{-1} (water) or less, the net accretion of coral reefs nears zero. This ion concentration is approached as atmospheric levels of CO_2 reach 450

ppm, a level predicted to be reached before 2100 (IPCC, 2007; Gattuso & Hansson, 2011; Hoegh-Guldberg, 2011).

As pH and Ω fall, many reef species and communities are predicted to experience a net decrease in calcification (Langdon *et al.,* 2000; Andersson *et al.,* 2009; De'ath *et al.,* 2009; Silverman *et al.,* 2009; Price *et al.,* 2012). Controlled laboratory studies have documented a reduction in calcification rates of a wide range of marine organisms, including corals, under future predictions for OA (see Table 1.2, Gattuso *et al.,* 1998; Marubini & Thanke 1999; Ohde & vanWoesik, 1999; Langdon *et al.,* 2000; Leclercq *et al.,* 2000; Leclercq *et al.,* 2002; Gazeau *et al.,* 2007; Fabry *et al.,* 2008; Ries *et al.,* 2010; Porzio *et al.,* 2011; Ries, 2011a; Kroeker *et al.,* 2013; Crook *et al*., 2013). Laboratory studies have documented decreases in coral calcification ranging from 3 % to 79 % (Gattuso *et al.,* 1998; Marubini and Thanke, 1999; Ohde and vanWoesik, 1999; Leclercq *et al.,* 2000; Leclercq *et al.,* 2002; Crook *et al*., 2013), and have found juvenile recruitment and post-settlement particularly threatened to decreasing Ω (Albright *et al.,* 2008; Albright *et al.,* 2010; Albright & Langdon, 2011; de Putron *et al.,* 2011). Other studies however, have revealed species-specific responses to OA that include negative, neutral and positive responses to increasing levels of CO_2 (Reynaud *et al.,* 2003; Jury *et al.,* 2010; Ries, 2011b; Kroeker *et al.,* 2013). Examples of physiological mechanisms that can influence coral species abilities to tolerate changes in seawater chemistry include: (i) the control of carbonate chemistry at the site of calcification (Ries, 2011b; McCulloch *et al.,* 2012) through the ability of coral species to modify H^+ concentrations within the calicoblastic fluid (Jokiel *et al.,* 2013), and/or (ii) their ability to utilise HCO_3^- within calcification (Comeau *et al.,* 2012).

Table 1.2 | A summary of the response of major taxa to predicted ocean acidification conditions

Taxa	Response					References
	Calcification	Growth	Photosynthesis	Development	Abundance	
 Coral	- 32 %				- 47 %	Kroeker *et al*., 2013
 Seagrass			+ 85 % + 260 %			Durako, 1993 Zimmerman *et al*., 1997
 Flesy algae		+ 17 %				Kroeker *et al*., 2013
 Molluscs	- 40 %	- 17 %		- 25 %		Kroeker *et al*., 2013
 Echinoderms		- 10 %		- 11 %		Kroeker *et al*., 2013
 Coccolithophores	- 23 %					Kroeker *et al*., 2013
 Diatoms		+ 17 %	+ 28 %			Kroeker *et al*., 2013
 Fish		+ 15 % + 18 %			- 50 % - 90%	Munday *et al*., 2009 Munday *et al*., 2010
 Crustaceans		- % - %				Kurihara *et al*., 2008 Wickins, 1984

For the major taxa known to currently be affected by ocean acidification, a summary of their response to a pH change of up to 0.5 pH units is shown. For all taxa other than Fish, Crustaceans and Seagrass the values provided come from Kroeker *et al*., 2013 who conducted a meta-analysis on 155 studies. The values provided are the mean affects measured. Fish, Crustaceans and Seagrass were excluded from the Kroeker *et al.*, 2013 summary due to confidence intervals overlapping at the 95 % level, or due to data deficiency. Relevant data for these taxa are shown to illustrate what is currently known. For Crustaceans due to data deficiency only general trends were reported.

Investigating the impacts of OA on taxa is complex due to the number of abiotic factors that can interact to influence a species response to changes in pH. Also, changes in other abiotic parameters, e.g. temperature, occurring alongside pH can influence a species response. For example, a moderate rise in temperature is associated with increased metabolic rates in corals that potentially enhances growth and off-sets some of the negative effects associated with low pH (Lough & Barnes, 1999; Reynaud-Vaganay *et al.* 1999; Bessat & Buigues 2001; McNeil *et al.* 2004), while a larger rise in temperature can push corals to their physiological limits resulting in catastrophic mortality (Hoegh Guldberg *et al.* 2007; Hoegh-Guldberg & Bruno 2010). The interactive effects of pH and temperature are explored further in Chapter 5. Light has also been shown to enhance calcification (Suggett *et al*., 2013) as has the addition of nutrients (Langdon and Atkinson, 2005) or the upregulation of heterotrophy (Cohen and Holcomb, 2009). Conversely, threats from OA can be exasperated by other global (e.g. deoxygenation and increased UVB damage) or local stressors (e.g. over-fishing, pollution) (IGBP- IOC- SCOR, 2013).

The interactive effect of biological and abiotic factors provides a challenge to researchers studying the impacts of OA on marine life. A criticism of almost all OA experimental studies is natural timescales with which OA as a stressor operates. Consequently, to overcome this, researchers have moved towards working in naturally acidified systems, e.g. CO_2 vents (Fabricius *et al.*, 2011). However, this has provided additional complexities in: (i) being able to deconvolve out the role of other stressors and/or, (ii) confidently dialling in "realistic scenarios" (e.g. abiotic interactions, specifically OA and elevated temp). A combination of both laboratory and field studies are thus required to try and resolve these complexities and has been the approach adopted within this thesis (see Chapter's 2 through 5).

1.4| Approaches to study the threats of ocean acidification

Coral reef climate research has to-date disproportionately focused on species-specific responses under controlled laboratory conditions (Wernberg *et al.,* 2012). Whilst this research has provided valuable insight into the capacity of individual taxa to tolerate stress, it largely fails to account for the complex interactions that exist between all biological components of the system. Therefore, in an attempt to more confidently predict the future of reef community structure and functioning research approaches have diversified to overcome such limitations through increased emphasis on ecosystem level studies (e.g. Kleypas *et al.*, 2011; Anthony *et al.,* 2013), *in situ* experimentation (e.g. Klein *et al.,* 2012; Okazaki *et al*., 2013), experimentation involving multiple climatic stressors (e.g. Anthony *et al*., 2011a; Dove *et al.,* 2013), experimentation across natural climate gradients (Dunne *et al*., 2004), as well as opportunistic experiments (e.g. temperature induced gradients from thermal outfall of a power station: Schiel *et al*., 2004). The international research community has also attempted to optimise and standardise sampling practices to minimise sampling error and increase confidence in the results obtained. Similarly, efforts have been directed into establishing new methodologies to limit destruction and stress on test colonies. These topics are expanded upon in Chapter 2.

Complementary to these various approaches has been the growing popularity of examining the nature and extent with which corals persist within environments that are considered extreme and towards their physiological limits for growth and survival (e.g. Fabricius *et al.,* 2011; Price *et al.,* 2012; Hume *et al.,* 2015); specifically, broad scale latitudinal limits of coral growth (e.g. elevated temperature, Rodolfo-Metalpa *et al.,* 2014), reef habitats that are considered atypical (e.g. CO_2 vents, Fabricius *et al*., 2011) or typical (reef-flat, Price *et al.,* 2012; Andersson *et al.,* 2013) and non-reef habitats such as mangroves (Yates *et al.,* 2014) and

seagrasses (Manzello *et al*., 2012). Recent interest in coral populations within mangroves and seagrasses is particularly intriguing since these habitats typically experience large diel variability in temperature and light conditions that included periods that would over longer-durations lead to bleaching-induced mortality within a classical reef setting; they also routinely experience pH conditions (daily average and/or variance) expected for many reefs under future OA scenarios (Price *et al.,* 2012; Guadayol *et al*., 2014; Yates *et al.,* 2014). Previous studies investigating environmental extremes have focused on the presence or absence of coral species in relation to their local carbonate chemistry conditions, however, the function and viability for coral survival remains largely unknown and is explored in Chapter's 3 and 4 of this thesis.

1.5| Coral habitats with large natural pH variability

Non-reef habitats that house corals, such as seagrass beds and mangroves, are part of the main reef complex and are typical habitats found globally, however, they experience very different abiotic conditions to the main reef. Both seagrass and mangrove habitats are important primary producers and nursery habitats for fish and crustacean species (Dawes, 1998; Mumby *et al*,. 2003; Harborne *et al*., 2006) as they provide shelter from predation (Nakamura & Sano, 2004) and an abundance of food. They are also often situated in locations that receive high larval supply (Parrish, 1989). Both systems also provide important coastal protection. In addition, seagrass beds provide support in biogeochemical cycling and substrate stabilisation (Dawes, 1998; Duarte, 2000; Abecasis et *al.*, 2009). Despite the range of important ecological services provided by seagrass beds and mangroves they are among the world's most threatened habitats (Valiela *et al.,* 2001; Short *et al*., 2011). Mangrove habitats are being lost at a rate of 1.8 % $year^{-1}$ (Valiela *et al.,* 2001) whilst seagrass habitats are being lost at an estimated rate of 1.5 % $year^{-1}$

(Waycott *et al.,* 2009). Persistence of corals within non-reef habitats such as mangroves and seagrass beds is receiving renewed attention (Manzello *et al.,* 2012; Hendriks *et al.,* 2014; Yates *et al*., 2014) and is particularly intriguing for two reasons:

Firstly, the capacity of corals to grow under highly-variable sub-optimal growth conditions (Price *et al.,* 2012; Yates *et al*., 2014) demonstrates their ability to adapt or acclimatise, and potentially tolerate wider environmental conditions. Several studies support the notion that highly-variable environments are important to enhancing coral tolerance against climate change (e.g. temperature: Baker *et al*., 2004; pH: Comeau *et al*., 2014). However, other studies have demonstrated no improved tolerance to stress despite prior regular exposure to environmental extremes (e.g. temperature: Rodolfo-Metalpa *et al*., 2014; pH: Crook *et al.,* 2013; Okazaki *et al.,* 2013). As such, it remains unclear to what extent corals currently persisting in highly-variable environments will actually provide added tolerance to future stressors.

Secondly, the ability for certain non-reef habitats to locally buffer or off-set the negative impacts of OA. Inherent biogeophysical processes of seagrass habitats have been proposed to significantly alter the intrinsic carbonate chemistry so as to buffer coral populations by off-setting future decreases in seawater pH (Hoegh-Guldberg *et al.,* 2007; Manzello *et al.,* 2010; Anthony *et al*., 2013), and thus effectively operate as a refuge. Throughout the thesis refugia will refer to the ability of a habitat to maintain favourable chemical conditions that are being lost elsewhere (*sensu* Keppel & Wardell-Johnson, 2012). Mangroves have similarly been proposed as potential coral 'refugia' against climate change (Yates *et al.,* 2014) but whether they could provide the same protective role (i.e. buffering) as determined for seagrass beds remains unclear.

Whilst corals clearly demonstrate some form of tolerance to survive within highly variable habitats (Price *et al.,* 2012; Yates *et al.,* 2014) the physiological properties that govern tolerance remain unknown. Similarly, it is unknown whether species are selected for within these systems and thus are adapted, or are they just part of the wider species pool and have acclimatised (a species has adjusted to its local environmental conditions, *sensu* Folk 1966). Whether corals can persist in non-reef habitats, across bioregions and independent of taxa is also unclear and will be studied in this thesis across four bioregion sites (see Chapter 4).

1.6| Study locations

Within this thesis four study locations were selected each contributing differently to the overall project (Table 1.3). Methods development both in terms of analysis techniques, sampling strategies and respirometry were carried out in the Atlantic (Cayman Islands and Brazil) whilst biological assessments were studied at three sites (Cayman Islands, the Seychelles and Hoga/Kaludepa Islands) within the three ocean basins (Atlantic, Pacific and Indian). The three sites were selected to assess whether similar coral species occupied the marginal non-reef habitats across ocean basins, and whether the species found in non-reef systems was proportional to the diversity of the region. For example, in the Atlantic region site, are the species present in non-reef habitats restricted due to the species pool.

Table 1.3| Study site information

Bioregion	Location	Site	GPS N/S	GPS E/W
Atlantic Ocean	Little Cayman, Cayman Islands	Back-reef 1	19°41.767	80°06.066
		Back-reef 2	19°42.479	80°00.161
		Back-reef 3	19°42.503	79°.98.256
		Seagrass 1	19°41.810	80°03.775
		Seagrass 2	19°42.470	79°98.250
		Seagrass 3	19°42.489	79°98.231
		Outer-reef 1	19°41.815	80°04.122
		Outer-reef 2	19°42.627	80°09.241
		Outer-reef 3	19°42.623	79°98.344
Atlantic Ocean	Salvador, Brazil	Patch-reef	12°59.570	38°31.500
Indian Ocean	Curieuse, Seychelles	Mangrove	04°17.290	55°43.898
		Seagrass	04°17.059	55°44.059
		Outer-reef	04°17.081	55°44.219
Pacific Ocean	Hoga, Indonesia	Mangrove	05°28.427	123°43.645
		Seagrass	05°28.384	123°43.746
		Outer-reef	05°28.382	123°43.738

The study sites for each location indicated by their general habitat type and their GPS location.

1.6.1| Atlantic Ocean: Little Cayman, Cayman Islands, British West Indies

Little Cayman geography and oceanography – The Cayman Islands are made up of three low-lying subtropical islands located in the northwest Caribbean Sea, approximately 145 km south of Cuba. Little Cayman is located 120 km northeast of Grand Cayman, and 10 km southwest of Cayman Brac (Manfrino *et al.,* 2013). Little Cayman is the smallest island (17 x 2 km) and has a low resident population (< 200) meaning it is subject to minimal local anthropogenic stress (Turner *et al.,* 2013). The Cayman Islands experience complex currents that can vary in duration, velocity and intensity across small spatial scales (Turner *et al.,* 2013). The location of the Cayman Islands within the Caribbean Sea means that they are subject to a spin-off of the north Atlantic Gyre (Kinder, 1983). Typically currents move in a north-westerly direction (Stoddard,

1980; Turner *et al.*, 2013), however, unpublished data from the Department of the Environment for the Cayman Islands shows that the local currents around Little Cayman head southeast and then loop back towards Little Cayman before they reach Jamaica. The currents then move west towards Grand Cayman (Turner *et al.,* 2013).

Little Cayman climate – The Cayman Islands have a tropical marine climate governed by two distinct seasons: the wet-summer season (May-November, average rainfall *ca.* 7.5 in per month), and the dry-winter season (April-December, average rainfall *ca.* 2.0 in per month) (Turner *et al.*, 2013). Average air temperature is 24.8 °C in February and 28.4 °C in July, with northeast trade winds predominant most of the year. The Cayman Islands are vulnerable to hurricanes between August and early November, with the islands typically receiving a direct hit every ten years. The reefs of the Cayman Islands were impacted by hurricane Ivan in 2004, which severely damaged soft coral communities. Hurricane Gilbert in 1989 decimated *Acropora* colonies (Turner *et al.*, 2013).

Little Cayman marine habitats **–** The dominant coastal boundaries of Little Cayman are lagoons, seagrass beds, coral reefs, iron-shore and mangroves. The lagoons are shallow saltwater systems locally known as "Sounds". Sounds typically have a back-reef habitat with marginal coral reef growth compared to the deeper spur-and-groove and reef flats. On the landward side of the back-reefs the substrate is a mix of small coral colonies, hard-ground, seagrass, sand, sediment, and algae (calcareous, macroalgae and filamentous) (Turner *et al.,* 2013). Seagrass beds are dominant around the lagoons of Little Cayman covering 4.5 km^2 (Turner *et al.,* 2013). Ironshore (white limestone with a hard calcrete crust) is another common habitat, with coral reefs constituting the last major substrate type surrounding the coastal marine habitats of Little Cayman.

Little Cayman coral reefs – The Cayman Islands are the peaks of underwater mountains and consequently have a very narrow coastal shelf (< 1 km) where reef development occurs. At the edge of the coastal shelf a deep wall system exists that drops off to depths of greater than 2000 m. Little Cayman has a northeast to southwest orientation which results in a high-energy and moderate-energy zone. The high-energy zones impact spur-and-groove reef formation with the south and east coasts developing the greatest spur-and-groove reef formations (Mc Coy *et al.,* 2010; Dromard *et al.,* 2011).The north shelf of Little Cayman is comprised of two reef terraces: a shallow reef terrace (5-12 m), consisting of a lagoon and fringing reef, and a deeper reef terrace (12-25 m) which drops off into a vertical abyss. An exception to this general geomorphology is the northwest section of the Bloody Bay-Jackson Point Marine Park, where the shallow terrace extends to the vertical drop off into the abyss, and the deeper terrace is lacking (Fenner, 1993).

Little Cayman has documented live coral cover higher than, or equivalent to, other islands in the Caribbean (Figure 1.4, Gardner *et al.,* 2003). Between 1999 and 2004, Little Cayman experienced a decrease in live coral cover from 26 % to 14 % (Coelho & Manfrino, 2007), primarily due to white plague syndrome which occurred after the 1998 bleaching event (Eakin *et al.,* 2010; van Hooidonk *et al.,* 2012*).* However in 2013, coral cover around Little Cayman was reported to be on a positive trajectory, with coral cover returning to levels seen in 1999 (Manfrino *et al.,* 2013).

Little Cayman marine protected areas – Since 1986, approximately 50 % of Little Cayman's near-shore habitats have been managed as Marine Protected Areas and no-take Replenishment Zones (Dromard *et al.,* 2011). Bloody Bay Marine Park on the north coast of Little Cayman is one of the main diving attractions of the Cayman Islands. On Little Cayman, there are currently four environmental designations: Marine Park Zones, Designated Grouper

Spawning Areas, Replenishment Zone and Animal Sanctuaries/RAMSAR sites. There are four additional designations found elsewhere in the Cayman Islands: No Diving Zone, Environmental Zone, Wildlife Interaction Zone, and Prohibited Diving Zone (see Table 1.4).

Figure 1.4| A photograph of the coral reef on the deeper terrace of Little Cayman, Cayman Islands, BWI. The picture was taken in 2012 on the dive site known locally as Mixing Bowl within the Bloody Bay Marine Park.

Table 1.4| The environmental designations on Little Cayman. The Cayman Islands have had Marine Protected Areas in place since 1986 (DOE, 2015). On Little Cayman there are four environmental designations whose rules are detailed below:

Environmental designation	Rules
Marine Park Zone	• No taking of any marine life alive or dead, except: line fishing from shore is permitted; line fishing at depths of 80 ft or greater is permitted; taking fry and sprat with a fry or cast net is permitted. • Fish traps, spear guns, pole spears and other nets are totally prohibited. • No anchoring – use of fixed mooring only, except; boats of 60 ft or less may anchor in sand as long as no grappling hook is used, and neither the anchor of rope will impact coral; anchoring prohibitions are suspended during emergencies and by permission of the Port Director. • No commercial operations may use Bloody Bay Marine Park without a license from the Marine Conservation board.
Designated Grouper Spawning Areas	• East and west End of Little Cayman; no fishing for Nassau groupers 1st November through 31st March; No fish pots or spear fishing within one-mile radius of Designated Grouper Spawning Area during this period.
Replenishment Zone	• No taking of conch or lobster by any means; no anchors, lines or chains may touch coral; no spear guns, pole spears, fish traps or nets allowed.
Animal Sanctuaries/RAMSAR Sites	• No hunting; no littering; no collection of any species.

1.6.2| Atlantic Ocean: Salvador, Brazil

Salvador geography and oceanography – The city of Salvador is located on the east coast of Brazil within the Bahia region. Salvador has a large population estimated at three million people making it the third most populated city in Brazil (Lessa *et al.,* 2001). Located in the southern Atlantic, Salvador is situated at the entrance of Todos os Santos Bay (TSB). TSB is the second largest bay ecosystem of Brazil (Cirano & Lessa, 2007). There is low annual variation in currents, with tides primarily responsible for annual variations (Cirano & Lessa, 2007). The tides are semidiurnal with a tidal range of 0.1 to 2.6 m (Cirano & Lessa, 2007). Tides are amplified by a factor of 1.5 in the bay and are bi-directional. The tides are strongest during the ebbing tide (Lessa *et al.,* 2001).

Salvador climate – Salvador experiences a tropical humid climate with mean annual water temperatures of 25.2 ± 3.0 °C and an annual mean precipitation of approximately 2100 mm yr^{-1} (Cirano & Lessa, 2007). Salvador is subject to high-pressure weather cells due to its location within the south Atlantic trade wind belt (Bittencourt *et al.,* 2000). The Atlantic Polar Front occurs during the autumn and winter, increasing wind speed whilst changing the wind and wave direction (Bittencourt *et al.,* 2000).

Salvador coral reefs – Along the eastern coasts of Brazil and the small offshore islands, there are shallow fringing reefs that have low hard coral diversity (*ca.* 21 species) and are characterised by high endemism (38 %) (Leão *et al*., 2003). The basal structure of these patch reefs originates from the pre-Cambrian basement that outcrops along the Salvador Fault (Dutra, *et al.,* 2006). The TSB offers tropical shallow waters that house patch reefs *ca.* 1-4 m high (Leão *et al.,* 2003). These patch reefs are subject to a lot of direct human pressure from the city.

Salvador marine protected areas – There is an Área de Proteção Ambiental for the TSB which was designated in 1999. It falls under the class of a sustainable-use area under Brazilian law, meaning that activities are restricted rather than any significant levels of marine protection. However, local police monitor research activities.

Figure 1.5| A photograph of the fringing coral reef of Salvador, Brazil. The picture was taken in 2014 at a site in Todos os Santos Bay adjacent to the Yacht Clube da Bahia harbour.

1.6.3| Indian Ocean: Curieuse, Seychelles

Curieuse geography and oceanography – The Seychelles Archipelago on the northern edge of the Mascarene Plateau consists of 115 islands located 1600 km east of Africa within the Indian Ocean. The central islands are composed of granite, whilst the outlying islands are coral atolls (Stoddart, 1984). Curieuse is the fifth largest granitic island within the Seychelles Archipelago and has an area of 2.86 km^2 (Hill *et al.*, 2002). Curieuse consists of two large peaks (the highest 172 m above sea level) which enclose a shallow bay (Baie La Raie). The Seychelles are subject

to seasonal currents, with north-westerly currents approaching from the Somali Basin dominating between December and March; May through September the wind shifts south-easterly changing the direction of the currents and exposing sites previously sheltered.

Curieuse climate – The Seychelles Islands experience a seasonal humid tropical climate (Walsh, 1984), with temperatures typically ranging between 24-32 °C. Wind speed is normally 15-22 km h^{-1}. Most of the annual rainfall occurs during the months of December and February. April marks the end of the 'rainy season' and is the month of monsoon reversal; the shift from northwest Monsoon to the onset of the southeast Trade Winds. From May to October, the climate is drier with cooler weather and often rougher seas resulting from elevated wind speeds of 19–37 km h^{-1} (SNPA, 2014).

Curieuse marine habitats – Curieuse is surrounded by patchy fringing reefs that have submerged granitic boulders where corals directly grow and provide a suitable substrate for recruitment. Surrounding the island are intertidal seagrass and dense algal beds comprised of calcifying and non-calcifying algae as well as small coral colonies. Baie La Raie is a bay located on the south side of the island. In 1909, a seawall was built across Baie La Raie effectively separating the mangroves from the rest of the bay. The 40 acre pond now has some connectivity with the surrounding waters as the 2004 Tsunami broke down part of the wall. Baie La Raie contains six of the seven mangrove species found in the Seychelles and is an important fish nursery habitat for the surrounding reefs (Domingue *et al.*, 2010).

Curieuse coral reefs – The Seychelles have an estimated 1690 km of coral reefs, comprised of fringing reefs, platform reefs and atolls (Spalding *et al.*, 2001). The coral reefs of the Seychelles have species richness values between 47-59 % of the total species found in the western Indian Ocean (Figure 1.6, Obura, 2012). The Seychelles has granitic and carbonate reef

systems, both of which can be located around Curieuse. Historical monitoring data shows large variability in coral cover (1-40 %) for the shallow reef systems (Smith/Suggett, Unpublished data, pers. Comm.). Over the last two to three decades, an increase in development around the Seychelles has significantly altered parts of the coastline. The severe El Niño event of 1997/98 resulted in unprecedented coral bleaching in the Indian Ocean, which severely impacted the reefs of the Seychelles with certain reefs experiencing 95 % bleaching (Dominigu *et al.,* 2010). Graham *et al.* (2014) documented 12 out of 21 reefs around the Seychelles returning to pre-disturbance coral cover after a loss of greater than 90 % of coral cover. The remaining nine reefs underwent a phase-shift to a macroalgae reef which was attributed to other local stressors and reef conditions (e.g. structural complexity and water depth).

Curieuse marine protected areas – Curieuse and its surrounding waters make up one of the six National Marine Parks (NMP) of the Seychelles. A NMP is designated in the Seychelles as:

> *"An area set aside for the propagation, protection and preservation for wildlife or the preservation of places or objects of aesthetic, geological, prehistoric, historical, archaeological or other scientific interest for the benefit, advantage and enjoyment of the general public and includes in the case of a Marine National Park an area of shore, sea or sea-bed together with coral reef and other marine features"* (pg 30, Dominigue *et al*., 2010).

The Curieuse National Marine Park consists of 2.86 km^2 land and 13.70 km^2 sea (total 16.56 km^2) and stretches 200 m offshore around the island and all the way across to the westerly neighbouring island of Praslin. The Curieuse NMP was designated in 1979 and attracts many tourists annually (>21,000 tourists in 2007). Designation of this NMP has helped with socio-

economic development of the surrounding islands, with the local economy heavily reliant on the fishing industry and tourism (Campling & Rosalie, 2006; Dominigue *et al*., 2010).

Figure 1.6| A photograph of the *Acropora* beds found around Curieuse, the Seychelles. The picture was taken in 2014 on a site known locally as House Reef off the south coast of Curieuse within the Seychelles National Marine Park.

1.6.4| Pacific Ocean: Hoga and Kaledupa Islands, Wakatobi, southeast Sulawesi, Indonesia

Hoga and Kaledupa Islands geography and oceanography – Hoga and Kaledupa Islands are located in the Wakatobi, southeast Sulawesi. The Wakatobi was established as its own separate district in 2004 and consists of four main islands: Wangi, Kaledupa, Tomia and Binongko (the acronym "Wakatobi" formed by the first two letters of each island). Several smaller islands are located in the Wakatobi, including Hoga found to the north of Kaledupa. The Wakatobi is central within the Coral Triangle; a global centre of marine biodiversity and a priority for marine conservation (Tomascik *et al.*, 1997; Pet-Soede & Erdman, 2003; TNC, 2007). The Wakatobi region is subject to complex, highly variable currents (Clifton, 2010). During the north-westerly monsoon season (typically November to April) the currents run anti-clockwise around Sulawesi, whilst from May to November no clear pattern is apparent. On the Sulawesi side of the Makassar Straits the currents run southwards year-round. Along the northern Sulawesi coast there is a year-long eastward current (Whitten, 1987). Locally, the reefs are subject to a large semi-diurnal tidal cycle with the inter-tidal zone being largely exposed at low tide.

Hoga and Kaledupa Islands climate –The climate of Sulawesi is best described in relation to rainfall. Between September and March, north-westerly winds crossing the South China Sea pick up moisture and arrive in north Sulawesi around November time. After this period, variable and humid south-easterly winds blow towards the eastern side of Sulawesi, with rainfall peaking on the southeast coast between April and June. The east coast of Sulawesi where the Wakatobi is located experiences its wettest month around May (Whitten, 1987).

Hoga and Kaledupa Islands marine habitats – The Wakatobi Marine National Park (WMNP) contains coral reef, mudflats, algal beds, seagrass and mangrove habitats, all with high conservation value which provide vital resources to local communities (Unsworth *et al.*, 2007).

The coral reefs are some of the most diverse in the world and have been identified as a biodiversity hotspot. Mangroves are located on the northwest coast of Hoga, as well as the east and south coasts of Kaledupa (Pet-Soede & Erdman, 2003). 37 species of mangroves have been documented within the WMNP. The mangroves of the WMNP are unusual as they are able to develop on shallow calcium carbonate-rich sediments that contain fossilised corals that restrict root formation (Cragg & Hendy, 2010). Mangrove habitats throughout Sulawesi have unfortunately been cleared for timber or to create brackish aquaculture ponds (Whitten *et al.*, 1987). Around Hoga and Kaledupa, large areas of mangroves have been cleared over the last 30 years for timber, fuel, and to accommodate the increasing populations.

Seagrass beds dominate the intertidal zones of the WMNP, with numerous beds extending for several kilometres (Whitten *et al.*, 1987). The two dominant species found in the seagrass beds of the WMNP are: *Thalassia hemprichii* and *Enhalus acoroides* (Unsworth *et al*., 2007). However, a further seven species (*Thallasodendron cilliatum, Halodule uninervis, Halophila spinulosa, Halophila ovalis, Halophila decipiens, Cymodocea rotundata, Syringodium isoetifolium*) have been noted within the region. Like most intertidal areas of the Indo-Pacific, seagrass beds in the WMNP experience vertical zonation down the shore (Unsworth *et al.*, 2007). Seagrass beds are an important fishing location for the local communities and when in continuum with mangroves and reef environments they support significantly higher fish densities (Salinas De León, 2006). Unfortunately, seagrass beds are often overexploited locally with communities harvesting fish stocks and exposed invertebrates at low tides (Clifton, 2010).

Hoga and Kaledupa Islands coral reefs – The Wakatobi district is located within the Coral Triangle and contains 600 km^2 of the most biodiverse coral reefs in the world, with over 350 species of hermatypic corals and 590 species of fish (Figure 1.7, Pet-Soede & Erdmann,

2003). From 2002 to 2007 hard coral cover for the WMNP fell by an average of 45 % for all study sites except Hoga, this suggested that local point sources were to blame for the decrease (McMellor & Smith, 2010). Overall, the reefs have experienced little impact from coral bleaching, likely due to cool water upwelling from the south (Tun *et al*., 2004) and seasonal temperature fluctuations increasing thermal tolerance. During the 2010 El Niño-La Niña event, the reefs around Wakatobi showed a high tolerance to prolonged thermal stress with only sub-lethal bleaching documented, primarily on the branching *Acropora spp.* (Smith/Suggett, Unpublished data, pers. Comm). Certain reefs have been damaged by the illegal practice of blast fishing and other reefs close to local Bajo villages experience higher impact and exploitation.

Hoga Island Wakatobi marine protected areas **–** The WMNP was established in 1996 and covers 1.39 million ha making it the second largest national park in Indonesia (Tomascik *et al.,* 1997). Unfortunately, the WMNP has historically been regarded as having ineffective enforcement, inappropriate zonation, insufficient funding and a lack of community support in management activities (Elliott *et al.* 2001). More recently however, several organisations have worked with the National Marine Park authorities to develop the area's management and the added value of the WMNP has come from its worth as a 'living laboratory'. Operation Wallacea and other NGOs have supported significant scientific research projects in the region. Collectively, this has resulted in the designation of the WMNP as a World Biosphere Reserve in 2012. The biosphere reserve is recognised under UNESCO's Man and the Biosphere programme and it acknowledges the site as an area of excellence, with sound science and local community efforts to conserve and preserve biodiversity (UNESCO, 2014).

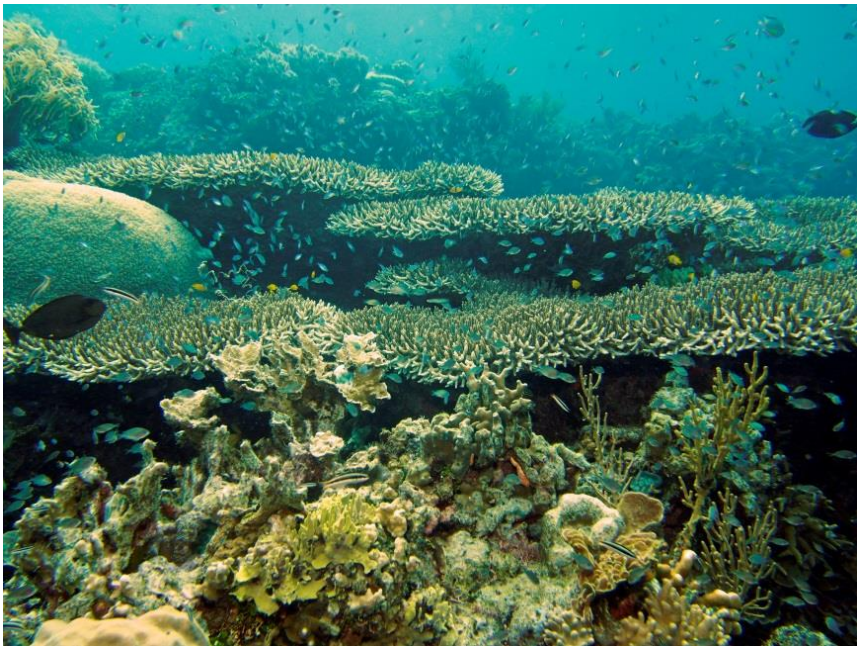


Figure 1.7| A photograph of the coral cover on the reef crest of Hoga's reefs, southeast Sulawesi, Indonesia. The picture was taken in 2014 on a site known locally as Pak Kasims off the north coast of Hoga.

1.7| Thesis aims

Coral reefs are threatened by OA. However, there are habitats (e.g. seagrass and mangroves) that house corals where conditions already are considered representative of the future under climate change. The distribution of these habitats across bioregions in tropical reef locations makes them a potentially important management option for corals threatened by climate change. However, the ability of corals to expand their niches into these environments, and the metabolic cost of living in these habitats is entirely undescribed. Similarly, whether the biogeochemical conditions of non-reef habitats, like seagrass beds and mangroves, are able to provide an ecological service in the form of refugia against OA is unresolved. The main aims of this thesis were therefore to:

1. Ascertain the natural levels of pH variability corals are already exposed to in reef and non-reef habitats;
2. Understand the ability of corals to persist in non-reef habitats;
3. Know the metabolic (photosynthesis, respiration and calcification rates) cost for dominant coral species living in non-reef habitats;
4. Understand whether corals living in non-reef habitats that have highly-variable pH experience the same metabolic (photosynthesis, respiration and calcification rates) response as corals from a more stable reef environment when subjected to pH and temperature stress predicted under future climate change.

In addressing these aims, a novel evaluation will be conducted to assess whether the local chemistry of non-reef systems can act as a buffer against future OA (see Chapter 6).

To achieve the thesis aims a systematic four-part approach was adopted; this approach is presented as each of four separate data chapters, all of which have been submitted or are in the final stages of preparation for submission, to peer review Journals:

1) Chapter 2: Methodological development was undertaken to determine the appropriate water sampling procedure necessary to capture the natural spatial and temporal variability in carbonate chemistry. A novel *in situ* respirometry chamber was also developed to assess the metabolic activity (photosynthesis, respiration and calcification) of corals.

Camp E, Krause S, Freitas L, Naumann MS, Kikuchi R, Smith DJ, Wild C, Suggett DJ. The "Flexi-Chamber": a novel cost-effective in situ respirometry chamber for coral physiological measurements. *PLoS One (Accepted).*

2) Chapter 3: A low biodiversity Atlantic Ocean site was used to assess in detail the spatial and temporal variability in carbonate chemistry, and to evaluate the biological versus abiotic control of non-reef habitats and the rates of photosynthesis, respiration and calcification of corals living within them.

Camp E, Lohr K, Dumbrell A, Manfrino C, Suggett DJ, Smith DJ. Coral recruitment into non-reef habitats: Consideration for coral refugia. *Marine Ecology Progress Series* (*Final draft).*

3) Chapter 4: To ascertain commonalities in the physiological responses of corals existing in non-reef habitats, three sites that were genetically disconnected and ranged in background diversity were compared.

Camp E, Suggett DJ, Gendron G, Jompa J, Manfrino C, Smith DJ. Pre-conditioning and buffering services of mangroves and seagrass beds for corals threatened by climate change. *Global Change Biology* (*In review).*

4) Chapter 5: The impact of future predicted changes in temperature and pH on the physiological responses of corals pre-adapted to habitats of differing inherent environmental variability was assessed by superimposing temperature and pH changes estimated for 2100 on top of the natural diurnal oscillations of each habitat through a laboratory manipulation study.

Camp E, Smith DJ, Evenhuis C, Enochs I, Suggett DJ. The roles of temperature and pH in non-reef habitats on coral calcification and metabolic activity: Can marginal systems buffer against change? *Proceedings of the Royal Society B (Final draft).*

A final general discussion (Chapter 6) considers these outputs to evaluate the role of non-reef habitats in providing some form of refuge for corals to future climate change. The discussion also considers what drives variability of non-reef habitats and how this variability may itself be changed in the future. The discussion considers the future for corals and coral reefs and concludes by identifying points for consideration for future OA research.

1.8| Thesis structure

The thesis studies different non-reef habitats that house corals and have natural variability in carbonate chemistry to: (i) determine the appropriate sampling regime to capture accurate and precise carbonate chemistry and coral mertabolic activity data (Chapter 2); (ii) understand the levels of variability corals are already exposed to within these systems (Chapters 3 & 4); (iii) determine which corals are currently living in these non-reef habitats (Chapters 3 & 4); (iv) measure what the metabolic cost is for the coral species found living in non-reef habitats (Chapters 3 & 4); (v) understand whether corals living in non-reef habitats experience the same metabolic cost as corals from a more stable reef environment when subjected to pH and temperature stress predicted under future climate change (Chapter 5). The thesis will then summairse the main findings and will include a discussion where the role of non-reef habitats to act as refugia to future climate change is considered (Chapter 6).

Chapter 2| Methodology for capturing the variance of carbonate chemistry and inherent metabolic activity of corals within reef and non-reef systems

Part of this chapter is in review in PLoS One as the manuscript: The "Flexi-Chamber": a novel cost-effective in situ respirometry chamber for coral physiological measurements.

2.1| Introduction

Assessing the variability of carbonate chemistry parameters between habitats fundamentally depends on accurate and precise measurements from a highly resolute sampling approach. Such an approach needs to critically capture the environmental mean and variance of any given habitat. Determination of carbonate chemistry parameters within ocean acidification (OA) studies form the basis for assessing the biological response of systems and species to future predicted levels of CO_2 (Ribas-Ribas *et al.*, 2014). Thus, the integrity of the data collected is imperative in ensuring maximum confidence in the results obtained. The Carbon Dioxide Information Analysis Centre (CDIAC) protocol and the Dickson *et al.* (2007) *Guide to Best Practice for Ocean CO_2 Measurements* provide guidelines on the levels of accuracy and precision within carbonate chemistry measurements that methods should strive for, as developed by the international community attempting to optimise and standardise sampling practice.

Similarly, when developing new methods and sampling techniques, it is imperative that their reliability is tested and that their outputs are compared to established methods that are considered the current "gold standard". Current *in situ* respirometry chambers typically require colony removal and are rigid vessels requiring artificial mixing to disrupt the outer coral

boundary layer (McCloskey *et al*., 1978). They are often expensive and difficult to travel with due to the fragility of transporting glass/plexi-glass vessels and their often large size for deployment, which limits the scale and resolution with which respirometry data can be generated. Established chambers are also typically a fixed size and thus a range of chambers may be necessary, or experimentation would otherwise be limited to a targeted organism size. The size of the chamber (i.e. volume of the incubation medium) relative to the organism to be incubated is critical in order to balance: (i) maximised signal strength as compared to, (ii) minimising potential toxicity via hypoxic or anoxic conditions (Orr e*t al.,* 2014). Thus, there has remained a long-standing need to develop a low-technology, cost-effective, non-destructive respirometry vessel to measure *in situ* coral metabolism. Note that metabolism throughout this thesis will refer to the physiological processes of photosynthesis, respiration and calcification. The objectives of this chapter are therefore to:

(1) Develop a discrete water sampling regime to capture the spatial and temporal pH variability of reef habitats;

(2) Establish a discrete sampling method for pH and total alkalinity (TA) that meets CDIAC standards;

(3) Construct a novel *in situ* bag respirometry chamber to test coral metabolism (respiration, photosynthesis and calcification) and verify against an established technique.

Through meeting these objectives the core methods for assessing both the carbonate chemistry as well as coral metabolic properties used throughout Chapters 3 through 5 are established.

2.2| Materials and Methods

Chapter two deals with a variety of methodological testing; figure 2.1 illustrates the layout of the material and methods section, whose format is followed in the results section.

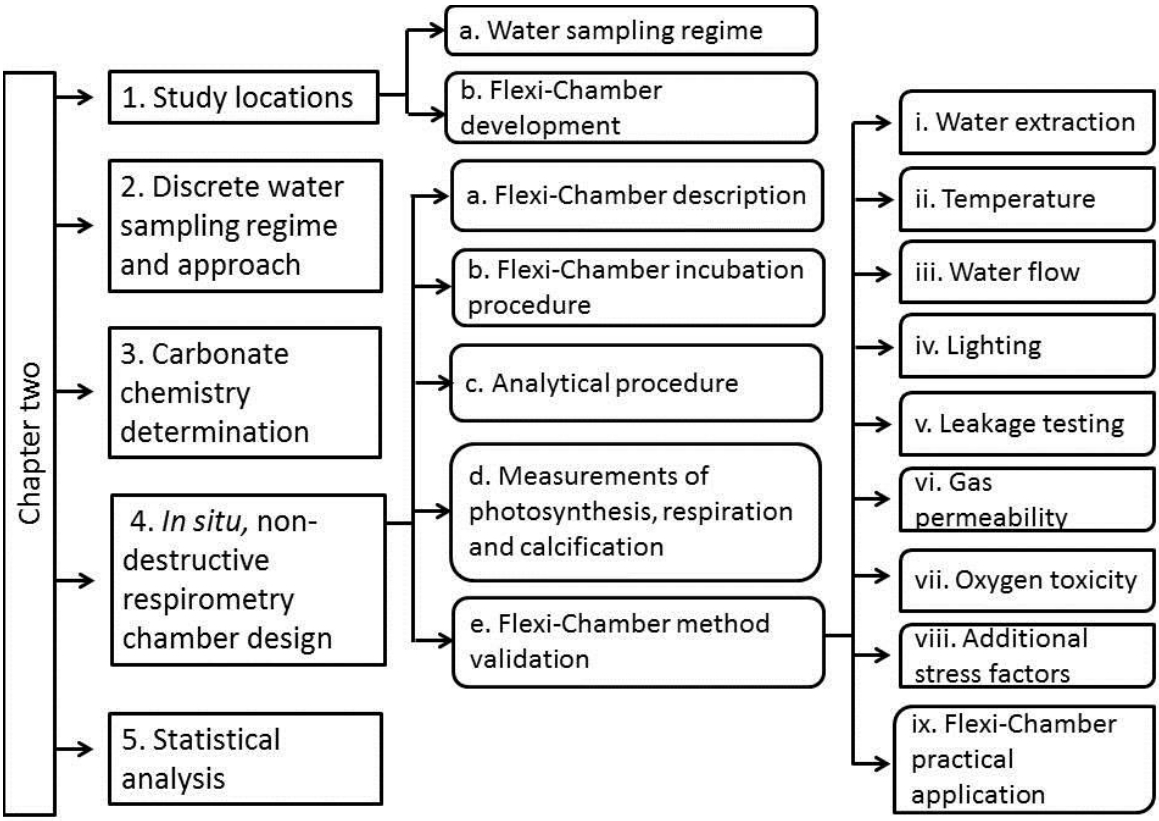


Figure 2.1| Flow-diagram of the material and methods section of chapter two. The sub-sections of each chapter are indicated to clarify the outline of the chapter. The results section follows this lay-out.

2.2.1| Study locations

Water sampling regime – Little Cayman, Cayman Islands, British West Indies (BWI) was used as the study location to identify an appropriate sampling regime to capture the spatial and temporal variability in pH of reef and non-reef coral habitats (see Chapter 1, section 1.6.1). Sampling took place between November 2011 and January 2012. Preliminary pH sampling was conducted around the five main lagoons of Little Cayman, known locally as: Grape Tree Bay

(GTB), Mary's Bay West (MBW), Mary's Bay East (MBE), Charles Bay (CB) and South Sound (SS) (Figure 2.2). High resolution pH sampling was conducted in a dense seagrass biomass site within GTB. All sites were 2-4 m in depth and situated away from any freshwater inputs. Sites experienced a tidal cycle range of 0.12-1.00 ± 0.03 m during sampling.

Respirometry chamber development – All laboratory testing of the Flexi-Chamber design was conducted using the aquarium facility at the Coral Reef Research Unit, University of Essex (January 2013- December 2014). Field validation was conducted in Salvador (Brazil) from March 26th to April 2nd 2014 (see Chapter 1, section 1.6.2). The study site was a near-shore fringing reef located at the entrance of Todos os Santos Bay adjacent to the Yacht Clube da Bahia harbour (Figure 2.3) at a depth of 3-5 m.

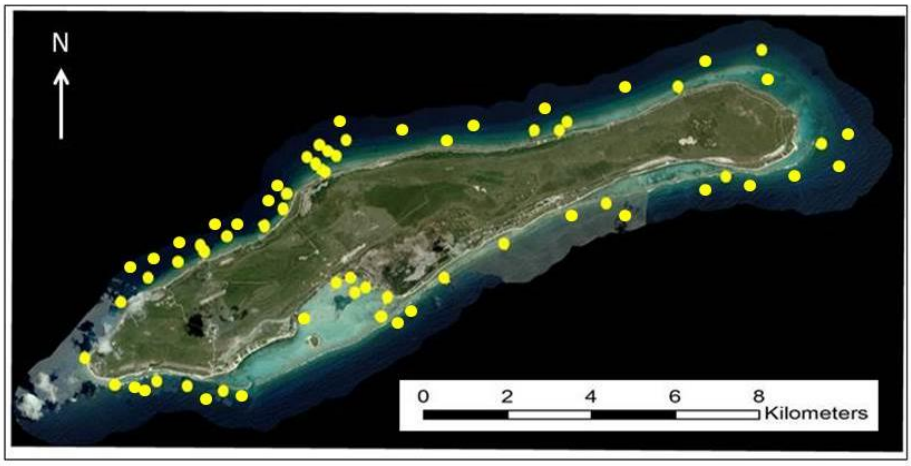


Figure 2.2| Preliminary sampling sites around Little Cayman, Cayman Islands, BWI. 66 sites (yellow dots) were sampled diurnally between November 2011 and January 2012 to identify different habitats with natural pH variability. pH was directly measured on discrete water samples collected at each site.

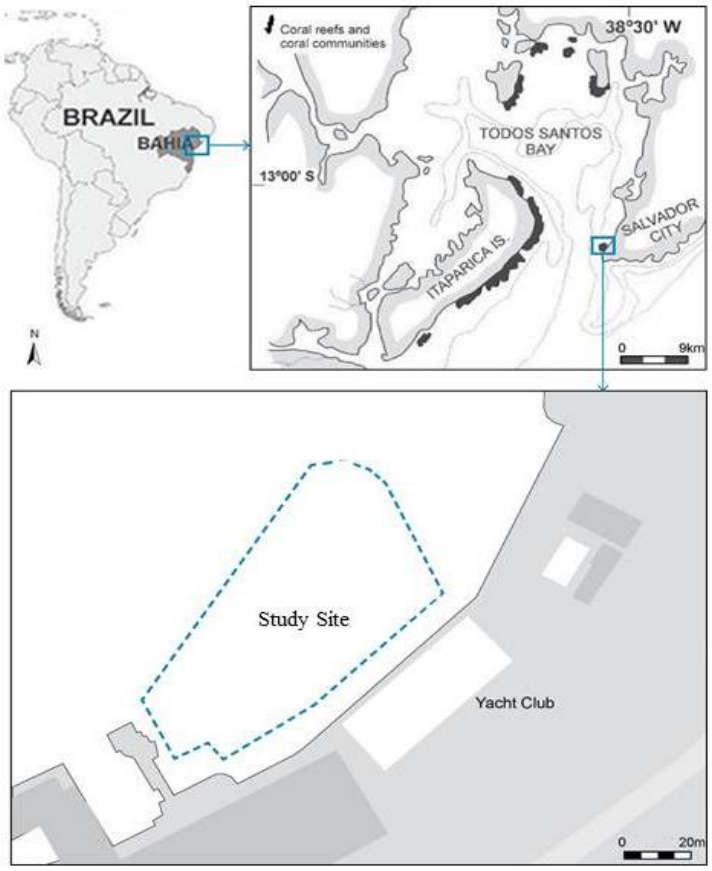


Figure 2.3| Study site location in Todo os Santos Bay (TSB) (modified from Ramos *et al.*, 2010). The study site was located at the entrance of TSB in depths between 3 and 5 m adjacent to the Yacht Clube da Bahia harbour in Salvador, Brazil.

2.2.2| Discrete water sampling regime and approach

To identify the spatial pH variability of different habitats, diurnal (one hour before sunrise and one hour before sunset) samples were collected at several sites (n= 66) inside and outside of the five main lagoons of Little Cayman (Figure 2.2). Basic abiotic data (depth, temperature, salinity, water velocity) was collected for each lagoon during the sampling period (Table 2.1). To quantify the resolution of sampling necessary to capture the diurnal variability in highly variable pH habitats, three 24 h sampling sessions were conducted in the seagrass habitat of GTB. pH was

determined every hour over the 24 h period starting at sunrise (*ca.* 7:00 h). Finally, to determine the extent of pH variability around sunrise and sunset, and thus whether initiating sampling at sunrise was the best time to capture the diurnal range in conditions, pH measurements were collected every 15 min for a 3 h window around sunrise and sunset to assess differences in mean and variability (coefficient of variation (Cv)) in pH values.

Table 2.1| Abiotic data for the five main lagoons of Little Cayman. Data (mean ± standard error, SE) was obtained during water sample collection between November 2011 and January 2012 (*n*= 66).

Lagoon	Dominant substrate*	Depth (m)	Salinity (ppm)	Temperature (°C)	Water velocity (cm/s)
Grape Tree Bay	Seagrass	1.4 ± 0.01	35 ± 0.01	26.3 ± 0.03	14 ± 0.01
Mary's Bay East	Seagrass	1.6 ± 0.03	35.5 ± 0.01	26.4 ± 0.02	12 ± 0.04
Mary's Bay West	Seagrass	1.9 ± 0.02	35 ± 0.01	26.4 ± 0.01	11 ± 0.02
Charles Bay	Seagrass	1.1 ± 0.01	35 ± 0.01	26.3 ± 0.01	13 ± 0.01
South Sound	Hard-ground/sand	1.9 ± 0.01	35 ± 0.01	26.2 ± 0.02	11 ± 0.01

*****Dominant substrate was assessed from GIS maps provided by the Cayman Islands Department of the Environment.

Discrete water samples were collected between 0.5-1 m directly into 250 ml acid washed (2 % HCL) borosilicate glass bottles (Manzello, 2010). Immediately before sample collection the bottles were rinsed twice with the sample water as recommended by CDIAC and filled to overflow to eliminate headspace for gas exchange (Dickson *et al.*, 2007). Mercuric chloride (0.05 ml of saturated (aq) solution) was added as the standard operating procedures for CDIAC to poison the samples and prevent any biological activity altering the carbonate chemistry parameters before measurement (Dickson *et al.*, 2007). Stoppers were inserted to ensure a gas

tight seal and where possible, samples were stored in the dark until returned to the laboratory (within 30 min) for further analysis.

During every sampling period, an additional seawater sample (250 ml) was collected to allow immediate measurements of pH, temperature, and salinity to obtain the *in situ* conditions needed for calculation of the carbonate parameters. The ORION 5 Star meter (model A329, Fisher Scientific, USA) with a pH/temperature probe (pH accuracy within ± 0.002 pH units, temperature accuracy within ± 0.1°C; combination probe Ross Ultra, Fisher Scientific, USA) and a handheld refractometer to measure salinity (accuracy within ± 0.5 ppm; model RF20, ExTech, USA) were used to obtain these measurements (pH measured in NBS scale). pH was measured in the field to ensure that it did not drift before samples were taken in the laboratory. Water flow was also measured using a mechanical flow meter (model 2030 series, General Oceanics, USA). All laboratory and field equipment were calibrated as recommended by their instruction manuals and/or CDIAC protocols (Dickson *et al.*, 2007).

2.2.3| Carbonate chemistry determination

pH was quantified on all samples returned to the laboratory using an Orion Ross Ultra Glass Triode Combination Electrode (accuracy *ca.* ± 0.002 pH units; Ross Ultra, Fisher Scientific, UK) using the potentiometric technique and the total scale (Dickson *et al.*, 2007). The pH probe was calibrated at 25 °C using 2-amino-2-hydroxy-1-3-propanediol (TRIS)/ HCL and 2-aminopyridine (AMP)/HCL buffers in synthetic seawater with a salinity of 35 ppm. The buffers were made up in the laboratory following the CDIAC recommendations and had their pH defined as (Dickson *et al.*, 2007):

TRIS (2-amino-2-hydroxy-1-3-propanediol)

$$\text{pH (s)}_{TRIS} = (11911.08 - 18.2499S - 0.039336S^2)\frac{1}{T/K} - 366.27059 + 0.53993607S + 0.00016329S^2 + (64.52243 - 0.084041S)\ln(T/K) - 0.11149858(T/K). \quad [1]$$

AMP (2-aminopyridine)

$$\text{pH (s)}_{AMP} = (111.35 + 5.44875S)\frac{1}{T/K} + 41.6775 - 0.015683S - 6.20815\ln(T/K) - \log_{10}(1 - 0.00106S). \quad [2]$$

From equations 1 and 2 the electrode response(s) was calculated:

$$s = \frac{EAMP - ETRIS}{\text{pH (s)}TRIS - \text{pH (s)}AMP} \quad [3]$$

s is the buffer, S is salinity, *T/K* is temperature in Kelvin and *E* is the e.m.f of the cell.

The electrode response was compared to the Nerst value (*RTln10*/*f*) to ensure that the difference was not above the acceptable difference of 0.3 % (Dickson *et al.*, 2007). The pH was then calculated:

$$\text{pH(x)} = \text{pH(s)} + \frac{Es - Ex}{RT\ln 10/f} \quad [4]$$

Where *x* is the sea water sample.

An open-cell potentiometric titration procedure was used to measure TA on the same sample as pH (Dickson *et al.*, 2007). The Gran method was used to determine the second end point of the carbonate system, from which TA was then determined:

$$TA = \frac{(\text{Normality of the titrant}) * (\text{mL of titrant added to reach eq2})}{(\text{Sample Volume in ml})} \quad [5]$$

TA of all samples was determined using a Titrino titrator (model 848, Metrohm, Buckingham, UK) or high precision burette (Fisher Scientific, USA). 0.1 N standardised HCL was used (Appendix 1). TA was measured with an accuracy and precision of *ca.* ≤ 2 μmol kg^{-1}. The remaining carbonate chemistry parameters (pCO_2, TCO_2, Ω_{arg}) were calculated with CO2SYS using TA and pH as determined in the laboratory (Riebesell *et al.*, 2010), *in situ* temperature and salinity, and sampling depth (m) (accuracy within ± 0.5 m) as a proxy for pressure (Lewis and Wallace, 1998). For CO2SYS the dissociation constants of Mehrbach *et al.* (1973) were used for carbonic acid as refined by Dickson and Millero (1987), and for boric acid (Dickson, 1990). Pressure effects, orthophosphate and silicate concentrations were assumed to be negligible (*see* Jury *et al.*, 2010).

To check the validity of the seawater sample storage method, a number of samples (n= 72) had their pH measured in the field and subsequently re-measured in the laboratory to check for any drifts. Samples were collected over the three 24 h sampling sessions in GTB, with three replicate samples collected every 3 h over the three 24 h periods. In addition, to test the precision of the pH and TA measurements, *R* charts were created, whilst $\bar{X}$ charts were used to assess the stability of the measurements (Dickson *et al.*, 2007). 15 sequential pH and TA measurements were taken on the initial 24 h sampling day in GTB to test the absolute differences of duplicate measurements to assess their precision. Over 15 sequential days, pH and TA were measured from a control sample collected from GTB on the initial day at 7:00 h to assess the stability of the measurement process. For each graph, an upper control limit (UCL), upper warning limit

(UWL), lower warning limit (LWL) and lower control limit (LCL) were calculated as shown below:

$\bar{X}$ Charts: x is the mean value, s is the standard deviation

$$\text{UCL} = x + 3\,s \quad [6]$$

$$\text{UWL} = x + 2\,s \quad [7]$$

$$\text{LWL} = x - 2\,s \quad [8]$$

$$\text{LCL} = x - 3\,s \quad [9]$$

R charts: $\bar{R}$ is the average range and is related to the short-term standard deviation:

$$\text{UCL} = 3.267\,R \quad [10]$$

$$\text{UWL} = 2.512\,R \quad [11]$$

$$\text{LWL} = 0 \quad [12]$$

$$\text{LCL} = 0 \quad [13]$$

The recommendations by CDIAC are that 95 % of the plotted points should fall between the UWL and LWL, and rarely should any fall outside of the control limits. TA was also compared to Dickson Standards (Scripps University) to test accuracy against a stable reference material.

A comparative analysis was finally conducted on the calculated pCO_2 values using CO2SYS to a direct pCO_2 measurement technique. Water samples were collected as previously described, every 3 h starting at sunrise (*ca.* 7.00 h) over the three 24 h sampling days in GTB (n= 24). For each sample, pH and TA were measured and pCO_2 derived using CO2SYS. The direct measurement technique used a custom-built gas diffusible membrane (standard silicone tubing, 0.31mm ID-0.64mm OD, Helix Medical, USA) attached to an external infrared gas analyser (IRGA, Li-820, Li-COR, Nebraska, USA) with a pump flow through system (see Suggett *et al.*, 2013). The gas analyser was also attached to a notebook computer with Li-820 software. Each

sample was transferred to a custom-built chamber and analysed for 60 min to allow the probe time to stabilise. A water bath was used to maintain the sample within 0.1 °C of the *in situ* temperature. After 60 min the stable probe reading for pCO_2 was used for comparison with the pCO_2 value derived from CO2SYS. To ensure that the direct measurement set-up was gas-tight, it was calibrated with CO_2 free air, created by stripping the CO_2 from air using Sodalime. If the computer readout from the IRGA read zero, then it could be confirmed that the direct measurement set-up was gas-tight and reading accurately.

2.2.4| *In situ,* non-destructive respirometry chamber design

In the following sections, a novel *in situ*, low-cost, high thorough-put incubation chamber, termed the Flexi-Chamber is described and tested, both in the laboratory and field.

Flexi-Chamber description – A transparent, gas-impermeable, 3 L urine bag (Vital Care, Essex) with a built-in heat-seam secured valve, formed the basis of the Flexi-Chamber (Figure 2.4a). The bottom of the bag was cut to create a fringe that was secured around the base of the test colony. A watertight seal was created by using a customised neoprene cuff and fastening (Figure 2.4a). The neoprene cuff was customised to fit securely around the base of the test colony, thereby minimising the possible contribution of the surrounding substrate and/or water column to the metabolic signal of the test specimen (Figure 2.4b). The internal water volume was adjusted to accommodate corals of different sizes: larger coral colonies (*ca*. 5 cm diameter) required the entire volume of the chamber, whereas smaller colonies (*ca.* 2-3 cm diameter) required a reduced volume (in this case 60 % of the bag volume) to optimise the metabolic signal.

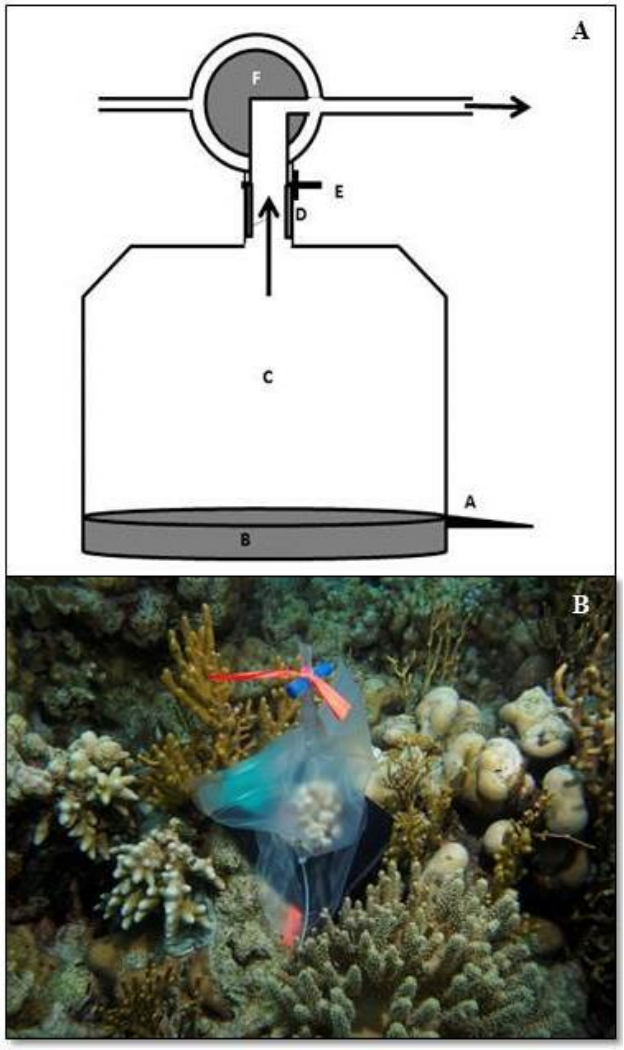


Figure 2.4| The Flexi-Chamber design: A) A schematic diagram of the Flexi-Chamber: A is the fastening mechanism, B is the neoprene cuff around the base of the coral, C is the urine bag (Vital Care, Essex), D is standard silicon tubing used to create a water tight seal with the valve, E is the valve of the urine bag and F is the three-way-valve. Figure 2.4 B) An example of the Flexi-Chamber set-up.

Flexi-Chamber incubation procedure – Prior to any experimental use all Flexi-Chambers were acid washed (2 % HCL). For each deployment, three Flexi-Chambers were filled with *in situ* water without any coral colonies to act as a control to correct for any planktonic metabolic activity within the surrounding water. Whilst colonies where haphazardly selected within any one habitat, colonies had to be *ca*. 3-5 cm in diameter, and chosen with no visible signs of

disease, bleaching, loss of colour (relative to the site mean), or excessive algal overgrowth (McCloskey *et al.,* 1978). Colonies also needed a suitable surrounding substrate to allow the attachment method, i.e. a basal area raised from the substrate for chamber attachment. Photographs were taken of each colony before incubations (Canon G12 in an underwater housing, Canon, WP-DC 34, Amazon, England).

Water was extracted from the chamber via the built-in three-way valve mechanism, which could be opened or closed to sample the internal water volume (Figure 2.4a). In this way, water could be conveniently extracted via a syringe without cross-contamination of the surrounding seawater. Two 100 ml syringes were attached to the three-way valve system; the first to remove *ca*. 30 ml of excess water from the bags valve which was discarded, and the second to extract 100 ml of the seawater sample from inside the Flexi-Chamber. For standardisation of the physiological measurements, water volume inside the chamber was accurately determined as the total of the water volume removed during the sampling process (100 ml) and the remaining water volume within the chamber (typically 950-1000 ml) determined by subsequent syringe removal of water until the bag was emptied. The swivel lock of the syringe to the valves as well as the watertight nature of the bag (see leakage testing) ensured that the water removed from the Flexi-Chamber was not contaminated with that from the surrounding seawater.

Sample water was syringed from each Flexi-Chamber and immediately transferred to pre-washed and labelled 250 ml borosilicate glass bottles (Naumann *et al.,* 2013). Sample bottles were kept at ambient temperature and under dark conditions until analysis (within 30 min of collection, as per Dickson *et al*., 2007). Chambers were left *in situ* for 3 h. After this period, water samples were re-collected from each chamber and stored as previously described. After all

samples had been collected, chambers were removed from each colony, the water was flushed with ambient water, and chambers were re-secured; control chambers were treated in exactly the same way. The whole process was then repeated at 3 h intervals for the duration of the study period. For the procedure validation, dark-cycle metabolism was examined by artificially darkening (opaque black polyester material) the Flexi-Chambers, but the technique could be used equally well during night-time sampling (see Chapter's 3 & 4).

Analytical procedures – pH, TA, temperature and salinity of each sample were measured as previously described for the carbonate chemistry determination (see section 2.2.3). To determine the oxygen (O_2) content (accuracy *ca.* 0.05 μmol/L) of each sample, a 100 ml aliquot of each water sample was transferred into a sealed chamber in the laboratory, where an O_2 and temperature probe (O_2 probe: Foxy-R, Temperature Probe: NeoFox TB, Ocean Optics, England) were attached to a bench-top fluorometer (NeoFox-FT, Ocean Optics, England) via a bifurcated fibre assembly (BIFBORO-10000-2, Ocean Optics, England) and attached to a PC running the O_2 sensing software (NeoFox Viewer, Ocean Optics, England). Samples were run in a climate-controlled laboratory until the O_2 concentrations stabilised. All probes were calibrated according to the Ocean Optics instruction manual.

Measurements of photosynthesis, respiration and calcification – For all samples, calcification was determined via the TA anomaly method (Jury *et al.*, 2010) corrected for any changes in TA of the seawater controls, to yield hourly calcification rates (G, mmol $CaCO_3$ m^2 h^{-1}) as:

$$G\ (t)\ = \left[\frac{(\Delta TA \cdot \rho \cdot 0.5)\cdot V}{I_t \cdot SA}\right]/1000 \qquad [14]$$

Where TA= total alkalinity (μmol kg^{-1}), V = volume of water (L) surrounding the coral within the respirometry chamber, I_t (h) is incubation time, SA is the coral surface area (m^2), ρ is the

density of seawater and 0.5 accounts for the decrease of TA by two equivalents for each mole of $CaCO_3$ precipitated. Calcification rates were not corrected for any changes in nutrients as Langdon et al. (2000), with Chisholm and Gattuso (1991) demonstrating that the assumptions of the TA method without the correction for nutrients are correct in obtaining accurate calcification rates for tropical coral reefs.

Net photosynthesis and respiration rates were determined for several time points (*t*) throughout the light and dark cycles respectively as the change in O_2 in the respirometry chamber corrected for any changes in O_2 of the seawater controls to yield hourly rates (mmol O_2 m^2 h^{-1}) as:

$$P_N \; and \; R\;(t) = \left[\frac{(\Delta O_2)\cdot V}{I_t \cdot SA}\right] /1000 \qquad [15]$$

Integration of all photosynthesis and respiration measurements during the light (dark) yielded the daily daily photosynthesis (P_N) and respiration (mmol O_2 m^2 d^{-1}) as:

$$P_N = \sum_{Light-end}^{Light-start} P\;(t)\;\Delta t \text{ and } R = \sum_{Dark-end}^{Dark-start} R\;(t)\;\Delta t \qquad [16]$$

Gross photosynthesis (P_G) was calculated by the addition of P_N and R. All values of R are subsequently multiplied by the factor -1 to convert to positive values. The surface area of all colonies was determined by the advanced geometric technique (Naumann *et al.*, 2008). Measurements of length and diameter were taken *in situ* using callipers, and the respective surface area calculations were calculated using the area formula of the most fitting geometric shape.

Flexi-Chamber method validation –Acropora sp. coral specimens supplied by Tropical Marine Centre Ltd. (Chorleywood, UK) was the test organism since this genus is present across bioregions and widely used in physiological process measurements (Connell, 1973). Five colonies were used and were secured into 1 cm^2 PVC plugs with a non-toxic epoxy resin

(Milliput-Standard) and left to acclimatise for 24 h prior to experimentation. Colonies were not fragmented and therefore did not need to heal before experimentation. Aquarium tanks were supplied with Tropic Marine PRO REEF salt-based seawater supplemented with $NaHCO_3$ maintained at 28.0 ± 0.9 °C (using Aquael Neo Heaters, IPX8, Poland), 35 ppm, a 4 L min^{-1} flow rate circulating between the tanks and a common biological sump of Fijian live rock (Tropical Marine Centre Ltd., Chorleywood, UK) and were kept under daylight conditions (*ca.* 80.2 μmol photons m^2 s^{-1}) using 150 W Metal-Halide lamp (Arcadia Products PLC, Redhill, UK). A series of laboratory tests were conducted:

Water extraction – To determine the average error of the water extraction method a Flexi-Chamber was filled with exactly 250 ml of seawater and subsequently extracted via the syringe method described above; the volume removed was measured and subtracted from the 250 ml to quantify the amount of seawater unaccounted for. The process was repeated 30 times to gauge the mean and error for this step.

Temperature – Possible temperature drifts as a result of incubation were tested. A Flexi-Chamber was filled with synthetic seawater and maintained in an aquarium of a known constant temperature of 28.0 ± 0.9°C (Aquael NeoHeater, IPX8, Poland). The internal temperature within the Flexi-Chamber was then determined every hour over an 8 h period (repeated three times) by opening the valve and inserting a temperature probe (NeoFox TB, Ocean Optics, England). An *in situ* temperature comparison between the Flexi-Chamber and surrounding seawater was also conducted in Salvador, Brazil, with a HOBO Pendant Temperature/Light Loggers (model UA-002-64, Microdaq, USA) set to log every 30 s, placed inside the Flexi-Chamber, and one outside the Flexi-Chamber for three 24 h periods.

Water flow – To test the extent of water movement (circulation) within the Flexi-Chamber, the relative acceleration in the X, Y and Z planes (Figure 2.5a) was determined inside and outside of the Flexi-Chamber during a 1 h period (n= 3) using a G Pendant HOBO logger (UA-004-64; Microdaq, USA) set to log every second. The logger had tethered anchored beams in fixed locations that had centre plates. As the beams move, the centre plate is displaced creating a change in capacitance proportional to the applied acceleration (Onset, 2012). This change in capacitance is converted to an output voltage which is processed with calibration data to produce an equivalent acceleration value where 1G =9.8 m/s^2. Prior to launch, both loggers were inter-calibrated by comparing their readings relative to one another when moving them through a series of set orientations (Figure 2.5b) (Onset, 2012). Both loggers were initiated at the same time and set to log at 1 s intervals. To establish a reading in all three planes, the logger had to be free to move without touching the inside of the chamber. As the logger is positively buoyant, a 6 cm length of cable of 1 mm thickness was used to tie the logger to a weight, resulting in the HOBO being orientated upside down. A small piece of glass was then attached to the underside of the logger to make it neutrally buoyant so that it sat centrally within the Flexi-Chamber. High water velocity could cause the HOBO to make contact with the Flexi-Chamber, constraining its movement and thus the acceleration; however this was never observed (as per Camp observation) and any restrictions associated with the cabling would influence both HOBOs equally and is therefore negligible.

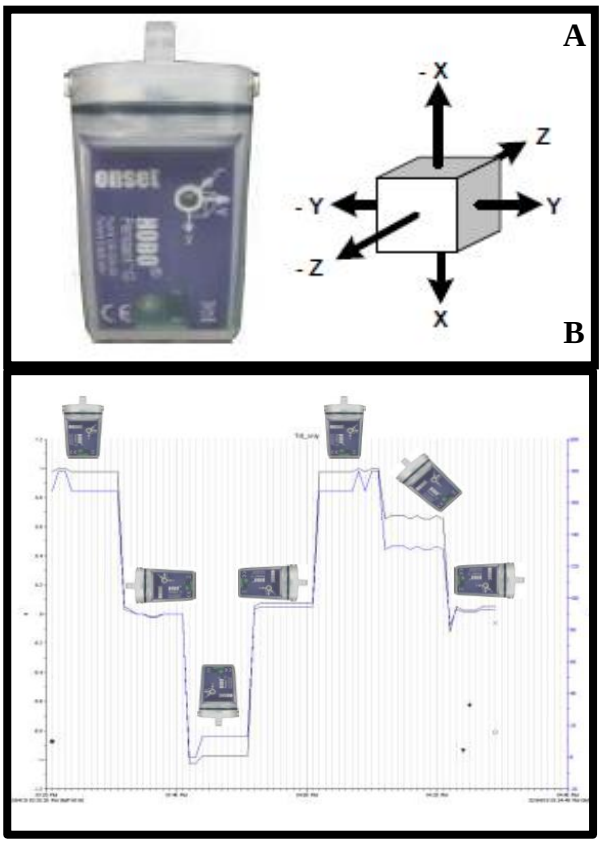


Figure 2.5| The HOBO Pendant G Logger. A) The orientation of the three orthogonal axis. The HOBO logger measures acceleration along three planes: X, Y and Z. When the logger is orientated as shown, the direction of the three planes are as illustrated. B) The calibration orientations used for the two Pendant G HOBO Loggers.

Lighting – Light penetration through the Flexi-Chamber was measured using a spectroradiometer (M/A-COM, model SR9910-UF, Lamington, Scotland), and converted from energy (W/m^2) to photons ($\mu mol\ m^2\ s^{-1}$) by multiplying by the light source coefficient (Kirk, 1994). Percent transmission was then determined as the amount of light transmitted through the respirometry chamber relative to no chamber present. Artificial lighting (240-800 nm) consisted

of LED arrays covering the Photosynthetically Active Radiation (PAR) (400-700 nm) spectra (Heliospectra, Göteborg, Sweden) and UV fluorescent 20 w tubes (Philips, Netherlands) covering UVB and UVA radiation (240-400 nm).

Leakage testing – To ensure that a watertight seal could be created around coral colonies, the Flexi-Chamber was secured around a colony of *Acropora sp*. and natural red food colouring was syringed into the Flexi-Chamber via the three-way-valve mechanism. Five replicate colonies were sampled. The Flexi-Chamber was situated inside an aquarium with a pendant temperature/light HOBO placed inside the aquarium and Flexi-Chamber. An initial 30 mL aliquot of water was syringed out of the Flexi-Chamber and also from the surrounding aquarium water. The Flexi-Chamber was left in the aquarium for 3 h, before another water sample was collected from inside the Flexi-Chamber and aquarium. Absorbance of each sample was then measured using a USB 2000^{+} Spectrometer (Mikropack Halogen Light Source (HL-2000), 1 cm Cuvette Holder, serial fibre optic probes (727-733-2447) Ocean Optics, England) to determine if any dye had transferred from inside the Flexi-Chamber to the surrounding aquarium water.

Gas permeability – Whilst the Flexi-Chamber material is designed to be gas impermeable, we verified negligible permeability for both CO_2 and O_2 by filling five Flexi-Chambers with *in situ* seawater (Salvador, Brazil). Mercuric chloride (as per CDIAC protocols) was added to prevent any further biological activity within the water and an initial 100 ml water sample was taken from each Flexi-Chamber. Flexi-Chambers were then secured *in situ* and left for 3 h. After the 3 h period, an additional 100 ml of water was removed from each Flexi-Chamber (end sample). For the initial and end samples taken, O_2 and CO_2 were measured. O_2 was measured using Foxy-R O_2 probe (Ocean Optics, England) and CO_2 was measured using a

custom-built gas diffusible membrane attached to an external infrared gas analyser (see Suggett *et al*., 2013).

Oxygen toxicity – Prior to any incubation procedure, a sensitivity analysis is first required to establish the optimum vessel size-to-organism biomass ratio, relative to the flushing time procedure to prevent anoxic or hypoxic conditions; time taken to reach anoxic or hypoxic conditions will be highly variable as a result of inherent differences in metabolism across taxa and growth environments. A benefit of the Flexi-Chamber is that the internal volume of water can be adjusted to accommodate different volumes of water to help mediate the balance of biomass-to-water required. An example of the sensitivity analysis required and how the Flexi-Chamber can easily be adjusted to accommodate different water volumes was undertaken on *Acropora sp.* 18 colonies of *Acropora sp.* of similar size (mean ± SE) (12 ± 0.13 cm^2) were enclosed in the Flexi-Chambers with a volume of either 250 ml, 500 ml, 750 ml, 1000 ml, 1250 ml or 1500 ml of surrounding seawater to compare when anoxic or hypoxic levels were reached. Chambers were maintained in the aquaria under light-dark cycles (conditions previously described). Initial water samples were collected to measure the O_2 levels at time zero. An aliquot of 30 ml of seawater was then removed every hour over a 4 h period to examine for changes in $[O_2]$.

Additional stress factors –To ensure that no unforeseen factors were stressing the coral, such as chemicals leaching from the plastic, we further incubated *Acropora sp*. nubbins (*n*= 5) in separate Flexi-Chambers for 9 h with regular 3 h flushing to observe for any visible signs of stress, in the form of mortality, excessive mucus formation or loss of pigmentation. Zooxanthellae counts were taken from tissue stripped from the base of each nubbin (Berkelmans & van Oppen, 2006). Coral tissue was removed using a water pik (Waterpik Inc, England) in 5

mL of GF/F–filtered seawater, and the area of tissue removed was quantified via the advanced geometric technique (Naumann *et al.,* 2008). The tissue slurry was homogenised using a pipeta pasteur and a small aliquot subsequently taken for cell quantification via microscopy using a neubauer haemocytometer (Berkelmans & van Oppen, 2006)

Flexi-Chamber practical application **–** The performance of the Flexi-Chamber design was compared *in situ* with that of an established glass respirometry vessel routinely used in metabolic activity measurements (Naumann *et al.,* 2013). Colonies of the commonly occurring *Siderastrea cf. stellata* were sampled from the entrance of Todos os Santos Bay in 3-5 m water depth. In total 40 colonies of *S. cf. stellata* were examined for P, R and G throughout a one-week period. All incubations occurred at a water depth of *ca.* 2-3 m. For 30 of the colonies, measurements using the Flexi-Chamber and glass chamber were made on separate colonies (15 colonies per chamber type). For the remaining ten colonies, metabolic measurements for both chambers were made on the same colony but for different days, with a minimum rest period of 24 h for any one colony between measurements, and random allocation to the initial chamber.

Colonies tested in the glass chamber were removed from the seafloor at least 48 h prior to measurements (Naumann *et al.,* 2013). During collection, all coral colonies were handled without any air exposure or direct tissue contact and gloves were worn throughout the handling process. Any extensive epibionts or endolithic boring organisms were excluded and any remaining overgrowth was cleaned using a soft toothbrush. Similarly, any air bubbles or large particulates in the water were removed from the chambers, to minimise O_2 fluxes from non-coral sources (McCloskey *et al*., 1978). Colonies were chiselled from their substrate and transferred using individual zip-lock bags to avoid mechanical damage during transport (Naumann *et al.,* 2013). The chambers were fixed to a custom-made metal frame, to facilitate transport and rapid

deployment *in situ* (Figure 2.6). Chamber incubations were conducted under no-flow conditions so hourly manual stirring (magnetic stirring bars) was necessary to break-up the boundary layer (Patterson *et al.*, 1991).

In this case study, a 3 h light incubation was followed by a 3 h dark incubation. All incubations were run around the daylight maximum (*ca.* 11:00-14:00 h) for the light incubations for P_N and G_L. Corresponding dark rates, i.e. R and G_D, were obtained during daylight hours by covering the Flexi-Chambers with an opaque black polyester material bag. During the dark sampling periods, the Flexi-Chambers were left for 1.5 h before the 3 h sampling session to ensure steady state respiration rates that were consistent with prolonged maintenance in darkness (Appendix 2).

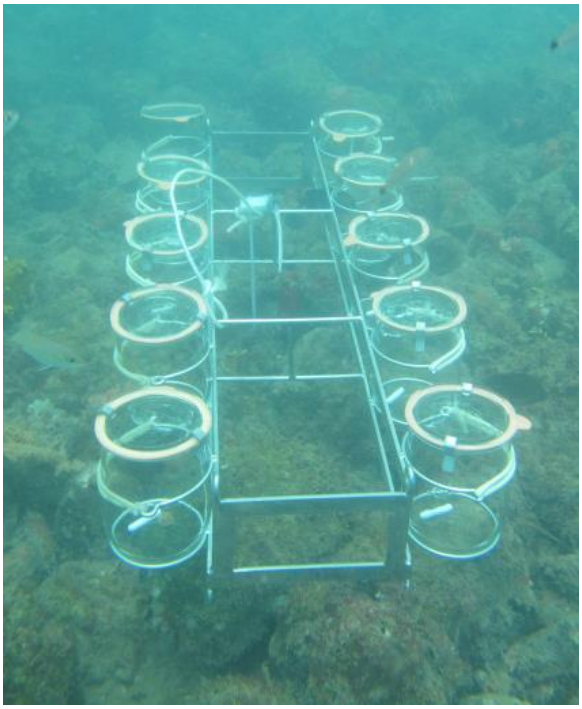


Figure 2.6| Example of a traditional incubation procedure. A photograph of the glass respirometery chambers on the metal frame used in incubations in Salvador, Brazil.

2.2.5| Statistical analysis

Discrete water sampling regime **–** An independent t-test was conducted to compare pH variability **(**Cv) for sites inside versus sites outside of the lagoons. To establish the extent of spatial variability, the pH of all of the major lagoons was compared using an ANOVA with post-hoc Tukey HDS. To determine the appropriate sampling frequency to capture the mean Cv in pH of highly variable habitats, the values of mean pH and Cv were compared using an ANOVA with post-hoc Tukey HDS for different sampling frequencies (diurnal data (2-points), data collected every 6 h (4-points), 4 h (6-points), 3 h (8-points), 2 h (12-points) and 1 h (24-points). Coefficients of variation (C_V) was determined as:

$$C_V = \frac{\sigma}{\mu} \qquad [17]$$

Where σ is the standard deviation and μ is the mean.

All parametric test assumptions were met, with the Levene's test used to check for homogeneity of variance and qq-plots to assess the normality of the data. Tests were conducted in R software (R 237 Development Core Team, 2011).

Verification of pH and TA analytical procedures **–** To verify that the pH measured *in situ* did not shift when re-measured in the laboratory, a Perason's correlation was conducted in R software (R 237 Development Core Team, 2011). *R* and $\bar{X}$ charts for pH and TA were visually assessed to see the placement of sample points on the graph in relation to the warning limits. Finally, the relationship between derived pCO_2 values and measured pCO_2 values were assessed using a linear regression conducted in R.

Flexi-Chamber laboratory validation – Water velocity within the Flexi-Chamber was measured in three planes: X, Y and Z and compared inside and outside of the Flexi-Chamber using a t-test in SPSS 17 (SPSS Inc, 2008). Paired t-tests were used to compare the start and end O_2, CO_2, mean Hobo light data, and zooxanthellae densities to assess whether there were any

differences between the start and end of incubations. The test assumptions for homogeneity of variance (Levine's test) and normal distribution (Sharpiro-Wilk test) of the data were met. To test the integrity of the watertight seals, start and end absorbance for inside the Flexi-Chamber and in the aquarium were compared using a Pearson's correlation conducted in R software (R 237 Development Core Team, 2011). Similarly, temperature inside the respirometry chamber was compared to the surrounding water *in situ* using a Pearson's correlation.

Flexi-Chamber practical application – For the ten *S. cf. stellata* colonies whose P, R and G were compared using both chamber designs, a paired t-test was used to compare each metabolic variable. Differences in mean photosynthesis, respiration and calcification integrated daily rates for the remaining 30 *S. cf. stellata* were compared between the two respirometry chamber designs using an independent t-test.

2.3| Results

2.3.1| Discrete water sampling regime and approach

Wide-scale sampling from the five main lagoons of Little Cayman identified minimal diurnal variability (range: 0.023 pH units) in pH outside of the lagoons, but large pH variability inside of the lagoons (range: 0.474 pH units) independent of site (t_{64} = -5.87, P= 0.001, Figure 2.7). Sites inside of the lagoons all exhibited similar mean and Cv pH with the exception of South Sound (SS), which had lower Cv ($F_{4,61}$ = 3.24, P= 0.05, *post hoc* Tukey, P= 0.05) and mean pH ($F_{4,61}$ = 9.82, P= 0.01, *post hoc* Tukey, P= 0.05).

A 3 h sampling schedule for the high-variability pH habitats resulted in mean pH and Cv pH the same as higher resolution (1 h and 2 h) sampling frequencies (mean pH: $F_{5,12}$ = 268.60, P= 0.001, *post hoc* Tukey, P= 0.001, Cv pH: $F_{5,12}$ = 7.14, P= 0.001, *post hoc* Tukey, P= 0.001, Figure 2.8). Sampling at a 4 h, 6 h, and 12 h frequency did not capture either the daily pH Cv or mean pH with values significantly different to the higher resolution sampling periods (*post hoc* Tukey P= 0. 01, Table 2.2).

The high-resolution sampling around sunrise and sunset indicated that initiating sampling within a 30 min window around sunrise captured the greatest diurnal range in pH (Figure 2.9a). Sampling initiated at sunrise and using the 3 h sampling schedule meant that dusk sampling fell *ca.* 1 h after sunset. Around sunset pH changed rapidly with pH varying by 0.005-0.008 units between 15 min sampling increments (Figure 2.9b).

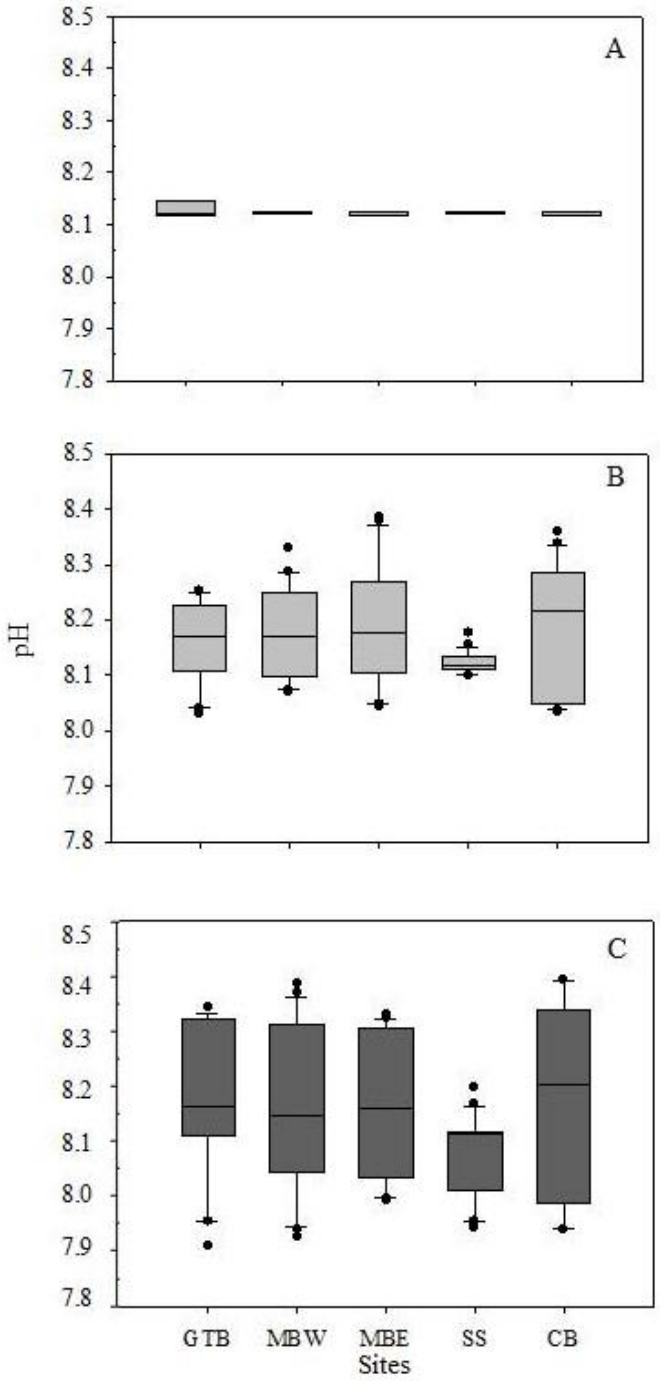


Figure 2.7| Preliminary diurnal pH sampling inside and outside of the five main lagoons around Little Cayman, Cayman Island, BWI: A) sites outside of the lagoons, B) back-reef sites and C) sites inside of the lagoon. Lagoons are identified along the x-axis by their initials: Grape Tree Bay (GTB), Mary's Bay West (MBW), Mary's Bay East (MBE), South Sound (SS), and Charles Bay (CB). Sampling was conducted diurnally (1 h before sunrise and 1 h before sunset) between November 2011 and January 2012. The black-dots represent outliers at the 95 % confidence interval.

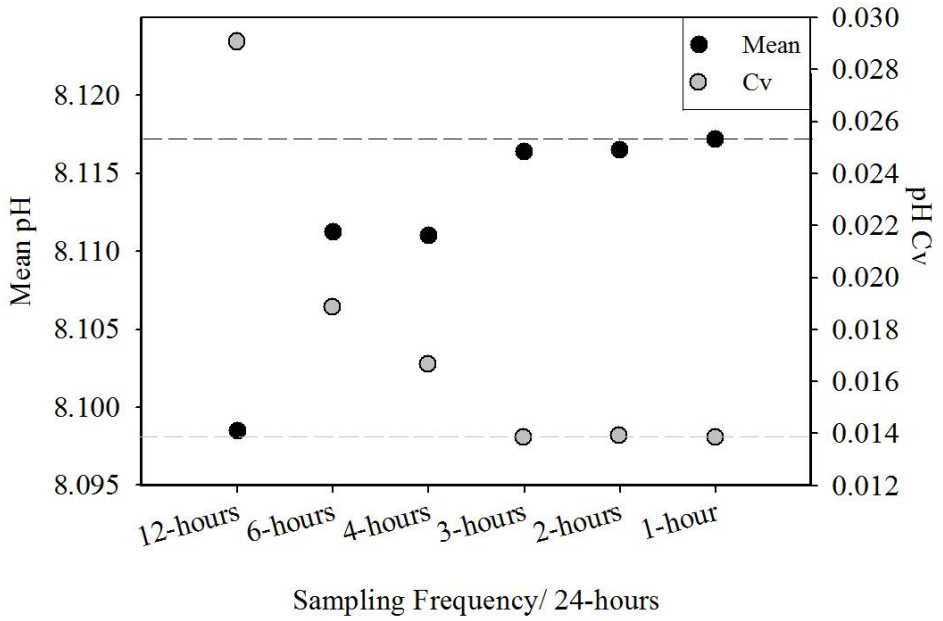


Figure 2.8| A comparison of mean and Cv pH obtained using different sampling frequencies for the high-variability pH habitats. Values for mean and coefficient of variation (Cv) pH were compared for different sampling frequencies: diurnal data (2-points), data collected every 6 h (4-points), 4 h (6-points), 3 h (8-points), 2 h (12-points) and 1 h (24-points). Data was collected from the seagrass of Grape Tree Bay located on the north coast of Little Cayman, Cayman Islands, BWI, and is averaged for three 24 h replicates. The grey dashed lines indicate when sampling points level out and there was no significant difference between mean and Cv pH values.

Table 2.2| ANOVA with post hoc Tukey HDS results for the frequency of sampling pH in highly variable pH habitats.

Variable	ANOVA	Comparison Group (Sampling Frequency)	Post hoc *P*-value
Mean pH	$F_{5,12}$= 268.60	12h Vs 6h	0.0010***
	P= 0.001	12h Vs 4h	0.0010***
		12h Vs 3h	0.0010***
		12h Vs 2h	0.0010***
		12h Vs 1h	0.0010***
		6h Vs 4h	0.9941
		6h Vs 3h	0.0020**
		6h Vs 2h	0.0001***
		6h Vs 1h	0.0010**
		4h Vs 3h	0.0010**
		4h Vs 2h	0.0020**
		4h Vs 1h	0.0010**
		3h Vs 2h	0.0727
		3h Vs 1h	0.6326
		2h Vs 1h	0.6327
pH Cv	$F_{5,12}$= 7.14	12h Vs 6h	0.0010***
	P= 0.001	12h Vs 4h	0.0010***
		12h Vs 3h	0.0010***
		12h Vs 2h	0.0010***
		12h Vs 1h	0.0010***
		6h Vs 4h	0.0010***
		6h Vs 3h	0.0010***
		6h Vs 2h	0.0010***
		6h Vs 1h	0.0010***
		4h Vs 3h	0.0010***
		4h Vs 2h	0.0010***
		4h Vs 1h	0.0010***
		3h Vs 2h	0.9988
		3h Vs 1h	0.9976
		2h Vs 1h	0.9921

*** indicates a significant statistical test with p<0.001,
** indicates a significant statistical test with p<0.01,
* indicates a significant statistical test with p<0.05.

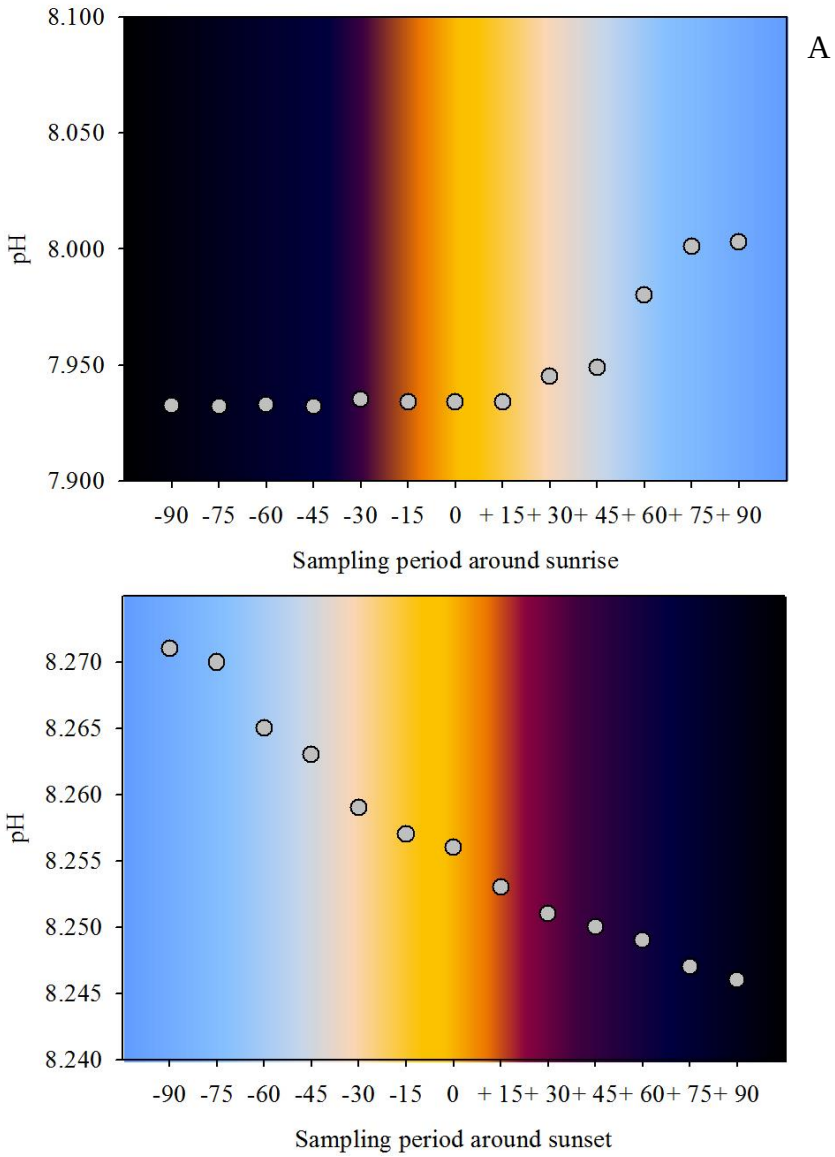


Figure 2.9| pH variance around: A) sunrise and B) sunset for a seagrass habitat on Little Cayman, Cayman Islands, BWI. pH was measured at 15 min increments around sunrise and sunset to determine if mean pH and coefficient of variation (Cv) of pH was captured by initiating sampling at sunrise. Data was collected from the seagrass of Grape Tree Bay located on the north coast of Little Cayman, Cayman islands, BWI, and is averaged for three 24 h replicates.

2.3.2| Carbonate chemistry determination

pH measured in the laboratory was similar to pH measured in the field (r= 0.999, n= 72, P= 0.001). The absolute difference (R) charts for TA and pH showed that all values were within the UWL apart from one TA measurement (Figure 2.10). The $\bar{x}$ charts showed that all pH measurement fell within the LWL with one exception that fell between the LWL and LCL (Figure 2.11). Derived values of pCO_2 (from pH and TA) were similar to direct measurements of pCO_2 (r^2= 0.998, n= 72, P= 0.01, 1:1 ratio, Figure 2.12), however directly measured values were consistently higher (average ± SE; 5.5 ± 0.4 μatm). Samples analysed directly were able to detect the diurnal range in pH, demonstrating the system's ability to measure pCO_2 at a variety of ppm and at different *in situ* temperatures (Figure 2.12).

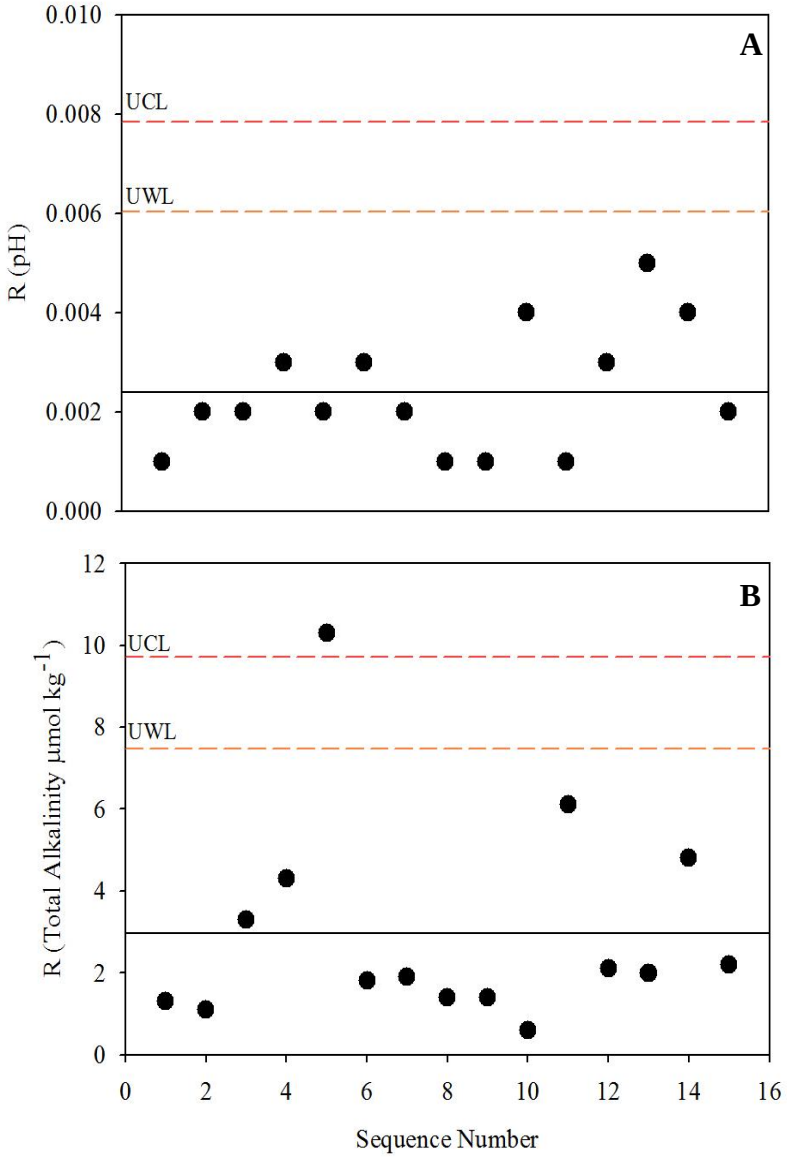


Figure 2.10| The absolute differences (*R*) charts for: A) pH and B) Total Alkalinity. The *R* charts have 15 duplicate measurements plotted sequentially to evaluate precision of the measurement prcoess. The Upper Control Limits (UCL) and Upper Warning Limits (UWL) were calcuated as: UCL = 3.267 $\bar{R}$, UWL = 2.512 $\bar{R}$, where $\bar{R}$ is the average range between sequential measurements. All but one total alkalinity measurement fell within the UWL, showing that the sampling is in accordance with CDIAC protocols.

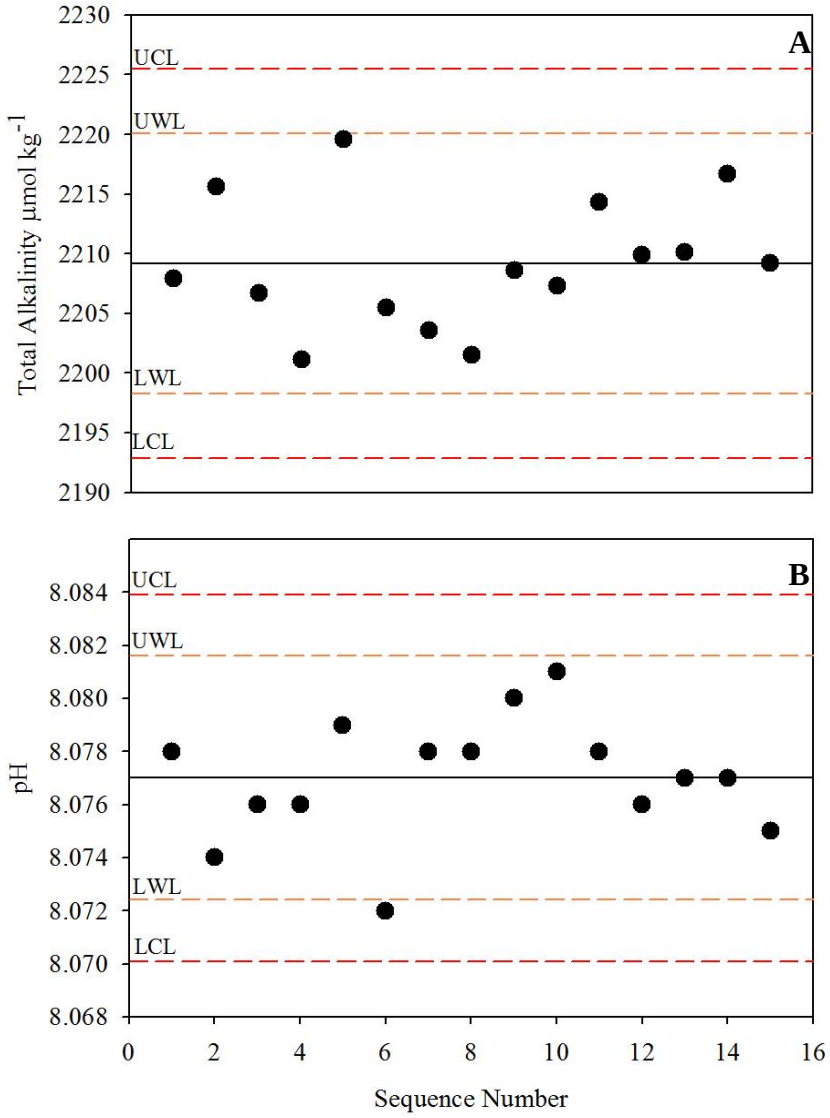


Figure 2.11| The $\bar{x}$ charts to evaluate stability of: A) Total Alkalinity and B) pH. The $\bar{x}$ charts have 15 repetitive measurements of a control sample taken over 15 days, to evaluate the stability of the measurement process. The Upper Control Limits (UCL), Upper Warning Limits (UWL), Lower Control Limits (LCL), and Lower Warning Limits (LWL) were calcuated as: UCL= $\bar{x}$ + 3 *s*, UWL= $\bar{x}$ + 2 *s*, LWL= $\bar{x}$ – 2 *s*, and LCL= $\bar{x}$ – 3 *s*, where $\bar{x}$ is the mean value and *S* is the standard deviation. All but one pH measurement fell within the LWL, showing that the sampling is in accordance with CDIAC protocols.

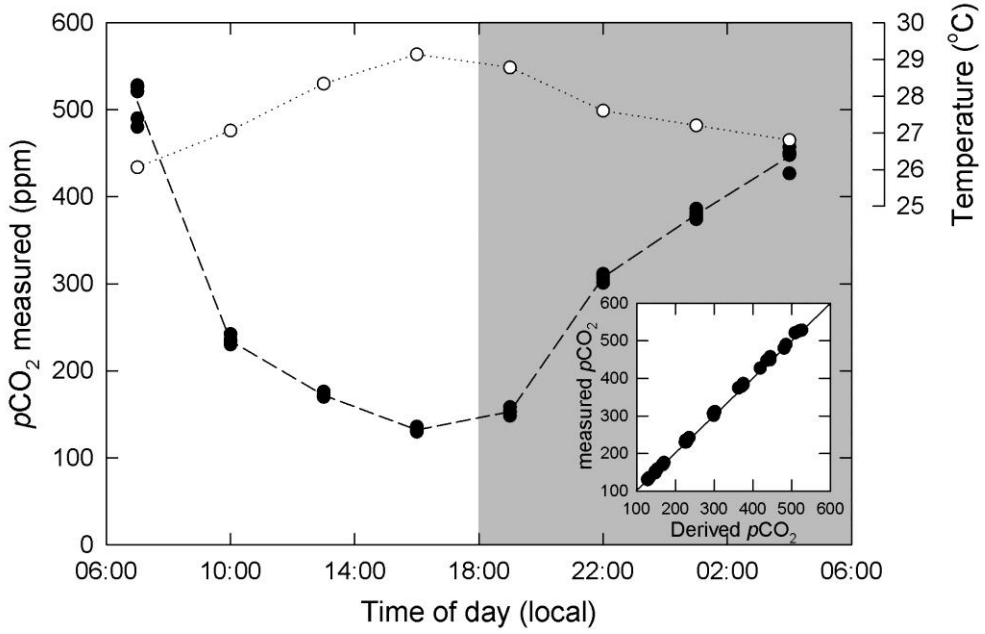


Figure 2.12| Comparison of derived and directly measured pCO_2 values. Direct values of pCO_2 were obtained from a custom-built gas diffusible membrane attached to an external infrared gas analyser and compared to pCO_2 calculated in CO2SYS using total alkalinity and pH. The two methods demonstrated high co-variability between derived and measured pCO_2 r^2= 0.998, n= 72, P= 0.01. The direct values of pCO_2 were able to detect the diurnal variability in pH of the seagrass habitat.

2.3.3| Flexi-Chamber method validation

Water extraction – A 0.5 % error was induced onto the metabolic rate (P, G, R) measurements per ml of water not re-extracted from the Flexi-Chamber. From the 30 replicates, the range of water volumes unaccounted for in the syringe removal ranged from 0.5 – 5 ml of water, with an average of 2.0 ± 0.4 ml. Consequently, the average error of the system is 1 % but never >2.5 %), based on the water extraction procedure.

Temperature – Temperature for the first 3 h of the 8 h laboratory test remained within 0.1 °C of the ambient temperature, however, after 4 h the temperature within the Flexi-Chamber started to rise considerably, with a 1.4 °C increase relative to the surrounding water after 8 h (Figure 2.13). The temperature of the Flexi-Chamber *in situ* exhibited high co-variability with the surrounding water temperature (r= 0.991, n= 947, P= 0.001). There was no measurable temperature difference between the Flexi-Chamber and *ex situ* water during the day, however, during the hottest period of the day (12.00-17.00 h) the temperature in the Flexi-Chamber increased up to 0.3 °C greater than the surrounding water.

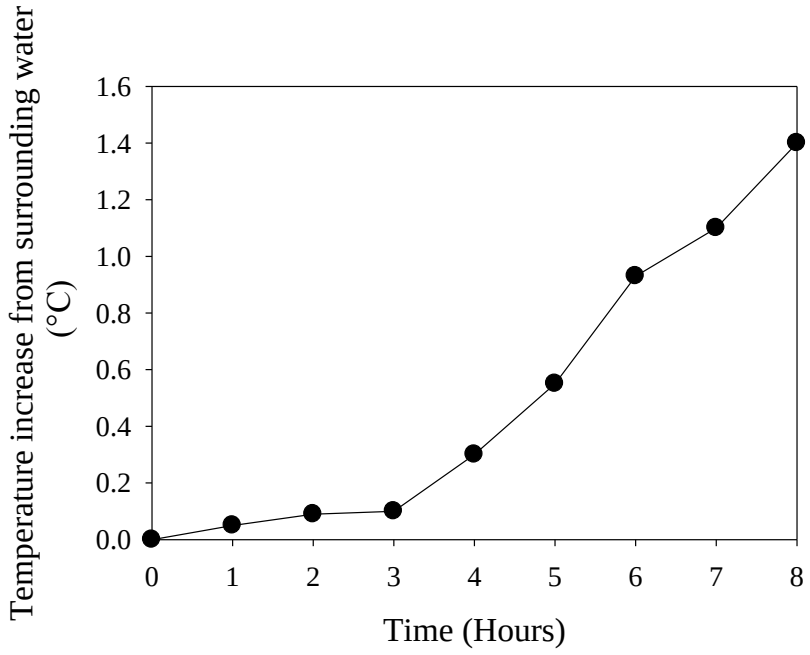


Figure 2.13| The internal temperature of the Flexi-Chamber relative to the surrounding water. Over an 8 h period, the temperature inside the Flexi-Chamber was compared to the surrounding aquarium water. The aquarium was at a constant temperature of 28.0 ± 0.9 °C. The graph shows the average temperature change per hour with standard error for the three 8 h replicates.

Water Flow – Overall water acceleration along the three axes (X, Y and Z) within the Flexi-Chamber was between 78.0-80.1 ± 2.0 % of the surrounding water acceleration (Figure 2.13). Despite the overall reduction in water acceleration experienced inside the Flexi-Chamber, there were no detectable differences between each individual plane of movement. The undulations visible on Figure 2.14b represent periods of restricted water movement within the Flexi-Chamber relative to the ambient water, which is represented by a smoother contour in Figure 2.14a. The greatest loss of movement was in the X dimension due to the way in which the HOBO logger was tethered and the Flexi-Chamber fixed to the substratum.

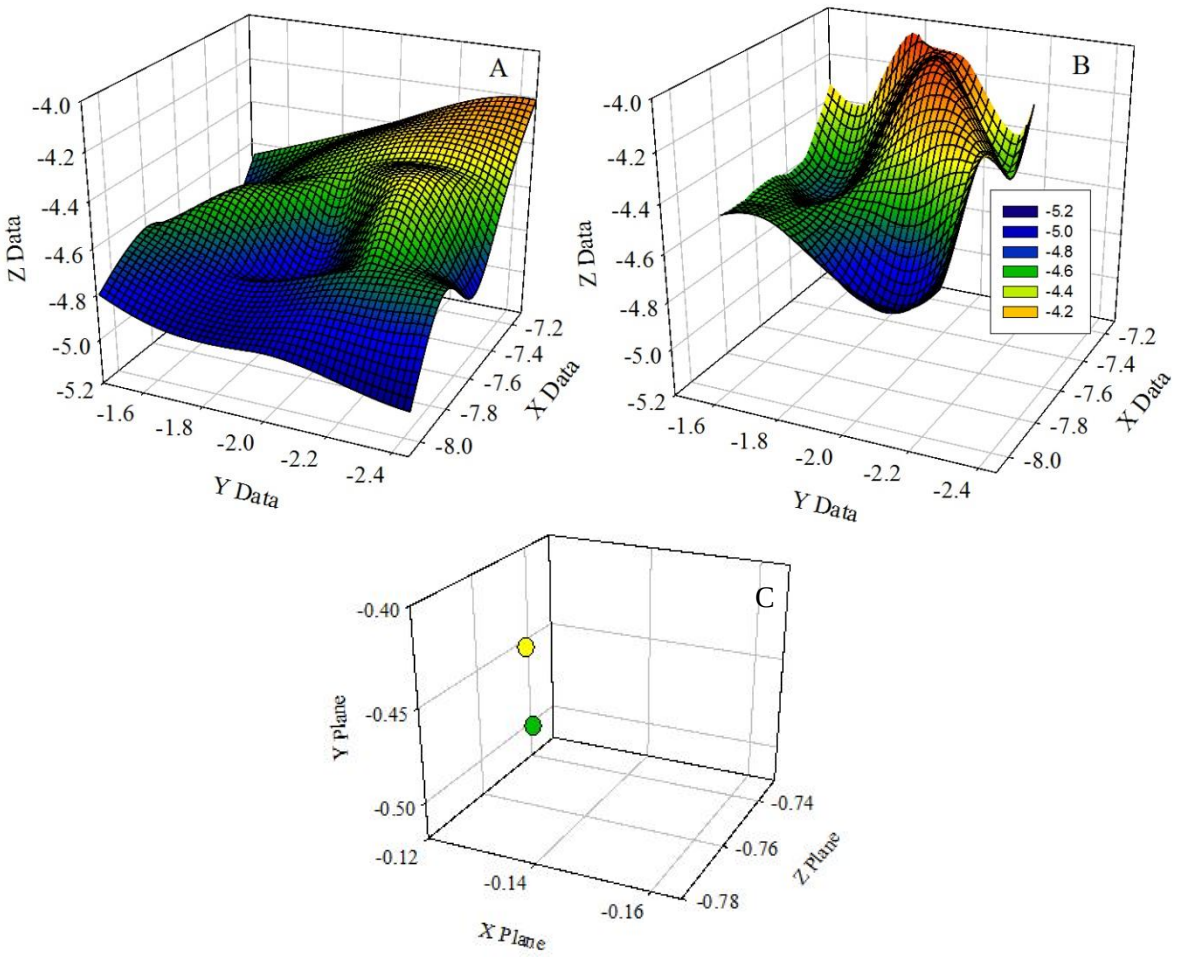


Figure 2.14| Mesh-contour graphs comparing the water acceleration (m/s^2) along the X, Y and Z planes inside and outside the Flexi-Chambers. The graphs show the location of each HOBO in 3D space over the one hour incubation time. At each logging interval (1 s) an X, Y, Z coordinate was generated and is plotted onto the graph. Graph A shows the external water acceleration; graph B shows the water acceleration within the Flexi-Chamber measured with a Hobo Pendant G data logger. Graph C is an example of how graphs A and B were generated. Each square on graph A and B is one data point consisting of an X, Y and Z coordinate. For example, the yellow dot on graph C shows that at that logging interval the HOBO had the following coordinates: Y = -0.45, X= -0.12, Z= -0.74. One second later, the HOBO had accelerated to the location indicated by the green dot and had the coordinates: Y= -0.50, X= -0.12, Z= -0.74. Over the one hour incubation all the acceleration points were plotted and form the mesh contour graphs shown in figures A and B.

Light – Light transmission through the Flexi-Chamber was independent of wavelength within PAR and averaged 84 % (Figure 2.15); transmissions for the major wavelengths of blue, green and red were: 83.7 ± 0.2 %, 83.8 ± 0.1 %, and 85.8 ± 0.2 % respectively. However, there was greater loss of light within the UV range (64 % and 30 % for UVA and UVB respectively).

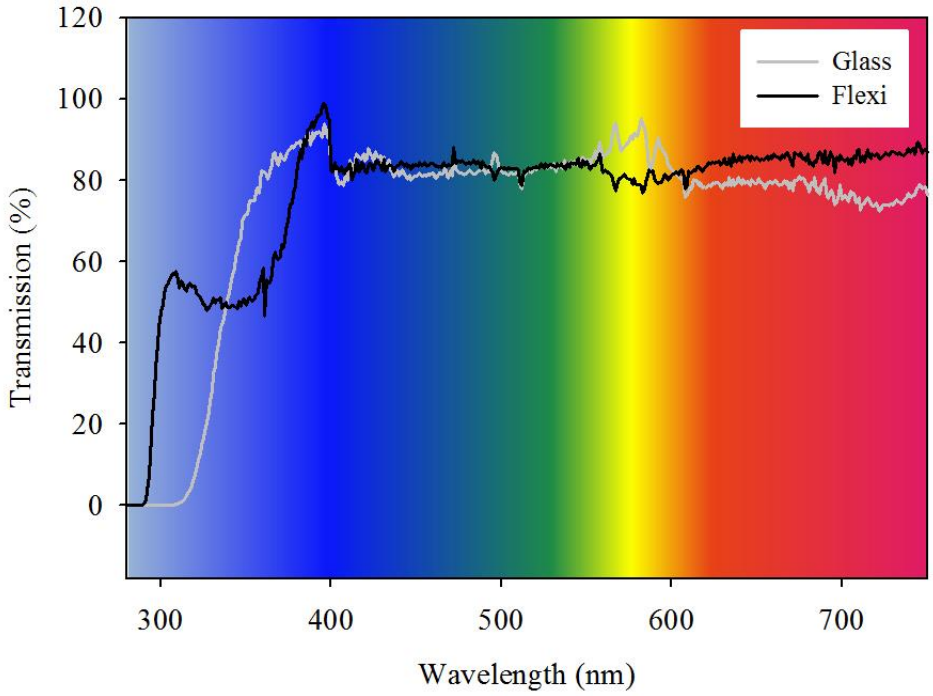


Figure 2.15| The percent light transmission through the Flexi-Chamber and a traditional glass respirometry chamber. Transmission was tested using a spectroradiometer for PAR and UVB and UVA light.

Leakage testing –A strong correlation between the start and end absorbance both inside and outside of the Flexi-Chamber suggested none-to-minimal transfer of dye between the Flexi-Chamber and surrounding aquarium water (*r* ranging from 0.240-0.200, *n*= 2048 per replicate, *P*= 0.01), thus confirming that a watertight seal was created.

Gas permeability – No detectable difference was measured in the mean (SE) O_2 (168 ± 0.2 μmol) and CO_2 (321 ± 11 ppm) levels inside the Flexi-Chamber for any of the replicates over the 3 h incubation period.

Oxygen toxicity – Hypoxia occurred for all colonies kept within 250 ml of water within 1 h of sampling (Table 2.3a). Subsequent hourly sampling showed that all samples surrounded by 500 ml of water showed hypoxia at 2 h (and 750 ml at 3 h). For the volumes that were not super-saturated after 3 h (1000 ml, 1250 ml and 1500 ml), a detectable change in metabolism was identified. As with hypoxia trends, anoxic conditions were observed under dark conditions for all colonies incubated in 250 ml and 500 ml of water within 1 h (and two colonies incubated in 750 ml anoxic within 3 h) (Table 2.3b). From these results, it was established that for *Acropora sp.* at the biomass used, an optimum volume of 1000-1250 ml of water was necessary to ensure a detectable change in metabolic response without hypoxia during daylight hours or anoxic conditions during darkness.

Table 2.3| Anoxic and hypoxic sensitivity testing for *Acropora sp*. in the Flexi-Chamber.

Table 2.3a| Oxygen hypoxic conditions (O_{2Sat}) and detectable metabolic change (Δ_{Met}) for internal water volume of the Flexi-Chamber during light conditions for *Acropora sp*.

Volume of water within the Flexi-Chamber	Water sample collection (Time in hours)								Decision
	1		2		3		4		
	O_{2Sat}	Δ_{Met}	O_{2Sat}	Δ_{Met}	O_{2Sat}	Δ_{Met}	O_{2Sat}	Δ_{Met}	
250 ml	+	+	+	+	+	+	+	+	X
500 ml	±	+	+	+	+	+	+	+	X
750 ml	±	+	±	+	+	+	+	+	X
1000 ml	–	±	–	+	–	+	±	+	✓
1250 ml	–	+	–	±	–	+	–	+	✓
1500 ml	–	–	–	±	–	±	–	+	X

2.3b| Oxygen anoxic conditions (O_{2Sat}) and detectable metabolic change (Δ_{Met}) for the internal water volume of the Flexi-Chamber during dark conditions for *Acropora sp*.

250 ml	+	+	+	+	+	+	+	+	X
500 ml	+	±	+	+	+	+	+	+	X
750 ml	–	+	–	+	±	+	+	+	X
1000 ml	–	±	–	+	–	+	±	+	✓
1250 ml	–	–	–	±	–	+	–	+	✓
1500 ml	–	–	–	–	–	–	–	±	X

Results are based on three colonies of *Acropora sp*. (surface area *ca*. 12 cm^2) at each water volume. O_{2Sat} is whether the water is hypoxic (2.3a) or anoxic (2.3b) in oxygen and Δ_{Met} is whether there is a detectable metabolic change (total alkalinity and oxygen). The – indicates a unanimous negative response among the three coral replicates, i.e. no hypoxic/anoxic conditions or no detectable metabolic change, ± indicates a mixed response within the three colonies tested. Finally, + indicates a unanimous positive response, so all colonies hypoxic/anoxic or all colonies showing a measurable metabolic change. The green shaded boxes indicate the sample volumes at 3 h that have a

detectable metabolic change without hypoxic or anoxic conditions.

Additional stress factors – No visual signs of stress (excessive mucus release, loss of pigmentation) were detected for any of the colonies and zooxanthellae concentrations (3.5 ± 0.7 x 10^6 cells cm^2) remained similar between the beginning and end of the incubation.

2.3.4| Flexi-Chamber practical application

The Flexi-Chamber and glass chamber had comparable internal temperatures, visible light transmission and UVA light permeability, but not UVB light penetration or internal water movement. The Flexi-Chamber acted as a neutral density filter for the major wavelengths of blue, green and red and allowed 50 % more transmission of UVB, compared to the glass chamber, which exhibited relatively reduced transmission in the red (Blue: 82.2 %, Green: 84.3 %, Red 77.3 %, Figure 2.15). Furthermore, the Flexi-Chamber provided consistent water movement within the chamber (78.0-80.1 ± 2 % of the external water movement), whereas the glass chamber had zero water flow until the one-minute continuous hourly stirring was applied (89.2-131.0 ± 4 % of the external water movement). Daily integrated rates of photosynthesis, respiration and calcification showed no significant statistical difference for the glass and Flexi-Chamber across the ten replicate colonies of *S. cf. stellata* (Figure 2.16). Similarly, the ranges in rates obtained for the 15 colonies measured in the Flexi-Chamber versus the 15 colonies measured in the glass chamber were not statistically different.

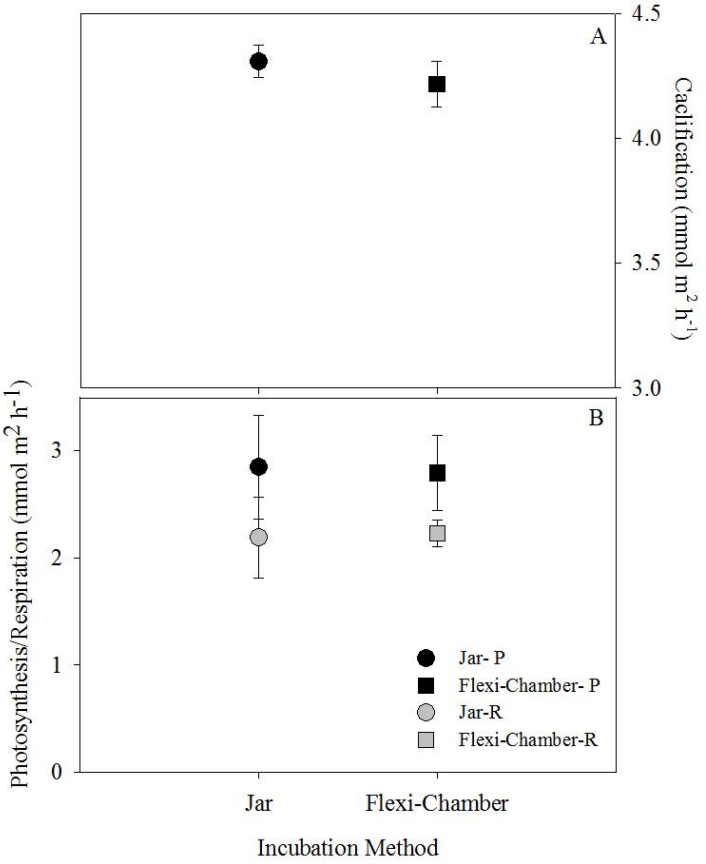


Figure 2.16| Physiological measurements for *Siderastrea cf. stellata* in Salvador Brazil using a conventional glass chamber and the Flexi-Chamber. A) Mean calcification (G) rates and B) mean photosynthesis and respiration rates for 30 colonies of *S. cf. stellata* are plotted with standard error (*n*= 10). Colonies were sampled from a site at the entrance of Todos os Santos Bay, Salvador, Brazil during March-April, 2014.

2.4| Discussion

Techniques developed in this chapter are used repeatedly throughout the thesis (Table 2.4) and therefore the extensive methodological development performed for this chapter was essential to ensure the quality and integrity of data collected.

Table 2.4| Summary table of techniques used throughout the thesis.

Chapter	Discrete water sampling	pH measurement	Total alkalinity measurement	pCO_2 derived	pCO_2 directly measured	*In situ* respiro-metry
3	✓	✓	✓	✓	✓	✓
4	✓	✓	✓	✓		✓
5	✓	✓	✓	✓	✓	

2.4.1| Discrete water sampling regime and approach

Evaluation of the pH sampling regime identified a 3 h discrete water sampling schedule as appropriate to capture the mean and Cv in pH. As anticipated, the greatest rates of change in pH occurred around sunrise and sunset. A 1 h window was identified around sunrise where by sampling could be initiated and the overall mean and Cv in pH were not significantly different. Outside of this window pH change occurred rapidly over a short period of time, which could bias the diurnal range measured, demonstrating the importance of using a consistent sampling schedule.

The island-wide pH sampling of Little Cayman demonstrated that inshore areas have higher diurnal variability in pH and elevated mean pH relative to sites outside of the lagoons, which suggest that inshore sites are influenced by local biogeochemical forces (Anthony *et al.*, 2011b; Manzello *et al.*, 2012; Anthony *et al*., 2013). A similar inshore-to offshore gradient has been observed in the Florida reef tract (Manzello *et al.*, 2012) and has been attributed to the

dominance inshore of seagrass beds. Spatially, similar habitats within this study revealed analogous mean and Cv in pH. The inshore results of SS were the only exception, having lower mean and Cv pH compared to all other inner lagoon sites. SS was the only lagoon not dominated by seagrass and thus highlights the potential influence of photoautotrophs on elevating local seawater pH. Prior studies in the field (Manzello *et al*., 2012) and laboratory (Kleypas *et al., 2011*; Anthony *et al.,* 2013) have suggested that photoautotrophs may be able to offset locally the negative impacts of OA. The initial diurnal pH results around Little Cayman suggest that changes in local pH are reflective of the habitat type, however it remains unclear what the capacity is for photoautotrophs to influence local carbonate chemistry, and whether seasonal or lunar cycles influence the extent of the inshore to offshore gradient. Further examination across habitats, over longer temporal scales and at higher resolution will be necessary to address these questions and is conducted in Chapter 3.

2.4.2| Carbonate chemistry determination

Following CDIAC protocols, the methods employed generated pH and TA measurements that had an accuracy and precision acceptable for the sampling undertaken. Importantly, the two outliers on the *R* and $\bar{x}$ charts confirm the importance of repeated measurements and updating the control charts throughout sampling. Similarly, the derived and directly measured pCO_2 levels were comparable, adding confidence to the use of CO2SYS to derive the other carbonate parameters.

2.4.3| The Flexi-Chamber

A novel method of adapting transparent urine bags to incubate corals *in situ* was developed and tested. *In situ* non-destructive incubations provide several benefits including: (i) the opportunity to study corals in their natural environment, (ii) minimising stress on the organism, (iii) eliminating the need to remove corals from their natural environment, and (iv) potentially providing more accurate results without the artefacts of colony removal. In addition to these benefits, the Flexi-Chamber overcomes some of the limitations of traditional respirometry chambers:

Firstly, due to its non-rigid design the Flexi-Chamber is able to move with the ambient water flow which allows internal mixing within the chamber, thus removing the need for cumbersome artificial mixing (e.g. via stir bars and magnets (Davies, 1980) or via semi-continuous automatic flushing systems (McCloskey *et al*., 1978)). The boundary layer must be disrupted to prevent a decrease in passive diffusion and to ensure continued advective exchange (Lesser *et al.,* 1994; Baird & Atkinson, 1997; Schutter *et al.,* 2011).

Secondly, the Flexi-Chamber size can be adjusted to help establish the appropriate volume of incubation medium relative to the test colony size, which is important in minimising toxicity via hypoxic or anoxic conditions (McCloskey *et al*., 1978). In shallow environments, similar to those where the respirometry chambers are most often deployed, studies have measured zooxanthellae raising host tissue O_2 levels to >200 % saturation (Dykens *et al.,* 1992; Kuhl *et al*., 1995; DeBeer *et al.,* 2000), and photosynthetically produced O_2 can be catalysed by UV radiation to produce harmful Reactive Oxygen Species (Foote, 1976). Thus, the Flexi-Chamber design helps to minimise these threats by appropriate chamber-incubation size. The *Acropora sp.* sensitivity analysis demonstrated the importance to initially establish the correct

water volume to biomass ratio and incubation time for any given incubation configuration. Before full and final experimental application of the Flexi-Chamber, users should identify the optimal time, coral biomass, and water volume, through such sensitivity analysis to ensure anoxic or hypoxic conditions do not occur for their organism or growth environment of interest. Differences in *Symbiodinium spp*., coral heterotrophy-to-autotrophy balance and local abiotic and/or experimental conditions all have the potential to influence metabolic rates and thus the time taken for anoxic or hypoxic conditions to occur.

In addition, a third advantage of the Flexi-Chamber is the opportunity it provides to establish relatively "high throughput" and therefore, large-scale replication with high temporal and spatial resolution without the need for large and costly infrastructure in deployment and sampling. Importantly, the Flexi-Chamber returned similar coral metabolic data to a conventional glass chamber (Naumann *et al.,* 2013). Consequently, datasets obtained by the two techniques can be compared historically or in the future, thus this new method developed will not detract from the ability to examine longer terms trends in the metabolic function of reef building corals or other benthic organisms. The comparison of the glass and Flexi-Chamber identified differences in their physical properties, however, despite these differences, they still provided similar metabolic rates suggesting that their inherent limitations carry an equal amount of inaccuracy. Whilst this inaccuracy was unable to be measured and benchmarked, the results suggest that the error between approaches is lower than the differences expected between organisms. Further research is needed to benchmark these methods against a 'gold standard' that can fully replicate the *in situ* environment.

Despite the benefits of the Flexi-Chamber there are still some limitations: (i) Only 84 % of incident PAR and 54 % of UV is currently transmitted and it may be possible to source other

materials to enhance light transmission, (ii) some small error is induced from the water extraction methods, (iii) the current 3 L maximum volume of the Flexi-Chamber and the existing attachment method limits the type of colony that can be examined. Although this current volume limitation is similar to traditional glass chambers there are options to significantly increase the Flexi-Chamber through the production of bespoke bags. This would be an important advancement in metabolic analysis since current respirometry methods are limited to smaller colonies, which do not represent the bulk of coral biomass in most classical reef systems (Vollmer and Edmunds, 2011). For example, colonies that have reached a size where they become reproductively active may well have different metabolic characteristics compared to newly-formed smaller colonies. It remains to be seen whether the improvements of the Flexi-Chamber identified here are scalable.

Consideration of the advancements and limitations of the Flexi-Chamber along with the comparative metabolic results of *S. cf. stellata* in Salvador, Brazil, with a conventional glass vessel ultimately demonstrates that the Flexi-Chamber design offers an alternative respirometry chamber for coral metabolic studies. The low-cost, ease of transport and non-destructive technique will allow wider scope for experimentation within this thesis; importantly including sampling where coral removal is prohibited (see Chapters 3 and 4).

2.5| Key Findings

2.5.1| Sampling regime

- A 3 h discrete water sampling schedule captures the diurnal mean and Cv pH across habitats.
- Greatest variability in carbonate chemistry occurs around sunrise and sunset.
- A 3 h sampling regime initiated at sunrise captures the extent of diurnal Cv and mean pH of highly variable seagrass habitats. There is an approximate 1 h window around sunrise where sampling can occur without an influence on the daily mean and Cv pH values recorded.

2.5.2| Sampling protocol

- pH measured in the laboratory was comparable to pH measured in the field.
- TA and pH measurements provided precision and repeatability within CDIAC recommendations.
- Direct and calculated pCO_2 values were comparable.

2.5.3| Novel *in situ* respirometry chamber

- Major Flexi-Chamber characteristics:
    - Acts as a neutral density filter for visible light, and allow 54 % of UV light through.
    - Allows internal water movement in all three planes (*ca*. 80 % of the surrounding water velocity).
    - Gas impermeable and watertight seal.
    - Maintains internal temperature (within 0.1 °C of surrounding water temperature) with regular 3 h flushing.
- The Flexi-Chamber produced comparable photosynthesis, respiration and calcification rates for *Siderastrea cf. sp.* as compared to a traditional glass chamber.
- The Flexi-Chamber provides an alternative *in situ* method to traditional established glass/plexi-glass respirometry vessels.

Chapter 3| Biological versus abiotic control of coral habitats and the metabolic expenditure of corals living within them

Part of this chapter is in final draft for Marine Ecology Progress Series as the manuscript: Coral recruitment into non-reef habitats: Consideration for coral refugia.

3.1| Introduction

Shallow reef and non-reef habitats are highly dynamic, where reef bathometry (Ohde & Woesik, 1999), local benthic composition (Hoegh-Guldberg *et al.*, 2007; Manzello *et al.*, 2012; Anthony *et al*., 2013), and tidal cycles (Ohde & Woesik, 1999; Manzello *et al.*, 2012) can create natural oscillations in pH and temperature. The scale of fluctuations is habitat and/or site specific (e.g. reef-flat versus seagrass/mangrove, Ohde & Woesik, 1999; Manzello *et al.*, 2012; Yates *et al*., 2014), with corals within these systems frequently experience periods of pH (Manzello *et al.*, 2012; Price *et al.*, 2012) and temperature (Manzello *et al.*, 2012) already considered representative of future reef conditions under IPCC climate change scenarios (Guadayol *et al.*, 2014). Therefore, the impact of future ocean acidification (OA) on these habitats remains unclear and they are increasingly becoming an important 'natural laboratory' (Okazaki *et al.*, 2013) as they allow scientists to examine what range of environmental conditions corals can survive under, and at what physiological cost.

The body of literature on *in situ* carbonate chemistry variability within different coral habitats is growing; however, there are still significant gaps in our understanding that need to be addressed for us to better manage the impacts of climate change on coral reef ecosystems. Primarily, coral habitats that experience large pH variability need to be assessed using a holistic

approach to examine their physiochemical and benthic properties, and to evaluate the physiological cost for coral species persisting there. More specific gaps include addressing the extent of ecophysical variability over different temporal scales (e.g. seasonal, lunar tidal cycle, and diurnal) and assessing the sampling timeframe necessary to capture this variability. In order to address these gaps, the following questions need to be answered: (i) what is the frequency and diversity of coral recruitment into these highly variable environments? (ii) Is there a physiological cost for corals to expand their niches into non-reef environments? (iii) What resolution of sampling is necessary to obtain robust measures of coral physiology? Consequently, the objectives of this chapter are to:

1) Measure how the key physiochemical properties of reef and non-reef habitats change over temporal scales (seasonal, lunar tidal cycle, diurnal);
2) Test whether a five-day resolution of sampling captures the mean and variability of the key physiochemical properties within a given season;
3) Measure the influence of benthic and biogeochemical drivers on local carbonate chemistry;
4) Quanitfy what the established and recruited coral populations of reef and non-reef habitats are;
5) Test whether photosynthesis, respiration and calcification rates are stable for a coral species during the daytime or nighttime and thus, what sampling frequency is necessary to capture coral metabolic activity.

In addressing these objectives, the data obtained will contribute important information on the extent of natural spatial and temporal variability in carbonate chemistry of reef and non-reef habitats. It will explore the ability of corals to expand their niches into highly-variable

habitats, and the potential susceptibility of different habitats to future OA. In addition, the resolution of daily sampling necessary to capture seasonal trends in the carbonate chemistry and the daily metabolic activity of corals will be determined, which will inform the methods employed in Chapters 4 and 5.

3.2| Materials and Methods

3.2.1 | Study location

Little Cayman, Cayman Islands, British West Indies (BWI) was used as the study location to explore how the key physiochemical properties of reef and non-reef habitats change over temporal scales, and to assess the differences in their local benthic community composition. Six non-reef sites (three back-reef and three seagrass) and three outer-reef sites were established where seawater carbonate chemistry varied but coral colonies were still present (Figure 3.1). The non-reef sites were situated within lagoons, whilst the outer-reef sites were situated outside the lagoons on the outer-reef. The back-reef habitat consisted of inter-dispersed seagrass and small patch reefs (15-30 % cover of seagrass) and was not a true reef, thus is referred to as a non-reef habitat within this chapter. All sites were sub-sampled three times, with each sub-sample a minimum of 50 m apart from one another (Figure 3.2). All sites were situated away from any freshwater inputs.

The study sites were located in bays known locally as Grape Tree Bay and Mary's Bay on the north coast of Little Cayman. Mary's Bay is the largest lagoon covering almost 50 % of the north coast of the island at *ca.* 4.3 km long and *ca.* 0.2 km wide, with depths ranging from 0.5-3.0 m. More than 50 % of Mary's Bay is designated as a Replenishment Zone (see Chapter 1, section 1.6.1). Grape Tree Bay is *ca.* 2.3 km long by *ca*. 0.15 km wide, and ranges in depth from 0.5-3.0 m. The bay gently slopes from east to west with more than 50 % of the bay designated a Marine Protected Area.

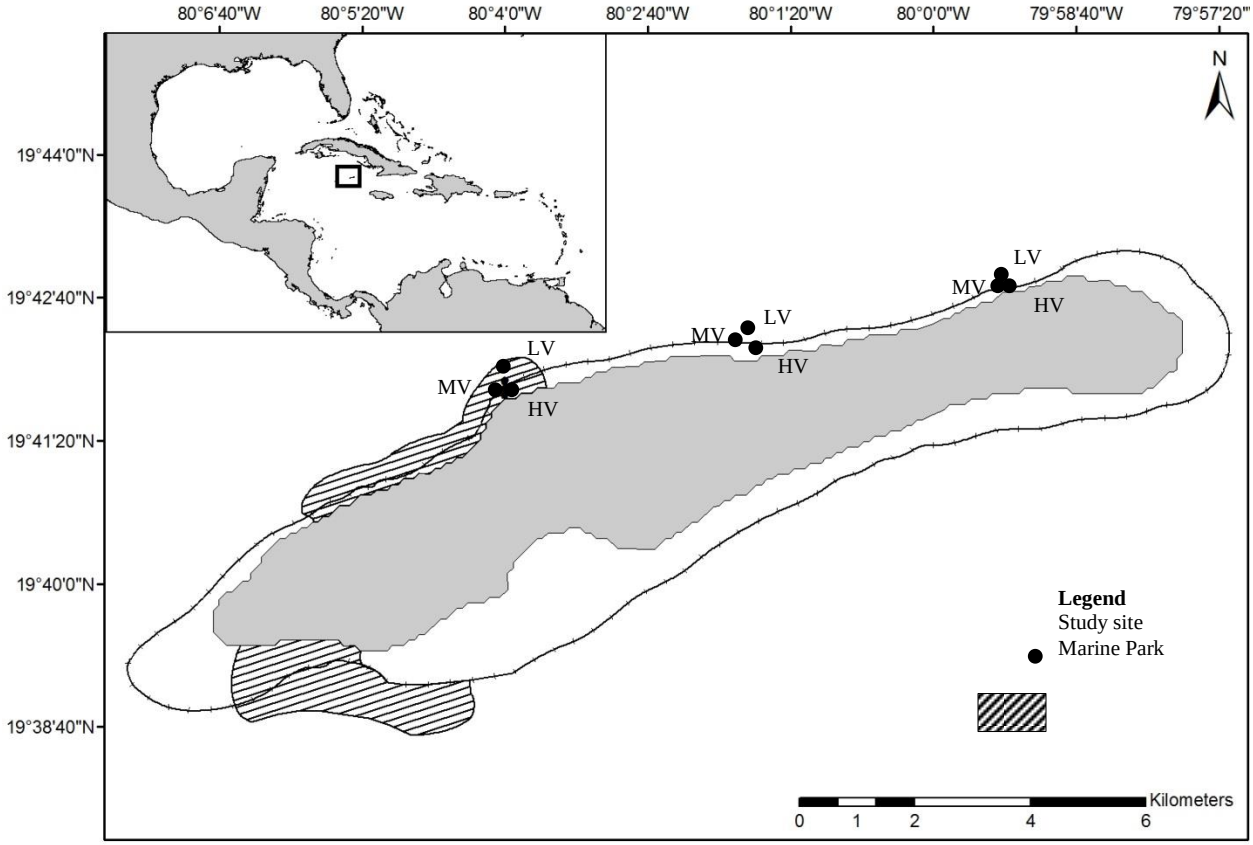


Figure 3.1| A close-up map of Little Cayman and a map of the Cayman Islands within the wider Caribbean region. The Cayman Islands are located 145 km south of Cuba and are made up of three islands. Little Cayman is the smallest of the three islands (17 x 2 km) and is shown as the large grey island. The nine study sites were all located on the north coast, with three high-variability (HV) sites situated in the seagrass habitat, three medium-variability sites (MV) located within the back-reef habitat and three low-variability (LV) sites located on the outer-reef subject to open-ocean chemistry.

Figure 3.2| An example from Grape Tree Bay of the sub-sampling regime used in Little Cayman, Cayman Islands, BWI. All sites were sub-sampled as illustrated. Habitat type is shown by data symbol: outer-reef= black squares, the back-reef= black triangles and the seagrass= black dots). Each sub-site was a minimum of 50 m apart.

3.2.2| Abiotic assessment

Temperature and light **–** Temperature (°C, accuracy ± 0.53 ºC) and Light (Lux, accuracy relative to light levels: see Onset, 2012) were measured using a HOBO Pendant Temperature/Light 64k Logger (Model UA-002-64, Microdaq, USA). One HOBO was located in each of the six non-reef study sites, and one in the outer-reef. Each HOBO was screwed face-up onto a 20 cm length of PVC piping which was placed over a galvanized nail and epoxied into position. The HOBOs were set to log temperature and light every 5 min. The HOBOs were installed in January 2012 for 20 months. The HOBOs were cleaned and downloaded monthly *in situ* using a Waterproof Shuttle Data Transporter Optic Base Station with Couplers (Model U-DTW-1, Microdaq, USA). Due to fouling on the HOBO sensor, light quality decreased by *ca.* 11-34 % after the first ten days *in situ*, so only data from this initial ten-day period was used. Light was measured in Lux and converted to PAR by diving Lux by the appropriate constant (*as per* Long *et al*., 2012). Light spectrum data (*see* Hennige *et al*., 2010) ranging from the main reef to the non-reef

habitats was compared to the spectrum data for each coefficient to determine the most appropriate constant in the conversion of PAR to Lux. Daily Light Integral (DLI) was also calculated for each site by:

$$\text{DLI} = PAR \cdot (3{,}600 \cdot P)/1{,}000{,}000 \quad [1]$$

Where PAR is μmol m^2s^{-1}, 3,600 is the number of seconds in an hour, *P* is the photoperiod (i.e. the period of time in hours of light exposure per 24 h) and 1,000,000 is the number of μmols per mole.

Nitrates – In the spring of 2013, nitrate sampling was conducted using an ORION Nitrate electrode (accuracy ± 0.01 μM, Model 9307, Fisher scientific, USA) attached to the ORION 5 Star meter. Over a two-week period, seven diurnal (early morning and late afternoon) samples were collected (*n*= 14 total per site) at every study site.

Carbonate chemistry sampling regime and approach – Water samples were collected at the six non-reef sites over a six-week period during the two seasons: a) summer (wet season: July-August) and b) winter (dry season: February-March) (2012-2013). Within each season, three 24 h sampling sessions were conducted around both the neap and spring tides. Seawater samples were collected as described in chapter 2 (section 2.2.2). Sampling was initiated around sunrise, with samples collected from three locations within each site (each 50 m apart), every three hours over a 24 h period (total *n*= 24 per site per sampling day). In addition, three outer-reef sites were sampled twice daily with samples collected from three sub-sites within each site (each 50 m apart, total *n*= 6 per site per sampling day)

3.2.3| Benthic measurements

To assess differences in the highly variable non-reef habitats, a series of benthic assessments were conducted:

Benthic community composition – To determine the percentage cover of the major benthic taxa, continuous line intercept transects were conducted in February 2012 and February 2013. Within each site, 3 x 50 m permanent transects (marked by rebar at either end), each separated by a minimum of 50 m were established. The initial transect was randomly located, and transects ran parallel to shore. Transects were completed in *ca*. 5 min and the video camera was held at a distance of 30 cm above the transect tape. Data were recorded using a high definition video-camera (G12, Canon, Amazon, England) in an underwater housing (WP-DC 34, Canon, Amazon, England) and footage was later analysed to determine benthic community composition (species level). The GPS location of the start and end of each transect was recorded (Garmin etrex 20, Amazon, England).

Established coral community – At the start of each transect a 20 m^2 quadrat was installed to quantify the established coral (all corals greater than 1 cm) community composition, size-frequency distribution and disease prevalence (visual assessment). Surveys were conducted in February 2012 and February 2013. Every coral colony within the quadrat was counted and identified to species level. Each colony's maximum height, width and diameter were recorded with callipers or a measuring tape (accuracy ± 1 mm) depending on colony size. If any colonies could not be identified *in situ*, pictures were taken using a digital underwater camera and were later identified with reference materials on land. In addition, corals that were: (i) de-pigmented, and/or showed evidence of, (ii) recent or (iii) past mortality were also recorded (defined by AGRRA protocol version 5.4, Lang *et al*., 2010, Chapter 3, Table 3.1).

Table 3.1| Atlantic and Gulf Rapid Reef Assessment (AGRRA) mortality definitions

Mortality Type	AGRRA Definition
Old mortality	Is defined as any non-living parts of the coral in which the corallite structures are: (a) covered over by organisms that are not easily removed (e.g., thick turf algae, dense macroalgae, attached invertebrates, possibly including other corals); or (b) the overgrowing organisms (and perhaps the outer corallite structures) have been removed by a scraping herbivore (e.g., the stoplight parrotfish *Sparisoma viride*), or abraded by a storm, exposing the underlying skeleton. The coral's soft tissues are presumed to have died within the previous months-years or decades.
Transitional mortality	Is defined as any non-living parts of the coral in which the corallite structures are slightly eroded at most, unless they have just been bitten by a fish or abraded, and its surface is covered by a thin layer of sediment, or by biofilms of bacteria (including photosynthetic cyanobacteria) and possibly diatoms or other microalgae, or by tiny turf algae. The coral's soft tissues are presumed to have died within the previous day(s)-months.
New mortality	Is defined as the non-living part(s) of the coral in which the corallite (= surficial skeletal) structures are still intact, unless they have just been bitten by a fish or abraded, and the freshly exposed, white surface is free of any sediment, microbial/diatom biofilms, other microalgae, etc. The coral's soft tissues would have died within the previous minutes-several days at most and, in some cases, may not have completely sloughed off the skeleton.

Definitions of Old Mortality, Transitional Mortality and New Mortality are taken from the Atlantic Gulf Rapid Reef Assessment (AGRRA) Protocol Version 5.4 (Lang *et al.*, 2010). These definitions were used to assess mortality in the 20 m^2 quadrats to assess coral community composition and health at each site and habitat in February 2012 and 2013.

Coral recruitment – To assess coral recruitment rates within each non-reef habitat, settlement arrays were deployed in January 2012 for 20 months. Within each of the six non-reef sites, three locations each 50 m apart had three settlement arrays randomly installed. Arrays were spaced a minimum of 5 m apart as per Salinas De León *et al.* (2011). In total, 54 settlement arrays were installed (324 tiles). The settlement arrays were constructed as a modification of Mundy (2000) and Salinas De León *et al.* (2011) (Figure 3.3). Arrays consisted of six unglazed limestone tiles (10 x 10 x 1 cm), as per Schmidt-Roach *et al*. (2008), that had two small holes (3/16 inch) drilled in the centre. The six tiles were zip-tied through the holes to a length of PVC with a 5.0 cm space between each tile. Tiles were labelled one through six for identification. The PVC settlement arrays were then attached to the substrate at either end using two galvanized nails. The diameter of the PVC provided a 2.5 cm gap between the tile and ground substratum, which is known to facilitate coral settlement (Harriott & Fisk, 1987). Each array was labelled with a tag stating that it was for research and letters were sent to the local community and resorts informing them of the study to minimise unwanted human interactions.

Before installation, tiles were conditioned for one month in the shallows of GTB. Tiles were then installed and left *in situ* for one month. At the end of the month, a daytime visual survey was conducted *in situ* of the top-side of every tile, recording any coral spats or coral fragments. A modification of the traditional fluorescence census techniques (Piniak *et al.*, 2005; Baird *et al*., 2006; Salinas De León *et al.*, 2011) was used during the daytime using a flashing blue light (BW-1 Dive Light, Nightsea, Bedford, USA) and yellow barrier filter over a dive mask. This technique allows visual fluorescent detection during the daytime, improving visual census.

After the monthly visual census, tile numbers two, three and six (randomly selected at the start of the study) were removed and replaced with new tiles that had been seasoned for two weeks in seawater. The removal of half of the tiles on a monthly basis allowed the timing and prevalence of recruitment to be investigated. The removed tiles were re-surveyed in the dark within the laboratory using the blue light on a continuous light mode. Tiles were also scanned under a microscope (20 x) (EZ4, Leica, Davie, USA). Any tiles with coral growth were treated for 48 h with chlorine solution to remove organic matter. Tiles were then re-surveyed using the microscope to count and identify any corallites as per Schmidt-Roach *et al.* (2008). Recruits were identified as belonging to the families: *Acroporidae, Agaricidae, Faviidae, Poritidae* or *Siderastreidae* using photomicrographs described by English *et al.* (1997), Babcock *et al.* (2003), Putron (2005), and Putron (2007). The remaining recruits, including any too damaged to identify, were labelled as 'other' (Schmidt-Roach *et al.*, 2011). At the end of the 20-month study, all of the tile arrays were removed and analysed following the same protocol as the monthly analysis.

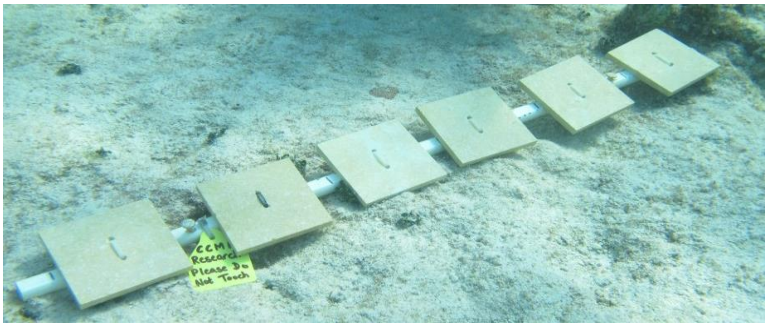


Figure 3.3| Photograph of a settlement array. The settlement array consisted of six unglazed limestone tiles zip-tied 5 cm apart onto a length of PVC. The PVC was secured into the reef substrate at either end using galvanized nails.

To analyse the recruited community composition over the 20 month period, the three tiles (one, four and five) on each array that were left in the water for the duration of the study were analysed using a modification of Coral Point Count with Excel extensions (CPCe) (Kohler & Gill, 2006) (total tiles *n*= 162). A high-definition picture was taken of each tile to allow CPCe analysis. Instead of using the CPCe data input, the data was entered into Excel where defined headings had been pre-determined as suitable for this project. From the CPCe points, the observed percentage covers were determined for each benthic component. The percentage of each polymorph (calcite, aragonite, high Mg-calcite) of calcium carbonate was also determined.

A power analysis was conducted to ensure that the correct point density (number of points per image frame) was used for the CPCe analysis, to ensure robust estimates of percent cover (Appendix 3). The actual percentage cover was determined for three tiles from the back-reef and three tiles from the seagrass. To determine the number of points needed on CPCe to provide an estimate of percentage cover with 95 % confidence of the actual percentage cover, 5, 10, 20, 40, 80, 160 and 320 points were initially overlaid on each image using CPCe. The estimated percentage cover for each number of points was compared to the actual percentage cover using the Chi-squared test (of association) in r (R 237 Development Core Team, 2011). From this analysis it was determined that the optimal point density was 150 (Appendix 3).

The metabolic expenditure of the dominant coral species across habitats – From the coral community composition analysis, the five dominant coral species living within non-reef habitats were identified: *Porites astreoides, Porites divericata, Siderastrea radians, Stephanocoenia intersepta* and *Dichocoenia stokeii*. To assess the metabolic expenditure for these five coral species within each habitat (outer-reef, back-reef and seagrass), *in situ* respirometry analysis was conducted during the summer season of 2013 over three 24 h periods.

Flexi-Chambers were deployed as described in Chapter 2 (section 2.2.4). Sampling began at 7:00 h (sunrise) with incubations running for 3 h (as deemed appropriate from Flexi-Chamber validation in Chapter 2) throughout the 24 h period (*n*= 8 incubations). Sample extraction and analysis followed the procedures outlined in Chapter 2 (section 2.2.4), from which photosynthesis, respiration and calcification daily rates were obtained.

3.2.4| Statistical analysis

All statistics were conducted in either R software (R 237 Development Core Team, 2011), Sigma Plot 10.0 (Systat Software, San Jose, CA), or SPSS 17 (SPSS Inc, 2008). Parametric test assumptions were all met unless stated, with the Levene's test used to check homogeneity of variance and qq-plots to assess the normality of the data.

Abiotic assessment – Linear Mixed Effects (LME) models were applied, with site as a random effect, to examine the seasonal and lunar tidal cycle differences in mean and Cv between habitats for all abiotic variables measured (pH, TA, pCO_2, Ω_{arg}, temperature, salinity, light and water velocity; Appendix 4), with the exception of nitrates. Nitrates were only measured during the summer season and were therefore analysed via a two-way repeated ANOVA with post-hoc Tukey's HDS. Within habitats, differences in abiotic parameters across sites were also compared using a two-way repeated ANOVA with post-hoc Tukey's HDS.

For LME models, Cleavland dot-plots were used to determine outliers and boxplots and scatterplots were used to check for co-linearity within the dataset (Zurr *et al.*, 2010). Assumptions of linearity, independence, homoscedasticity and normality were met. Models were fitted using the lme function in the nlme package in R software (R 237 Development Core Team, 2011). Model simplification was undertaken using ANOVA to compare models with progressively simplified fixed effects, thus ensuring correct *P* values (Crawly, 2007). The

acceptability of the models was tested by plotting the residuals against: (i) fitted values to check for homogeneity and (ii) each explanatory variable in the model (including those dropped during model selection) to check for violations of independence (Zuur *et al.,* 2007). Parameter estimation in LME models was done based on Restricted Maximum Likelihood (REML).

Mean inshore carbonate chemistry (TA, pH, pCO_2 and Ω_{arg}) values were subtracted from the mean offshore values for each sampling period to establish the magnitude of the inshore to offshore gradient (as Manzello *et al.,* 2012). Values of TA and pCO_2 were normalised to salinity (S= 36) to allow a comparison between seasons and sites:

$$nTA = TA*36/S \quad [2]$$

$$npCO_2 = pCO_2*36/S \quad [3]$$

Where *n*TA is total alkalinity standardised by salinity, *S* is salinity, and npCO_2 is pCO_2 standardised by salinity.

A positive gradient indicated that the inshore non-reef sites had a higher value than the outer-reef sites, with a negative value showing the opposite (see Manzello *et al.,* 2012). The magnitude of each gradient was compared across habitats, for each season and lunar tidal cycle again applying LME models.

Diurnal ranges in all abiotic parameters were calculated, with detailed analysis conducted on the diurnal trends in pH of each habitat. Histograms were used to evaluate the typical duration of time per day sites within each habitat spent at a given pH. Each histogram bin represented the percentage of time each site spent at a given pH class (classes each 0.1 pH units) and skewness (G_1) for each habitat was determined. To evaluate whether a low-resolution (5-diel cycles) sampling schedule provided comparable mean and variability (Coefficient of Variation (Cv) determined as described in Chapter 2, section 2.2.5) values of pH, temperature, and TA, to a

high-resolution (18-diel cycles) sampling schedule for a given season, a one-way ANOVA was conducted. Data was compared from the summer season and the 5-diel cycle was randomly selected from the total diel cycles collected.

Biogeochemical controls of habitats – Salinity-normalised TA (nA_T) to dissolved inorganic carbon (nC_T) plots were generated (Suzuki & Kawahata, 2003; Kleypas *et al*., 2011; Yates *et al*., 2014) to assess the biogeochemical control of each habitat. Biochemical processes including photosynthesis (P), respiration (R), carbonate precipitation (G) and dissolution (D) all occur with a predictable gas exchange which can be theoretically modelled (Suzuki & Kawahata, 2003). Calcification decreases TA by two moles for each mole of $CaCO_3$ precipitated. If $CaCO_3$ is precipitated without photosynthesis then the line of the theoretical slope would be two. Photosynthesis lowers DIC whereas respiration increases DIC. Neither process alters TA so in theory the P-R line of the graph could be horizontal. However, NO_3^- and PO_3^{-4} accompanying changes in photosynthesis and respiration resulted in the slight gradient in the line established from both models and experimentation (see Yates *et al.,* 2014). The ratios of net ecosystem calcification to net community production (NEC:NEP) were derived from these nA_T-nC_T plots as:

$$\text{NEC:NEP} = 1/[(2/m)-1] \qquad [4]$$

where m is the regression slope from the corresponding linear regression equation of nA_T vs nC_T) (Suzuki & Kawahata, 2003; Kleypas *et al*., 2011). Finally, the threshold of calcification to dissolution (G-D) was determined. G-D is the level of Ω_{arg} below (and/or pCO_2 above) which dissolution of carbonate sediments exceeds rates of calcification.

Physiochemical controls of non-reef habitats – Finally, to assess any major trends in mean and Cv of the abiotic parameters (Note: nitrates were not included due to the limited

sampling replication), between non-reef habitats, two Principal Component Analysis' (PCA) were conducted. From each PCA, two main components were extracted (PC1 and PC2). PC1 and PC2 scores were compared between habitats and seasons using a two-way ANOVA (as Dumbrell & Hill, 2005).

Benthic composition – Within habitat differences in abiotic parameters were independent of site, thus, benthic analysis focused on habitat differences. Inter-habitat benthic composition was analysed using a MANOVA (Wilk's Lambda) after arcsine transformation with post-hoc Tukey HDS. In the case that the MANOVA was not significant, ANOVA's or t-tests were conducted between each benthic taxa and Bonferroni correction was added to see if any taxa differences existed despite no overall statistical difference detected in benthic composition.

Established coral community – For each habitat, actual and relative percentage coral cover, coral density per 20 m^2, species richness, and Shannon-Wiener Index and Evenness were calculated (equations: 5 & 6) and compared via a two-way ANOVA.

$$H' = -\sum_{i=1}^{S} pi \cdot \ln(pi) \quad [5]$$

$$J \ \frac{H\prime}{HMAX} \quad [6]$$

Where *H'*= Shannon-Wiener Diversity Index, *S*= number of species, *i*= total number of species, *pi* = relative abundance of each group of organisms, *J* = Evenness.

Visual disease prevalence was compared between habitats with a one-way ANOVA and post-hoc Tukey HDS. Size-frequency graphs were created to compare the sizes of coral species within each habitat. The greatest width or length data was used, as per Suggett *et al.* (2012), to ensure some standardisation among colonies with different growth forms.

Coral recruitment – The number of coral spats recorded over the 20-month period were totalled within each habitat and their density per m^2 was calculated. A t-test was used to compare the total density of recruits per habitat, with the three locations within each site used as replication (*n*= 9 replicates per habitat). The diversity of recruits per coral family was determined, and the relative percentage cover of each family was calculated. The relative percentage coral cover per family was compared to the relative percentage coral cover of the established coral community within each habitat using a MANOVA as described for the benthic composition analysis.

Benthic community recruitment – CPCe analysis was used with 150 points to estimate the actual percentage cover of the major benthic taxa (Kohler & Gill, 2006). Actual percentage cover of recruited benthos was compared between habitats using a MANOVA as described for benthic composition analysis. The three locations within each site were used as replication (*n*= 9 replicates per habitat). Similarly, for the calcareous benthic taxa (green, red and brown calcareous algae, corals, serpulid worms, crustose coralline algae and bivalves) the type of calcium carbonate polymorphs were determined and again compared between habitats using a MANOVA.

The relationship between pH and local benthic community composition –A series of linear regressions were conducted to assess the relationship between calcifying benthos (corals, hydrocorals, crustose coralline algae, calcifying algae) and photoautotrophs (seagrass and non-calcifying algae) to mean and Cv pH for each non-reef habitat. pH was the carbonate chemistry parameter used for this assessment as it was directly measured.

The metabolic expenditure of the dominant coral species across habitats – LME models with coral species as a random effect, were applied as described in the abiotic assessment section

to examine the effect of habitat on daily net photosynthesis, respiration and calcification (see Appendix 4). Two linear regressions were run to compare mean and Cv pH to G rates. Finally, two-way ANOVA's were used to assess if there were differences in photosynthesis, respiration and calcification between each 3 h sampling period (n= 8 per day).

3.3| Results

3.3.1| Abiotic assessment

Non-carbonate chemistry parameters – All non-reef sites experienced greater variability in the abiotic parameters measured compared to the outer-reef sites (all values of $P = 0.01$, see Appendix 7 for *t* values). Between the two non-reef habitats, no differences in mean or Cv were evident between any of the measured non-carbonate chemistry abiotic variables, with the exception of salinity and light intensity (Figure 3.4). In the seagrass habitat, salinity was more variable ($P = 0.001$, *t*= see Appendix 7) and had lower mean values ($P = 0.01$, *t*= see Appendix 7) relative to the back-reef (Table 3.2). Light intensity was elevated during the summer season in the seagrass habitat, where the mean DLI was *ca.* 2.0 mol m^2d^{-1} greater than the back-reef ($P = 0.01$, *t*= see Appendix 7, Table 3.3). Seasonally, mean temperature and water velocity were elevated during the summer season independent of habitat (Temperature: $P = 0.001$, Water velocity: $P = 0.01$, *t*= see Appendix 7, Table 3.1). Salinity was also more variable in the seagrass habitat during the summer season ($P = 0.01$, *t*= see Appendix 7).

Table 3.2| Summary table of the physiochemical characteristics of the outer-reef, back-reef and seagrass habitats on Little Cayman, Cayman Islands, BWI

Abiotic Variable	Season	Outer-reef	Back-reef	Seagrass
pH	Summer (Wet)	8.111 ± 0.01	8.165 ± 0.03	8.130 ± 0.02
(total scale)	Winter (Dry)	8.115 ± 0.01	8.200 ± 0.02	8.188 ± 0.04
Total alkalinity	Summer (Wet)	2373 ± 2.31	2257 ± 3.82	2247 ± 3.82
(μmol/kgSW)	Winter (Dry)	2309 ± 3.74	2236 ± 6.21	2231 ± 1.64
pCO_2	Summer (Wet)	320 ± 1.71	280 ± 1.71	290 ± 1.83
(μatm)	Winter (Dry)	319 ± 2.88	220 ± 1.72	247 ± 2.23
Ω_{arg}	Summer (Wet)	3.5 ± 0.01	4.3 ± 0.01	4.0 ± 0.02
	Winter (Dry)	3.5 ± 0.02	5.2 ± 0.02	5.1 ± 0.02
Salinity	Summer (Wet)	35.5 ± 0.01	35.5 ± 0.03	35.0 ± 0.02
(ppm)	Winter (Dry)	35.5 ± 0.01	35.4 ± 0.03	35.0 ± 0.03
Temperature	Summer (Wet)	28.6 ± 0.02	29.8 ± 0.12	30.0 ± 0.19
(°C)	Winter (Dry)	27.3 ± 0.09	26.9 ± 0.10	27.3 ± 0.11
Daily Light Integral	Summer (Wet)	330.8 ± 9.12	320.8 ± 4.44	334.1 ± 6.44
(mmol m^2 s^{-1})	Winter (Dry)	301.2 ± 8.45	311.5 ± 4.03	284.9 ± 9.03
Water velocity	Summer (Wet)	17 ± 0.16	18 ± 0.31	16 ± 0.38
(cm^1s^{-1})	Winter (Dry)	15 ± 0.27	13 ± 0.20	13 ± 0.18
Nitrates	Summer (Wet)	1.12 ± 0.04	0.76 ± 0.04	0.80 ± 0.04
(μM)	Winter (Dry)	N/A	N/A	N/A

Data shown are seasonal means (18-diel cycles) ± standard error (*n*= 432 per site) obtained from discrete water samples. Sampling was conducted in 2012-2013.

Table 3.3| The seasonal daily light integral (mol m^2d^{-1}) for non-reef habitats on the north coast of Little Cayman, Cayman Islands, BWI

Site	Season	Daily Light Integral (mol m^2 d^{-1})
Grape Tree Bay (Back-reef 1)	Summer	18.06
	Winter	16.24
Grape Tree Bay (Seagrass 1)	Summer	16.68
	Winter	13.26
Mary's Bay West (Back-reef 2)	Summer	20.76
	Winter	18.72
Mary's Bay West (Seagrass 2)	Summer	20.12
	Winter	14.98
Mary's Bay East (Back-reef 3)	Summer	24.03
	Winter	18.65
Mary's Bay East (Seagrass 3)	Summer	19.57
	Winter	16.56

The Daily Light Integral was calculated for 18-23 days for both the summer (July-August) and winter seasons (March) of 2012.

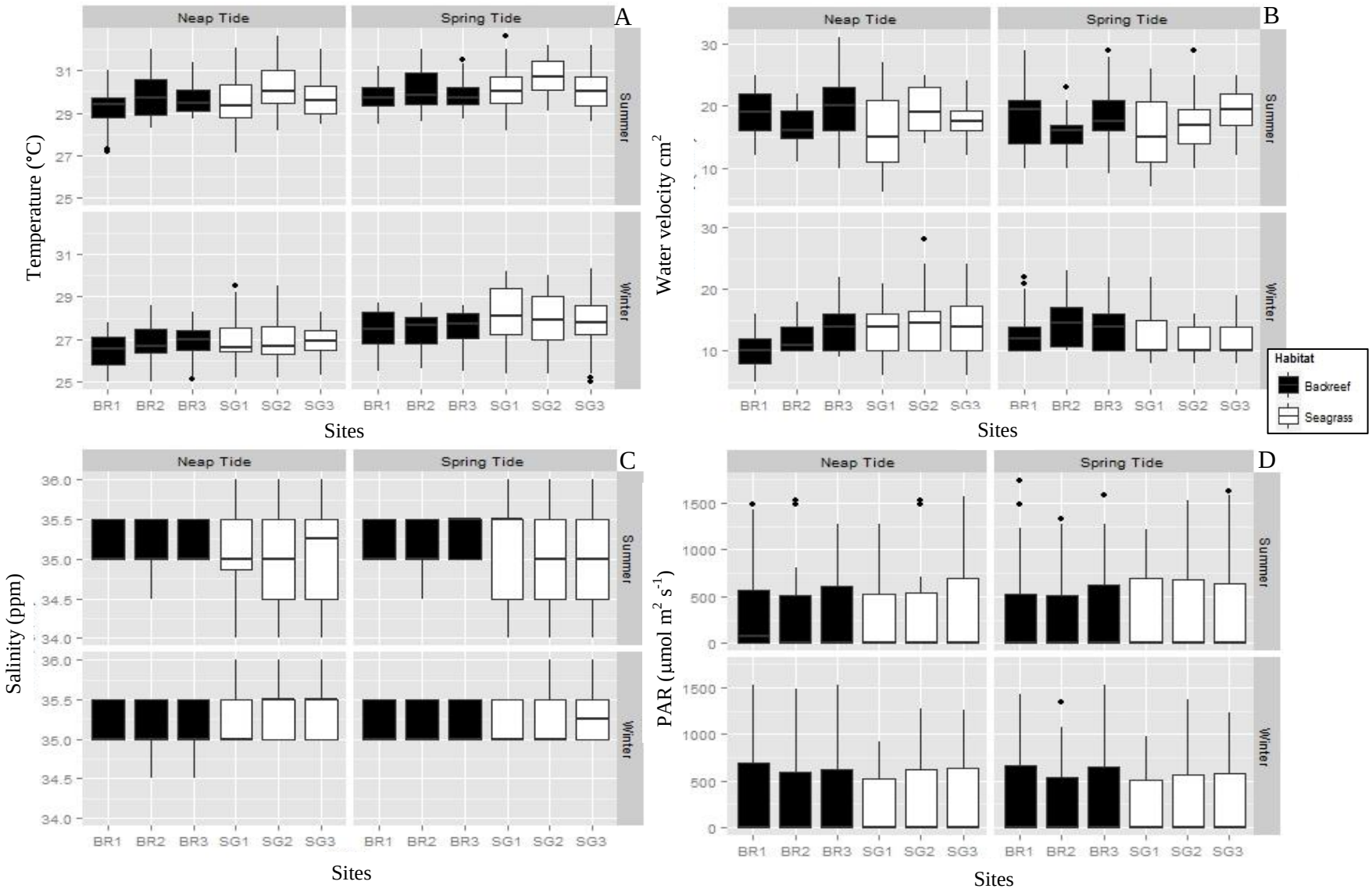


Figure 3.4| The seasonal and lunar cycle trends in: A) temperature, B) water velocity, C) salinity and D) light for the two inner non-reef habitats (back-reef (BR) and seagrass (SG) on the north coast of Little Cayman, Cayman Islands, BWI. Sampling took place over a six-week period in both the wet-summer (July-August) and dry-winter (March-April) seasons in 2012 (*n*= 12 diurnal cycles). Box-plots show the range in parameters within the 95 % confidence interval with the median values shown as the solid black line. Outliers are shown as the solid black dots.

Carbonate chemistry (inshore to offshore gradient) **–** An inshore to offshore gradient was present for all three lagoons with average pCO_2 and TA depleted in inshore waters relative to the outer-reef (P= 0.01, t= see Appendix 6, Figure 3.5). The depletion in pCO_2 corresponded with an average increase in pH sufficient to elevate the mean Ω_{arg} relative to the outer-reef (Back-reef: P= 0.01, Seagrass: P= 0.05, t= see appendix 6). Inshore values were highly dynamic with pCO_2 levels sometime elevated, thus pH and Ω_{arg} depleted inshore relative to the outer-reef (Figure 3.5, Appendix 5). This result is indicative of diurnal variability within the non-reef habitats (*see* Inshore non-reef habitats results section).

Seasonally, TA depletion was greatest within the non-reef habitats during the winter season (P= 0.01, t= see Appendix 6). Similarly, the inshore depletion of pCO_2, and resulting elevation in pH was greater during the winter season, which resulted in a significant *ca*. two-fold increase in the mean $\Delta\ \Omega_{arg}$ (P= 0.01, t= see Appendix 6). Offshore, the outer-reef habitat showed no difference in the mean or Cv of carbonate chemistry parameters between seasons or stage of the lunar tidal cycle, independent of site. The outer-reef habitat experienced less variability in all carbonate chemistry parameters measured compared to the non-reef habitats, for both seasons and lunar tidal cycles (P= 0.01, t= see Appendix 6).

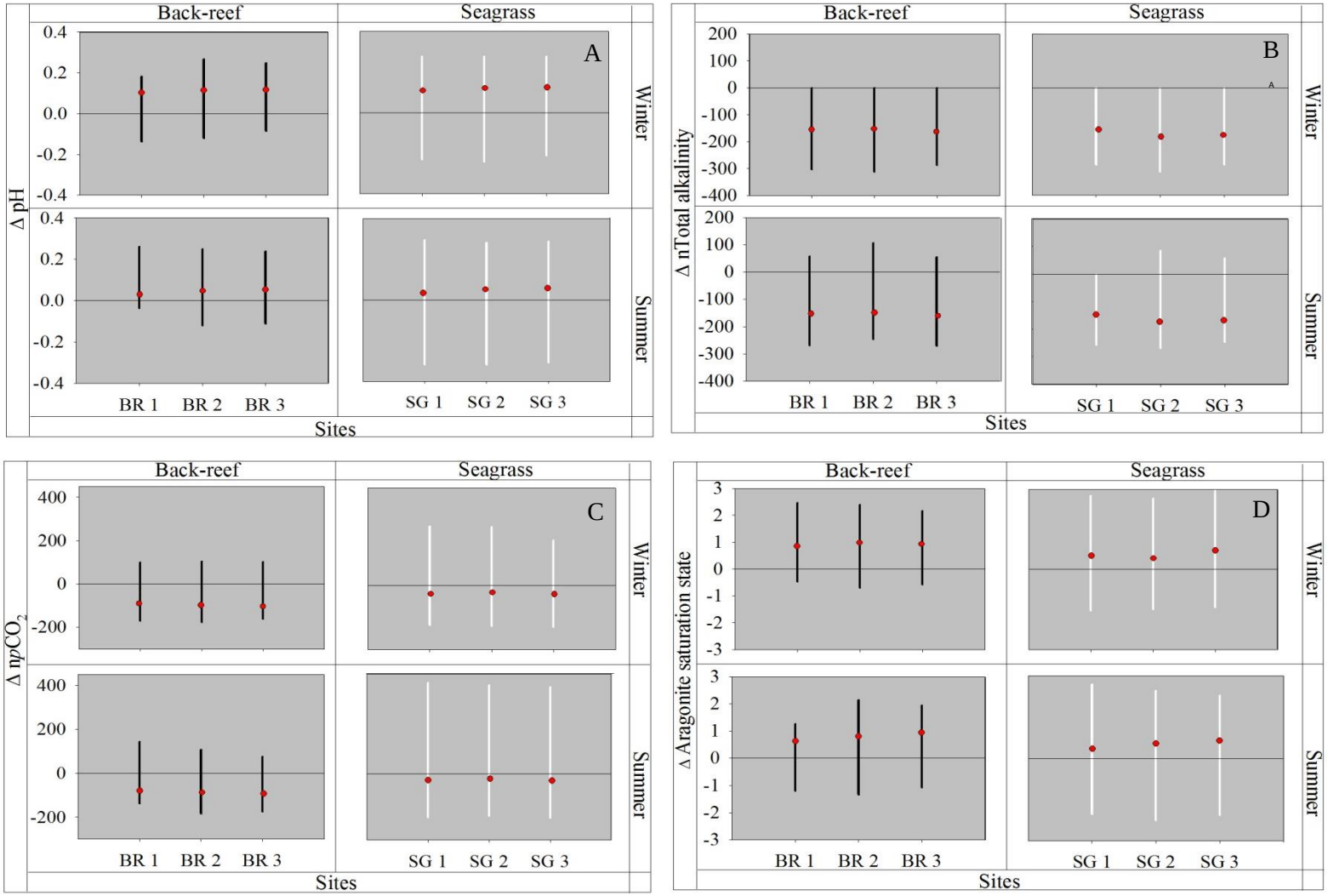


Figure 3.5| The inshore to offshore seasonal carbonate chemistry gradient for the north side of Little Cayman, Cayman Islands, BWI. The seasonal gradient (Δ) between the inner non-reef habitats (back-reef and seagrass) and outer-reef for: A) pH, B) n total alkalinity, C) n pCO_2, and D) Δ Ω_{arg} are shown. Mean inshore carbonate chemistry values were subtracted from the mean offshore values for each sampling period to establish the magnitude of the inshore to offshore gradient (*as* Manzello *et al.*, 2012. Total alkalinity and pCO_2 were standardised to salinity (S= 36). The mean values are indicated by the red dots.

Carbonate chemistry (inshore) non-reef habitats – The inshore non-reef habitats were highly dynamic across different temporal scales. For example, the seagrass habitat recorded a pH range (mean ± SE) over a season of 0.588 ± 0.09 pH units, had a range of 0.554 ± 0.09 pH units over a lunar tidal cycle and experienced a range of 0.554 ± 0.16 pH units over a diel cycle (see Table 3.4). Despite the large range in carbonate chemistry parameters recorded over a season, habitats experienced a very consistent trends in their carbonate chemistry with the low-resolution (5-diel cycles) dataset generating mean and Cv values of pH, TA and temperature that did not significantly differ from values determined over the higher-resolution sampling period (18-diel cycles) (Figure 3.6). Inshore carbonate chemistry mean values were similar between stages of the lunar tidal cycle (Figure 3.7), however, pH and pCO_2 Cv were elevated during spring tides (P= 0.05, pH: t= see Appendix 6). Within habitat differences were also minimal for all carbonate chemistry parameters, with the exception of pH and pCO_2 Cv being more variable at seagrass site one compared to seagrass site three (P= 0.05, Appendix 5; Figure 3.7).

Seasonally, inshore pH, pCO_2 and Ω_{arg} were more variable during the summer (P= 0.01, t= see Appendix 6). Mean pCO_2 values were elevated during the summer season for both habitats, which corresponded with lower pH and Ω_{arg} (Figure 3.5). Importantly, at no point during sampling did the Ω_{arg} fall below one within either of the non-reef habitats, showing that both habitats remained supersaturated in aragonite.

Table 3.4| pH temporal variability for non-reef habitats of Little Cayman

Temporal Scale	Habitat	Mean (±SE)	Range
Seasonal	Back-reef	0.035 (0.09)	0.378 (0.12)
	Seagrass	0.060 (0.08)	0.588 (0.09)
Lunar Tidal Cycle	Back-reef	0.001 (0.01)	0.366 (0.13)
	Seagrass	0.008 (0.06)	0.554 (0.09)
Diurnal	Back-reef	0.002 (0.01)	0.343 (0.13)
	Seagrass	0.004 (0.01)	0.554 (0.16)

Mean indicates the differences between temporal scale (i.e. between summer and winter) whereas range indicates the largest range of values obtained over the specified period. Means are based on the 24 h sampling replicates: Seasonal *n*= 18, lunar tidal cycle *n*= 18, diurnal *n*= 36.

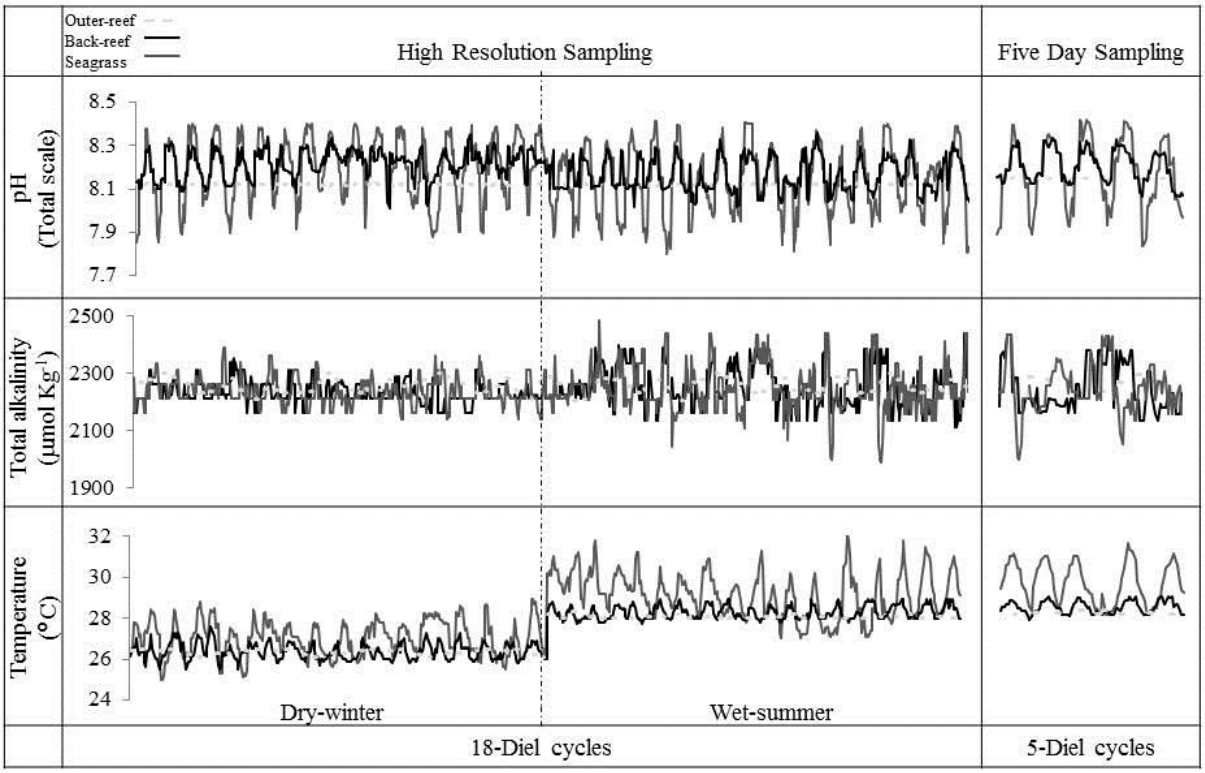


Figure 3.6| High-resolution (18-diel cycles) versus low-resolution (5-diel cycles) sampling of pH, total alkalinity and temperature for an outer-reef, back-reef and seagrass habitat on Little Cayman, Cayman Islands, BWI. High-resolution (*n*= 18 diel cycles) samples were collected in both seasons: 18-diel cycles during the dry-winter season and 18-diel cycles during the wet-summer season of 2012. The five-diel cycles were randomly selected from the 18-diel cycles collected during the summer seasons of 2012.

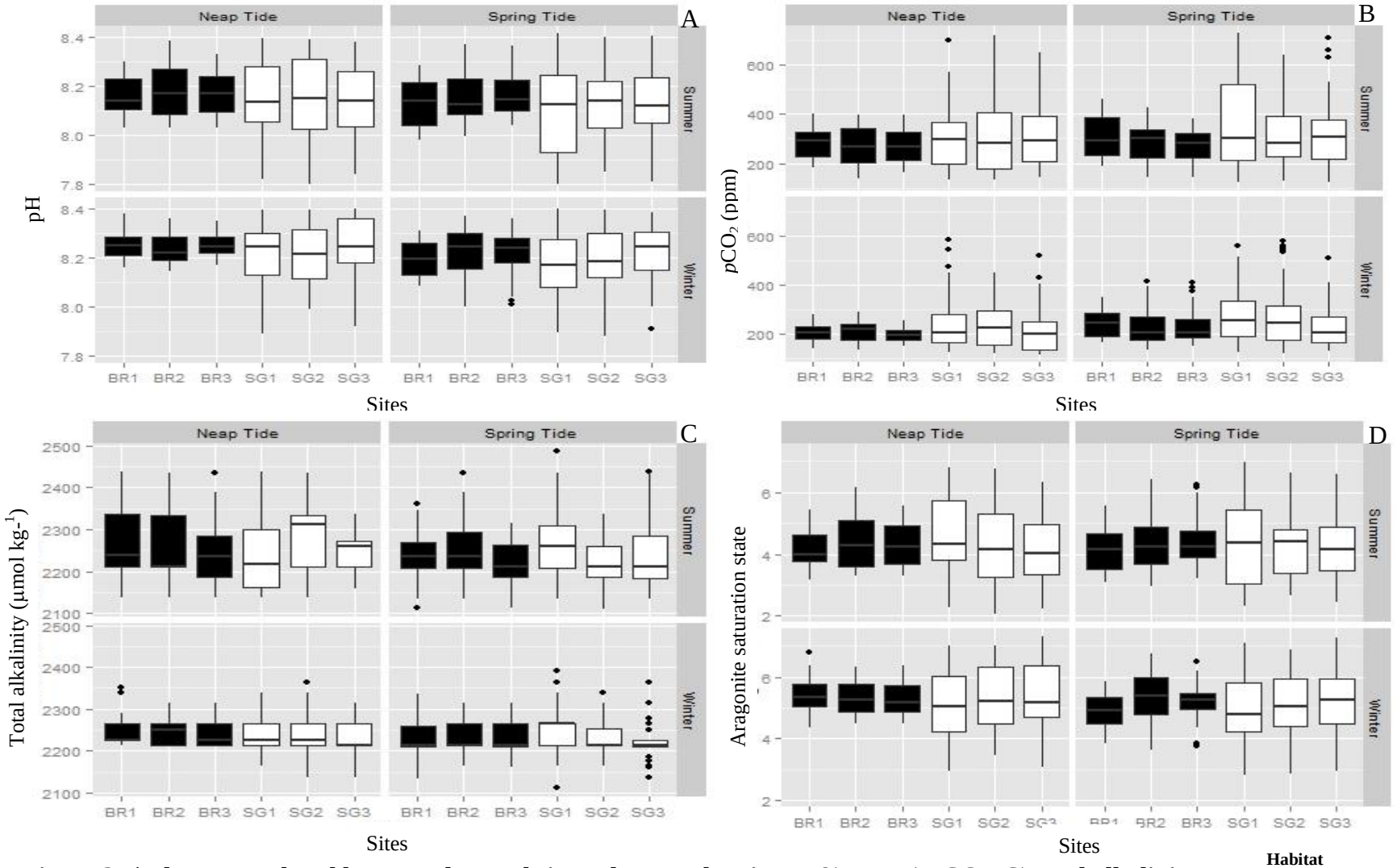


Figure 3.7| The seasonal and lunar cycle trends in carbonate chemistry: A) pH, B) pCO_2, C) total alkalinity and D) Ω_{arg} for the Back-reef (BR) and Seagrass (SG) habitats on the north coast of Little Cayman, Cayman Islands, BWI. Sampling took place over a six-week period in both the wet-summer (July-August) and dry-winter (March-April) seasons in 2012 (*n*= 12 diurnal cycles). Boxplots show the range in parameters within the 95 % confidence interval with the median values shown as the solid black line. Outliers are shown as the solid black dots.

Patterns of seasonal and diurnal variability were very different between non-reef habitats. The seagrass habitat experienced the largest seasonal variability in all carbonate chemistry parameters (P= 0.05, t= see Appendix 6), with the exception of TA, which remained similar between habitats. Diurnal variability was also greatest in the seagrass habitat (0.554 pH units) compared to the back-reef habitat (0.343 pH units). The differences in pH range between habitats was generated by the elevated daytime pH values for the seagrass habitat between 13:00 h and 16:00 h and then the extreme low night-time pH values between 22:00 h and 7:00 h (Figure 3.8). The peaks and troughs of pH correspond with maximum and minimum PAR values (Figure 3.9).

Daytime and nighttime mean pH values varied between the back-reef and seagrass habitats. For the back-reef habitat, the average daytime pH was 0.1 units higher than the nighttime (daytime= 8.263, night-time= 8.130), whereas the daytime seagrass average pH was 0.245 units higher (daytime= 8.279, nighttime= 8.034). These different daytime and nighttime averages resulted from the different durations of time each habitat spent at specific pH values. The back-reef habitat spent the majority of time (50-63 %) at a pH between 8.101-8.200 (Figure 3.8). The seagrass habitat however, experienced much more variability, with 25 % of time spent between a pH of 7.900-8.100, 25 % spent between 8.101-8.200, 20-30 % of time spent between 8.201-8.300, and up to 30 % of time spent between 8.301-8.400 (Figure 3.8). Consequently, the seagrass habitat experienced more extreme daytime and nighttime pH averages and a more variable diurnal trend.

Seasonal differences in diurnal trend were also apparent; both habitats showing relative symmetry for the amount of time spent at low and high pH values during the winter season (back-reef G_1=0.27, seagrass G_1= 0.12). However, in the summer season, both habitats spent a greater time at low pH values (back-reef G_1= -0.41, seagrass G_1= -0.43).

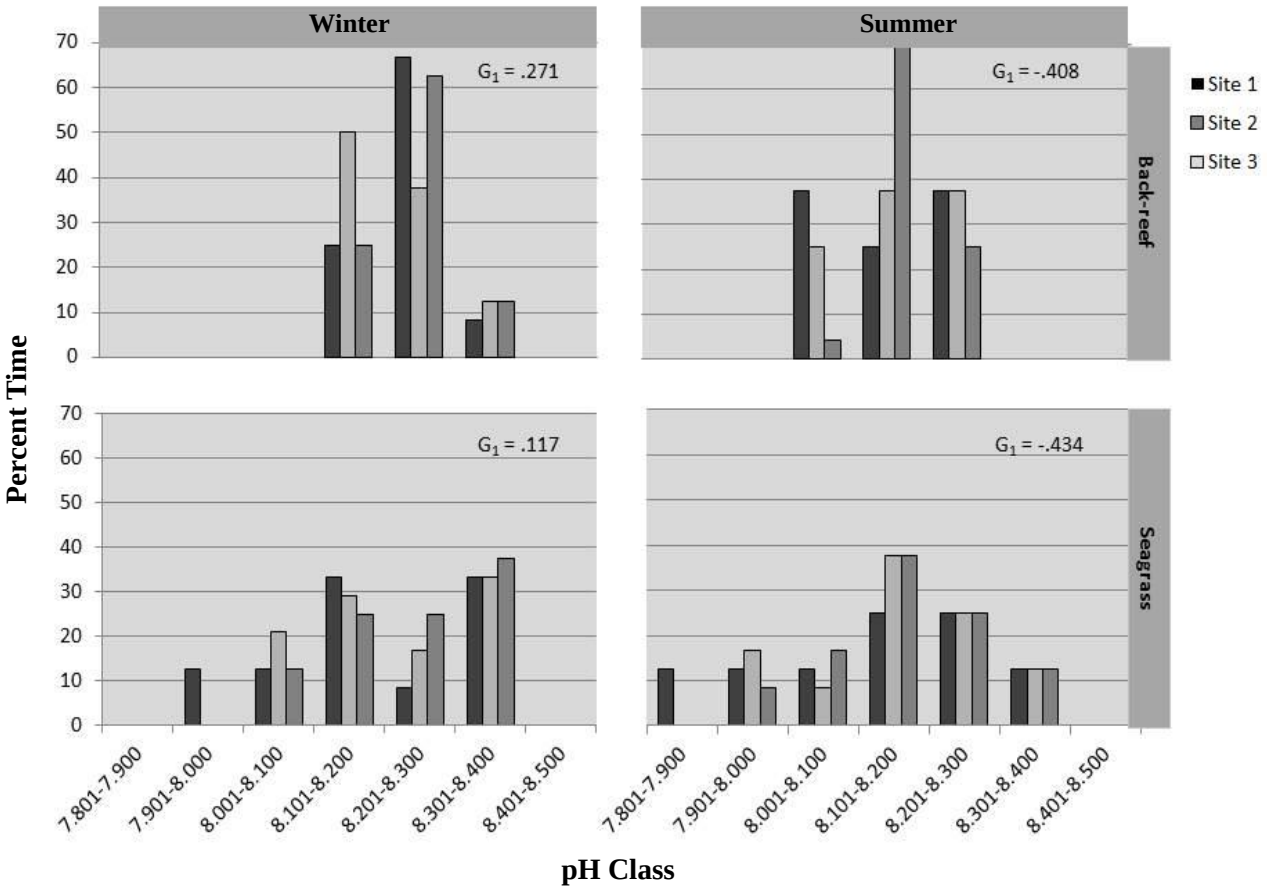


Figure 3.8| Daily proportion of time non-reef habitats on Little Cayman, Cayman Islands, BWI spent at set pH classes. The proportion of daily time was plotted by habitat (back-reef and seagrass), sites (site 1= Grape tree Bay, site 2= Mary's Bay West, site 3= Mary's Bay East) and season (summer-wet and winter-dry). The proportion of daily time was determined using 12-diel cycles. G_1 is the collective skewness for the habitat during the particular season.

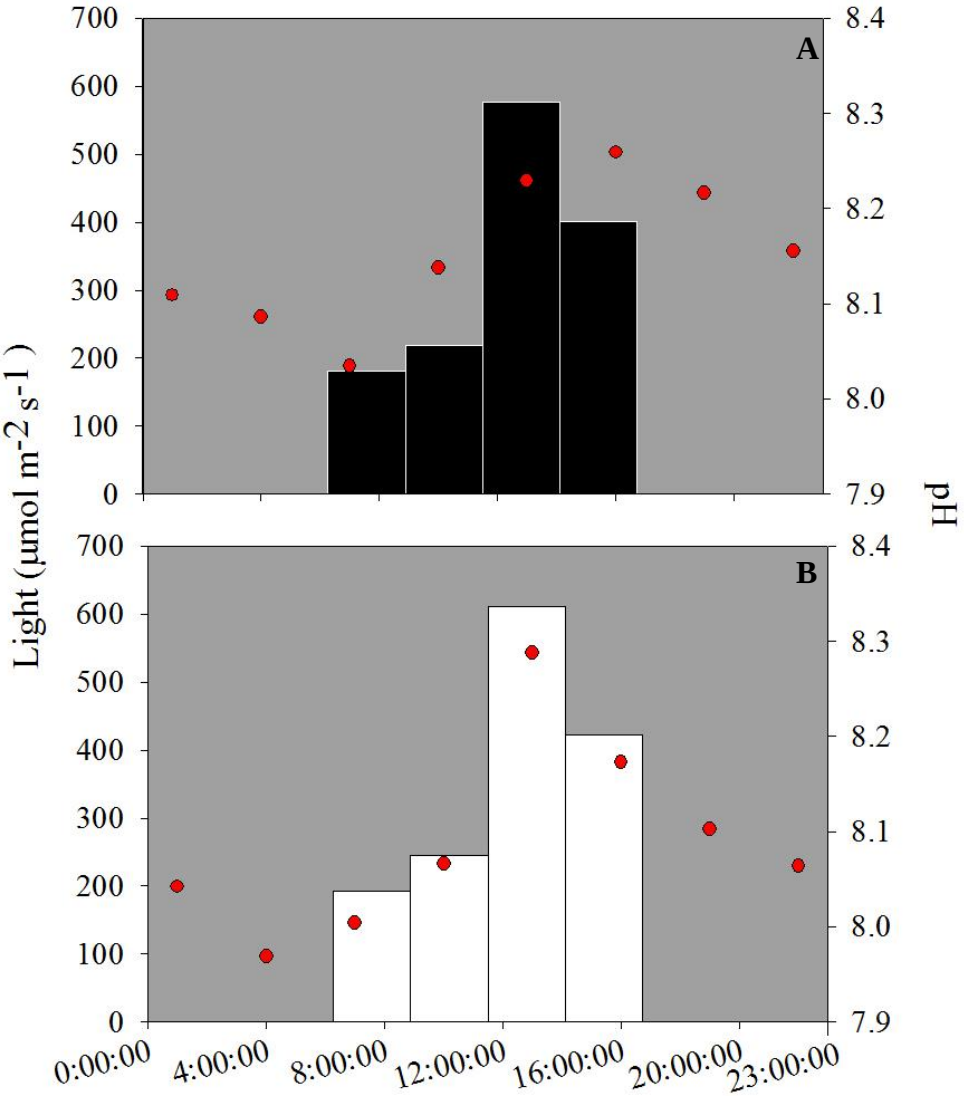


Figure 3.9| Diurnal pH trend for the: A) back-reef and B) seagrass habitats on the north coast of Little Cayman, Cayman Islands, BWI. The red dots indicated the mean (± SE) pH per time period. Data is based off of discrete samples collected every 3 h during the summer and winter seasons of 2012 (*n*= 12 diel cycles). Average PAR values for each time period are shown as the bar charts.

Biogeochemical control of habitats – Across all sites, the outer-reef control habitat showed strongest co-variability between nA_T and nC_T and was influenced most by calcification-carbonate dissolution (Figure 3.10). Calcification decreases TA by two moles for each mole of $CaCO_3$ precipitated and dissolution increases TA by two moles for each mole of $CaCO_3$ dissolved. If $CaCO_3$ is precipitated without photosynthesis then the line of the theoretical slope would be two. All non-reef habitats exhibited co-variability between nA_T and nC_T that was more strongly influenced by photosynthesis-respiration (and thus CO_2 uptake-release). Habitat differences in net ecosystem calcification to net community production (NEC:NEP, Table 3.5) were evident ($F_{2, 22}$= 167.67, P= 0.001), with values consistently lowest for seagrass habitats (range: 0.05-0.55) and highest for the outer-reef control habitats (range: 1.10-1.20). The NEC:NEP ratios are influenced by the slope of the nC_T-nA_T plots and consequently, the outer-reef habitats had a slope closer to two than all the non-reef habitats, which demonstrated less influence from photosynthesis and more influence from calcification.

All outer-reef control sites showed a relationship between nA_T and nC_T for both seasons ($r^2 > 0.90$, Table 3.5). A large seasonal effect was seen within the non-reef habitats, with the summer season experiencing the largest variability in nA_T and nC_T, showing greater influence from photosynthesis and respiration. Higher summer NEC:NEP values ($F_{2,22}$= 18.64, P= 0.001) across sites also shows greater seasonal influence from calcification-carbonate dissolution.

Table 3.5| NEC:NEP ratios for the nine study sites located within: Grape Tree Bay (GTB), Mary's Bay West (MBW) and Mary's Bay East (MBE)) with nA_T vs. nC_T

Site	Habitat	Season	NEC:NEP	LRE	R^2	*P*-value
GTB	Seagrass	Summer	0.40	0.5765x + 3303.2	0.35	0.0001
	Seagrass	Winter	0.17	0.2852x + 2832.5	0.17	0.0001
	Back-reef	Summer	0.42	0.5920x + 3335.4	0.41	0.0001
	Back-reef	Winter	0.20	0.3338x + 2899.4	0.24	0.0001
	Outer-reef	Summer	1.20	1.0889x + 224.8	0.98	0.0001
	Outer-reef	Winter	1.19	1.0847x + 234.5	0.97	0.0001
MBW	Seagrass	Summer	0.33	0.4967x + 3130.8	0.37	0.0001
	Seagrass	Winter	0.05	0.0989x + 2494.5	0.03	0.0001
	Back-reef	Summer	0.50	0.6672x + 3467.6	0.37	0.0001
	Back-reef	Winter	0.10	0.1843x + 2653.1	0.19	0.0001
	Outer-reef	Summer	1.10	1.0496x + 303.0	0.97	0.0001
	Outer-reef	Winter	1.10	1.0458x + 311.5	0.98	0.0001
MBE	Seagrass	Summer	0.55	0.7080x + 3528.4	0.57	0.0001
	Seagrass	Winter	0.14	0.2430x + 2733.9	0.18	0.0001
	Back-reef	Summer	0.63	0.7698x + 3641.1	0.54	0.0001
	Back-reef	Winter	0.15	0.2595x + 2764.9	0.18	0.0001
	Outer-reef	Summer	1.13	1.0599x + 283.7	0.94	0.0001
	Outer-reef	Winter	1.40	1.1672x + 72.2	1.00	0.0001

Ratios of net ecosystem calcification to net community production (NEC:NCP) were calculated from the slopes of best-fit linear regression with all sites showing a correlation between nA_T and nC_T. NEC:NEP was calculated using the expression $1/[(2/m) - 1]$, where *m* is the slope from the corresponding linear regression equations (LRE). Calcification and dissolution are dominant processes when a linear regression slope approaches two. There was variation in NEC:NEP within all habitats as expected due to variation in community structure.

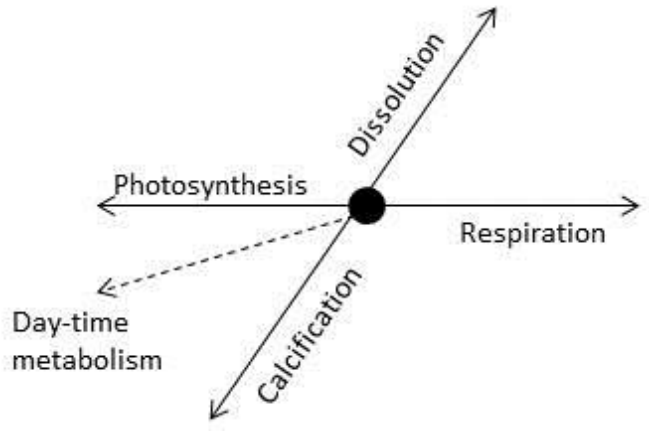


Figure 3.10.A| A guide to understanding the nA_T-nC_T plots. The diagram illustrates how net photosynthesis, respiration, calcification and dissolution affect location of points.

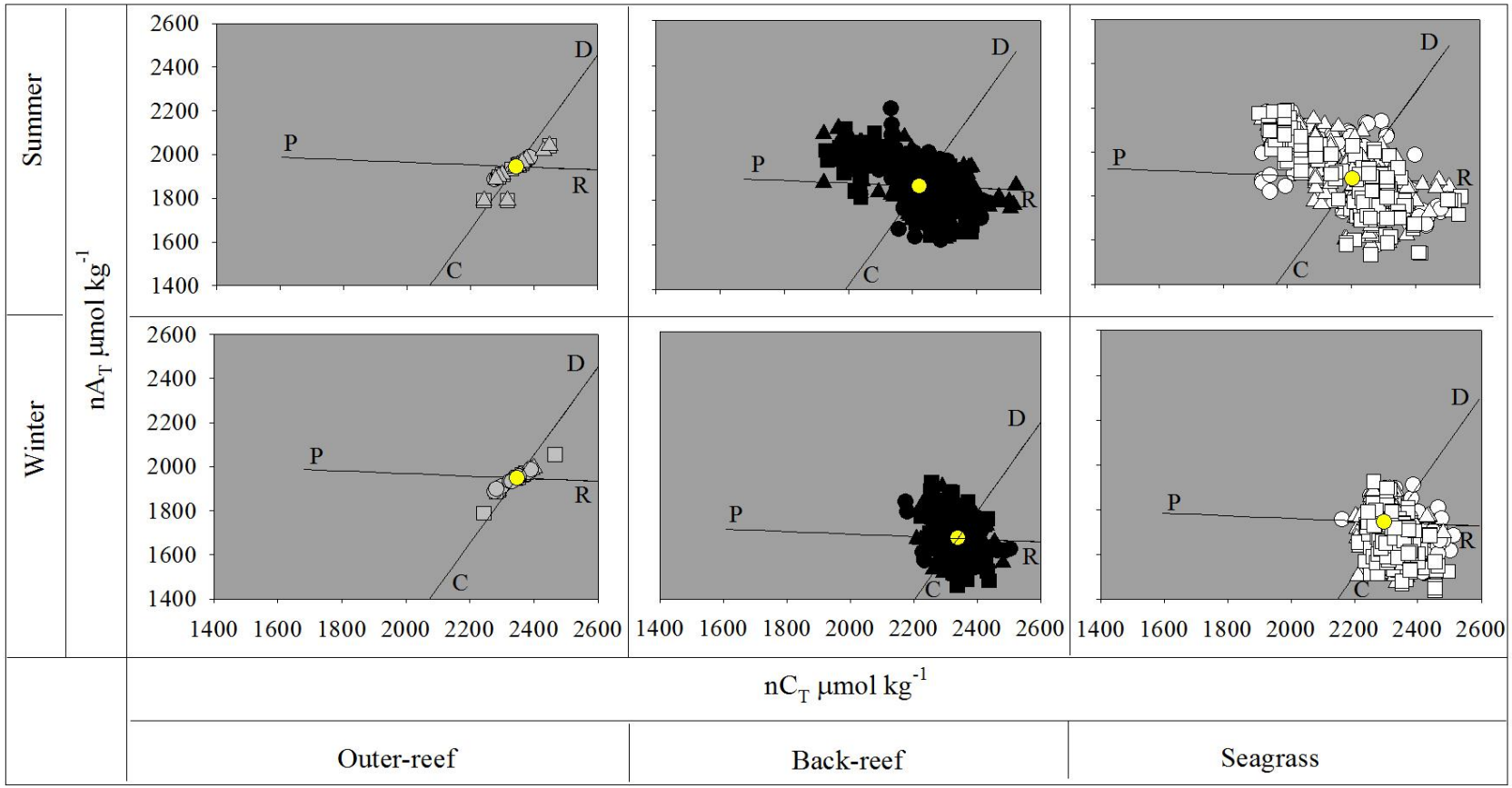


Figure 3.10.B| Salinity-normalised total alkalinity (nA_T) and total carbon (nC_T) plots with best-fit linear regression. Average data is shown for all habitats (outer-reef control, back-reef, seagrass) on Little Cayman, Cayman Islands, BWI (Table 3.5 - r^2 and equations). Sites are indicated by data symbol: Grape Tree Bay (circle), Mary's Bay West (square) Mary's Bay East (triangle). Sampling took place over a six-week period in both the wet-summer (July-August) and dry-winter (March-April) seasons in 2012. Black lines represent the theoretical impact of calcification (C), carbonate sediment dissolution (D), photosynthesis (P), and respiration (R) CO_2 on A_T and C_T. Average nA_T and nC_T is indicated by a yellow dot. Calcification and dissolution are dominant processes when a linear regression slope approaches two.

Physiochemical controls of non-reef habitats – Two Principle Component Analyses (PCA) were conducted to evaluate the variation in mean and Cv of the physiochemical conditions between non-reef habitats. For each PCA, two main components were extracted (PC1 and PC2). For Cv, the two components combined accounted for 54.0 % of the variation in the physiochemical conditions between non-reef habitats; 35.8 % of the variation was explained by elevated variation in the seagrass habitat of pH, pCO_2 and Ω_{arg} ($F_{2,72}$= 265.22, P= 0.01, Figure 3.11a), whilst 18.2 % of the habitat differences in Cv were explained by higher variance in TA and salinity within the seagrass habitat ($F_{2,72}$= 53.06, P= 0.001). Seasonally, greater variance in the two components occurred during the summer season (PC1: $F_{2,72}$= 10.84, P= 0.01, PC2: $F_{2.72}$= 7.70, P= 0.01, Table 3.6). An additional 12.8 % of Cv of the physiochemical conditions between habitats was explained by temperature differences (PC3).

For mean, the two components combined to account for 57.3 % of the variance in the physiochemical conditions between non-reef habitats (Figure 3.11b); 44.7 % of the variation was explained by higher mean values of pH, pCO_2, Ω_{arg}, temperature and salinity in the seagrass ($F_{2,72}$= 5.89, P= 0.05, Table 3.6), whilst 12.6 % of the mean habitat differences were explained by elevated light in the seagrass habitat ($F_{2,72}$= 18.10, P= 0.01). Again, seasonal effects were pronounced during the summer (PC1: $F_{2,72}$=331.01, P= 0.01, PC2: $F_{2,72}$=3.99, P= 0.05). Differences in mean water velocity accounted for an additional 10.7 % of the variation between habitats (PC3).

A comparison between the two PCA's conducted demonstrates the importance of considering both Cv and mean in physiochemical habitat analysis. For example, in the Cv PCA, temperature contributed minimally in PC3 (weight 0.886, Eigin value 12.8 %); however, in the mean PCA, temperature contributed largely in PC1 (PC1, weight 0.825, Eigin value 44.7 %);

thus highlighting how physiochemical factors can influence a habitat differently depending on whether their mean or Cv values are considered. Comparison of the dispersal of each PCA (Figure 3.11a & b) demonstrates how seasonality is dominant in the mean PCA, whereas habitat differences are more evident in the Cv PCA.

Table 3.6| The PCA weightings for A) physiochemical variance (Cv) and B) physiochemical mean values for the non-reef habitats on Little Cayman, Cayman Islands, BWI

A | Physiochemical Cv: The contributions of the first three principle components (PC1, PC2 and PC3) that combined explained 66.8 % of the differences within the non-reef habitats.

Variable	Weighting		
	PC1	PC2	PC3
pH	**0.824**	0.000	0.022
pCO_2	**0.875**	0.005	0.000
Total alkalinity	0.059	**0.509**	0.000
Aragonite saturation state (Ω_{arg})	**0.915**	0.016	0.000
Temperature	0.016	0.004	**0.886**
Salinity	0.032	**0.558**	0.010
Light	0.122	0.021	0.034
Water Velocity	0.020	0.343	0.072

B | Physiochemical mean: The contributions of the first three principle components (PC1, PC2 and PC3) that combined explained 80.1 % of the differences within the non-reef habitats.

Variable	Weighting		
	PC1	PC2	PC3
pH	**0.878**	0.363	0.066
pCO_2	**0.885**	0.368	0.118
Total alkalinity	0.378	0.144	**0.643**
Aragonite saturation state (Ω_{arg})	**0.963**	0.068	0.065
Temperature	**0.825**	0.202	0.195
Salinity	**0.801**	0.056	0.205
Light	0.367	**0.521**	0.117
Water Velocity	0.654	0.435	0.195

Data was collected during a discrete water sampling procedure during the summer and winter of 2012 (n= 18 diel cycles, see main text). The major contributing variables are shown in bold.

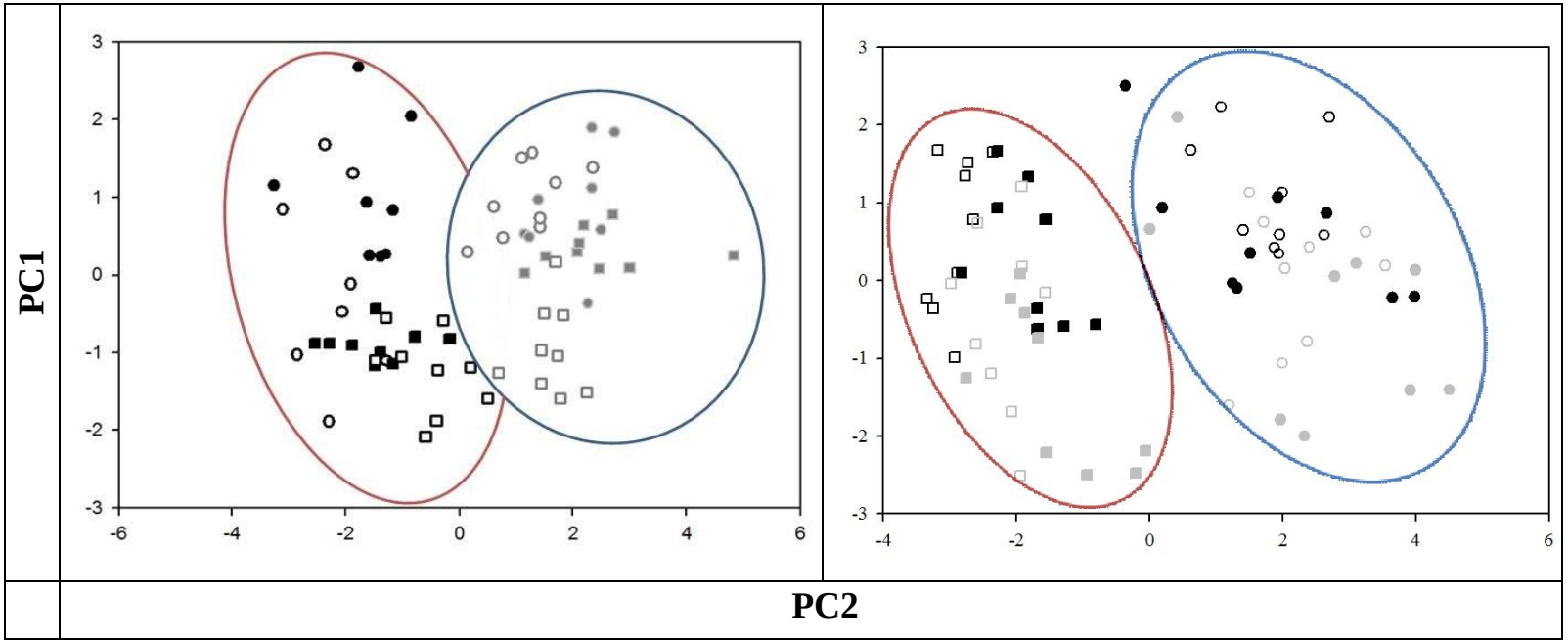


Figure 3.11| Principal component analysis (PCA) plot for: A) Coefficient of variation (Cv) and B) mean physiochemical data from non-reef habitats on the north coast of Little Cayman, Cayman Islands, BWI. The first two principle components are plotted (PC1 and PC2). The shape of the points indicates seasons, with circles representing the summer season and squares representing the winter. Filled or unfilled data points show lunar cycle; filled points representing the spring tide and no fill indicating the neap tide. The plot illustrates differences in variance between the two non-reef habitats. The red and blue circles distinguish the major clustering of habitats. Figure 3.11a: PC1 accounted for 35.8 % of the variance, and PC2 accounted for 18.2 % of the variance. Figure 3.11b: PC1 accounted for 44.7 % of the variance, and PC2 accounted for 12.6 % of the variance.

3.3.2| Benthic assessment

Benthic community composition – No differences at the major benthic taxa level (e.g. corals, chlorophyta etc, see Figure 3.18 for all classifications) were detected within habitats between the 2012 and 2013 benthic sampling data. Overall community composition was also similar between habitats, with no significant inter-habitat variation observed between sites (Figure 3.12). However, comparisons of individual taxa revealed subtle differences. For example, in both 2012 and 2013, seagrass cover was lowest in the back-reef habitat (2012: t_4= 22.22, P= 0.001, 2013: t_4= 52.82, P= 0.01, Figure 3.13) and abiotic cover was largest (2012: t_4= 40.12, P= 0.01, 2013: t_4= 52.69, P= 0.01, Figure 3.13). Coral cover (mean ± SE) was low across habitats independent of year, with the back-reef (6.5 ± 1.2 %) having *ca.* double the coral cover of the seagrass habitats (2.7 ± 0.4 %).

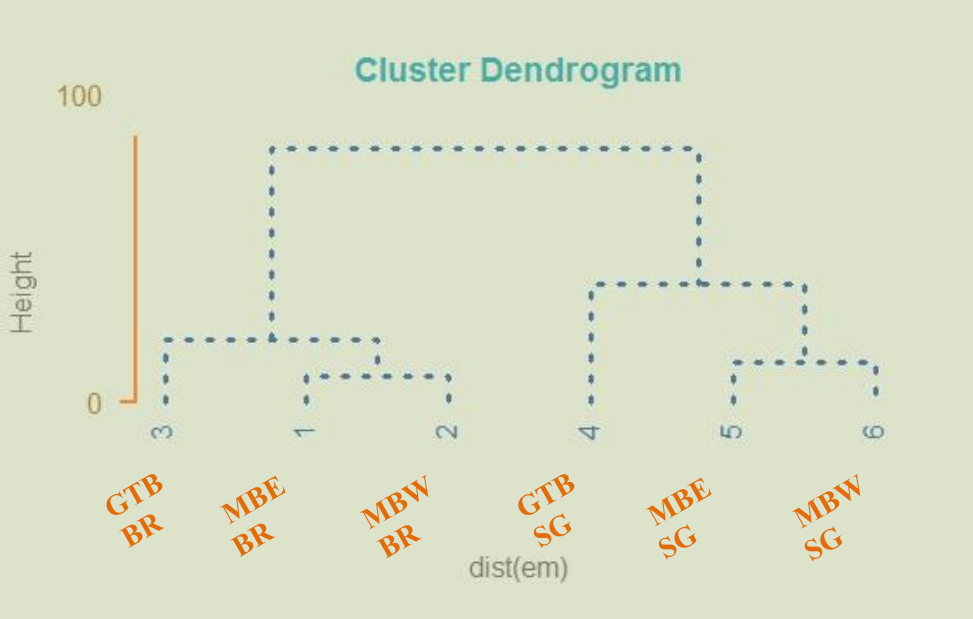


Figure 3.12| A dendrogram showing site clustering based on benthic composition for the six non-reef habitats on Little Cayman, Cayman Islands, BWI. Average percent benthic composition for 2012 and 2013 were used. Two habitats (back-reef (BR) and seagrass (SG)) were compared at the three sites: Grape Tree Bay (GTB), Mary's Bay West (MBW) and Mary's Bay East (MBE).

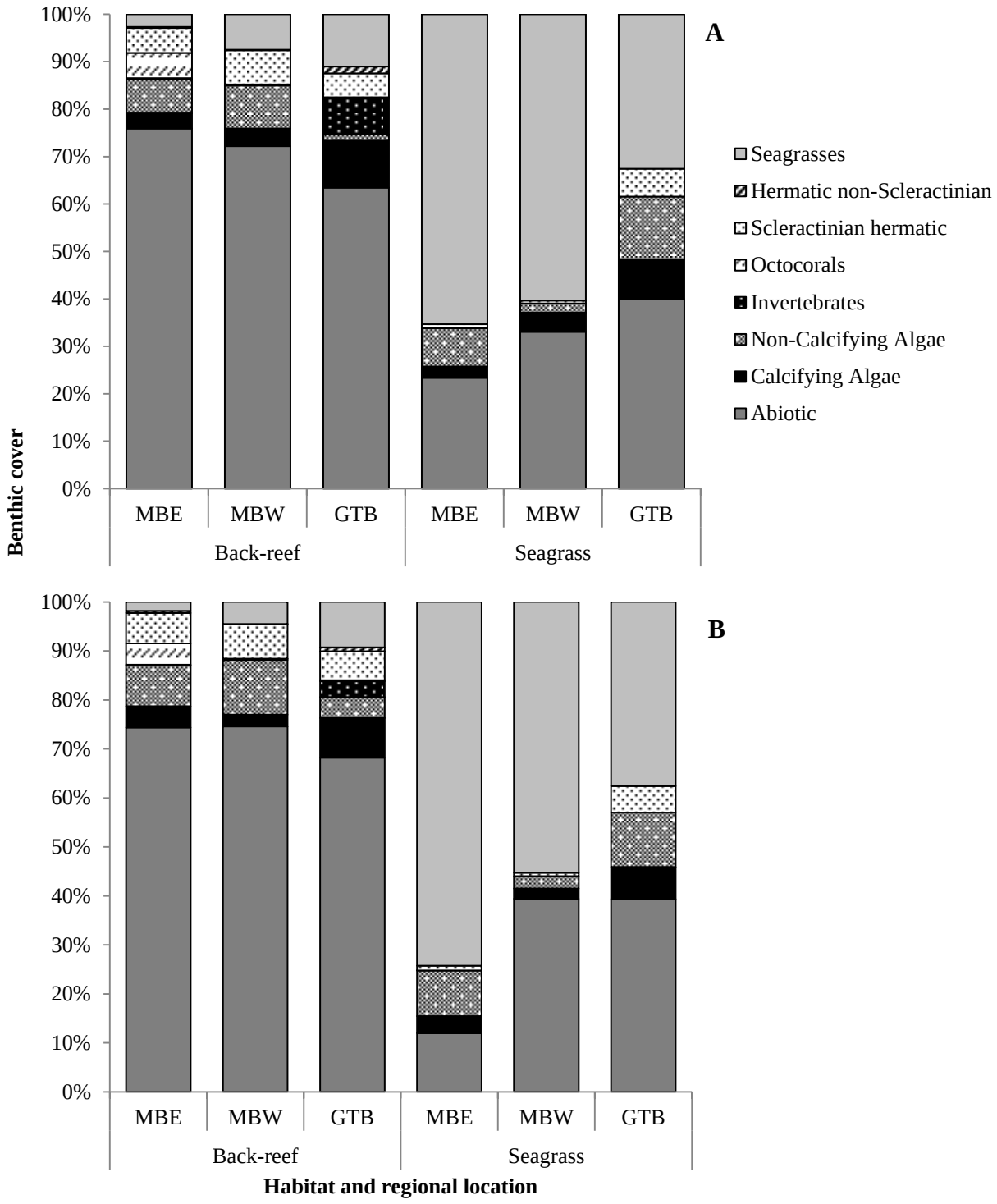


Figure 3.13| The percentage cover of major benthic taxa for the seagrass (SG) and back-reef (BR) habitats on the north coast of Little Cayman, Cayman Islands, BWI during: A) 2012 and B) 2013. Surveys were conducted in February at three sites: Grape Tree Bay (GTB), Mary's Bay West (MBW) and Mary's Bay East (MBE). Data is averaged for three 50 m transects per site.

Established coral community – Relative percentage coral cover (± SE) was similar for both years, with *Porites astreoides* the dominant species in the back-reef habitat (2012: 32.0 ± 0.7 %, 2013: 36.0 ± 0.4 %, Figure 3.14a), and *Siderastrea radians* the dominant species in the seagrass habitat (2012: 49.0 ± 0.3 %, 2013: 52 ± 0.5 %). The seagrass coral community belonged almost solely to the two families: *Poritidae* and *Siderastreidae* (99 %), whereas the back-reef habitat had 65 % of corals in these families, with 18 % in the Faviidae and Meandrinidae families (Figure 3.14b). Although biomass of corals was higher in the back-reef habitat, the actual number of coral colonies was greatest in the seagrass due to high densities of small *P. divericata* colonies spread-out through the seagrass beds (2012: 209 ± 112.5, 2013: 262 ± 98.3 colonies, Table 3.7). However, densities of *P. divericata* were highly variable between transects, as illustrated by the large standard error.

The size of coral colonies was low at both habitats, with most corals ranging from 1-15 cm (Figure 3.15). The seagrass habitat contained smaller colonies than the back-reef in 2012 and 2013 (Seagrass, 2012: G_1 = 2.82, 2013: G_1= 2.84; Back-reef, 2012: G_1= 1.70, 2013: G_1= 1.85). In 2013, both habitats had a greater number of coral colonies observed, falling into the 0-5 cm bin. Between years, there was no significant decrease in the number of larger (21-50^+ cm) coral colonies. In both 2012 and 2013, the back-reef habitat had the highest species richness, species evenness and Shannon-Wiener Index (Table 3.7). The seagrass habitat did however have relatively large species richness, housing *ca.* 70 % of coral species found within the neighbouring back-reef habitat. Visually, disease prevalence was low across habitats (< 1 %), however there were obvious signs of old-mortality (back-reef: 14 %, seagrass: 2 %) with the back-reef showing greater amounts ($F_{2,36}$= 253.46, P= 0.001). No differences in new or transitional-mortality were detected.

Table 3.7| Summary table of established coral communities of the back-reef and seagrass habitats of Little Cayman, Cayman Islands, BWI

Year	Habitat	Average Density (per $20m^2$)	Species Richness	Shannon-Wiener Index	Shannon-Wiener Evenness
2012	Back-reef	107 ± 12.4	18	1.18	0.48
2013	Back-reef	132 ± 10.2	21	1.45	0.58
2012	Seagrass	209 ± 112.5	13	0.78	0.35
2013	Seagrass	262 ± 98.3	14	0.84	0.37

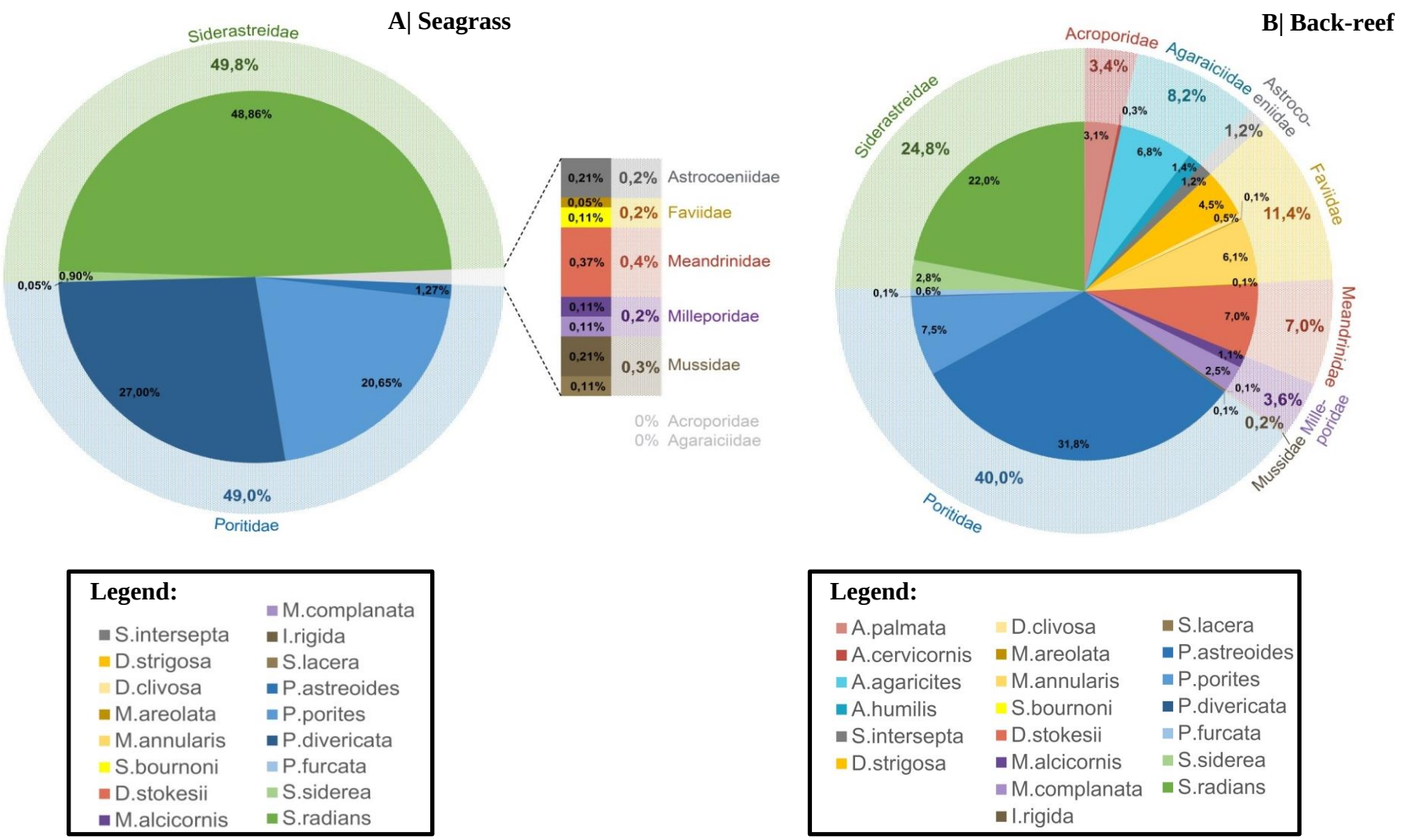


Figure 3.14| The average relative coral cover for the seagrass and back-reef habitats of Little Cayman, Cayman Islands, BWI. Pie charts show the relative cover of each coral family around the edge, followed by the species coverage in the internal pie-chart. Data was obtained from 9 x 50 m benthic video transects analysed to species level conducted in 2012 and 2013.

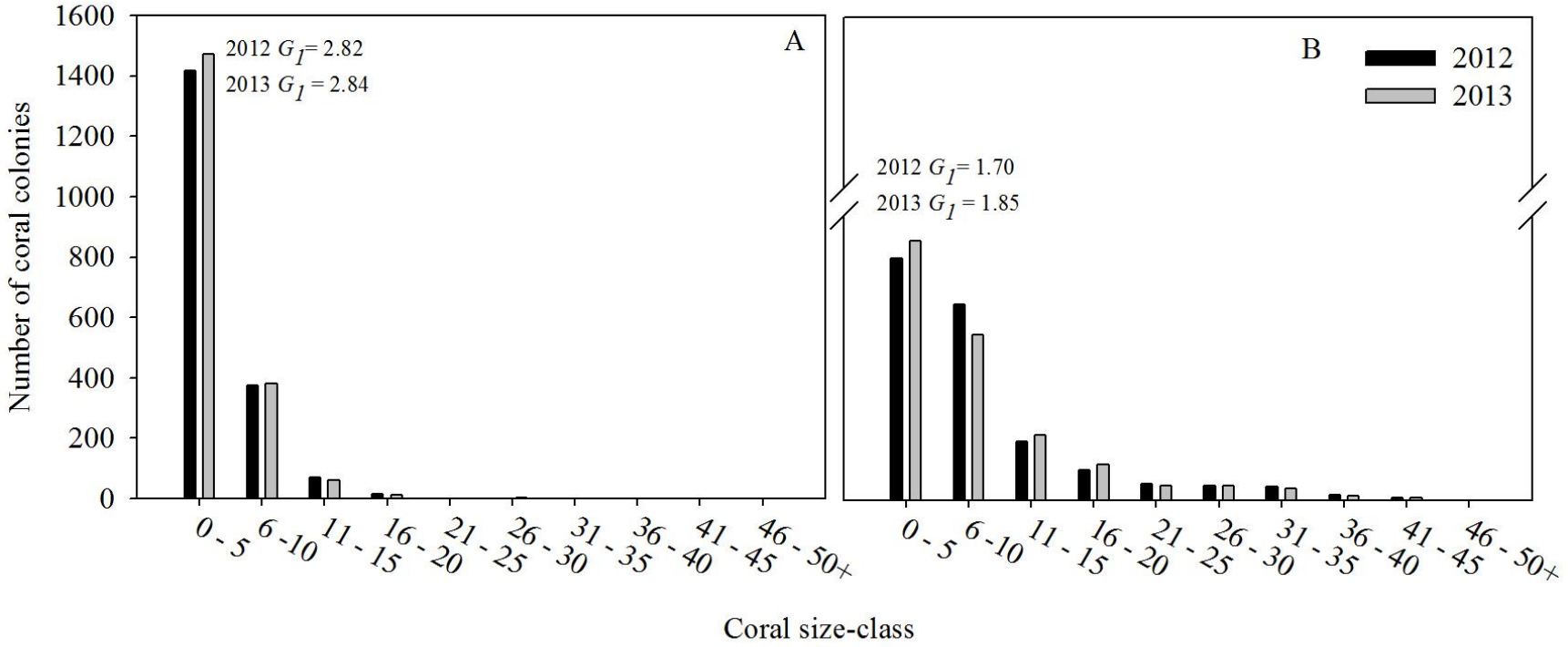


Figure 3.15| The coral size-frequency distribution in the: A) seagrass and B) back-reef habitats in 2012 and 2013 around Little Cayman, Cayman Islands, BWI. Annually data was obtained from 9 x 20 m^2 quadrats within each habitat during the month of February. Skewness (G_1) is shown for each habitat and year.

Coral recruitment – Monthly analysis of the settlement tiles at both non-reef habitats indicated that recruitment only occurred during the months of August and September (in both 2012 and 2013). The back-reef habitat had the highest number of coral spats counted over the 20-month period (back-reef: 181, seagrass: 81), averaging 1.2 ± 0.05 m^2 compared to 0.5 ± 0.03 m^2 in the seagrass habitat. Along with a higher recruitment frequency in the back-reef habitat (t_{17}= 8.84, P= 0.001), a greater diversity of corals recruited (Figure 3.16). Across habitats, most recruits belonged to the families *Poritidae* and *Siderastreidae.* The back-reef had recruits identified across five families and 'other', whilst the seagrass had recruits identified to two families and 'other'.

The newly recruited coral populations established from the recruitment tiles mirrored the adult coral populations for both habitats, with similar relative abundance of the major families (Figure 3.17). Analysis of individual families between the established and newly recruited coral populations revealed two differences: firstly, in the back-reef habitat *Faviidae* recruits were 8.1 % higher than the relative established coral population (t_4= 52.66, P= 0.01), and secondly, more corals fell into the 'other' category within the established coral population of the seagrass habitat compared to the recruited population (t_4= 150.22, P= 0.001).

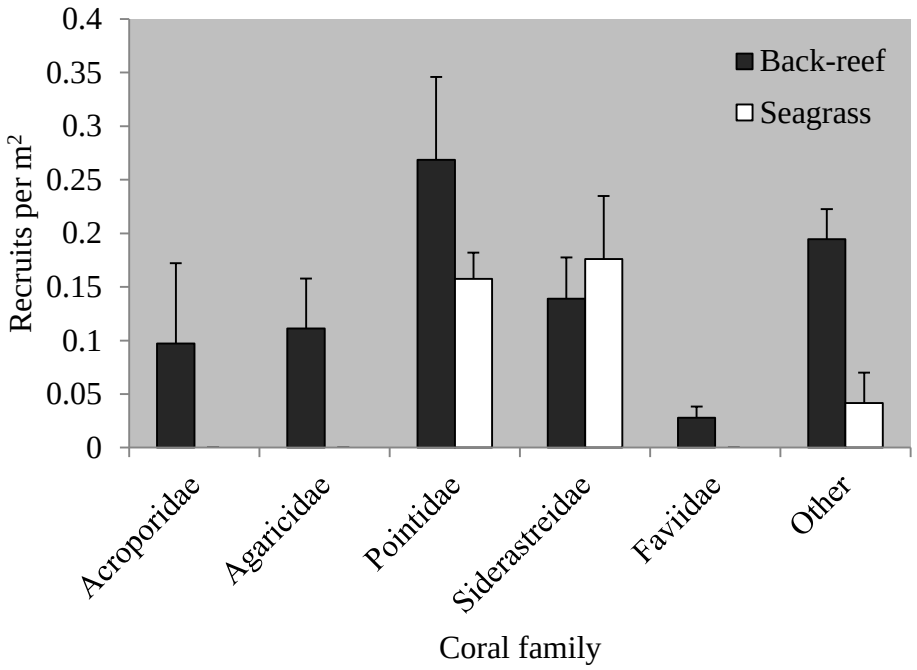


Figure 3.16| Coral recruitment per family per m^2 for the seagrass and back-reef habitats of Little Cayman, Cayman Islands, BWI. Data was collected from recruitment tile analysis over 20-months initiated in January 2012 from three back-reef and three seagrass sites. Data is the mean with standard error).

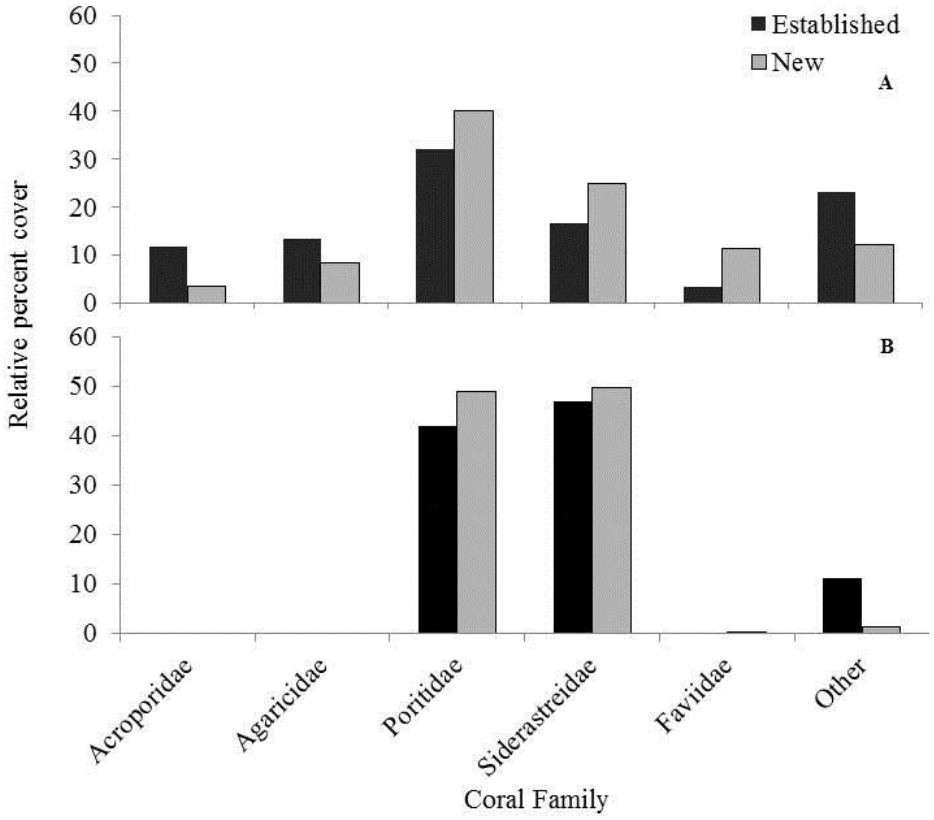


Figure 3.17| A comparison of the established and newly recruited coral communities of: A) the back-reef and B) the seagrass habitats of Little Cayman, Cayman Islands, BWI. The relative percentage cover of the major coral families was established for the counted recruits on settlement tiles (20-month period from January 2012), and established colonies on 9 x 50 m transects within each habitat (surveyed in February 2012 and 2013).

Benthic recruitment composition – The recruited benthic composition was different between the seagrass and back-reef habitats ($F_{4,13}$= 200.73, P= 0.01), with the majority of taxa varying with the exception of tunicates, serpulid worms, corals, coralline algae, rhodophyta and chlorophyta (Figure 3.18). Recruited coral cover was very low (< 0.7 %) across habitats. A comparison of the percentage cover of recruited calcium carbonate polymorphs between habitats, revealed that there was a difference between the back-reef and seagrass habitats (t_{12}= 114.14, P= 0.01). Specifically, there was less aragonite (t_{12}=5.24, P= 0.05), and high Mg-calcite (t_{12}= 20.98, P= 0.001) in the seagrass habitat with more carbonate sediment (F_{12}=1165.65, P= 0.001) than in the back-reef (Figure 3.19). Interestingly, for the least soluble polymorphy (calcite) there was no difference between habitats, however, for the more soluble polymorphs of high Mg-calcite and aragonite, we see less accretion in the seagrass habitat.

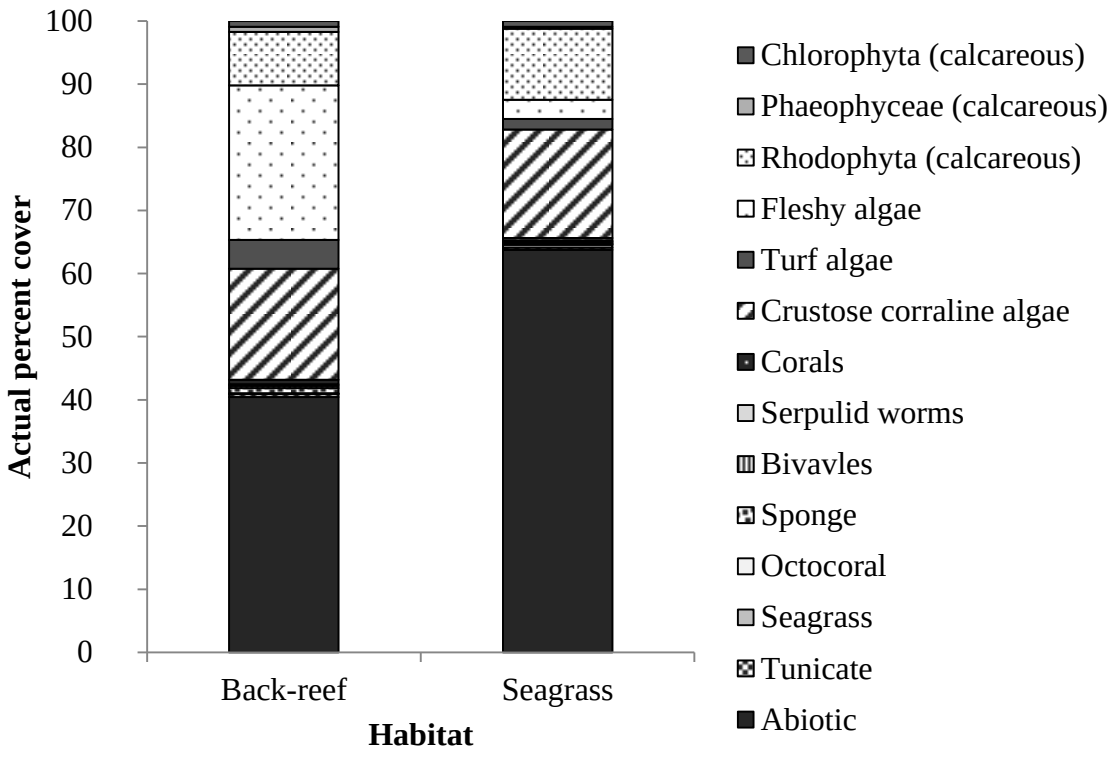


Figure 3.18| The percent cover of the major benthic taxa recruited over a 20-month period in the seagrass and back-reef habitats of Little Cayman, Cayman Islands, BWI. Starting in January 2012, for a 20-month duration, settlement tiles (*n*= 81 per habitat) were installed to record recruitment. Data presented is actual percentage cover estimated from CPCe counts (150 points) of high-definition pictures of each tile.

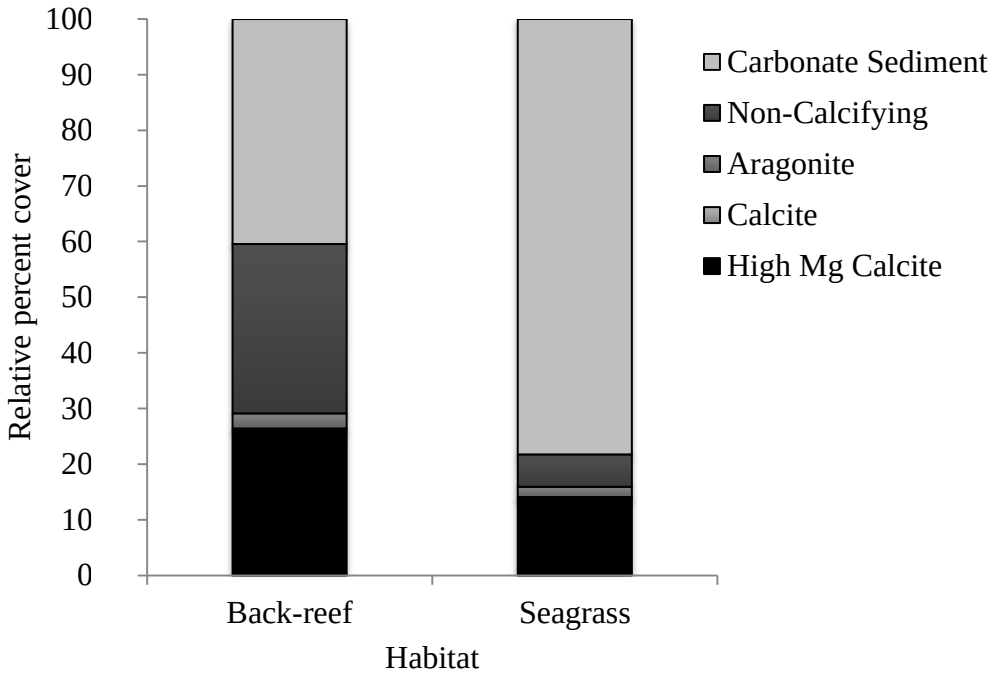


Figure 3.19| The relative percentage cover of calcium carbonate polymorphs recruited over a 20-month period in the seagrass and back-reef habitats of Little Cayman, BWI. Starting in January 2012, for a 20-month duration, settlement tiles (*n*= 81 per habitat) were installed to record recruitment. Data presented is relative percentage cover estimated from CPCe counts (150 points) of high-definition pictures of each tile.

3.3.3| The relationship between pH and benthic community composition

An increase in pH Cv resulted in an increase in the percentage cover of photoautotrophs (r^2= 0.846, *n*= 18, *P*= 0.001, Figure 3.20a, Photoautotroph cover= 0.0002pHCv +0.0043) whilst calcifying benthos decreased (r^2= 0.780, *n*= 18, *P*= 0.001, Calcifying benthos= -0.0009pHcv + 0.0125, Figure 3.20b). An increase in mean pH resulted in a decrease in the percentage cover of photoautotrophs (r^2= 0.872, *n*= 18, *P*= 0.001, Photoautotroph cover= -0.0009pHCv +8.205, Figure 3.20c) and an increase in the percentage cover of calcifying benthos (r^2= 0.802, *n*= 18, *P*= 0.001, Calcifying benthos cover= 0.0038pHCv +8.129, Figure 3.20d).

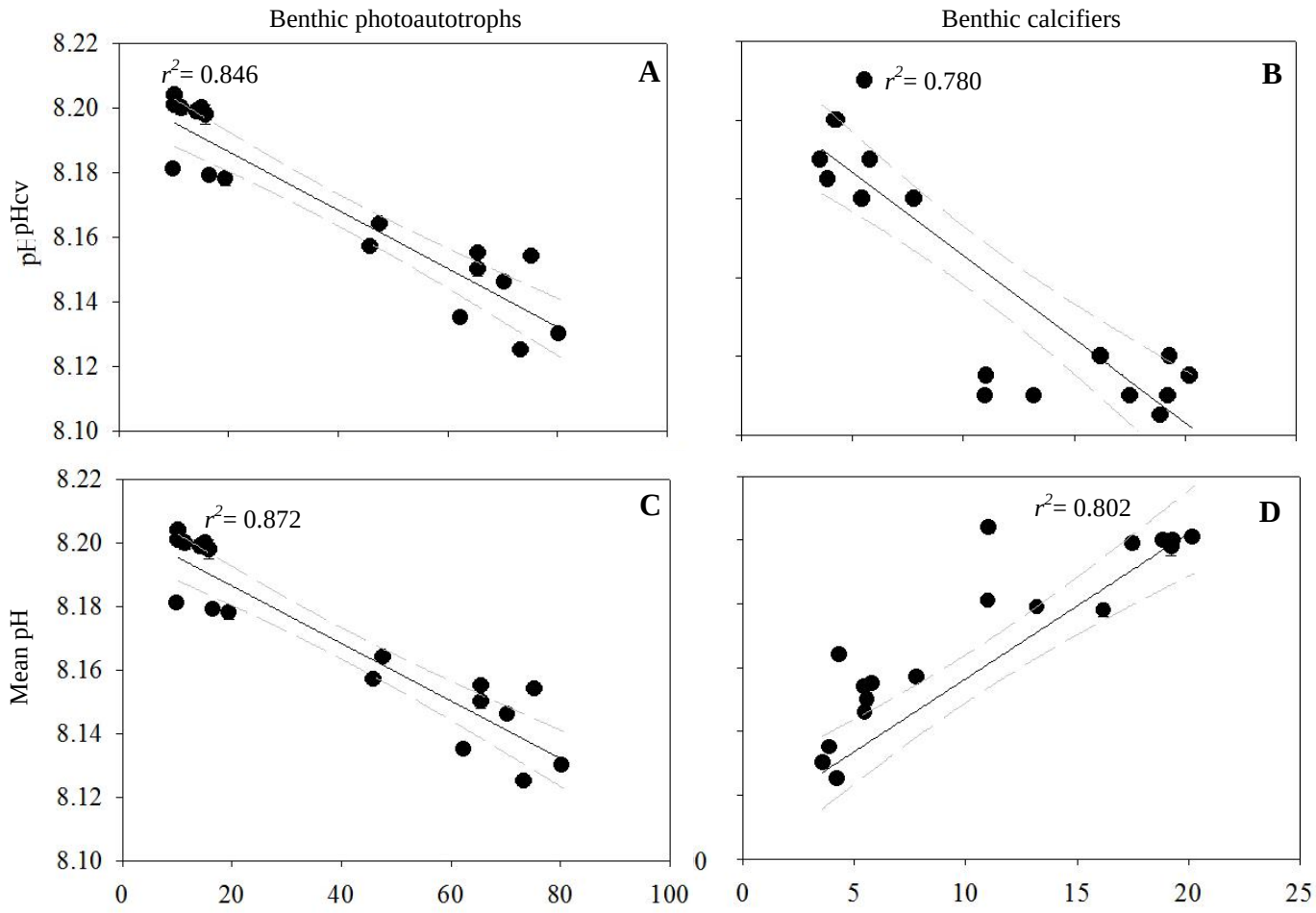


Figure 3.20| Plots of mean and Coefficient of Variation (Cv) pH versus calcifying (coral, hydrocoral, calcifying algae) and non-calcifying (seagrass and non-calcifying algae) benthos for the non-reef habitats of Little Cayman, Cayman Islands, BWI. A) pHcv versus photoautotrophs (r^2= 0.846, *n*= 18, *P*= 0.001, Photoautotroph cover= 0.0002pHcv +0.0043), B) pHcv versus calcifying benthos (r^2= 0.780, *n*= 18, *P*= 0.001, Calcifying benthos= -0.0009pHcv + 0.0125), C) mean pH versus photoautotrophs (r^2= 0.872, *n*= 18, *P*= 0.001, Photoautotroph cover= -0.0009pHcv +8.2055), D) mean pH versus calcifying benthos (r^2= 0.802, *n*= 18, *P*= 0.001, Calcifying benthos cover= 0.0038pHcv +8.129). Data is from three back-reef and three seagrass sites, with data collected in 2012 and 2013. Regression is shown with 95 % confidence interval.

3.3.4| The metabolic expenditure of the dominant coral species across habitats results

Calcification was maintained across the non-reef habitats remaining similar to the outer-reef control (Figure 3.21a, Table 3.8), even with the elevated variance in pH of the non-reef habitats, (Figure 3.21b). The greatest rates of calcification were recorded for *P. astreoides* across habitats (10.4 ± 0.9 mmol cm^2 d^{-1}, Table 3.9). Rates of calcification were consistent over the daylight sampling period, however, calcification rates at dusk (16:00-20:00 h) were significantly higher than rates obtained during the other night-time sampling periods ($F_{2,54}$= 11.53, P= 0.001, *post-hoc:* P= 0.01). Rates of photosynthesis were elevated in the outer-reef control relative to the non-reef habitats ($F_{2,54}$= 3.32, P= 0.01), however, no differences in respiration rates were observed. Rates of photosynthesis varied over the day-light sampling period ($F_{2,54}$= 11.60, P= 0.01), whilst respiration rates again remained stable.

Table 3.8| Model parameters to estimate daily net Photosynthesis (P), Respiration (R) and Calcification (G) as a function of habitat (back-reef, outer-reef control, seagrass) for corals in reef systems around Little Cayman, Cayman Islands, BWI

Metabolic Parameter	Model terms	Estimate	SE	*t*-value	*P*-value
P	Intercept	18.03	4.16	4.33	0.005
	Habitat	5.37	1.62	3.32	0.01
R	Intercept	35.88	1.18	30.46	0.0001
	Habitat	-0.42	0.42	-1.00	N/S
G	Intercept	29.18	5.97	4.89	0.001
	Habitat	-2.80	2.36	-1.19	N/S

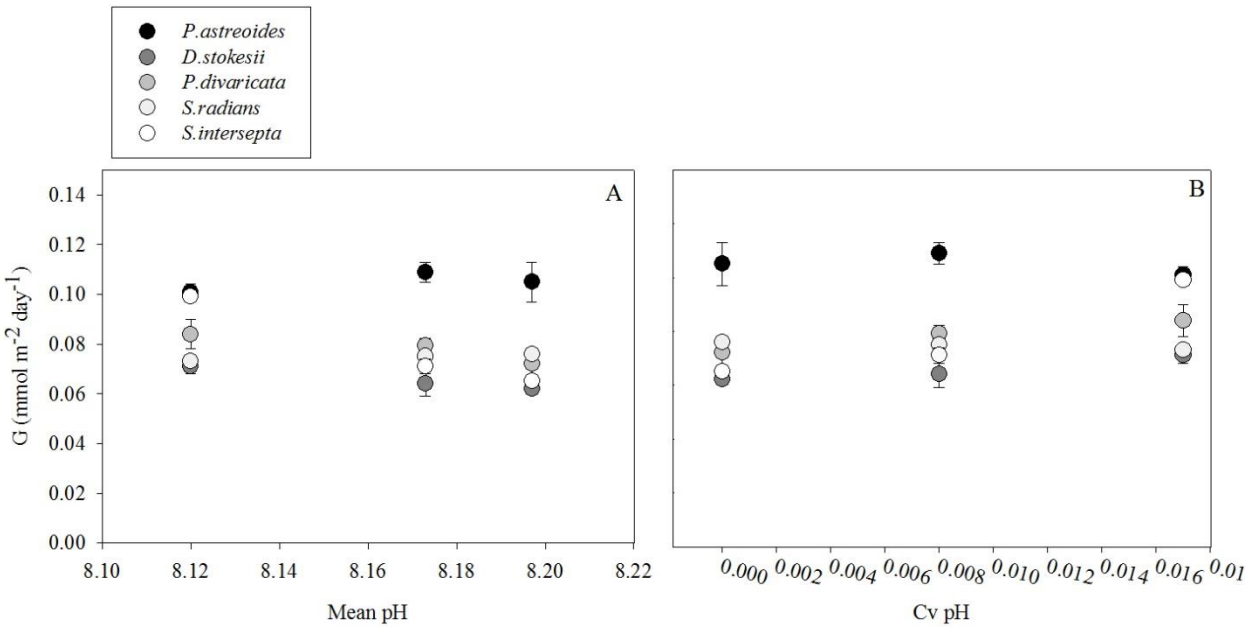


Figure 3.21| The mean daily integrated net calcification (G) (mmol m^2 day^{-1}) versus: A) mean pH and B) pH Coefficient of Variation (Cv) for five dominant coral species in reef habitats of Little Cayman, Cayman Islands, BWI. All data plotted are mean values ± standard error (SE, *n*= 3) for the five dominant coral species examined across non-reef habitats (seagrass, back-reef) and outer-reef habitat. Regression analysis was non-significant for both mean and Cv pH versus calcification.

Table 3.9| The metabolic response (Photosynthesis (P), Respiration (R) and Calcification (G) for five dominant coral species in reef systems around Little Cayman, Cayman Islands, BWI

Habitat	Metabolism (Mean ± SE)	*P.astreoides*	*D.stokesii*	*P.divaricata*	*S.radians*	*S.intersepta*
Seagrass	P (μmol O_2 cm^2 d^{-1})	30.2 ± 0.5	20.2 ± 0.4	25.6 ± 0.2	23.2 ± 0.6	23.2 ± 0.8
	R (μmol O_2 cm^2 d^{-1})	38.2 ± 1.2	35.2 ± 0.6	35.7 ± 0.3	34.2 ± 0.4	35.3 ± 0.1
	G (mmol cm^2 d^{-1})	10.5 ± 0.8	6.2 ± 0.2	7.2 ± 0.5	7.6 ± 0.1	6.5 ± 0.2
Back-reef	P (μmol O_2 cm^2 d^{-1})	31.2 ± 0.3	23.2 ± 0.4	26.9 ± 0.3	25.3 ± 0.1	26.5 ± 0.8
	R (μmol O_2 cm^2 d^{-1})	37.3 ± 0.4	33.2 ± 0.4	34.2 ± 0.1	30.1 ± 05	37.4 ± 0.1
	G (mmol cm^2 d^{-1})	10.9 ± 0.4	6.4 ± 0.5	7.92 ± 0.3	7.5 ± 0.2	7.1 ± 0.3
Outer-reef	P (μmol O_2 cm^2 d^{-1})	55.2 ± 0.1	30.1 ± 0.1	34.2 ± 0.6	29.2 ± 0.5	27.4 ± 0.1
	R (μmol O_2 cm^2 d^{-1})	36.2 ± 0.5	35.2 ± 0.1	34.1 ± 0.5	32.9 ± 0.2	36.2 ± 0.1
	G (mmol cm^2 d^{-1})	10.1 ± 0.3	7.1 ± 0.3	8.4 ± 0.6	7.29 ± 0.1	9.9. ± 0.2

Data was collected during May 2013, over three 24 h sampling sessions, with rates calculated from eight 3 h incubations per 24 h period. All rates were corrected for any metabolic activity of the seawater, and were normalised to the volume of the respirometry chamber and surface area of the coral.

3.4| Discussion

3.4.1| Spatial and temporal variability of physiochemical properties across habitats

Non-reef habitats are highly variable in all physiochemical conditions relative to the outer-reef, which remained comparatively stable through all measured temporal and spatial scales. Globally, inshore habitats like seagrass beds are being identified as having highly variable carbonate chemistry conditions (Manzello *et al*., 2012; Price *et al.,* 2012; Yates *et al.,* 2014) which is raisings questions over how these sites will be influenced by OA in the future, due to the range of conditions they already experience on a regular basis (Durate *et al.,* 2013).

Inshore, the main physiochemical differences between habitats were pH, pCO_2 and Ω_{arg}. Sites within habitats had very similar local physiochemical conditions. To know whether habitat trends identified on Little Cayman apply more broadly, further investigation is necessary across other study locations situated across bioregions. Stage of the lunar tidal cycle did not appear to influence the carbonate chemistry, potentially due to the minimal tidal range experienced in the Cayman Islands (range during sampling: 0.4-0.9 m, NOAA, 2015). Seasonal influences were evident within the non-reef habitats with both habitats experiencing greater variation during the summer season. Interestingly, the range of pH measured over a season was not dissimilar to the average daily range experienced by each habitat.

Diurnal trends in pH and pCO_2 corresponded with the time-of-day and average PAR, showing a large drawdown of CO_2 during the day when PAR is high and photosynthesis is the dominant metabolic process, with the reverse seen at night when respiration is prevailing. Local photosynthetic activity has been proposed through models (Unsworth *et al.,* 2012) and field studies (Manzello *et al.,* 2012; Anthony *et al*., 2013; Buapet *et al*., 2013; Hendricks *et al*., 2014) to influence the local chemistry, potentially buffer impacts of OA. Importantly however, the high drawdown of pCO_2 measured in the seagrass habitat during the daytime was slightly offset by

periods of extreme low pH recorded at night. At some points, nighttime pH was recorded below 7.8, which represents conditions predicted for the future open-ocean under OA scenarios (IPCC, 2015). The periods of extreme low pH measured on Little Cayman are similar to the findings of Price *et al.* (2012) in the northern Line Islands, suggesting that our observations are not limited to this bioregion. The relationship between daytime draw-down of CO_2 versus nightime production of CO_2 will likely influence the response of these non-reef habitats to future OA.

3.4.2| Influence of benthic and local biogeochemical drivers on carbonate chemistry

The stability of the outer-reef suggests that it experienced minimal influence from local biogeochemical drivers and thus, its chemistry is driven primarily by that of the open-ocean. Conversely, the elevated variance experienced in the non-reef habitats, combined with seasonal influences (elevated variance in the summer-wet season) suggests that local chemistry is heavily influenced by the ambient benthic composition, a trend also observed in the Florida Reef Tract (Manzello *et al*., 2012). The seagrass habitat had greater variability in pH and pCO_2 than the back-reef, likely resulting from the higher coverage of photoautotrophs (Anthony *et al.*, 2011b). The shallow water depth (*ca*. 1m) also likely magnifies the chemical response as there is a smaller body of water. The nA_T vs nC_T plots corroborate this observation, with the seagrass habitats experiencing periods influenced significantly by photosynthesis and calcification, as well as respiration and carbonate dissolution, characteristic of the diurnal influence of pCO_2 on the biogeochemistry of this habitat.

Differences between the inshore non-reef habitats and the outer-reef created an inshore to offshore gradient in the carbonate chemistry. The gradient was driven primarily by changes in pCO_2, which suggests that net primary productivity is again responsible. The gradient resulted in

depleted pCO_2 and consequently, elevated pH and Ω_{arg} in the non-reef habitats. The gradient was predominant during the dry-winter season. Conversely however, other studies documenting an inshore to offshore gradient have only documented it during the local summer season (e.g. Manzello *et al.,* 2012). Within this study, the gradient was present across seasons, and the larger magnitude during the winter-dry season corresponded with reduced storm activity and rainfall (Turner *et al.,* 2013), a pattern consistent with most other study-locations during the summer season. No differences in PAR were detected between seasons, however, greater water velocity and higher variability in salinity were observed during the summer season. Elevated water velocity reduces the residency time of water and can increase mixing (Ohde & Woesik, 1999; Unsworth *et al*., 2012), which potentially dilutes the inshore to offshore gradient. Lower salinity can also increase pCO_2 absorption as seawater can hold more gas (Feely, 2010).

3.4.3| Coral populations of reef and non-reef habitats

In both the back-reef and seagrass habitats, coral cover was low. However, surprisingly the seagrass habitat supported a high diversity of corals despite typically being considered a poor habitat for coral survival (Jackson, 1985) and experiencing periods of extremely low pH. Yates *et al.* (2014) recorded a high diversity of corals in mangrove systems (which experienced variable pH), around St John's in the US Virgin Islands, adding to the growing body of evidence that corals can survive under a wide range of conditions, including periods of extreme low pH predicted under future OA (Fabricius *et al.*, 2011). Furthermore, minimal visual signs of coral stress (e.g. bleaching/disease/partial-mortality) were observed, suggesting that the coral species found within these habitats are coping with the highly variable and extreme conditions. It was however noted that the overall benthic recruitment of the more soluble polymorphs of calcium

carbonate were reduced in the seagrass habitat. The reduced recruitment of aragonite and high-Mg-calcite organisms could be coincidence, however, it is plausible that the extreme low pH conditions, experienced at night (*ca.* 25 % of the total time of day), may be challenging carbonate genesis of the more soluble polymorphs; an observation reported in laboratory conditions (Kleypas *et al.,* 2006).

Importantly within this study, both non-reef habitats received coral recruits, with evidence that spawning events occur around August and September. In 2013 there were smaller colonies than (< 5 cm) in 2012 suggesting a potential recruitment event or an increase in recruitment survival from the preceding year. To-date, minimal research has been conducted globally on the recruitment of corals into non-reef habitats, despite the importance of determining the ability for corals to recruit into these systems when assessing their viability to act as a potential refuge against future climate change. The seagrass habitat only received recruits from two main families: *Poritidae* and *Siderastreidae*, which represents the established adult community of this habitat. However, what is not understood is whether these are the only families that are able to tolerate and survive the conditions of the seagrass habitats, or whether the seagrass population results from a larval supply limitation? Further investigation is necessary to address these unknowns, and they warrant exploration to help us understand the ability of these systems to act as a potential refuge.

Previous studies have commented that seagrass beds are poor habitats for coral growth due to their soft substrate (Jackson, 1985) and the stunted growth of colonies recorded (Glynn, 1974), therefore, any buffering effects of OA have been proposed downstream of seagrass beds (Manzello *et al*., 2010, Anthony *et al.,* 2013). However, our findings demonstrate that patches of intermittent hard-ground within seagrass beds can house corals themselves, which in turn offers a

different form of refuge, directly within seagrass beds. Under this form of refugia, corals are: (i) exposed to a high-variability in pH conditions which potentially pre-conditions them to lower pH, and (ii) provided optimal conditions for calcification with mean pH and Ω_{arg} elevated relative to the outer-reef despite the periods of low pH at night.

3.4.4| Metabolic expenditure of dominant coral species across habitats

For the coral species whose metabolism was investigated, the maintenance of calcification across non-reef habitats corresponds with the elevated mean pH and Ω_{arg} for these habitats. Despite large variability in pCO_2 resulting in pH as low as 7.8, calcification was sustained. These results suggest that the duration of time spent at a given pH is what influences overall rates of calcification. Daylight calcification was consistent over the daylight-sampling period, as was nighttime calcification rates. However, during the dusk-twilight period, calcification varied from the daytime and nighttime calcification rates, a consideration necessary for sampling frequency.

Rates of calcification were sustained without a change in respiration, however, rates of photosynthesis were slightly reduced relative to the outer-reef. Rates of photosynthesis also varied during the day, which means that several daytime samples are needed to encapsulate this variance. Comparatively, high calcification within the back-reef and seagrass habitats supports the hypothesis that these habitats play a buffering role for resident corals possibly from OA through biologically-mediated elevation of mean pH. Understanding whether these habitats, across different bioregions, have similar biogeophysical conditions and can provide similar ecosystem services remains unknown. Similarly, it is unknown whether reef/non-reef habitats whose mean pH is lower than the outer-reef control, support the same metabolic functions as the habitats described in this chapter.

3.4.5| Consequences for future research

The mean and range of pH, temperature, salinity and TA for each habitat remained similar over a 5-day and 18-day sampling period within an individual season, thus highlighting the similarity in the contribution of these physiochemical factors on a daily, weekly and monthly scale. This finding was further supported by the similarities in the range of pH measured over a season, lunar tidal or diel cycle. Moving forward with the research to address questions raised within this chapter, confidence can be taken in a shorter sampling period to capture the physiochemical conditions of a habitat, as long as seasonality is considered.

When assessing the metabolic activity of coral *in situ*, differences in photosynthesis during daytime sampling demonstrates the need to have replicate samples throughout this period. However at night, no differences in respiration were recorded and calcification remained similar after the dusk sampling, therefore, a minimum of one dusk and one nighttime sample is necessary within each sampling replicate to estimate the metabolic activity of corals.

3.5| Key Findings

3.5.1| Spatial and temporal variability of physiochemical properties across habitats

- Non-reef habitats are highly variable in all physiochemical conditions relative to the outer-reef.
- Carbonate chemistry was highly dynamic in the non-reef habitat, with large diel variation and increased variability during the summer season.
- pH, pCO_2 and Ω_{arg} were the most variable physiochemical factors between the two non-reef habitats.

3.5.2| Influence of benthic and local biogeochemical drivers on carbonate chemistry

- Inshore depletion of pCO_2 created an inshore to offshore gradient, which was greatest during the winter season.
- The non-reef habitats saw the greatest range in nA_T and nC_T and exhibited co-variability between nA_T and nC_T more strongly influenced by photosynthesis-respiration (and hence CO_2 uptake-release).
- The percent cover of photoautotrophs increased, whereas the cover of calcifying benthos decreased, with lower mean pH and increased pH Cv.

3.5.3| Coral populations of reef and non-reef habitats

- Coral cover was low across both habitats, however, a surprisingly large diversity (70 % of the back-reef diversity) of coral species was recorded in the seagrass habitat.
- Corals recruited to both non-reef habitats.
- Recruited coral populations were similar to the established adult coral populations.

3.5.4| Metabolic expenditure of dominant coral species across habitats

- For corals, net daily calcification was sustained across habitats, with no change in respiration and despite a slight decrease in photosynthesis rates.

3.5.5| Consequences for future research

- A 5-diel sampling schedule can capture the mean and Cv of a habitat within a given season.
- *In situ* analysis of coral metabolism requires replicated daytime sampling and a minimum of one dusk and one nighttime sampling period within each sampling replicate to encapsulate the daily variance in coral metabolic activity.

Chapter 4| Spatial variability of carbonate chemistry and the *in situ* metabolic response of dominant coral species

Part of this chapter is in review in Global Change Biology as the manuscript: Pre-conditioning and buffering services of mangroves and seagrass beds for corals threatened by climate change.

4.1| Introduction

Coral reefs are typically restricted to the photic zone (< 60 m) of the tropics (Lesser, 2004) and tolerate relatively narrow ranges of abiotic variables (salinity, light, temperature, turbidity, pH) (Done, 1999; Kleypas *et al.*, 1999; Sheppard *et al.*, 2010). However, some species of corals have demonstrated great plasticity in surviving in conditions that would typically be considered sub-optimal, maybe even extreme for coral growth. For example, *Siderastrea siderea* is routinely exposed to varying salinity (< 10 and/or > 37 ppm) in the coastal lagoons of south Florida (Lirman & Manzello, 2009) and corals living around CO_2 vents in Papua New Guinea survive in seawater of 7.7-7.8 pH units (Fabricius *et al*., 2011). Consequently, our understanding of the fundamental niche of reef building coral is expanding as researchers look to suboptimal habitats.

Worldwide environments are changing, partly as a response to global anthropogenic threats (e.g. ocean warming and acidification, Hoegh-Guldberg *et al*., 2007; Dove *et al.*, 2013) and partly due to local point source stressors such as increased sedimentation due to coastal destruction (Hughes *et al.*, 2003). Understanding the physiological tolerance of corals to highly-variable, sub-optimal conditions are important for determining the vulnerability of reefs threatened by global environmental change.

Researchers are starting to examine the characteristics and traits of corals that persist within environments that were traditionally considered extremes and representing growth

limiting conditions (e.g. Fabricius *et al.*, 2011; Price *et al.*, 2012; Hume *et al.*, 2015). However, whilst corals clearly demonstrate some form of tolerance to survive within these highly variable habitats (Price *et al.*, 2012; Yates *et al.*, 2014), the biological mechanisms that infer tolerance remain unknown. Similarly, whether patterns in corals ability to biologically perform under variable environments today are common across taxa remains untested. It is also unknown if corals biological performance within these habitats is a localised phenomenon or characteristic of corals across larger spatial scales. Therefore the objectives of this chapter are to:

1) Quantify the mean and variance of pH experienced by reef and non-reef habitats at sites in both high and low biodiversity regions;
2) Measure the association between mean and variability (Cv) in pH and species richness;
3) Quantify the ability of corals to expand their niches into non-reef habitats;
4) Measure the physiological responses (photosynthesis, respiration, calcification) of corals existing in different regions to ascertain commonalities;
5) Measure how coral calcification rates change between habitats and sites in relationships to photosynthesis and respiration;
6) Test whether certain coral species or coral growth forms are superior in sustaining metabolic activity across different habitats.

In addressing these objectives this research will deliver a novel dataset quantifying the metabolic costs for dominant coral species living in suboptimal environments that may become the norm in coral reefs in the near future. The data will provide insight in to the relationship between photosynthesis to respirtion and calcification. Combined the data will help in the identification of tolerant coral species and will expand our knowledge of their environmental niche and the metabolic costs of living at the extremes of their environmental distribution.

4.2| Materials and Methods

4.2.1| Study locations

Three study locations situated across three bioregions (Atlantic Ocean (AO), Pacific Ocean (PO) and Indian Ocean (IO)) were investigated (Figure 4.1). These three sites were selected to allow a comparison across three geographically separate locations that experience different species and levels of diversity. Each site was similar in that it was a small island that received minimal local anthropogenic impacts. All sites were also a similar size and had no direct impact from freshwater at the study location. At each location, an outer-reef control site subject to open-ocean seawater chemistry was compared to two non-reef habitat sites, with all sites 2-4 m in depth and situated away from any freshwater inputs. All sites experienced a tidal cycle range of 0.1-1.0 ± 0.1m during sampling. All non-reef habitats were situated within lagoons that had high water retention making them ideal locations to study the effects of local water chemistry.

The Atlantic Ocean study location was situated on the north coast of Little Cayman, with the two non-reef habitats (back-reef and seagrass) situated in Grape Tree Bay (Figure 4.1, see Chapter 1, section 1.6.1). The outer-reef habitat was adjacent to the bay. The back-reef habitat consisted of inter-dispersed seagrass and small patch reefs (15-30 % cover of seagrass) and was not a true reef, thus is referred to as a non-reef habitat within this chapter. This transitional site was selected to assess the continuum of carbonate chemistry changes from the outer-reef control, to the inshore seagrass habitat in the absence of tidal flooded mangrove shrubland (Turner *et al.*, 2013). The outer-reef site was subject to the ocean currents around Little Cayman which move in a northwesterly direction (Stoddard, 1980; Turner *et al*., 2013), while the two non-reef habitat sites experienced a western current. Sites were subject to a mix of diurnal and semi-diurnal tidal cycles.

The IO study location was situated around the island of Curieuse within the Seychelles archipelago (Figure 4.1, see Chapter 1, section 1.6.3). The two non-reef habitat sites consisted of a seagrass dominated habitat and a mangrove habitat situated within a bay known locally as Baie La Raie. The mangrove site was not directly under the mangrove canopy but in close proximity on the seaward side so it was not shaded from light. The outer-reef site was located on the reef flat adjacent to the reef crest. All sites were located within the Curieuse National Marine Park and subject to a semi-diurnal tidal cycle that ran in an anti-clockwise direction within the bay throughout sampling.

The Pacific Ocean study location was situated in the Wakatobi, southeast Sulawesi (Figure 4.1, see Chapter 1, section 1.6.4). The outer-reef site was situated on the reef flat known locally and in previous publications as “Pak Kasims” off Hoga Island. One of the non-reef habitat sites was an adjacent inshore seagrass habitat also off the south coast of Hoga Island.The second non-reef habitat was immediately adjacent to the Langeria mangroves, located off the north coast of Kaledupa Island. As for the Indian Ocean site this non-reef habitat site was situated outside of the mangrove canopy. The carbonate reef systems here experience good water quality with minimal impact from sediment load (Bell & Smith, 2004). During sampling currents ran in a southeast direction but were driven by tides, with sites exposed to a semi-diurnal tidal cycle.

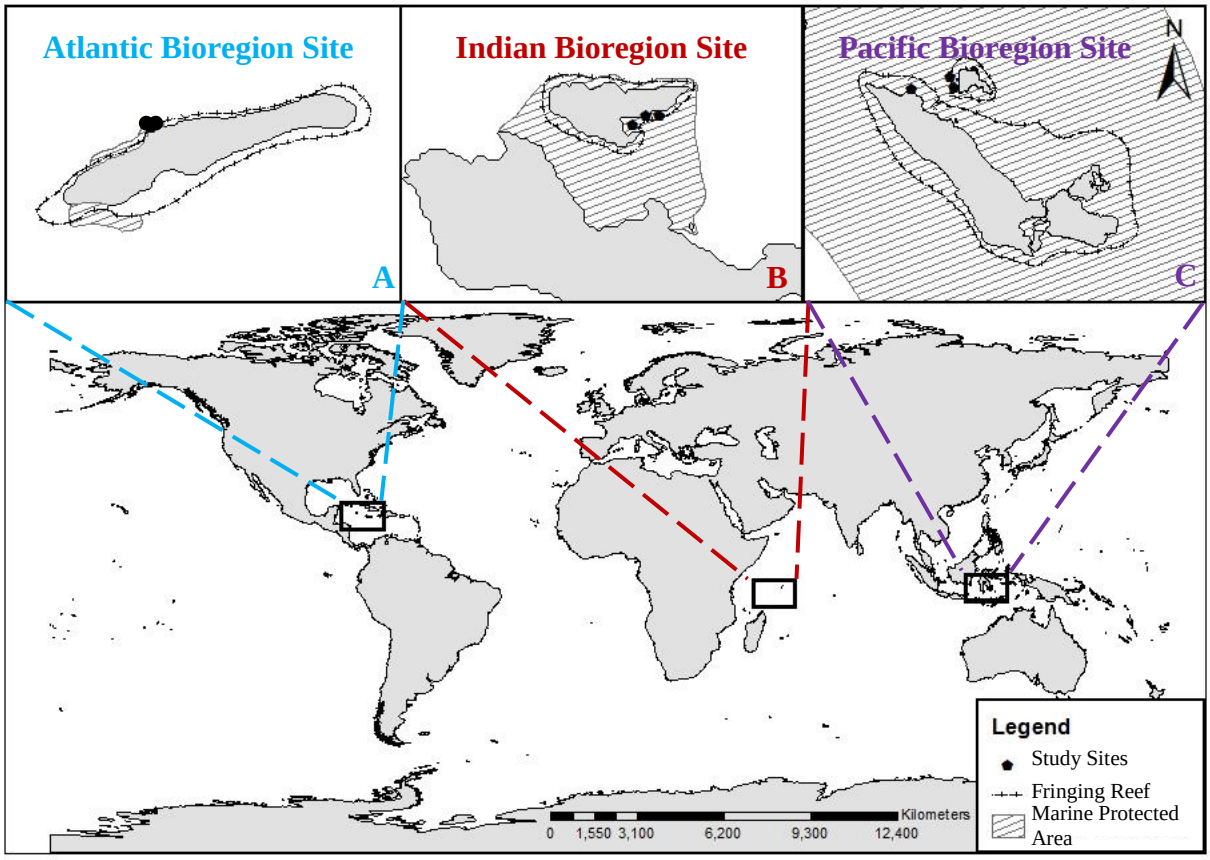


Figure 4.1| The three study locations indicated on a global map. A) Little Cayman, Cayman islands, BWI situated in the Atlantic Ocean, B) Curieuse, the Seychelles located in the Indian Ocean, and C) Hoga and Kaledupa located in the Wakatobi, southeast Sulawesi in the Pacific Ocean. At each location one outer-reef habitat and two non-reef habitats (Atlantic: seagrass and back-reef, Pacific and Indian: seagrass and mangrove) were sampled.

4.2.2| Sampling regime

Environmental conditions and *in situ* coral metabolic activity for select coral species were measured over five 24 h sampling days within a two-week period. Sampling took place during the annual dry seasons of each region. Based on results from Chapter 3 it was determined that the mean and variance (coefficient of variation (C_V)) of environmental conditions for this short period dataset did not significantly differ from values determined for a longer-term study across a full neap-spring cycle (18-days) within the same season. As expected (Albright *et al.*, 2013), a seasonal affect (overall difference of 0.07 pH units) was identified and thus we subsequently focused on the dry season within each bioregion (Atlantic Ocean: March 2014, Indian Ocean: April 2014, Pacific Ocean: August 2014).

4.2.3| Abiotic assessment

Temperature, light and nitrates **–** Temperature (°C, accuracy ± 0.53 ºC) and Light (Lux, accuracy relative to light levels: see Onset, 2012) were measured using a HOBO Pendant Temperature/Light 64k Logger (Model UA-002-64, Microdaq, USA). Light was measured in Lux and converted to PAR as described in Chapter 3 (section 3.2.2). Three HOBOs were situated within each habitat (at each study location) and data was averaged, providing an accurate (*ca*. 3 %) conversion to PAR (see Long *et al*., 2012). Each HOBO was temporarily secured *in situ*

using zip-ties and set to log every 30 s over a continual seven day period within the two week sampling period. Nitrate sampling was conducted using an ORION nitrate electrode (accuracy ± 0.01 μM, Model 9307, Fisher scientific, USA) attached to the ORION 5 Star meter. Samples were taken diurnally (7.00 and 16:00 h) on the days of the *in situ* coral incubations (*n*= 10 total per habitat per location).

Carbonate chemistry sampling regime and approach – Over the five sampling days, discrete water samples were collected every 3 h starting at 6:00 h and ending at 18:00 h. A total of four daytime samples were collected and one dusk sample (18:30-19:00 h). An additional evening water sample was collected (*ca*. 22:00 h). Seawater samples were collected as described in Chapter 2 (section 2.2.2).

4.2.4| Benthic measurements

Benthic habitat assessments were conducted using continuous line intercept transects. Within each habitat three 30 m transects were randomly located, with each being separated by a minimum of 50 m. The initial transect was randomly located, and transects ran parallel to shore. Data were recorded using a high definition video-camera (Canon, G12 in underwater housing WP-DC 34) and footage was later analysed to determine benthic community composition (species level). One 20 m^2 quadrat was examined at the start of each transect to determine species specific coral colony density. In addition corals that were: (i) de-pigmented, and/or showed evidence of, (ii) recent or (iii) past mortality were also recorded (defined by AGRRA protocol version 5.4, Lang *et al*., 2010, Chapter 3, Table 3.1).

4.2.5| *In situ* metabolic incubations

In situ incubations were conducted to assess the metabolic expenditure of dominant coral species existing across habitats within each study location. The metabolic function (daily-integrated calcification, photosynthesis and respiration) was determined for the dominant coral species: Atlantic Ocean site: *Dichocoenia stokesii, Porites astreoides, Porites divaricata, Siderastrea radians, Stephanocoenia intersepta*. Indian Ocean site & Pacific Ocean site: *Acropora austera, Pocillipora damicornis, Porites lutea.* Indian Ocean site only*: Porites attenuata*. Together these species represented the majority (55-70 % Atlantic Ocean site, 56-72 % Indian Ocean site and 49-70 % Pacific Ocean site) of the total coral abundance within the non-reef habitats. *Acropora palmata*, the iconic coral species of the Atlantic Ocean, was also examined due to its critically endangered status and functional importance in the Caribbean (Aronson *et al.*, 2014).

Incubations were performed using the Flexi-Chamber protocol outlined in Chapter 2 (section 2.2.4). At each location five separate colonies of each species were examined in each habitat over a period of two weeks. Sampling was conducted from 7:00 to 16:00 h with incubations (n= 3) running for 3 h (as deemed appropriate from Flexi-Chamber validation in Chapter 2). From 16:00 to 19:00 h one dusk incubation was conducted. From 19:00 to 20:00 h one nightime incubation was conducted, as validated in Chapter 3 (section 3.2.2). Sample extraction and analysis followed the procedures outlined in Chapter 2 (section 2.2.4), from which daily rates of photosynthesis, respiration and calcification were obtained.

4.2.6| Statistical analysis

All statistics were conducted in either R software (R 237 Development Core Team, 2011), Sigma Plot 10.0 (Systat Software, San Jose, CA), or SPSS 17 (SPSS Inc, 2008). Parametric test

assumptions were all met unless stated, with the Levene's test used to check homogeneity of variance and qq-plots to assess the normality of the data.

Environmental characteristics were compared between habitats using 2-way ANOVA with post-hoc Tukey HDS. Linear regression was used to assess the relationship between daily net calcification rates to the daily photosynthesis-to-respiration ratio (P:R). Liner regression was also used to assess the relationships between calcification rates to pH mean, calcification rates to pH C_V, percent cover of calcifying and non-calcifying species to pH C_V and mean pH in relation to photosynthesis and respiration rates. A Pearson's correlation was used to compare light with pH over a diel cycle for the non-reef habitats, as well as comparing changes in calcification with changes in photosynthesis.

Salinity-normalised TA (nA_T) to dissolved inorganic carbon (nC_T) plots were generated (Suzuki & Kawahata, 2003; Kleypas *et al*., 2011; Yates *et al*., 2014) to assess the biogeochemical control of each habitat. The ratio of net ecosystem calcification to net community production (NEC:NEP) were derived from these nA_T-nC_T plots as $1/[(2/m)-1]$ (where m is the regression slope from the corresponding linear regression equation of nA_T vs nC_T) (Suzuki & Kawahata, 2003; Kleypas *et al*., 2011), as described in Chapter 3 (section 3.2.4). Finally, the threshold of calcification to dissolution (G-D) was determined. G-D is the level of Ω below (and/or pCO_2 above) when dissolution exceeds rates of calcification as established from models and verified through experimentation (*see* Yates *et al.,* 2014).

Linear Mixed Effects (LME) models with bioregion as a random effect were applied to examine the influence of habitat on daily net photosynthesis and respiration (Appendix 3). All model testing was conducted as described in Chapter 3, section 3.2.4.

4.3| Results

4.3.1| Abiotic assessment

Across bioregions and habitats there were significant differences (see Table. 4.1 & Appendix 8) in carbonate chemistry (pH, TA, salinity and Ω_{arg}) and in salinity, nitrate and temperature. Mean nitrate levels were slightly elevated on the outer-reef relative to the non-reef habitats and temperature Cv was greater in the lagoon habitats relative to the outer-reef (Table 4.1). Across all outer-reef sites seawater carbonate chemistry exhibited minor variability with similar mean (± SE) pH (8.120 ± 0.03), pCO_2 (323 1.42 ppm), and TA (2372.1 ± 15.2 μmol kg^{-1} SW) (Figure 4.2). Greater variance in carbonate chemistry parameters was inherent within all non-reef habitats, with seagrass beds experiencing the greatest pH C_V (seagrass pH Cv: 0.02 ± 0.01 compared to 0.01 ± 0.01 for the other non-reef habitats, Appendix 8).

The seagrass habitats had elevated mean pH (8.150 ± 0.01) and lower TA (2082.4 ±1.1 μmol kg^{-1} SW) relative to the outer-reef (pH: P= 0.001, TA: P= 0.001, Appendix 8). The elevation in pH and corresponding reduced pCO_2 (290.0 ± 7.8 ppm) was significant enough to elevate mean Ω_{arg} in the seagrass above the outer-reef (4.5 ± 0.1, P= 0.01). The back-reef (Atlantic) habitat exhibited similar mean pH and pH C_V trends to the seagrass habitat, however,

pH C_V was less extreme and mean pH was slightly reduced (8.130 ± 0.01). Mangroves experienced moderate pH C_V (0.015) and lower TA (1987.7 ± 1.3 μmol kg^{-1} SW). However in contrast to the seagrass beds, mangroves had a mean pH significantly lower than the outer-reef (8.04 ± 0.01, *P*= 0.001), which corresponded with elevated pCO_2 (352.0 ± 6.7 ppm) and lower Ω_{arg} (3.5 ± 0.1, *P*= 0.001, Table 4.1, Appendix 8).

On average, the calcification-to-dissolution threshold (G-D) never fell below the Mg-calcite Ω threshold levels of 3.0-3.2 for any the habitats (Table 4.2, Langdon and Atkinson, 2005; Yates and Halley, 2006; Silverman *et al*., 2009; Yamamoto *et al*., 2012). However, non-reef habitats came close-to, or breached the carbonate-sediment G-D of 3.7. The carbonate sediment values have been calculated by Yamamoto *et al*., 2012 experimentally. Carbonate sediment has been shown to typically consist of a mixture of low Mg-calcite, Mg-calcite and aragonite (Yamamoto *et al*., 2012). The mixture of these different structural forms of $CaCO_3$ results in the Ω threshold of carbonate-sediment occurring at a higher Ω. All mangrove habitats mean Ω remained just above the carbonate sediment G-D. Mangroves experienced minimal variability in pCO_2 and consequently Ω levels rarely fell below this threshold. However, seagrass habitats experienced diurnal variability in pCO_2 which resulted in the threshold being breached (seagrass pCO_2 C_V: 0.4 ± 0.01), resulting in times (within nighttime hours) when dissolution of carbonate-sediment would exceed rates of calcification. The maintenance of average Ω above the carbonate-sediment G-D demonstrated that daytime elevation of Ω off-set nighttime lows; where dissolution exceeds rates of calcification. The pH diel variability of each habitat was similar independent of bioregion location (Figure 4.3), with all non-reef habitats exhibiting pH peaks and troughs that correspond with maximum and minimum PAR values (*r*= 0.519, *n*= 36, *P*= 0.001). The tidal cycle for the Pacific Ocean sites corresponded with pH peaks and troughs. In

the Atlantic Ocean and Indian Ocean sites pH peaks and troughs did not correspond with the tidal cycles (Figure 4.3).

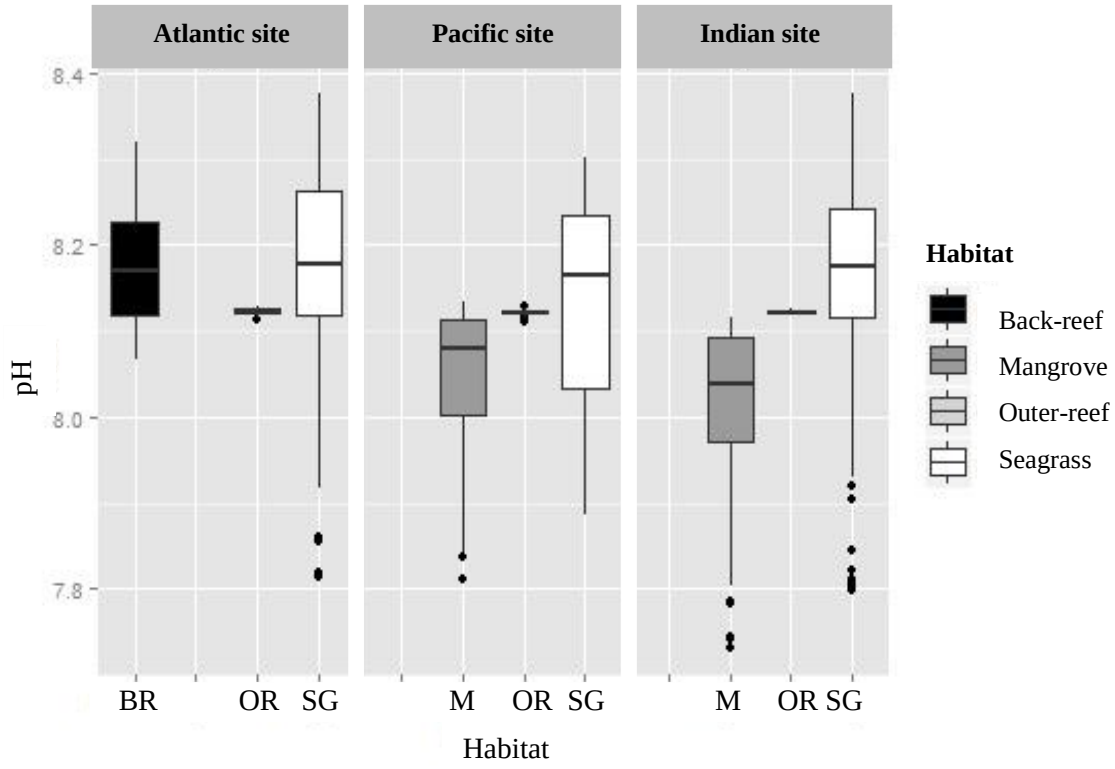


Figure 4.2| The pH variability for habitats across the three study locations. The study locations were within the Atlantic Ocean (Little Cayman, Cayman Islands, BWI), Pacific Ocean (Hoga and Kaledupa Islands) and Indian Ocean (Curieuse, the Seychelles). At each study location an outer-reef control site (OR) was compared to two non-reef habitats (Atlantic: seagrass (SG) and back-reef (BR), Pacific and Indian: SG and mangrove (M)). pH (total scale) was averaged from discrete water samples collected over five-days within the dry season of each location (*n*= 30 per habitat per location).

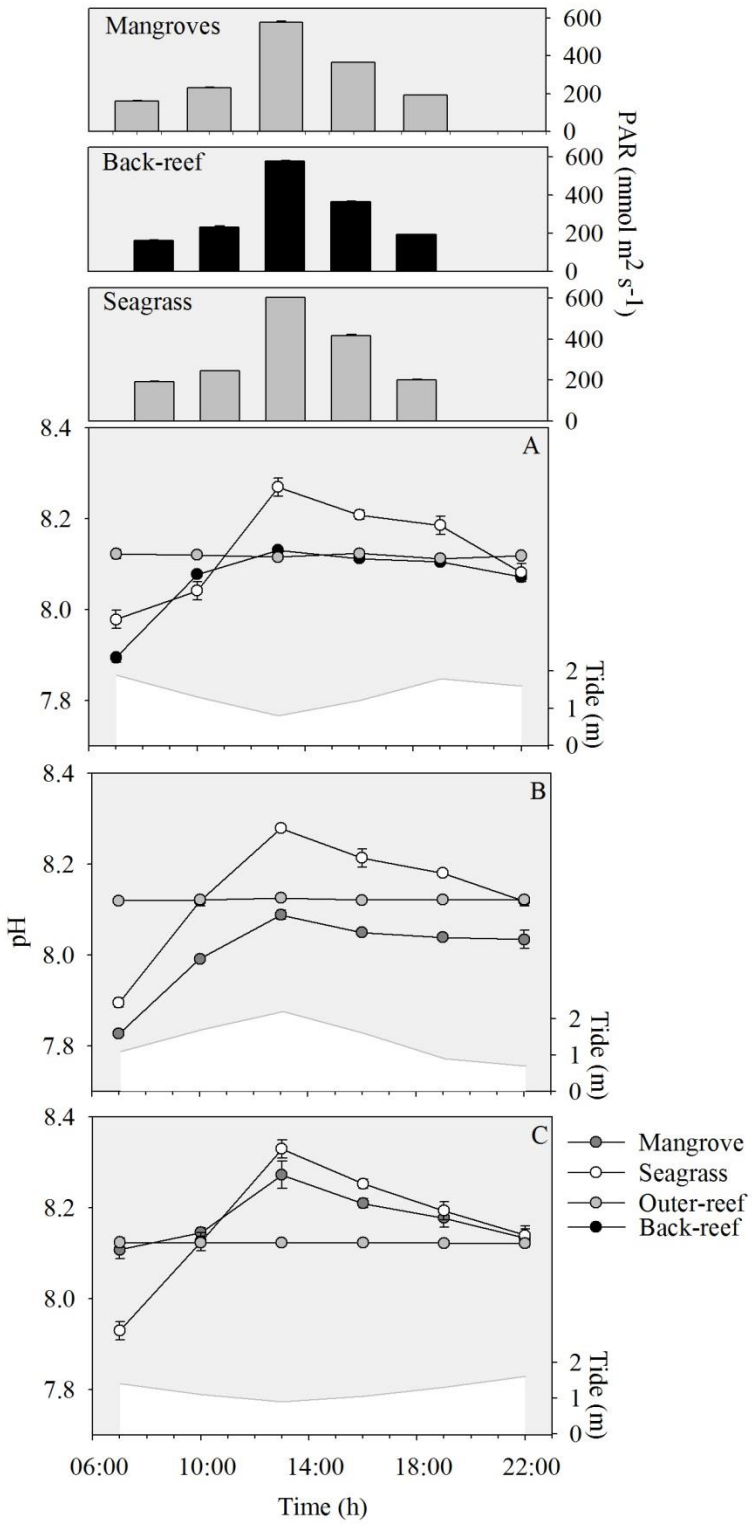


Figure 4.3| The average diel trends in pH, PAR and tidal cycles for habitats within each bioregion site. The study locations were within: A) the Indian Ocean (Curieuse, the Seychelles), B) the Pacific Ocean (Hoga and Kaledupa Islands) and C) the Atlantic Ocean (Little Cayman, Cayman Islands, BWI). At each study location an outer-reef control site was compared to two non-reef habitats (Atlantic: seagrass and back-reef, Pacific and Indian: seagrass and mangrove).

Table 4.1| Bio-physiochemical data for all habitats (outer-reef control, seagrass, back-reef and mangrove) and bioregion sites (Atlantic, Indian and Pacific Ocean). Data shown is the mean (± standard error, SE) and coefficient of variation (C_V) of 40 discrete water samples collected over five days. pH, temperature, salinity and nitrates were directly measured from each discrete water sample. Total alkalinity was determined for each water sample via an open-cell potentiometric titration. pCO_2 and Ω_{arg} were derived from pH and total alkalinity using CO2SYS. Light was measured in Lux at the sea floor (*ca.* 1 m) over five-days by *in situ* HOBOs and converted to PAR.

Abiotic Factor		Outer-reef			Seagrass			Back-reef	Mangrove	
pH	Mean	8.123	8.122	8.121	8.140	8.155	8.139	8.134	8.004	8.056
(total scale)		±0.01	±0.01	±0.01	±0.02	±0.01	±0.02	±0.01	±0.01	±0.03
	Cv	~0.00	~0.00	~0.00	0.01	0.02	0.02	0.09	0.01	0.01
Total alkalinity	Mean	2422.5	2358.5	2305.2	2167.1	2072.6	2087.3	2250.0	1955.7	2093.9
(μmol Kg/SW)		±0.63	±0.05	±0.03	±0.93	±1.56	±1.83	±0.66	±1.14	±0.04
	Cv	~0.00	0.02	0.01	0.03	0.05	0.06	0.02	0.04	0.04
pCO_2	Mean	322	323	326	290	259	323	261	372	333
(μatm)		±1.35	±1.02	±1.26	±26.86	±16.45	±24.36	±10.59	±19.30	±12.07
	Cv	0.02	0.02	0.02	0.51	0.35	0.41	0.22	0.28	0.20
Ω_{arg}	Mean	4.2	4.3	4.3	4.6	4.6	4.5	4.6	3.8	3.9
		±0.01	±0.01	±0.07	±0.16	±0.12	±0.03	±0.09	±0.13	±0.06
	Cv	0.02	0.02	0.02	0.19	0.15	0.18	0.10	0.20	0.10
Salinity (ppm)	Mean	36	35.5	35	36	36.5	36.0	36.0	35.5	34.5
		±0.01	±0.03	±0.02	±0.02	±0.06	±0.05	±0.02	±0.05	±0.15
	Cv	~0.00	~0.00	~0.00	~0.00	0.01	0.01	~0.00	0.01	0.02
Temperature (°C)	Mean	28.5	29.2	27.4	29.1	30.5	27.4	28.5	30.7	27.5
		±0.02	±0.02	±0.02	±0.11	±0.11	±0.05	±0.04	±0.16	±0.09
	Cv	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.02
Daily light integral (PAR)	Mean	21.96	20.79	21.18	17.76	17.70	17.14	18.02	17.00	17.10
		±0.24	±0.17	±0.27	±0.21	±0.20	±0.19	±0.27	±0.07	±0.12
	Cv	0.02	0.02	0.03	0.03	0.01	0.02	0.03	0.01	0.02
Nitrates	Mean	1.12	1.07	1.02	0.83	0.72	0.83	0.95	0.78	0.80
(μM)		±0.04	±0.04	±0.02	±0.03	±0.01	±0.03	±0.02	±0.01	±0.03
	Cv	0.07	0.07	0.05	0.08	0.03	0.08	0.05	0.03	0.08

Biogeochemical control of habitats – Across all bioregion locations, the outer-reef showed strongest co-variability between nA_T and nC_T via calcification-carbonate dissolution (Figure 4.4). In contrast, the non-reef habitats exhibited co-variability between nA_T and nC_T that was more strongly influenced by photosynthesis-respiration (and thus CO_2 uptake-release). The seagrass habitats showed the greatest range in nA_T and nC_T, with data indicating periods influenced significantly by photosynthesis and calcification, as well as respiration and carbonate dissolution. These characteristics are consistent with periods of extreme high (e.g. 8.402 pH units) and low (e.g. 7.809 pH units) pH as experienced in the seagrass habitats during the day (mean daytime pH= 8.246) and night (mean nightime pH= 8.032) respectively. The ratio of net ecosystem calcification to net community production (NEC:NEP, Table 4.2) was consistently lowest for seagrass/back-reef habitats (range: 0.27-0.55), highest for the outer-reef (range: 0.99-1.45) and intermediate for the mangroves (range: 0.75-0.79). The NEC:NEP ratios are influenced by the slope of the nC_T-nA_T plots and consequently, the outer-reef habitats had a slope closer to two than all the non-reef habitats, which demonstrated less influence from photosynthesis and more influence from calcification.

Table 4.2| NEC:NEP ratios for study sites with nA_T vs. nC_T.

Bioregion Site	Habitat	NEC:NEP	LRE	r^2	*P*-value
Atlantic Ocean	Seagrass	0.270	0.4253x + 1418.8	0.8101	= 0.0001
Atlantic Ocean	Back-reef	0.342	0.5101x +1363.9	0.8289	= 0.0001
Atlantic Ocean	Outer-reef	1.452	1.1843x + 169.8	0.9954	= 0.0001
Indian Ocean	Seagrass	0.546	0.7066x + 900.5	0.7948	= 0.0001
Indian Ocean	Mangrove	0.790	0.8826x + 496.1	0.4374	= 0.0001
Indian Ocean	Outer-reef	1.275	1.1208x + 158.8	0.9951	= 0.0001
Pacific Ocean	Seagrass	0.536	0.6982x+ 881.7	0.8785	= 0.0001
Pacific Ocean	Mangrove	0.753	0.8589x + 565.2	0.8744	= 0.0001
Pacific Ocean	Outer-reef	0.990	0.9952x + 333.2	0.8304	= 0.0001

Ratios of net ecosystem calcification to net community production (NEC:NEP) were calculated from the slopes of best-fit linear regression with all sites showing a relationship between salinity-normalized total alkalinity (nA_T) and total carbon (nC_T), with $P = 0.05$, and eight out of the nine sites $r^2 > 0.5$. NEC:NEP was calculated using the expression $1/[(2/m) - 1]$, where m is the slope from the corresponding linear regression equations (LRE). Calcification and dissolution are dominant processes when a linear regression slope approaches two.

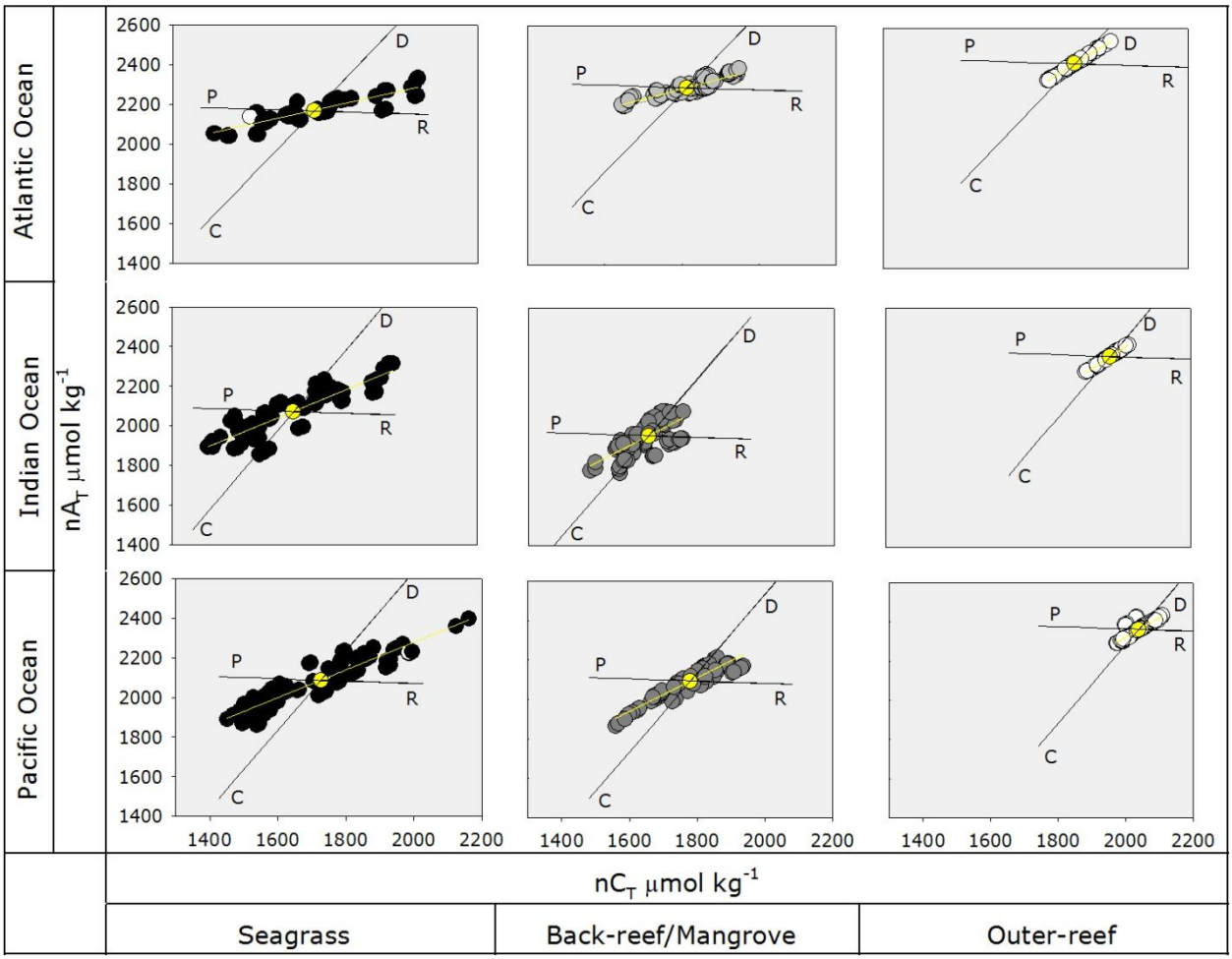


Figure 4.4| Salinity-normalised total alkalinity (nA_T) and total carbon (nC_T) plots with best-fit linear regression for three marine habitats in the Atlantic, Indian and Pacific Oceans. Data was collected over five days during a two week sampling period during the dry season for each bioregion (2013-2014). The Atlantic Ocean location consisted of a seagrass, back-reef and outer-reef control site, whilst the Indian Ocean and Pacific Ocean locations had seagrass, mangrove and outer-reef control sites. Black lines represent the theoretical impact of calcification (G), carbonate sediment dissolution (D), photosynthesis (P), and respiration (R) on nA_T and nC_T. Average nA_T and nC_T is indicated by a yellow dot. G and D are dominant processes when a linear regression slope approaches two.

4.3.2| Benthic assessment

Habitats showed relatively consistent cover of major benthic taxa across bioregion sites. The benthic surveys corroborating the nA_T vs nC_T plots with the outer-reef sites having the highest cover of benthic calcifiers (coral and coralline algae, 37.7 ± 2.2 %, Figure 4.5), and thus an environment where calcification-carbonate dissolution is the most influential process upon carbonate chemistry. Non-reef sites had greatest cover of photoautotrophs (seagrass, macro-and turf algae, 27.4 ± 2.2 %, Figure 4.5) and hence more heavily influenced by CO_2 uptake-release. Despite low cover of benthic calcifiers in the non-reef habitats (8.6 ± 0.1 %, Figure 4.5), corals were found persisting within these habitats with a surprisingly high coral species richness in the Atlantic Ocean site (7-15 species, Table 4.3).

The corals found within the non-reef habitats accounted for 28 – 86 % of coral cover on the main outer-reef (Table 4.3, Figure 4.6). In the Atlantic Ocean site, the coral species of the non-reef habitats collectively accounted for a large (back-reef= 86 % and seagrass= 48 %) percentage cover of coral species found on the outer-reef. In the higher diversity regions of the Indian Ocean and Pacific Ocean, the non-reef habitat coral species contributed between 28 – 40 % to the coral cover of the outer- reef habitats (Table 4.3). Coral cover at the Atlantic Ocean outer-reef site (13.5 ± 0.5 %) was *ca.* 60 % lower than the same habitat type in the Indian Ocean site (34.5 ± 1.4 %) and Pacific Ocean site (32.3 ± 0.9 %). Across bioregion locations, corals within the non-reef habitat sites showed minimal (2.2 ± 0.8 %) visual signs of stress (e.g. bleaching/disease/partial mortality).

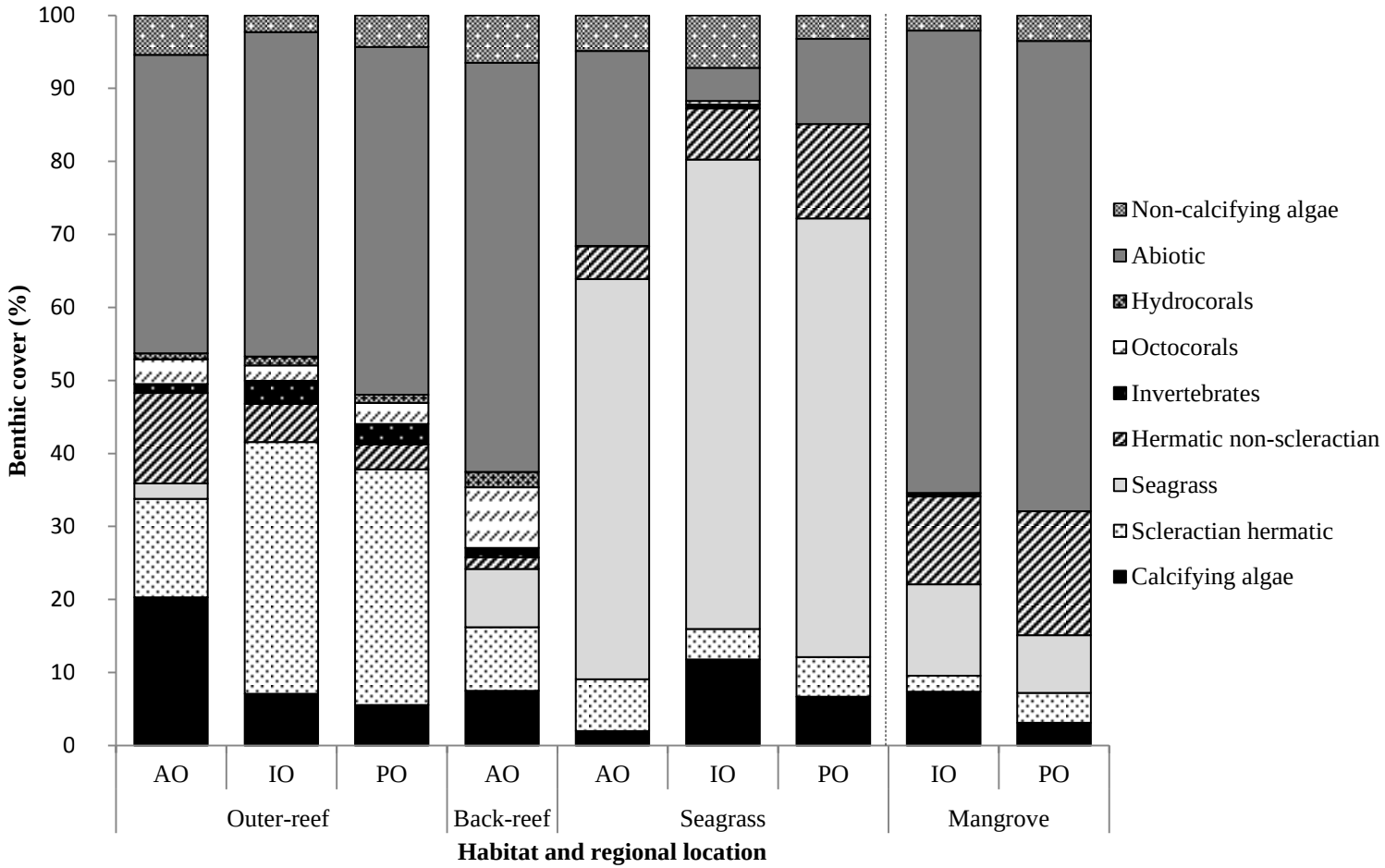


Figure 4.5| The percentage cover of major benthic taxa for the: A) Atlantic Ocean (AO), B) Indian Ocean (IO) and C) Pacific Ocean (PO) sites. Data is averaged from three by 30 m transects conducted within each habitat at each bioregion location. Surveys were conducted in the dry season of each region. Video transects were conducted and analysed to species level (see main text).

Table 4.3| Coral species list for non-reef sites in the Atlantic, Indian and Pacific Oceans. The percent cover within each non-reef habitat is indicated with the percent cover of that coral on the outer-reef in parentheses. Coral species with individual cover less than 1 % were represented by < 1; however their absolute values were included to get the total actual percent coral cover. Colonies not found within a particular region are indicated by a dash. X indicates that the species was not observed despite being present in that bioregion.

	Non-reef habitat and bioregion location					
	Seagrass			**Mangroves**		**Back-reef**
Species	Atlantic	Indian	Pacific	Indian	Pacific	Atlantic
Acropora austera		< 1 (< 1)	< 1 (1.0)	< 1 (< 1)	< 1 (1.0)	
Acropora formosa		< 1 (< 1)	1.0 (1.3)	< 1 (< 1)	1.0 (1.3)	
Acropora gemmifera		< 1 (< 1)	< 1 (< 1)	< 1 (< 1)	X	
Acropora palmata	X					< 1 (< 1)
Acropora sp 1.		X	< 1 (< 1)	X	X	
Agaricia agaricites	X					< 1 (< 1)
Agaricia humilis	X					< 1 (< 1)
Dichocoenia stokesi	< 1 (< 1)					< 1 (< 1)
Diploria strigosa	X					< 1 (< 1)
Favites abdita		X	< 1 (< 1)	X	X	
Fungia danai		X	< 1 (< 1)	X	< 1 (< 1)	
Galaxea cryptoramosa		X	< 1 (< 1)	X	X	
Goniastrea edwardsi		X	< 1 (< 1)	X	< 1 (< 1)	
Goniastrea pectinata		< 1 (< 1)		X	X	
Lobophyllia hataii		X	< 1 (< 1)	X	X	
Millepora alcicornis	X					< 1 (1.2)
Millepora sp.		< 1 (< 1)	X	X	X	
Montastraea annularis	X					1.1 (1.9)
Pavona varians		X	< 1 (< 1)		< 1 (< 1)	
Pocillopora damicornis		1.2 (2.5)	1.0 (1.8)	< 1 (2.1)	< 1 (1.8)	
Pocillopora verrucosa		X	X	< 1 (< 1)	X	
Porites astreoides	< 1 (3.1)					3.3 (3.1)
Porites attenuata		< 1 (< 1)	< 1 (< 1)	< 1 (< 1)	< 1 (< 1)	
Porites divaricata	1.3 (< 1)					< 1 (< 1)
Porites furcata	< 1 (< 1)					X
Porites lobata		X	< 1 (1.2)	X	< 1 (1.2)	
Porites lutea		1.3 (1.7)	1.4 (1.8)	< 1 (1.7)	1.6 (1.8)	
Porites porites	< 1 (1.2)					< 1 (1.2)
Scolymia lacera	X					< 1 (< 1)
Siderastrea radians	< 1 (< 1)					1.1 (< 1)
Siderastrea siderea	< 1 (1.2)					< 1 (1.2)
Solenastrea bournoni	X					< 1 (< 1)
Stephanocoenia intersepta	< 1 (< 1)					< 1 (< 1)
Total Number of Species	**8**	**8**	**14**	**7**	**9**	**15**
Actual percent cover	**3.10%**	**4.20%**	**5.40%**	**2.20%**	**4.10%**	**8.70%**
Relative percent coral cover of outer-reef	**48.00%**	**40.00%**	**35.70%**	**36.50%**	**28.30%**	**86.00%**

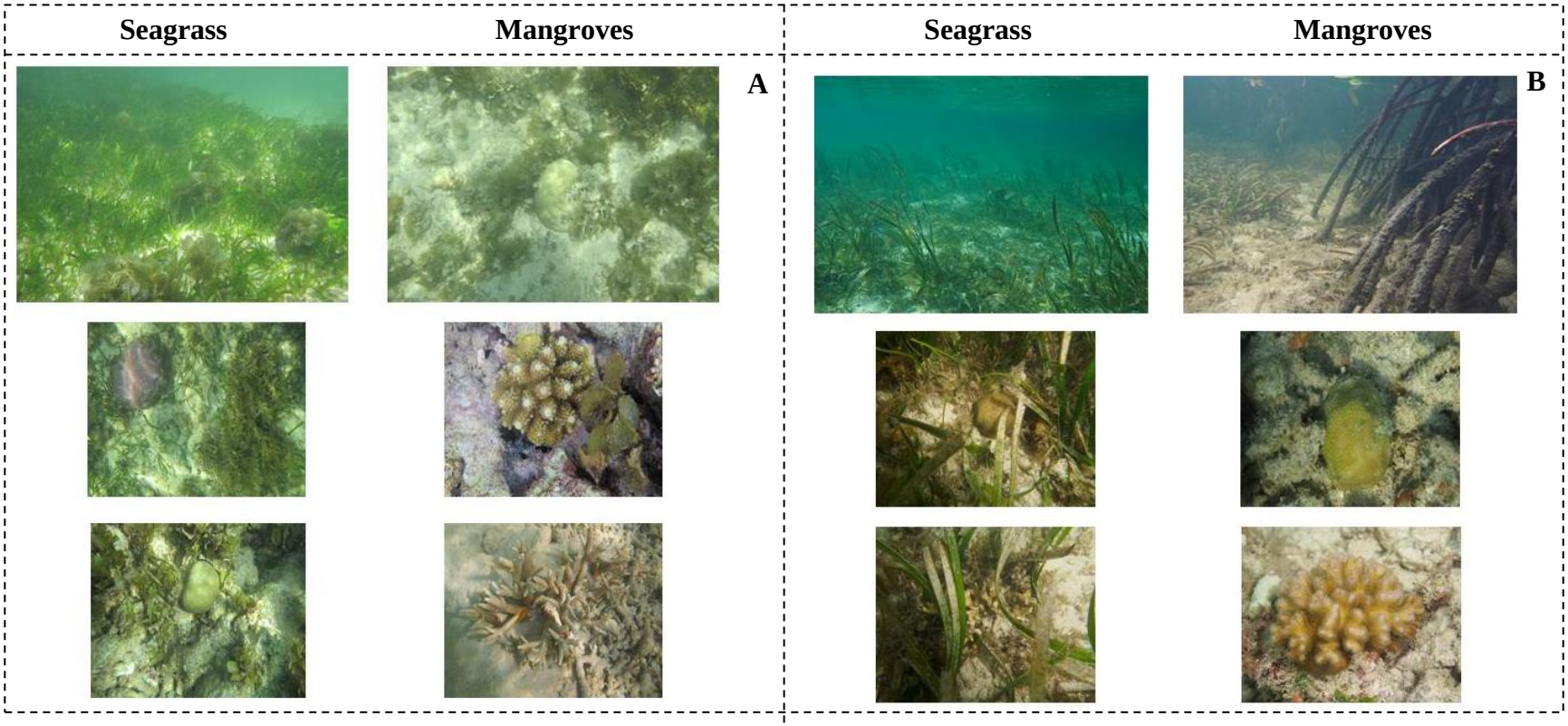


Figure 4.6| Examples of the non-reef habitats and corals present. A) Curieuse, the Seychelles, B) Hoga and Kaledupa Islands, Indonesia C) Little Cayman, Cayman Islands BWI. The larger picture show the general habitat type, whilst the small pictures are specific examples of some of the corals found within each habitat. Pictures were taken during each sampling period (see main text).

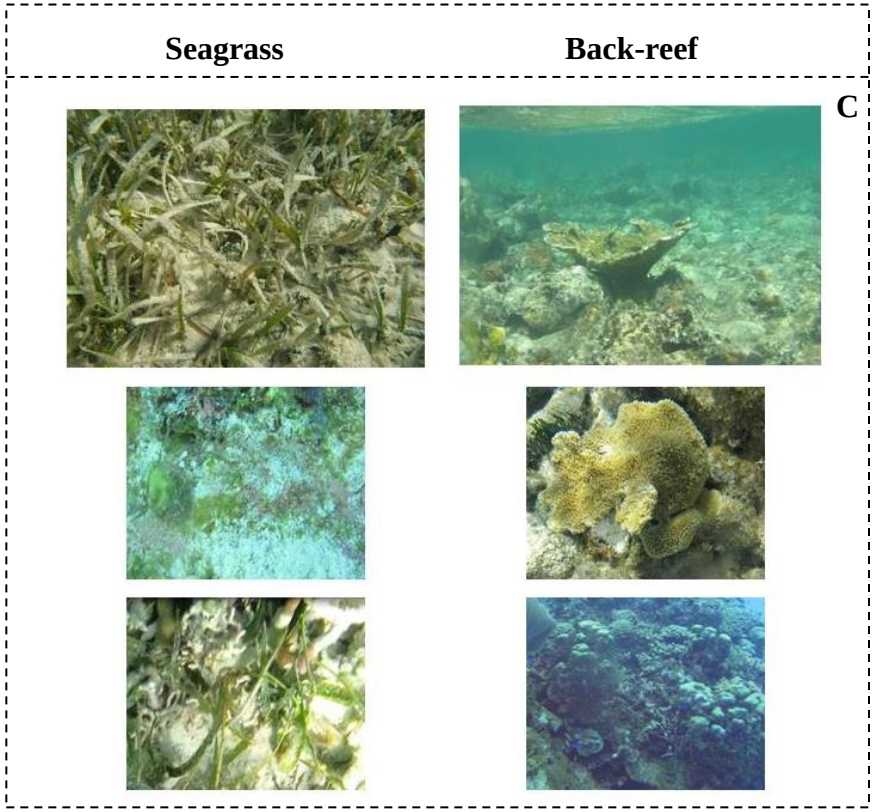


Figure 4.6| Examples of the non-reef habitat habitats and corals present. A) Curieuse, the Seychelles, B) Hoga and Kaledupa Islands, Indonesia C) Little Cayman, Cayman Islands BWI. The larger picture show the general habitat type, whilst the small pictures are specific examples of some of the corals found within each habitat. Pictures were taken during each sampling period (see main text).

4.3.3| The relationship between pH and benthic community composition

Benthic composition naturally varied between regions, however a consistent trend in percent cover (mean ± SE) of calcifying and non-calcifying phototrophs was observed within the seagrass (calcifying= 8.6 ± 0.7 %; non-calcifying= 36.3 ± 11.5 %), mangrove (calcifying= 14.1 ± 0.2 %; non-calcifying= 72.6 ± 1.0 %) and outer-reef sites (calcifying= 37.8 ± 2.2 %; non-calcifying= 7.8 ± 3.4 %). Consequently, the relative abundance of non-calcifying benthic phototrophs increased with increasing pH Cv (r^2= 0.713, n= 9, P= 0.001, non-calcifying benthic phototrophs cover= 0.0022pHCv + 0.0002, Figure 4.7c) whereas benthic calcifiers decreased (r^2= 0.864, n= 9, P= 0.001, benthic calcifiers cover= 0.0188pHCv – 0.0005, Figure 4.7a). However, when mean pH was considered there was no relationship with calcifying benthos or non-calcifying benthic phototrophs (Figure 4.7b & d). For the non-calcifying benthos, all habitats had relatively low (< 25 %) cover, irrespective of their mean pH, with the exception of the seagrass habitat (cover *ca*. 70 %, Figure 4.7d).

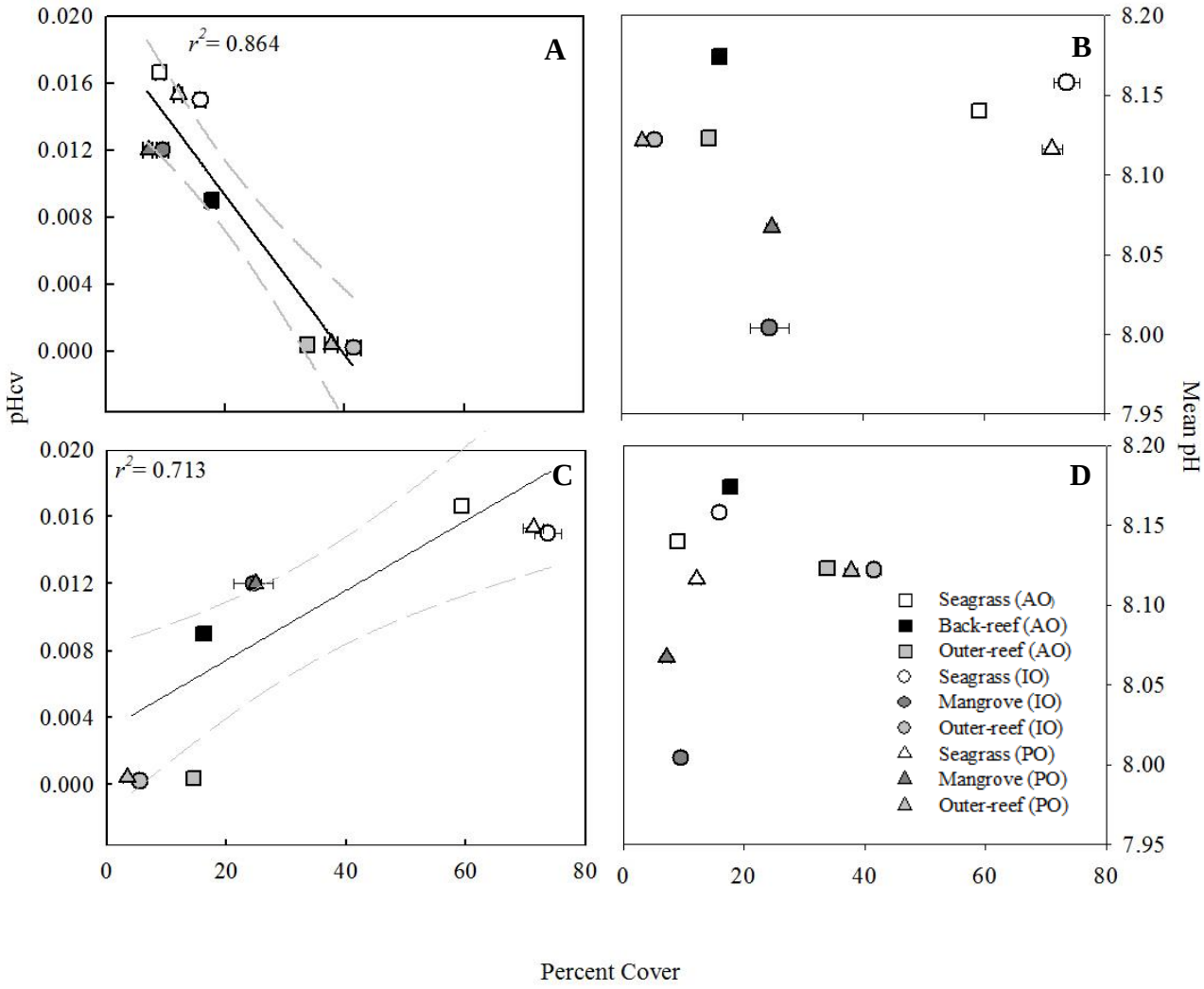


Figure 4.7| Plots of pH Coefficient of Variation (c_v) and mean pH versus the percent cover (±SE) of: A & C) calcifying (coral and calcifying algae) and B & D) non-calcifying (seagrass and fleshy macro algae) benthos for the non-reef habitat sites and an outer-reef site in the Atlantic, Indian and Pacific Oceans. Data is averaged from three by 30 m transects conducted within each habitat at each bioregion location. Surveys were conducted in the dry season of each region. Regression is shown with 95 % confidence interval.

4.3.4| The metabolic expenditure of the dominant coral species across habitats

Calcification rates were highest at the outer-reef habitat across regions (257.0 ± 15.93 mmol m^2 d^{-1}), in particular for the fast growing *Acropora spp.* (340.0 ± 2.87 mmol m^2 d^{-1}). Here, conditions were relatively stable compared to non-reef habitats (Table 4.1). Very different trends for coral calcification were observed between the non-reef habitats. Seagrass and back-reef habitats supported corals with relatively small decreases in calcification relative to the outer-reef (12.5-33.0 %, with the exception of *Acropora spp*. 68.0 %), whereas mangrove coral calcification rates were 63.0-81.0 % lower than the outer-reef.

Maintenance of relatively high calcification in the seagrass beds and back-reef corresponds with the elevated mean pH and Ω_{arg} for these habitats. Similarly, low rates of calcification within mangroves were consistent with higher pCO_2 levels and reduced Ω_{arg}. Overall calcification rates decreased in line with a decrease in mean pH (r^2= 0.372, n= 38, P= 0.001, Figure 4.8b), and to a lesser extent with increasing pH (C_V, r^2= 0.268, n= 38, P=0.01, Figure 4.8a). This potential regulatory function of mean pH is consistent with the corresponding change of NEC:NEP across habitats (Table 4.2). The similarity in mean and Cv of the abiotic factors (light, temperature, nitrates, Appendix 8) between the non-reef habitats suggests that differences in carbonate chemistry were the proximal driver in structuring coral biomass and growth between mangroves and seagrass habitats. Supporting this is the fact that no relationships were detected between calcification rates and mean or Cv temperature and light (Appendix 8 & 9).

Across all bioregion sites, an increase in the gross photosynthesis-to-respiration ratio (P:R) corresponded with a positive increase in calcification (r^2= 0.501, n= 38, P= 0.001, Figure 4.9). In the outer-reef, P:R remained above one, however, in the non-reef habitats P:R decreased

largely due to a decrease in photosynthesis (P= 0.05, Appendix 10) whilst respiration remained stable (within 8 %), a trend that was greatest within the mangroves. Photosynthesis rates decreased with lower site mean pH (r= 0.427, n= 38, P= 0.01), however, again respiration rates were found to remain constant (Figure 4.10). For the branching species, changes in respiration with mean pH were best represented by a sigmoidal graph, (r^2= 0.848, n= 9, P= 0.05, Photosynthesis= 15.269 + 42.676/(1 + exp(-(pHmean – 8.114)/ 0.020)) Figure 4.10). Within the non-reef habitats, massive and sub-massive species exhibited higher P:R than branching species ($F_{2,26}$= 4.18, P= 0.05) corresponding with increased calcification rates. P:R was lower in branching species because of a dramatic decrease in photosynthesis (60.0 ± 1.3 %) as compared to both massive (22.0 ± 0.5 %) and sub-massive (40.0 ± 1.4 %) species ($F_{2,26}$= 4.55, P= 0.05, see Figure 4.10).

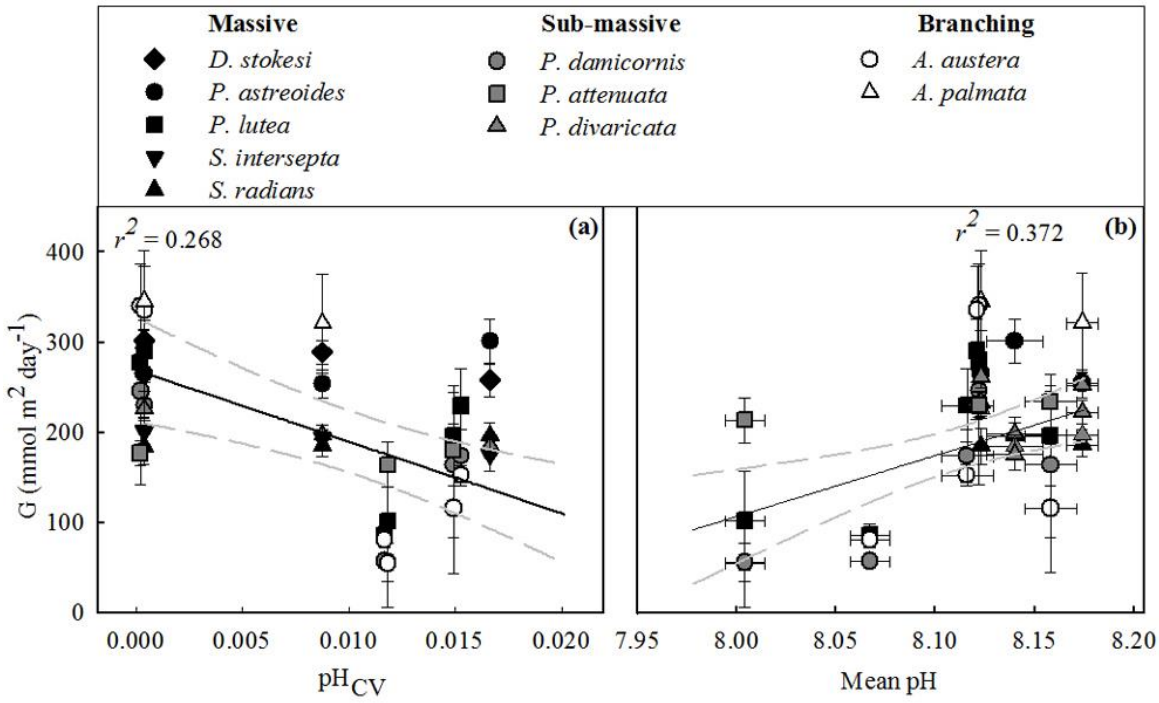


Figure 4.8| Mean daily integrated net calcification (G) (mmol m^2 day^{-1}) versus: A) pH Coefficient of Variation (C_V) and B) mean pH. All data plotted are mean values ± standard error (SE, *n*= 5) for the dominant coral species examined across non-reef habitat sites (seagrass, back-reef and mangrove) and outer-reef habitat sites for all bioregion locations (Indian, pacific and Atlantic Ocean sites). Sampling was conducted within the dry seasons of each region. Regression is shown with 95 % confidence interval.

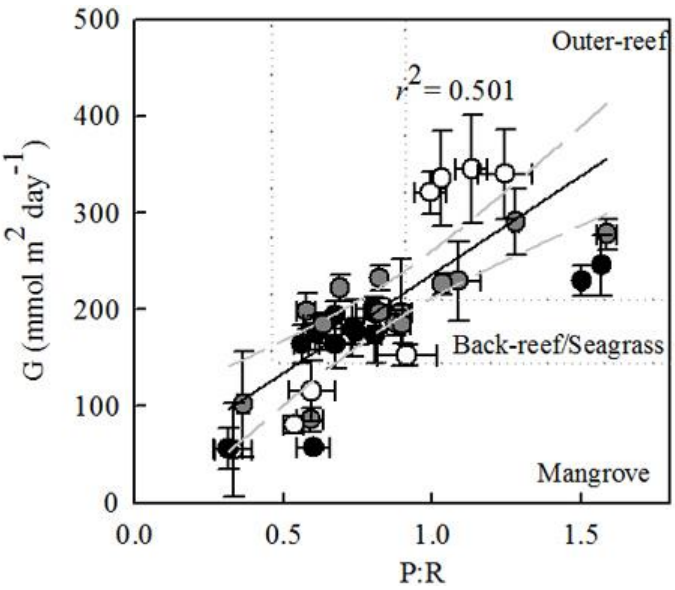


Figure 4.9| Mean daily integrated net calcification (G) (mmol m^2 day^{-1}) versus the ratio of daily net photosynthesis (P) to daily net respiration (R). Data was obtained from five replicate days of sampling on five individual colonies of each species, within each habitat and bioregion location (Indian, Pacific and Atlantic Ocean sites). Sampling was conducted within the dry seasons of each region. Across sites, G decreased with corresponding decreases in the gross photosynthesis-to-respiration ratio (P:R, r^2 = 0.501, n= 38, P= 0.001). Regression is shown with 95% confidence interval.

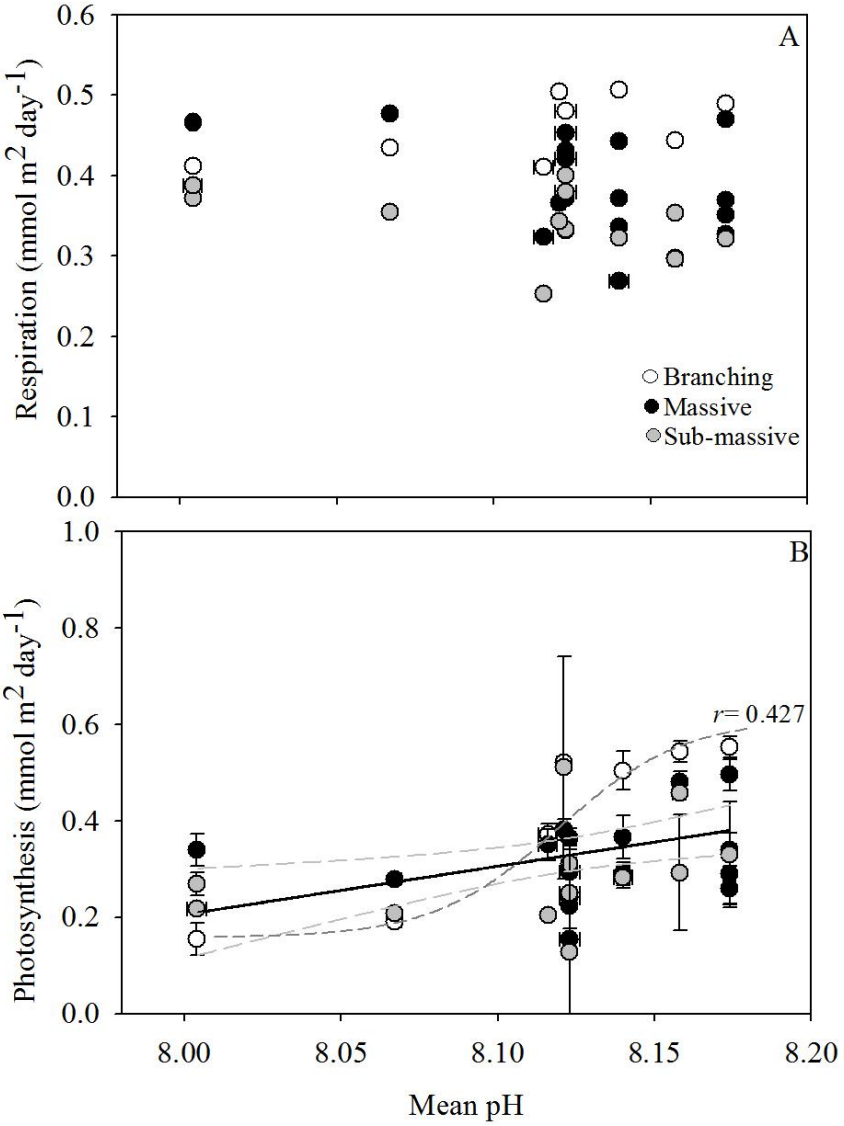


Figure 4.10| The relationship between: A) Respiration rates and B) Photosynthesis rates for the dominant coral species examined across non-reef habitats (seagrass, back-reef and mangrove) and outer-reef habitat sites for all bioregion locations. The bioregion locations were sites situated within the Indian, pacific and Atlantic Oceans. Sampling was conducted within the dry seasons of each region. Corals are indicated by their growth form. The solid black line is the correlation for the whole dataset with 95 % confidence interval (grey dashed line). The black dotted line is a polynomial quadric curve fitted to the branching species data. Data is mean (± SE, *n*= 5).

4.4| Discussion

4.4.1| pH mean and Cv trends across bioregion sites

The sites sampled across bioregions demonstrated that the same general trend in carbonate chemsitry is apprent for similar habitats in both high and low biodiversity regions. All inshore habitat sites within this study had more variable carbonate chemistry than the outer-reef sites, however mean values of pH, pCO_2 and Ω_{arg} were very different between the non-reef habitats. Seagrass sites and the back-reef site within this study consistently had elevated mean pH, reduced pCO_2, and therefore elevated Ω_{arg} relative to the outer-reef. Periods of elevation and depletion in pCO_2 corresponded with time of day and light intensity. Seagrass habitats also experienced low pH at night which corresponded with periods of under-saturation of carbonate sediment resulting in dissolution. Dissolution has been proposed as a self-regulatory function of marine habitats to buffer some of the negative impacts of future OA, by raising pH and TA (Anthony *et al.*, 2011a; Andersson *et al.*, 2013). Andersson *et al.* (2013) demonstrated a partial offset of future OA due to dissolution by increasing pH and Ω_{arg} by 9 % and 11 % respectively. The ability of seagrass habitats to buffer future OA will depend in part on the fine balance of G-D over diel cycles.

In contrast, mangrove habitats consistently experienced a lower mean pH relative to the outer-reef, which corresponded with elevated pCO_2 and a reduction in Ω_{arg} when compared to both the outer-reef and seagrass habitats. Despite overall low mean pH, the mangrove habitats did not experience the magnitude of diel variability experienced in the seagrass habitat. Consequently, Ω levels rarely resulted in the dissolution of carbonate-sediment which would elevate TA (as evidenced in the nA_T-nC_T plots) and thereby self-regulate or "buffer" the local system. Failure to maintain favourable conditions suggests that mangroves therefore do not strictly operate as refugia, as it is currently defined (Keppel & Wardell-Johnson, 2012).

4.4.2| The relative influence of mean versus Cv pH on structuring the local coral populations

Characterising the relative importance of mean versus C_V pH on the local coral population is important in identifying potential refugia to OA (Guadayol *et al*., 2014). Results from this study demonstrate why it is significant to consider the actual amount of time a coral is exposed to a set of environmental conditions within any habitat. In this instance characterising the variability (C_V) as well as the mean in pH is important for understanding the buffering capacity of non-reef habitats and in therefore evaluating their role as potential refugia (Guadayol *et al*., 2014). Within this study, overall cover of benthic calcifies showed an increase with lower pH Cv. No difference in cover of benthic calcifies was identified with mean pH. However, *in situ* coral metabolic analysis suggested that mean pH had a greater association with calcification rates rather than C_V.

The fact that no overall relationship was observed with mean pH and the cover of benthic calcifies, despite coral calcification showing an increase with mean pH could be explained by a few different possibilities, including but not limited to: (i) the percent cover of benthic calcifers includes several different taxa and species that may have different pH regulatory mechanisms (Comeau *et al*., 2012), potentially facilitating different levels of survival within habitats and in turn the overall cover of benthic calcifers, and (ii) other factors besides the carbonate chemistry determine the benthic cover, for example available substrate and recruitment rates (Babcock & Mundy 1996; Salinas-de-León *et al*., 2011), which in turn have a feedback on the local system.

These results therefore suggest that both mean and Cv pH likely influence local populations, or that local benthic assemblages influence the dominant habitat characteristics (e.g. mean and Cv pH) which in turn influence local populations. Based solely on coral calcification, mean pH appears to be more influential on structuring the local coral population. Supporting this

is the fact that the site with the lowest mean pH (the mangrove habitat) had greatly suppressed coral calcification. Whether the supressed calcification is directly due to the influence of mean pH on calcification, or is associated with reduced productivity which in turn reduces calcification, remains unresolved. However, these findings support the growing consensus that low mean pH threatens the sustainability of reef accretion and therefore function (Dove *et al.*, 2013).

4.4.3| The ability of corals to expand their niches into non-reef habitats

The cross-bioregion dataset from this chapter demonstrates that a range of coral taxa can persist in non-reef habitats. Recent work by Yates *et al.* (2014) on Caribbean mangroves, has similarly documented numerous coral species living in sub-optimal non-reef habitats. Corals in this study were documented having a variety of different growth forms and included species of architecturally complex genera such as *Acropora* and *Pocillipora* that have demonstrated varied responses to environmental extremes (Hughes *et al.*, 2003; Baker *et al.*, 2004). Whilst the total number of coral species recorded in non-reef habitats was similar across regions, these total values represent very different proportions of the overall number of coral species found within each bioregion location. For example, corals found in the non-reef habitats of the Atlantic Ocean site represent *ca*. 20-30 % of the total number of coral species currently documented in the Atlantic region. However, in the Indian Ocean and Pacific Ocean sites, corals recorded in the non-reef habitats only represent 1-2 % of coral species found in the Indo-Pacific region. Clearly examining the ecophysiology of corals in these environmentally more extreme and variable habitats can inform our understanding of the potential for individual coral taxa to persist under future environmental change.

4.4.4| The metabolic expenditure of dominant non-reef coral species

In the seagrass habitats some corals sustained calcification and some experiencing a reduction, which averaged 17.0 ± 6.1 % relative to the outer-reef control. In contrast, corals found within the mangroves were pre-conditioned to generally low pH (and Ω) but with significant suppression to calcification (70.0 ± 7.3 % relative to the outer-reef control). Corals in mangrove habitats were metabolically impaired, evidenced through lower photosynthesis and calcification but with no net change in respiration rates. Both habitats also experienced variable temperature (diel range up to 2.5°C) relative to the outer-reef (diel range less than 0.7 °C) which did not correspond with changes in calcification rates but can potentially increase thermal tolerance of coral species making them important future stores of genetic diversity.

4.4.5| The relationship between coral calcification and photosynthesis/respiration rates

Across non-reef habitats, any decreases in coral calcification were accompanied by a reduction in photosynthesis without a change in respiration. Such a trend is consistent with experimental work of Anthony *et al.* (2008) on *Acropora spp.* and *Porites spp.* exposed to future IPCC IV and VI scenarios. An increase in light has been shown to enhance calcification (Suggett *et al.*, 2013) and a moderate rise in temperature is also associated with increased metabolic rates in corals that potentially enhance growth (McNeil *et al.* 2004; Bessat & Buigues 2001). Unsurprisingly there were no significant relationships observed between calcification and temperature or light (Appendix 9) in our study due to the similarity in mean conditions at all habitats (Table 4.1).

Increased heterotrophy (Cohen & Holcomb, 2009) and the addition of nutrient's (Langdon & Atkinson, 2005) have also been suggested to enhance calcification for some coral

species (Cohen & Holcomb, 2009). Nitrate concentration were higher in outer-reef control sites but differences in calcification rates observed in non-reef habitats are not explained by variability in nitrate concentrations (Appendix 8). It is possible that other nutrients may influence coral metabolic activity within non-reef habitats (Langdon & Atkinson, 2005). Collectively however our results suggest that photosynthesis and calcification were most likely impaired by the metabolic costs of maintaining cellular homeostasis within a low pH environment (Anthony *et al*., 2008; McCulloch *et al.,* 2012). Photosynthesis could be impaired by pH induced bleaching as observed by Anthony *et al*. (2008); however this will need to be explored further within the laboratory study of Chapter 5. Within the limitations of the study and the environmental factors measured the abiotic characteristic that appears to be most important in potentially influencing the abundance and productivity of corals in the non-reef habitats is the local carbonate chemistry. These findings across bioregion sites are consistent with the initial study results on Little Cayman (see Chapter 3).

4.4.6| Coral species and growth form responses to varying carbonate chemistry conditions

Acopora spp. experienced the largest decrease in calcification whilst *Porites spp*. were better able to maintain calcification across environments. McCulloch *et al.* (2012) modeling internal pH regulation also concluded that the calcification rates of *Acropora spp*. would be most sensitive to reductions in external pH and *Porites spp.* the least. Branching coral species documented the largest reduction in P:R driven by a decrease in photosynthesis at low pH habitats, irrespective of bioregion. The reduction was associated with a large decrease in calcification. The decrease in photosynthesis for branching species was significantly greater than that measured for massive and sub-massive species. Also, a non-linear response was observed

for the changes in photosynthesis for branching species. As pH increased there were dramatic increases in calcification rates of branching species but only moderate and quickly saturated calcification rates by massive and sub-massive species. Collectively these results suggest that the branching species have a different ability in maintaining photosynthesis under elevated pCO_2, compared to the massive and sub-massive corals. The difference in sustaining photosynthesis corresponds with the corals ability to maintain calcification.

4.4.7| Consequences for future research

Across bioregions locations, Poritidae and Acroporidae were the most common coral families with both found in eight out of the nine study sites. Specifically, massive *Porites spp.* and branching *Acropora spp*. were documented to experience very different abilities in maintaining their photosynthesis rates across the non-reef habitats, which corresponded with their abilities to sustain calcification. The results from this study suggest that photosynthesis and calcification were most likely impaired by the metabolic costs of maintaining cellular homeostasis within a low pH environment. Within field experimentations there are however, several possible reasons for changing calcification, not only pH. Therefore, to explore the different responses of *Porites spp.* and *Acropora spp.* observed in the field, a controlled laboratory experiment is necessary, whereby, corals can be subjected to natural variability in pH whilst controlling all other variables. Chapter 5 will therefore explore the response of these two genera (*Acropora* and *Porites*) to future predicted changes in pH. Temperature effects will also be explored as this is going to vary alongside pH under future climate change.

4.5| Key Findings

4.5.1| pH mean and Cv trends across bioregion sites

- All outer-reef sites experienced minimal carbonate chemitry variability.
- Seagrass habitats and the back-reef site within this study consistently elevated local mean pH (reduced pCO_2) and therefore elevated Ω_{arg} relative to the outer-reef.
- Periods of low pH within the seagrass habitat resulted in times of carbonate-sediment dissolution which increased TA and the buffering ability of the system.
- Mangrove habitats consistently experienced a lower mean pH (elevated pCO_2) relative to the outer-reef, with a decrease in Ω_{arg}.

4.5.2| The relative influence of mean versus Cv pH on structuring the local coral populations

- Overall benthic cover of calcifying organisms was related to pH Cv (higher cover of calcifiers in low pH Cv) and not mean pH.
- Coral calcification was enhanced under higher mean pH.

4.5.3| The ability of corals to expand their niches into non-reef habitats

- A range of coral species were recorded in the non-reef habitat that were not restricted to a specific growth form or genera.
- The total number of coral species recorded in non-reef habitats was similar across regions
- Very different proportions of the overall number of coral species found within each bioregion location were represented by the coral species within the non-reef habitats (Atlantic Ocean site= 20-30 %, Indian Ocean & Pacific Ocean sites= 1-2 %).

4.5.4| The metabolic expenditure of dominant non-reef coral species

- Generally higest calcification rates occurred on the outer-reef.

- In the seagrass habitats rate of coral calcification were sustained or experienced minimal reductions (<17 % relative to the outer-reef control) with the exception of *Acropora spp*. (68.0 % relative to the outer-reef control).
- Calcification was supressed in the mangrove habitats (>70 % relative to the outer-reef control).

4.5.5| The relationship between coral calcification and photosynthesis/respiration rates

- Within all non-reef habitats, coral metabolism was governed by reductions in photosynthesis and calcification but respiration was maintained. without an up-regulation in respiration.
- Of the environmental factors measured the abiotic characteristic that appears to be most important in potentially influencing the abundance and productivity of corals in the non-reef habitats is the local carbonate chemistry.

4.5.6| Coral species and growth form responses to varying carbonate chemistry conditions

- Calcification rates of branching species were negatively affected across non-reef habitats more than sub-massive and massive coral species.
- Productivity was most affected in branching species across non-reef habitats.
- Under low pH productivity of branching species were similar to massive and sub-massive species
- As pH increased there were dramatic increases in calcification rates of branching species but only moderate and quickly saturated calcification rates by massive and sub-massive species.

4.5.7| Connection between chapter 4 research findings and the research approach reported in chapter

- Across bioregions locations, Poritidae and Acroporidae were the most common coral families (present in 8 out of 9 sites).

- Massive *Porites* and branching *Acropora* documented very different abilities in maintaining their photosynthesis rates across the non-reef habitats, which corresponded with their abilities to sustain calcification.
- Photosynthesis and calcification were most likely impaired by the metabolic costs of maintaining cellular homeostasis within a low pH environment.
- Within field experimentations there are several possible reasons for changing calcification, not only pH. Therefore, a controlled laboratory experiment is necessary, whereby, corals can be subjected to natural variability in pH whilst controlling all other variables.

Chapter 5| The impact of future predicted changes in temperature and pH on the biological performance of corals from different variability habitats

Part of this chapter is in final draft for the Proceedings of the Royal Society B as the manuscript: The roles of temperature and pH in non-reef habitats on coral calcification and metabolic activity: Can marginal systems buffer against change?

5.1| Introduction

As atmospheric pCO_2 concentrations continue to rise tropical coral reefs are under threat from both ocean warming and lower global seawater pH (ocean acidification, OA) (Gattuso *et al.*, 1999; Hoegh-Guldberg & Bruno 2010). How these two factors will interact to drive future productivity of reef building corals (Anthony *et al.* 2008; Kroeker *et al.*, 2010, 2013) and in turn their distribution (van Hooidonk *et al.* 2014; Vergés *et al.* 2014; Cacciapaglia & van Woeosik 2015) is still unclear. For example, there is evidence that a moderate rise in temperature is associated with an increase in metabolic rates of corals that potentially enhances growth (Reynaud-Vaganay *et al.*, 1999; Lough & Barnes, 2000; Bessat & Buigues 2001; McNeil *et al.* 2004), whilst a larger rise in temperature can push corals past their physiological limits resulting in catastrophic mortality (Hoegh Guldberg *et al.* 2007; Hoegh-Guldberg & Bruno 2010). Similarly, lowered pH could potentially fuel the metabolism of the symbiont by increasing the concentration of CO_2 used in photosynthesis (Brading *et al.*, 2011; Suggett *et al.*, 2012). However, low pH also increases the energetic cost of calcification by lowering the concentration of HCO_3^- (Cohen & Holcomb 2009; McCulloch *et al.*, 2012). Consequently, research efforts have focused on attempting to deconvolute the impacts of these two factors whilst also trying to

understand the potential synergistic interaction of combined stressors to evaluate the net physiological and ecological responses of the coral holobiont to future climate change scenarios. As demonstrated throughout the thesis, shallow reef and non-reef habitats are highly dynamic, with some habitats already routinely experiencing periods of pH (pH 7.8: see Chapter 3 & 4, Manzello *et al*., 2012; Price *et al.,* 2012) and temperature (>34 °C; Manzello *et al*., 2012) considered representative of future reef conditions under business-as-usual IPCC climate change scenarios (Guadayol *et al.,* 2014). However due to the innate variability and the possible synergistic effect of multiple environmental variables, knowledge concerning the future implications of OA on fringe habitats remains confused and often contradictory.

Corals populating habitats with inherently greater environmental variability as compared to classic reef settings, appear to exhibit increased resilience to anomalous stressors (e.g. temperature, Baker *et al*., 2004, pH, Comeau *et al*., 2014), by expanding their physiological thresholds (Donner *et al.,* 2007). However, this seemingly is not always the case with several other studies demonstrating no improved tolerance to stress despite prior regular exposure to high environmental variance (e.g. temperature, Rodolfo-Metalpa *et al*., 2014; pH, Crook *et al*., 2013; Okazaki *et al.,* 2013). Reconciling such findings may reflect the fact that few stress-based studies have included both temperature and pH as experimental variables, and fewer experiments still have actually replicated the natural daily oscillations (frequency and range) of temperature and pH within coral environments.

Experiments that have accounted for ambient pH variability have recently demonstrated enhanced calcification rates for coral recruits (*Seriatopora caliendrum*, Dufault *et al.,* 2012) and adult corals (*Acropora hyacinthus*, Comeau *et al.,* 2014) when grown under oscillating rather than steady-state pCO_2 conditions. However, despite showing a positive role for pH variance in

potentially offsetting the negative impacts of OA on a local scale, they do not consider the likely simultaneous role of temperature variance. Other studies that have considered both temperature and OA on massive (Anthony *et al.*, 2008 (*Porites sp*.), Thompson & van Woseki, 2009 (*Siderastrea siderea*)) and branching (Anthony *et al*., 2008 (*Acropora sp.*)) corals, demonstrated a negative but variable response on coral calcification. These studies however, did not replicate the ambient variability, instead maintaining steady-state conditions. One study by Dove *et al.* (2013) has incorporated daily and seasonal variability for both temperature and pH within their study on patch reef communities, predicting serious implications for coral reef systems under future climate change. However, this study focused on one habitat only and does not allow for the elucidation of how natural habitat variability effects coral productivity.

Review of previously published experiments demonstrates that there has been a lack of studies that have compared the physiological response of corals from relatively "stable" and highly-variable coral habitats, considering both natural temperature and pH daily oscillations. Differences observed in the *in situ* metabolic responses of massive *Porites* and branching *Acropora* make them interesting genera to study (see Chapter 4). Therefore, the objectives of this chapter are to:

1) Re-create the natural diurnal oscillations of pH and temperature within a laboratory setting and superimpose their future predicted changes (as per 2100) for a reef and non-reef habitat;
2) Determine the relative influence of temperature and pH on the metabolic activity of corals;
3) Measure whether diel variability in experimental conditions influences the metabolic response of corals;

4) Test whether two common Caribbean species (*Acropora palmata* and *Porites astreoides*) exhibit the same metabolic response to enhanced temperature and low pH when superimposed on top of their natural variability;
5) Test whether corals from highly variable (pH & temperature) habitats are better at maintaining their metabolic activity under conditions predicted in 2100 (high temperature, low pH).

To address these objectives a multifactorial manipulative experiment was conducted. Importantly, the experiment: (i) superimposes predicted temperature and pH changes on top of the natural diurnal oscillations of each habitat (following Dove *et al.*, 2013) and (ii) cross-compared corals from each habitat by exposing them to present-day and future, high- and low-variance conditions. Using this approach the study demonstrates the relative influence of pH and temperature on key metabolic traits (photosynthesis, respiration and calcification) for corals in high-and low-variability habitats, and demonstrates how the biological performance of corals may be impacted under climate change.

5.2| Materials and methods

5.2.1| Study location, study organisms and collection

The study was conducted on Little Cayman, Cayman Islands, British West Indies. The Atlantic Ocean study location was selected for the laboratory study because the high-resolution abiotic and benthic analysis was conducted there (see Chapter 3). Two sites located on the north coast of Little Cayman were selected: an outer-reef site (low-variability site, LV) on the reef terrace and a seagrass site (high-variability site, HV) within the shallow adjacent lagoon (Grape Tree Bay, see Chapter 3, section 3.2.1). The manipulation study was conducted between May-July 2014. Baseline *in situ* chemistry data was collected over 18-days between July and August of 2012 (see Chapter 3) and was used as the target control conditions within the study. Spot sampling in June 2014 confirmed similarities in seawater chemistry to the measured values in 2012.

Two study organisms were used: *Acropora palmata*, which was only found at the LV site and *Porites astreoides* which populated both the HV and LV sites. For each species and site 40 fragments (> 5 cm total length) were collected, allowing for five replicates per experimental treatment. Corals were collected from the LV and HV habitats from a depth of *ca*. 1.5 m. Corals were never exposed to air and were handled underwater with PVC gloves and transported in individual zip-lock bags filled with *in situ* seawater back to the laboratory within 30 min of collection.

5.2.2| Experimental design

To incorporate the natural diurnal pH and temperature variability of each habitat, pH and temperature conditions predicted in 2100 were superimposed on the natural diurnal trends (as Dove *et al.*, 2013, Figure 5.1). The conditions predicted in 2100 were manipulated on top of the natural diurnal trends of each habitat. A mean (SE) temperature increase of 2.2 ± 0.03 °C and pH decrease of 0.3 ± 0.02 units were used based on A1B scenario estimates (IPCC, 2015). It should be noted that it remains unclear how systems like seagrass habitats will respond to future changes in carbonate chemistry due to the high cover of photoautotrophs and their use of DIC for photosynthesis (Langdon *et al*., 2005). As their response remains unclear a "worst-case" scenario has been used here where the predicted 0.3 unit decrease in pH for the open-ocean has been superimposed onto the seagrass HV present-day diel trends.

Seven sampling periods were identified from the natural diurnal trends in temperature and pH. These periods represent the greatest rates of change and were selected to best replicate the habitats natural oscillations (Figure 5.2). For each time period, the average *in situ* pH and temperature were determined, and the IPCC predicted changes for 2100 were superimposed onto these values. In total eight experimental treatment conditions were created within controlled laboratory conditions (Figure 5.3), including a control, temperature increase, pH decrease, and temperature increase with pH decrease, all superimposed over the ambient HV and LV habitat conditions. Experimental treatments were replicated five times, with one test organism from each site in an aquarium.

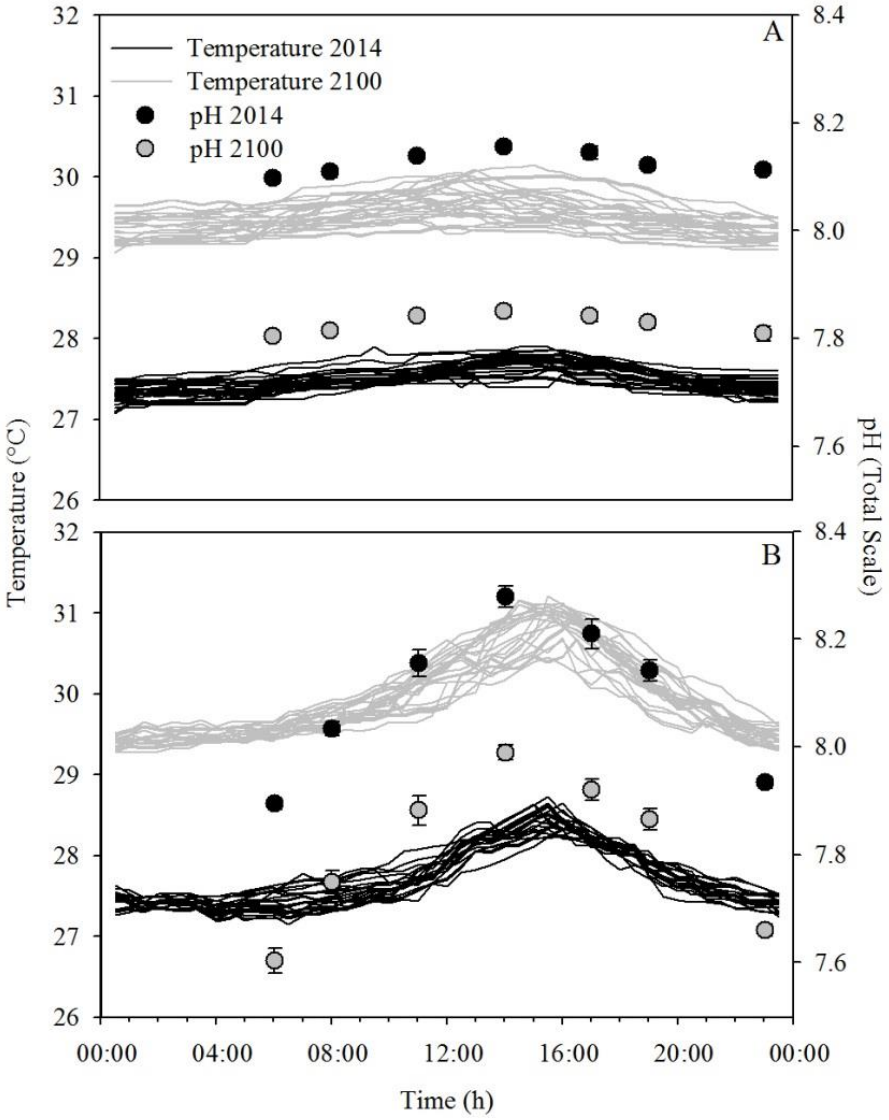


Figure 5.1| The average pH and temperature tank conditions for: A) the outer-reef and B) the seagrass habitats of Little Cayman, Cayman Islands, BWI, for both present day and 2100 (under IPCC A1B scenario estimates). The 2100 conditions (grey) represent an approximate mean (SE) temperature increase of 2.2 ± 0.03 °C and pH decrease of 0.3 ± 0.02 units. pH was measured from daily discrete water samples over the seven time periods, whilst temperature was measured continuously with a HOBO Pendant Temperature/Light 64K data logger set at a 30 min interval.

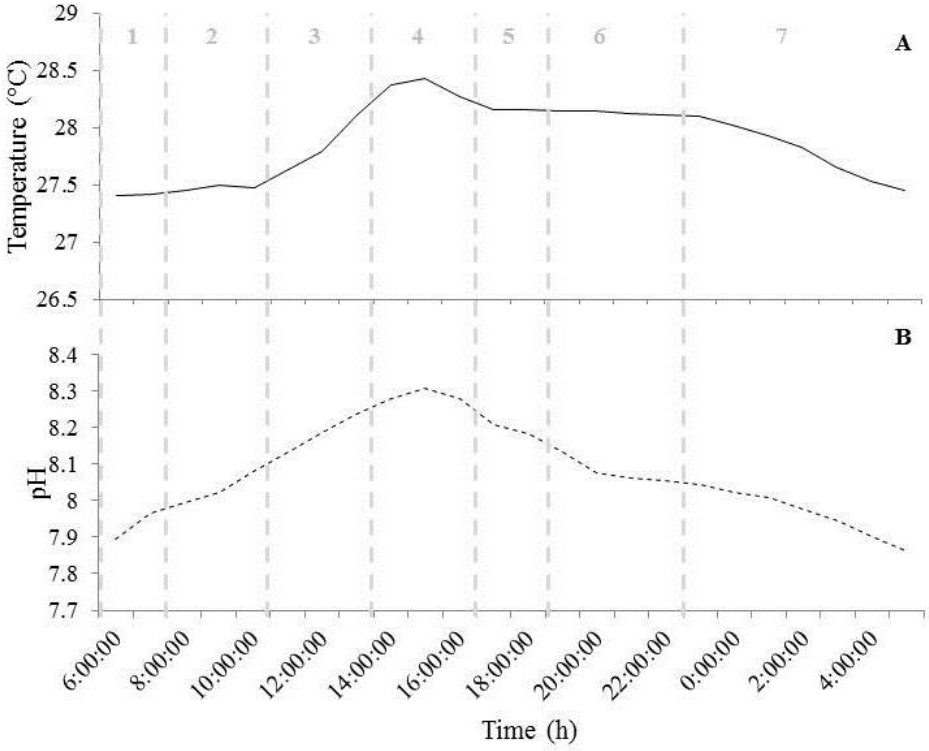


Figure 5.2| The natural diurnal oscillations in: A) temperature and B) pH for the seagrass habitat on Little Cayman, Cayman Islands, BWI. From the natural diurnal cycles, seven time periods were selected to manipulate the temperature and pH to conditions predicted in 2100. The seven time periods were selected to try and best-represent the natural diurnal conditions.

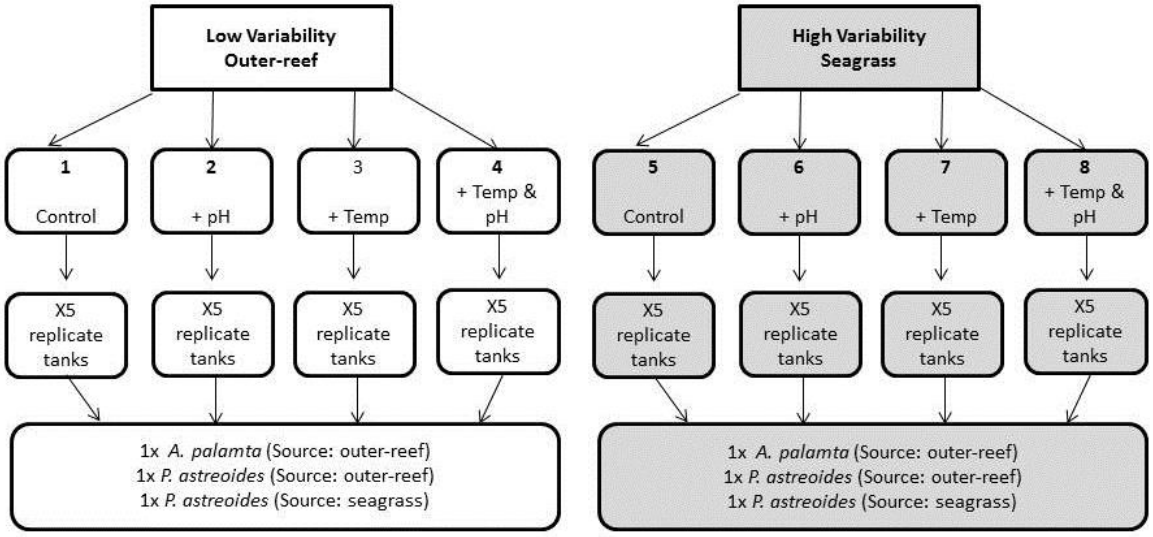


Figure 5.3| Schematic of the experimental design. Each treatment (1-8), which included a control and the effect of elevated pH and/or temperature, was performed relative to the background variance for the low variability outer-reef site (LV) and high-variability seagrass site (HV). Five replicate samples (n= 5) of the species chosen (*Acropora palmata* sampled from the LV site, and *Porites astreoides* form both the HV and LV sites) were simultaneously subjected to each of the eight treatments (total n= 40 per species).

The basis of the experimental set-up was a reservoir (4 x 55 gallon PVC Tote, Lowes, USA) of non-filtered outer-reef (LV) natural seawater that supplied the four LV experimental conditions. The reservoir was re-filled with fresh seawater every 2-3 days as necessary. Water from the reservoir was pumped (DC Utility pump connected to 5/8 in garden hose, Pacific hydrostar, USA) into a sump (10 gallon PVC Tote, Lowes, USA) containing seagrass and carbonate sediment (collected from the *in situ* seagrass habitat) to re-create the natural variability in pH experienced in the seagrass habitat. The sump contained a pump and aerator (EHEIM 200, EHEIM GmbH & Co. KG, Deizisau, Germany), which subsequently supplied water to the four HV experimental treatments. For this, water was tapped into additional aquaria where pH was altered by subsequently manipulating the seawater by equimolar additions of strong acid (1 mol

L^{-1} HCl, Thermo Fisher Scientific, NJ, USA), NaOH (Thermo Fisher Scientific) and Na_2CO_3 (Thermo Fisher Scientific) (Richier *et al.,* 2014). The volumes of HCL and HCO_3^- required to adjust pCO_2 and pH to the chosen target values were first calculated from the measured ambient state of the carbonate system in seawater using CO2SYS (Richier *et al.,* 2014). For each of the seven daily time periods, water within each aquarium was flushed with new manipulated seawater.

pH and total alkalinity (TA) were tested before water exchanges from discrete water samples. pH was measured using the Orion Ross Ultra Glass Triode Combination Electrode (Ross Ultra; Fisher Scientific, UK) calibrated with TRIS buffers (accuracy *ca.* ± 0.002 pH units) using the potentiometric technique and the total scale (see Chapter 2, section 2.2.2. and Dickson *et al.,* 2007). An open-cell potentiometric titration procedure was used to measure TA using the Gran method to determine the second end point of the carbonate system. TA of all samples was determined using a Titrino titrator (Model 848; *Metrohm*, Buckingham, UK) with accuracy and precision of *ca.* ≤ 2 μmol kg^{-1} as verified with certified reference materials distributed by A. Dickson (Scripps Institute of Oceanography). Temperature was controlled by a water bath incubating each aquarium to the desired temperature via heaters at either end (NeoHeater IPX8, Aquael, Poland). Temperature was continuously measured over the duration of the experiment using a HOBO Pendant Temperature/Light 64k Logger (Model UA-002-64; Microdaq, USA) set to log every 30 min. Experimental conditions were also monitored daily using a temperature Probe (NeoFox TB, Ocean Optics, England). All aquaria were exposed to natural daylight-dark cycles 12:12 cycle (Anthony *et al*., 2008), with average daylight PAR of 350-500 μmoles photons m^2 s^{-1} which was representative of *in situ* light conditions. Light was measured using three HOBO Pendant light loggers (Model UA-002-64, Microdaq, USA), with values averaged

and converted to PAR using the daylight coefficient (Long *et al.*, 2012, see Chapter 3 section 3.2.2).

The experiment was divided into three phases: 1) Recovery (3-days), where corals were removed from their *in situ* environment and left to recover in the laboratory under their ambient conditions; 2) Acclimate (21-days) where pH and temperature were manipulated for each tank and corals were able to adjust to the specific experimental conditions (as deemed appropriate from prior experimentation, see Suggett *et al.*, 2013), and 3) Experimentation (35-days) where corals continued to be exposed to the treatment condition via pH and temperature manipulation (total experimental duration = 59 days). During the acclimation period, regular (every 3 days) spot checks were conducted to assess the metabolic response (photosynthesis, respiration, calcification rates) of a sub-set (20 %) of the corals to assess if rates were becoming regular, i.e. had acclimation taken place. At the end of the recovery period (t_0) and at the end of the experimental period (t_e) zooxanthellae and chlorophyll α measurements were taken and a 24 h incubation (described in detail below in the *measurements of photosynthesis, respiration calcification and growth rates* section) was conducted to measure photosynthesis, respiration and calcification daily integrated rates. At the end of the acclimatory period (t_i) and t_e buoyant mass measurements were taken to establish an average daily growth rate over the 35 days.

Carbonate chemistry baseline – At each location discrete water sampling was conducted over 18-days between July and August 2012 to establish the natural diurnal trends in pH and temperature. See Chapter 3 (section 3.2.2) for detailed methods, but in brief, discrete water samples were collected at 3 h intervals starting at sunrise (n = 144 per site: three sub-sites each sampled eight times per 24 h sampling session, with six 24 h replicates). Seawater carbonate chemistry was measured through direct water sampling following the Carbon Dioxide

Information Analysis Centre (CDIAC) protocols (Dickson *et al.*, 2007), with carbonate parameters (pCO_2, TCO_2 and Ω_{arg}) calculated with CO2SYS from TA and pH (Riebesell *et al.*, 2010), *in situ* temperature and salinity, and sampling depth (m) as a proxy for pressure (Lewis and Wallace, 1998). For CO2SYS the dissociation constants of Mehrbach *et al.* (1973) were used for carbonic acid as refined by Dickson and Millero (1987), and for boric acid (Dickson, 1990). Pressure effects, orthophosphate and silicate concentrations were assumed to be negligible (Jury *et al*., 2010).

Measurements of photosynthesis, respiration calcification and growth rates – All nubbins were weighed in seawater (buoyant weight) at t_i and t_e with a Ohaus Scout Pro balance (accuracy 0.01 g). Skeletal dry weight was demined using the method described by Davies (1989) and normalised to surface area that was calculated via the advanced geometric technique (AGT) (Naumann, 2013). Growth rates established from buoyant mass closely coupled rates established from the TA method demonstrating that this method is a good indicator of mass (Figure 5.4). Consequently, data presented is obtained from the TA method as rates of calcification (mmol $CaCO_3$ m^2 h^{-1}).

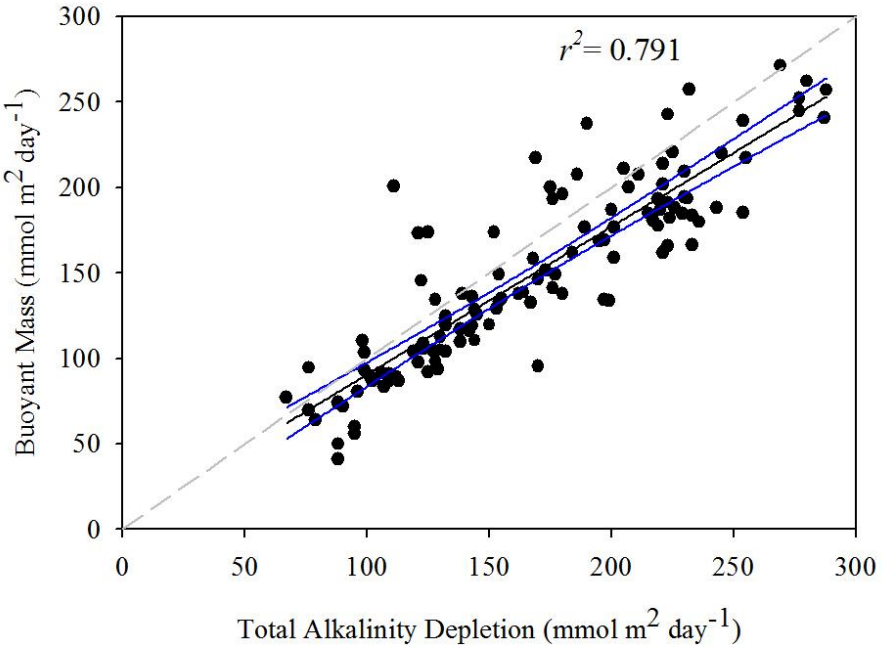


Figure 5.4| The relationship between growth rates based on the total alkalinity depletion and buoyant mass methods. Each point represents measurements from both methods on an individual coral fragment. Data is shown for the high-variability and low-variability treatments. The grey line illustrates a 1:1 ratio, the solid black line represents the best fit line (Buoyant Mass (mmol m^2 day^{-1}) = 4.32 + 0.864 * Alkalinity depletion (mmol m^2 day^{-1}), r^2= 0.791, n= 120, P= 0.001) and the blue lines represent the 95 % confidential internals. Rates were obtained from eight 3 h incubations conducted over a 24 h period at t_0 and t_e.

At all incubations every colony was incubated in a separate 750 ml closed plastic chambers filled with the experimental water. TA was measured at the start and end of the 3 h incubation. After this period, the water was flushed with new experimental water and chambers were re-secured. The next incubation was then initiated following the same sampling procedure and repeated at 3 h increments over the 24 h period. The same procedure was performed on three control chambers that contained only seawater. The change in TA in each respirometry chamber was corrected for any changes in TA of the seawater controls to yield rates of G as:

$$G\ (t)\ = \left[\frac{(\Delta TA \cdot \rho \cdot 0.5)\cdot V}{I_t \cdot SA}\right]/1000 \qquad [1]$$

Where TA= total alkalinity (μmol kg^{-1}), *V* = volume of water (L) surrounding the coral within the respirometry chamber, I_t (h) is incubation time, SA is the coral surface area (m^2), ρ is the density of seawater and 0.5 accounts for the decrease of TA by two equivalents for each mole of $CaCO_3$ precipitated.

G rates for the day (i.e. G light, G_L) and night (G dark G_D) were calculated as:

$$G_{DAY} = \left(\left(\sum_{dawn}^{dusk} G\ (t)\ \Delta t\right) + \left(\sum_{dusk}^{dawn} G\ (t)\ \Delta t\right)\right)/1000 \text{ ; i.e. } = G_L + G_D \quad [2]$$

Net photosynthesis (P_N) and respiration rates (R) were determined at the same incubation periods (8 x 3 h incubations at t_0 and t_e) as G. O_2 was measured at the start and end of each incubation using a Foxy-R O_2 probe (Ocean Optics, England). The change in O_2 in each respirometry chamber was corrected for any changes in O_2 of the seawater controls to yield hourly rates (mmol O_2 m^{-2} h^{-1}) as:

$$P_N \text{ and } R\ (t) = \left[\frac{(\Delta O_2) \cdot V}{I_t \cdot SA}\right] /1000 \quad [3]$$

Integration of all P and R measurements during the day (night) yielded the daily P_N and R (mmol O_2 m^{-2} d^{-1}) as:

$$P_N = \sum_{dawn}^{dusk} P\ (t)\ \Delta t \text{ and } R = \sum_{dawn}^{dusk} R\ (t)\ \Delta t \quad [4]$$

Gross photosynthesis (P_G) was calculated by the addition of P_N and R. All values of R are subsequently converted to a positive value as multiplied by a factor of -1. Surface area of all colonies was determined by the AGT.

Chlorophyll α and zooxanthellae counts – Coral tissue was removed from the base of each nubbin using a water pik (Waterpic Inc, England) in 5 mL of GF/F–filtered seawater; the area of tissue removed was quantified via the AGT method. The tissue slurry was homogenised

using a pipeta pasteur and a small aliquot subsequently taken for cell quantification via microscopy using a neubauer haemocytometer (Berkelmans & van Oppen, 2006). A second aliquot of sample was subsequently filtered and extracted in 3 mL methanol for 24 h at 4 °C. Chlorophyll α was subsequently quantified by measuring the pigment extracts on a USB 2000^{+} Spectrometer (Mikropack Halogen Light Source (HL-2000) and applying the equations of Ritchie (2006) for dinoflagellates.

5.2.3| Statistical analysis

All statistics were conducted in either R software (R 237 Development Core Team, 2011) or Sigma Plot 10.0 (Systat Software, San Jose, CA). Parametric test assumptions were all met unless stated, with the Levene's test used to check homogeneity of variance and qq-plots to assess the normality of the data. Within the results section means are shown with standard error (SE).

Linear regression was used to compare rates of calcification between t_0 and t_e, calcification rates calculated from the TA anomoly method compared to the buoyant mass technique. A paired *t*-test was conducted to assess whether rates of calcification at t_0 were different from rates of calcification at t_e. An independent sample *t*-test was used to compare the average ranges in temperature and pH recorded for experimental treatments, as well as differences in density between high- and low-variability treatments.

Multi-model comparison – Within the study *P. astreoides* was found at both sites whereas *A. palmata* was only found at one site, meaning that the experiment could not be fully factorial. In addition to being unbalanced, from exploratory analysis of the data it appeared that third-order interaction terms (such as pH, temperature and species) may be significant. The large number of

terms in a third-order ANOVA and the unbalanced design raised concerns that an ANOVA may be affected by over-fitting or ill-conditioning (Zurr *et al*, 2010). For these reasons the results have been analysed in two ways: (i) by ANOVA with restrictions on the variables (Zurr *et al*, 2010), and (ii) with a set of nested non-linear models using the multi-model selection framework (Burnham & Anderson, 2003).

ANOVA and non-linear analysis – Three ANOVA analyses were performed; main effects, second order interactions, and with third order interactions (Appendix 10). Non-linear models were also applied. Non-linear multi-model selection is a very general and flexible approach that estimates the parameters of non-linear functions by maximum likelihood, and compares different models using the Akaike Information Criteria (AIC). Using the guidelines from Burnham & Anderson (2003), a difference in AIC of 0-2 was considered negligible. The values of the coefficients were found by maximising the likelihood, which the case of normally distributed errors is equivalent to least squares fitting. Confidence intervals (95 %) were calculated by the log-profile method.

Exploratory data analysis was initially conducted. The saturating model had the maximum number of variables that can be used to describe the results (25 parameters). This model is not overly useful as it provides no insight into the relationships between the factors, however it is used to provide an upper-bound on model complexity. From these plots the relative size of the various effects were gauged (e.g. influence on photosynthesis, see Figure 5.5; Appendix 10).

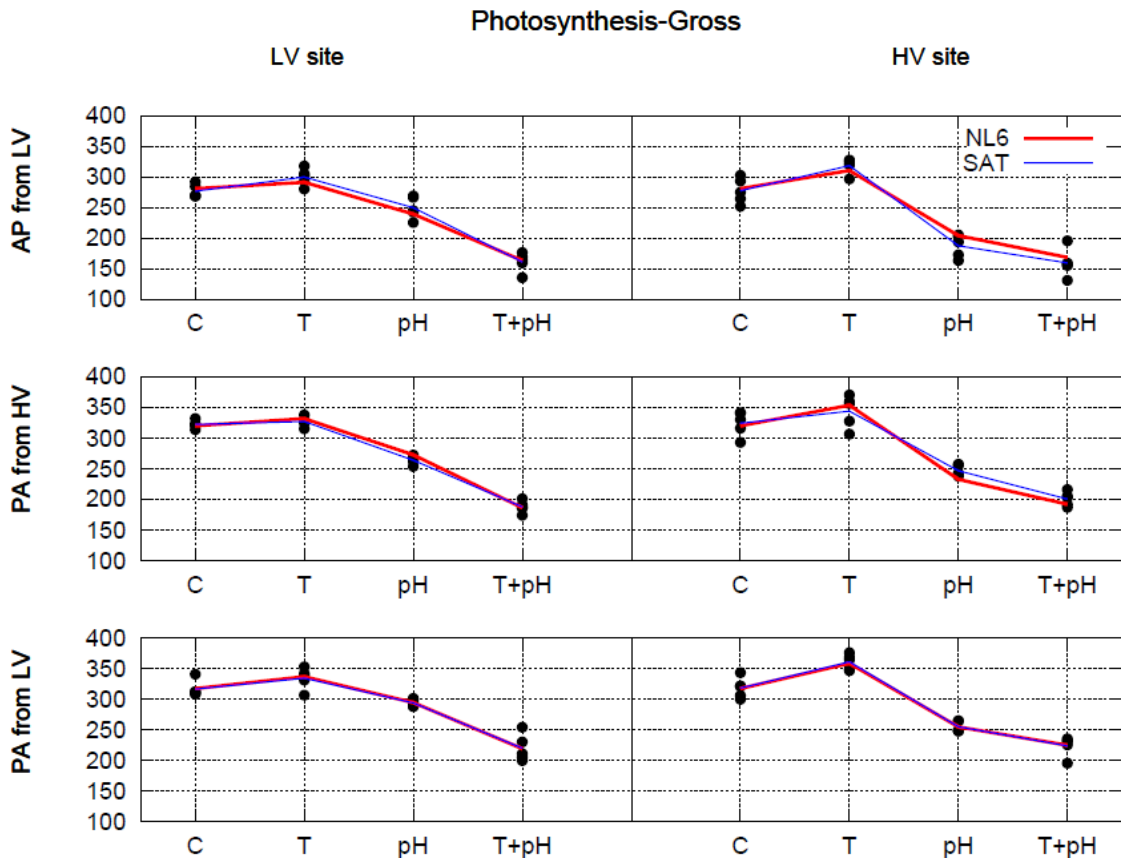


Figure 5.5| An example of data exploration for the non-linear models. The saturation model (blue-line) contains the maximum number of variables that can be used to describe the results, in this case 25 parameters. It is used to set an upper-bound on model complexity. The graph also shows the non-linear model 6 that was used and how this related to the saturation model.

From the initial analysis the structure of the non-linear model was:

$$V = V_0 \times \left[1 + a_s s + a_h h\right] \times \begin{bmatrix} 1 + T \left(b_T + b_{T,v} v + b_{T,h} h\right) \\ + pH \left(c_{pH} + c_{pH,v} v + c_{T,h} h\right) \\ + T \cdot pH \left(d_{T,pH} + d_{T,pH,v} v + d_{T,pH,h} h\right) \end{bmatrix} \quad [5]$$

The model describes the process of interest, say calcification (G), in terms of an average value for the two species (V_0). The second bracketed term describes the difference between corals under control conditions (i.e. no temperature or pH manipulation) using multiplicative term. The

third term describes the changes resulting from experimental manipulation as an additional multiplicative term. This term is organised first in terms of temperature, pH and an interaction between temperature and pH (b_T, c_{pH}, $d_{T,pH}$ coefficients). In addition there are coefficients to account for the differences due to the variability of experimental treatment and the habitat from which the coral were sourced ($b_{T,v,}$ $b_{T,h,}$ etc). There are several versions (1-6) of this model that correspond to dropping the terms to simplify the model (NL1-NL6, see Appenidx 10).

Model NL6 was selected to explain the data due to its low AIC value, the number of parameters that were included and its close relationship to the trends of the data shown with the saturation models. As recommended by Burnham & Anderson (2003) confidence intervals (CI) at the 95 % level are displayed next to model estimates rather than significance values. CI are beneficial for this study as it allows the size of a difference to be measured between experimental treatments, species, and habitats, rather than simply indicating whether or not there is a statistical difference. In attempting to de-convolve the relative influence of temperature and pH on coral metabolism, along with investigating some of the more intricate third order interactions, the approach of using CI is favoured (Gardner & Altman, 1986; Burnham &Anderson, 2003).

The ANOVA and non-linear models were compared using their AIC. The AIC of the linear (ANOVA) and non-linear models were compared with each other and were found to be consistent. The benefit of the non-linear model is that any terms with insignificant *p*-values or confidence intervals were excluded and the models are not affected by over fitting or ill conditioning. The non-linear model NL6 consistently had lower AIC scores than the linear AN3 model (2.7 vs 8.7), which indicates that the non-linear model has considerable more empirical support (Appendix 10).

5.3| Results

5.3.1| Carbonate chemistry manipulation

The method of manipulating the pH and temperature was able to recreate the predicted pH and temperature changes of 2100 under IPCC A1B scenarios and included the natural diel trends of each habitat (Figure 5.1). Under both the HV and LV treatments, more daily variability in both temperature and pH was experienced for the tanks exposed to the 2100 level conditions (temperature: t_6= 6.36, P= 0.001, pH: t_6= 4.44, P= 0.005). Growth rates from t_i were the same as t_e demonstrating that the experimental method sustained conditions in the control tanks throughout the experimental period (r^2= 0.859, n= 30, P= 0.001, Figure 5.6), showing no difference over the study duration (e.g. 1:1 ratio).

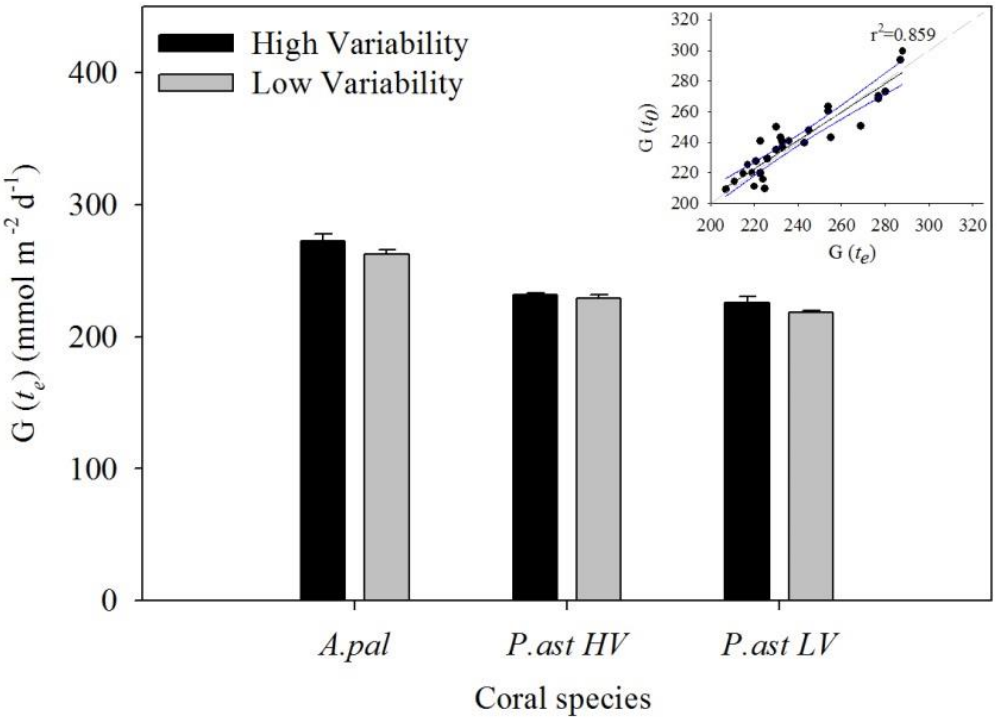


Figure 5.6| Avergae (SE) daily calcification rates (G) at the end of the experiment (t_e) for all three coral species: *Acropora palmata* (outer-reef, low-variability site (LV)), *Porites astreoides* (outer-reef, LV site), and *P. astreoides* (seagrass, high-variability site (HV)) within the high-variability and low-variability control tanks. A regression between rates of G at the start (t_0) and end (t_e) of the experiment show strong co-linearity between rates (r^2= 0.859, n= 30, P= 0.001, G(t_0) = 18.55 + 0.93* G (t_e).

5.3.2| Native habitat response

Reciprocal transplantation of corals to high/low variability control tanks did not induce a change in calcification for any species (Figure 5.6). Corals native to the LV outer-reef site sustained calcification in the HV seagrass control tanks. Corals originating from the HV seagrass site showed no enhanced rates of calcification under the more stable LV control conditions.

Prior exposure to variability in pH and temperature for *P. astreoides* originating from the seagrass habitat was found to have minimal influence on their response to all experimental treatments. Colonies native to the seagrass habitat experienced a reduction in calcification that

was similar (*ca.* 4 % lower reduction, Table 5.1) to colonies from the more stable outer-reef habitat. Similarly, rates of photosynthesis decreased proportionally for *P. astreoides* originating from both habitats under the pH (- 17.3 %, CI: -14.8 to -19.7 %) and pH and temperature (- 26.5 %, CI: -22.5 to -30.6 %) experimental treatments (Table 5.1).

5.3.3| Experimental variability response

Overall the variability in pH and/or temperature of the experimental treatment had minimal influence on the physiological response of each coral (Figure 5.7). One exception was observed under the pH treatment, where gross productivity was reduced by a further 6.3 % under the HV treatment (Table 5.1). Although all colonies experienced a decrease in calcification rates independent of the level of variability experienced (Figure 5.7), colonies exposed to the LV water treatment all experienced a larger decrease in skeletal density relative to the control colonies (t= -3.79, P= 0.005, Figure 5.8). Under the HV (seagrass) treatments, both *P. astreoides* species experienced an increase in skeletal density. No significant difference was detected in the skeletal densities of the controls between the HV and LV treatments, independent of species.

Although the net calcification response of *P. astreoides* and *A. palmata* were similar for both the HV and LV treatments, the conditions they were exposed to within each habitat were very different (Figure 5.9). Over a diel-cycle pH fluctuations resulted in daily oscillations in Ω_{arg} (Figure 5.9), where by daytime peaks (control tanks: HV 4.5 ± 0.02, LV 4.0 ± 0.01) occurred around mid-day and lows occurred during the night (control tanks: HV 2.7 ± 0.04, LV 3.8 ± 0.01). Diel cycles resulted in the HV habitat under current seawater conditions spending *ca.* 43 % of its time under the $CaCO_3$ sediment calcification-to-dissolution threshold (G-D). A further 20 % of time was spent below the Mg-calcite G-D. The outer-reef, LV habitat was exposed to

carbonate-sediment under saturation for less than 12 % of its daily cycle. When the -0.3 pH and 2.2 °C conditions were added, both habitats had a very similar reduction in their mean Ω_{arg} (Reduction of: HV = 1.4 and LV =1.5 units) although their exposure to over-and under-saturated conditions were very different. The HV habitat spent time (< 10 %) close-to and/or below the Ω_{arg} with 85 % of time below the $\Omega_{Mg\text{-}calc.}$ The LV habitat spent all time below the $\Omega_{Mg\text{-}calc}$ and remained above the $\Omega_{arg.}$

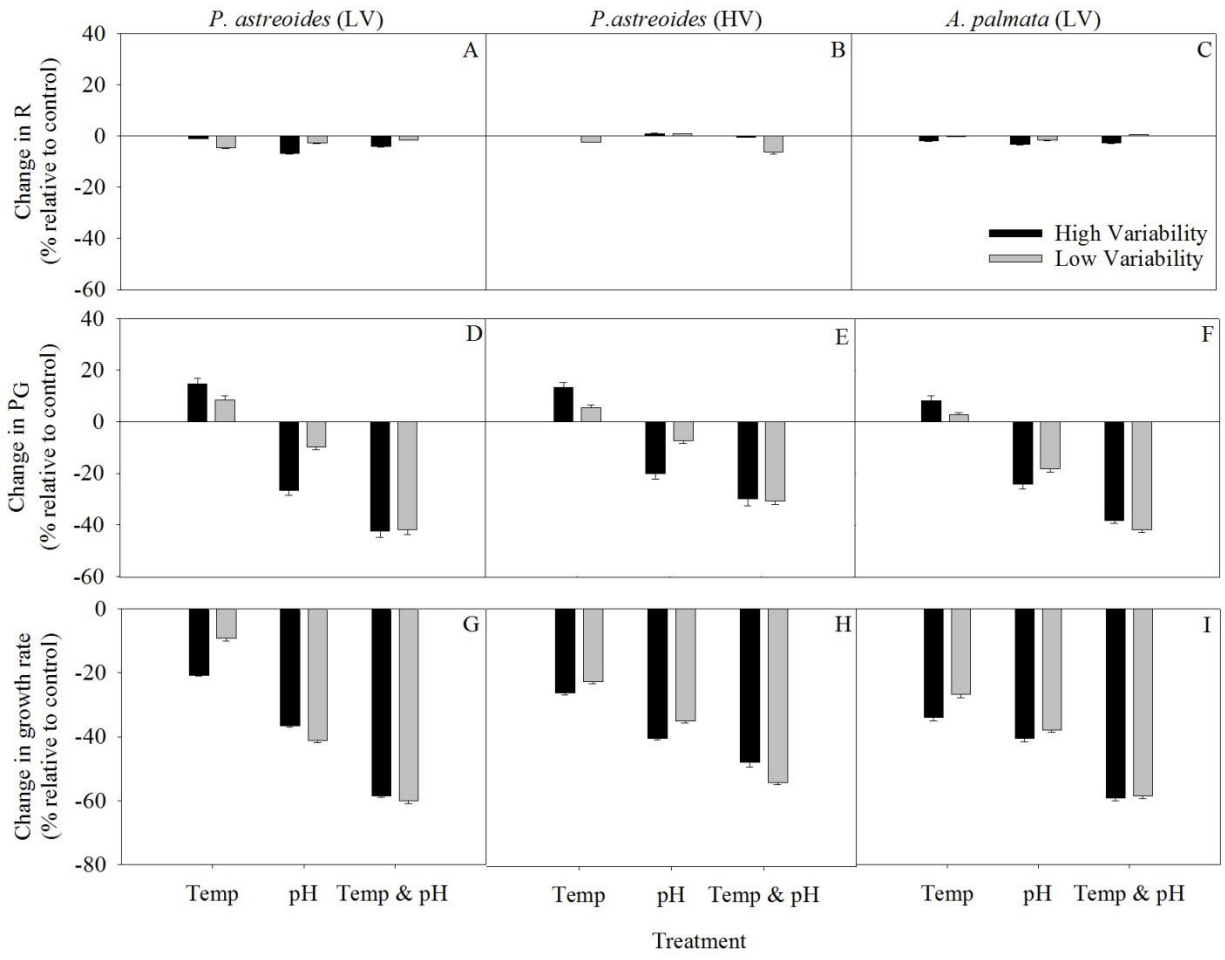


Figure 5.7| The metabolic response of corals across experimental treatments. The relative percent change in respiration (R), gross productivity (P_G) and calcification (G) for *Acropora palmata* (outer-reef, low-variability site) (4.a, d, g), *Porites astreoides* (seagrass, high-variability site) (4.b, e, h) and *P. astreoides* (outer-reef, low-variability site) (4.c, f, i) relative to the controls, within the high-variability and low-variability experimental tanks. Relative percent change (± SE) was detemined by averaging the experimental replicates per treatment (n= 5) and standardising by the control at the end of the experiment (t_e).Experimentation ran for five weeks, with a three week acclimitasation phase. Corals were from Little Cayman, Cayman Islands, BWI, with control conditions resprentative of present day *in situ* conditions for the seagrass and outer-reef, whilst experimental conditions best represent the temperature increases and pH decreases estimated under the IPCC A1B scenario.

Table 5.1| a) The non-linear model results (NL6).

		Photosynthesis (%)			Respiration (%)			Calcification (%)		
		est	CI lo	CI hi	est	CI lo	CI hi	est	CI lo	CI hi
V0		299	293	305	151	147	155	249	244	254
a	s	6.5	5.2	7.9	8.7	7.2	10.3	-9.3	-10.7	-7.8
	h	-0.4	-2.5	0.9	-0.6	-3.3	1.6	2	0	3.9
b	T	8.3	5.6	11.1	-1.5	-4.7	3.5	-20.4	-22.9	-17.8
	T,v	3.4	1.6	5.2	-0.4	-2.7	1	1.7	-0.1	3.5
	T,h	1.2	-2.8	4	0.3	-0.7	3.6	1.1	-2.6	3.6
c	pH	-17.3	-19.7	-14.8	-2.2	-5.5	1.1	-38.7	-41	-36.3
	pH,v	-6.3	-8.1	-4.5	-3.2	-5.4	0.9	-1.9	-3.7	-0.1
	pH,h	3.8	1.3	6.2	3.2	-0.1	6.5	3.7	1.4	6
d	T,pH	-26.5	-30.6	-22.5	1.5	-3.6	6.1	3.2	-0.8	7.1
	T,pH,v	3.7	0.6	6.9	4.4	0.5	8.3	3.8	0.7	7
	T,pH,h	0.5	-1.1	4.5	-4.6	-9.3	0	0.1	-0.2	4

Table 5.1| b) Legend to the model output shown in table 5.1a

Variable	Symbol	Values	Meaning
species	s	1	*P. astreoides*
		-1	*A. palmata*
habitat	h	1	High variability, seagrass
		-1	Low variability, Outer reef
variability	v	1	High variability, seagrass
		-1	Low variability, Outer reef
temperature	T	0	Control
		1	Manipulation, + 2.2 °C
pH	pH	0	Control
		1	Manipulation, -0.3 units

Colour coding:

- Small Increase ($x < 10$ %)
- Small decrease ($x < 10$ %)
- Medium decrease ($10 \geq x < 20$)
- Large decrease ($x \geq 20$)

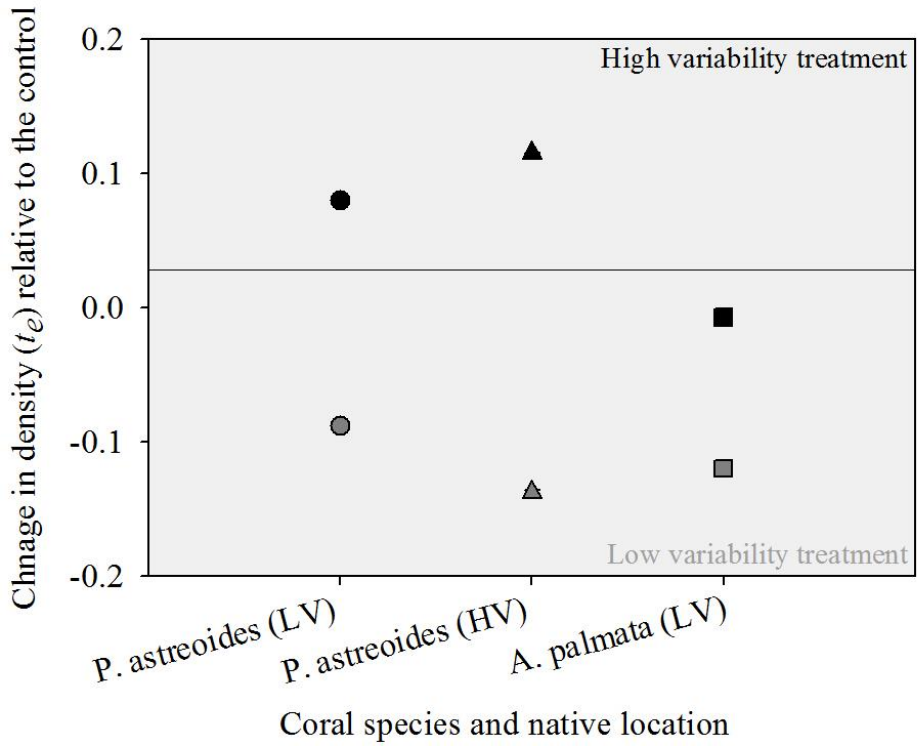


Figure 5.8| The changes in coral density over the experimental period. The average (SE) changes in density for each coral species, from each location (low-variability (LV) outer-reef, high-variability (HV) seagrass) for both the high and low variability treatments, relative to the controls at the end of the experiment (t_e). Density was determined on a sub-sample of corals from each experimental treatment, (n= 3 per treatment, total n= 24) using a 3D scanning process following the protocol of Enochs *et al*., (2014).

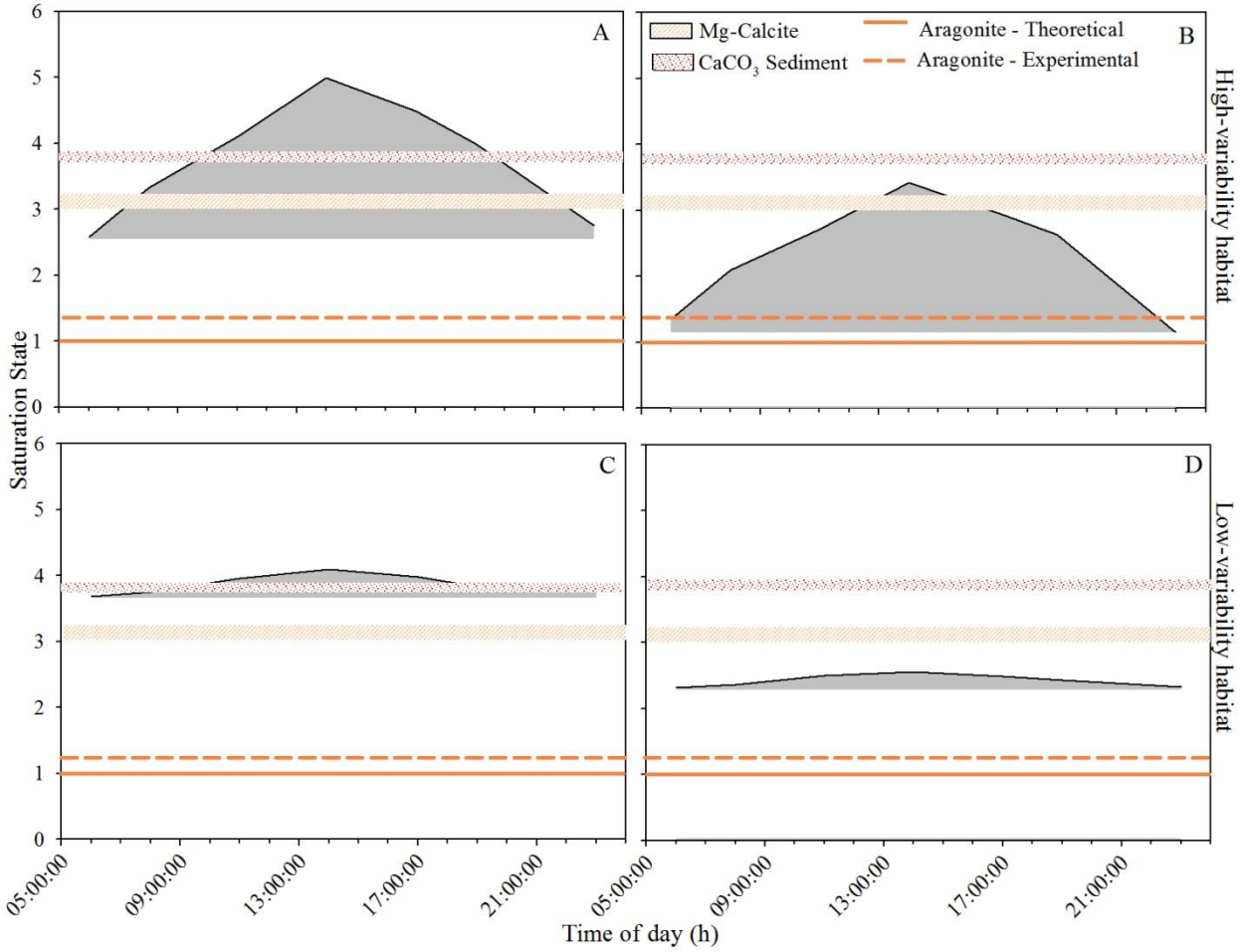


Figure 5.9| The time each habitat is exposed to a given saturation state. Data is averaged for each level of variability (high or low). The grey shaded area shows the daily trend in the saturation state of seawater within each habitat under current and future (2100) seawater conditions. A & C) show current ambient seawater conditions, B & D) show the effect a change of – 0.3 pH units and a 2.0 °C temperature increase has on the saturation states of each habitat. Calcification-to-dissolution thresholds are shown for Mg-calcite, $CaCO_3$ sediment, aragonite theoretical (e.g. the calculated level of one) and experimental aragonite (levels experiments have measured the aragonite threshold to occur at). The carbonate-sediment values were calculated by Yamamoto *et al*., 2012 experimentally based on sediment that contained a mixture of low Mg-calcite, Mg-calcite and aragonite. The mixture of these different structural forms of $CaCO_3$ results in the Ω threshold of carbonate-sediment occurring at a higher Ω.

5.3.4.| Species response

Under control conditions, growth rates were 9.3 % higher (CI: 7.8 – 10.7 %) for *Acropora palmata* (Table 5.1), with an average daily rate of 268.5 ± 1.2 mmol m^2 d^{-1}, compared to 228.0 ± 0.9 mmol m^2 d^{-1} for *P. astreoides* (Table 5.2). Comparison of the relationship between growth rates for all species based on the TA anomaly method versus buoyant mass showed that the data from each were significantly correlated (r^2= 0.791, n= 120, P= 0.001), with their relationship described by the equation: Buoyant Mass (mmol m^2 day^{-1}) = 0.864 * Alkalinity depletion (mmol m^2 day^{-1}) (Figure 5.4). Respiration (8.7 %, CI: 7.2 - 10.3%) and photosynthesis (6.5 %, CI: 5.2 - 7.9 %) rates were slight elevated for *P. astreoides* compared to *A. palmata.* Overall physiological responses to the experimental treatments were generally similar for both *A. palmata* and *P. astreroides* (Figure 5.7), however clear species differences were evident when gross photosynthesis was standardised to zooxanthellae counts:

pH influence – Under both levels of variability in environmental conditions, and across all species, lower pH under the 2100 scenario led to a reduction in daily gross photosynthesis (Figure 5.8). The reduction in gross photosynthesis resulted from a decrease in net productivity (17.3 %, CI: 14.8 - 19.7, Table 5.1) whilst respiration remained constant (within 5.5 % of the control, Figure 5.8). Decreased pH led to a significant reduction in calcification rates across all treatments (38.7 %, CI: 36.3- 41.0, Figure 5.8, Table 5.1).

Temperature influence – Elevated temperature under the 2100 scenario increased photosynthesis for all species and under both levels of habitat variability (8.3 %, CI: 5.6 -11.1 %, Table 5.1). Temperature had no influence on respiration rates, however, calcification decreased

for all species under elevated temperature independent of variability (20.4 %, CI: 17.8 – 22.9 %, Figure 5.7, Table 5.1).

Combined pH and temperature effect – The combined influence of elevated temperature and lower pH resulted in a large decrease in gross photosynthesis across species and habitats, which corresponded with a decrease in calcification (Figure 5.7). Within the non-linear model the combined effect of pH and temperature was negligible (3.2 %, CI: -0.8 - 7.1 %, Table 5.1) due to the additive response of temperature and pH independently explaining the majority of trends within the dataset. Consequently, an additive response of temperature and pH stress resulted in a decrease in calcification of 59.1 % (Table 5.1). Again, the decrease in gross photosynthesis resulted from a decline in net photosynthesis whilst respiration remained constant (Table 5.2).

Productivity and calcification standardised to zooxanthellae counts – Rates of calcification were closely coupled to rates of gross photosynthesis, independent of species and experimental treatment (r^2= 0.831, n= 90, P= 0.01, Figure 5.10a), with gross photosynthesis explaining 83 % of the variance in calcification. For *P. astreoides*, changes in gross photosynthesis were independent of the density of zooxanthellae (remaining within *ca*.10 % of the control (Figure 5.11)). Similarly, *A. palmata*, colonies exposed to the temperature treatment had a decrease in gross photosynthesis that was independent of the density of zooxanthellae. However, when *A. palmata* was exposed to both the pH, and pH and temperature treatments a decrease in gross photosynthesis occurred that corresponded with a loss in zooxanthellae cell density (Figure 5.10b) and a decrease in chlorophyll α (10-23 %, Figure 5.11). Visual observations support these measurements, with paling observed for *A. palmata* from day 14 of the experiment.

Table 5.2| Raw data measurements before experimental manipulation (t_0) and at the end (t_e) of the experimental period for the corals *Acropora palmata* and *Porites astreodies* from a high-variability (HV) seagrass habitat and a low-variability (LV) outer-reef location on Little Cayman, Cayman Islands, BWI. Measurements include: daily net productivity (P_N), daily respiration (R), daily calcification (G), and growth rates. Note: buoyant mass rates were calculated from the end of the acclimatory period t_i to t_e.

Table 5.2a| High-variability treatments

Variability	Treatment	Species	P_N (mmol m^2 d^{-1})		R (mmol m^2 d^{-1})		G (mmol m^2 d^{-1})		Buoyant Mass (mg d^{-1} g^{-1} coral)
			t_0	t_e	t_0	t_e	t_0	t_e	Rate (t_i to t_e)
High	Control	*A.palmata LV*	132.0 ± 1.7	138.0 ± 0.3	142.1 ± 2.1	139.5 ± 0.3	284.1 ± 0.3	272.2 ± 2.1	6.08 ± 0.2
		P.astreoides HV	157.0 ± 0.6	161.2 ± 0.1	165.2 ± 1.9	159.1 ± 1.7	226.1 ± 1.6	231.6 ± 0.6	3.93 ± 0.1
		P.astreoides LV	162.1 ± 0.2	157.8 ± 0.8	169.9 ± 3.1	163.8 ± 2.5	230.1 ± 1.0	221.4 ± 0.7	3.60 ± 0.1
	Temperature	*A.palmata LV*	129. 9 ± 3.2	173.7 ± 1.2	142.1 ± 3.2	138.1 ± 5.1	280.1. ± 0.7	220.2 ± 0.9	4.71 ± 0.1
		P.astreoides HV	142.1 ± 2.2	193.8 ± 0.8	150.1 ± 0.8	159.1 ± 3.7	231.9 ± 0.6	174.3 ± 1.1	2.71 ± 0.3
		P.astreoides LV	160.0 ± 2.1	179.3 ± 0.9	152.1 ± 0.4	160.9 ± 7.9	223.1 ± 0.2	146.5 ± 1.3	2.22 ± 0.2
	pH	*A.palmata LV*	127.0 ± 1.9	72.1 ± 2.2	137.2 ± 2.6	131.9 ± 3.6	277.3 ± 1.7	173.2 ± 1.7	3.58 ± 0.1
		P.astreoides HV	155.1 ± 3.8	100.1 ± 1.7	155.0 ± 0.7	160.6 ± 3.7	233.1 ± 0.2	155.6 ± 2.7	2.38 ± 0.1
		P.astreoides LV	162. 9 ± 0.5	188.5 ± 2.3	151.1 ± 0.3	158.7 ± 4.1	228.1 ± 0.3	111.2 ± 3.1	1.85 ± 0.1
	Temperature & pH	*A.palmata LV*	139.2 ± 0.2	28.1 ± 2.8	139.1 ± 0.4	138.5 ± 4.4	265.9 ± 0.4	118.6 ± 0.2	2.56 ± 0.2
		P.astreoides HV	141.2 ± 0.4	68.8 ± 4.1	151.6 ± 1.1	158.6 ± 3.3	225.5 ± 0.2	150.2 ± 0.2	1.78 ± 0.2
		P.astreoides LV	146.2 ± 0.9	42.1 ± 3.1	158.3 ± 0.5	164.9 ± 4.2	225.1 ± 0.4	89.2 ± 0.9	1.76 ± 0.1

Table 5.2b| Low-variability treatments

Variability	Treatment	Species	P_N (mmol m^2 d^{-1})		R (mmol m^2 d^{-1})		G (mmol m^2 d^{-1})		Buoyant Mass (mg d^{-1} g^{-1} coral)
			t_0	t_e	t_0	t_e	t_0	t_e	Rate (t_i to t_e)
Low	Control	*A.palmata LV*	129.2 ± 1.1	134.8 ± 1.9	135.1 ± 0.6	141.7 ± 1.9	272.0 ± 0.9	262.6 ± 1.7	6.21 ± 0.1
		P.astreoides HV	143.9 ± 2.1	149.3 ± 2.7	170.3 ± 1.3	167.3 ± 0.7	231.0 ± 0.6	229.0 ± 0.9	3.74 ± 0.1
		P.astreoides LV	160.1 ± 0.4	158.8 ± 4.9	160.1 ± 0.3	163.2 ± 1.8	221.1 ± 0.1	218.6 ± 0.9	3.46 ± 0.1
	Temperature	*A.palmata LV*	133.8 ± 0.1	164.5 ± 0.5	134.2 ± 0.3	135.0 ± 1.7	225.1 ± 0.4	236.8 ± 1.3	4.88 ± 0.4
		P.astreoides HV	149.3 ± 2.4	170.6 ± 0.2	167.7 ± 0.8	163.5 ± 2.0	272.0 ± 0.9	170.0 ± 1.4	2.64 ± 0.2
		P.astreoides LV	140.1 ± 0.1	163.9 ± 2.2	171.9 ± 1.6	163.0 ± 2.7	231.0 ± 0.6	157.8 ± 1.1	2.07 ± 0.2
	pH	*A.palmata LV*	163.9 ± 1.5	111.8 ± 3.4	144.3 ± 0.2	137.8 ± 5.2	221.0 ± 0.6	153.8 ± 2.7	3.53 ± 0.3
		P.astreoides HV	147.3 ± 3.1	125.3 ± 1.1	168.1 ± 1.1	168.8 ± 1.9	270.0 ± 1.2	168.8 ± 1.7	2.50 ± 0.2
		P.astreoides LV	139.9 ± 3.4	103.2 ± 5.5	152.3 ± 0.5	160.7 ± 3.5	235.0 ± 0.1	150.6 ± 3.9	2.06 ± 0.1
	Temperature & pH	*A.palmata LV*	137.9 ± 0.4	21.6 ± 2.2	144.7 ± 2.6	139.5 ± 3.8	229.6 ± 0.3	105.4 ± 2.3	2.40 ± 0.4
		P.astreoides HV	153.1 ± 0.8	63.4 ± 0.1	162.1 ± 0.7	156.8 ± 3.5	275.0 ± 0.1	129.0 ± 2.9	2.09 ± 0.3
		P.astreoides LV	146.2. ± 2.1	28.8 ± 2.9	150.3 ± 1.9	158.9 ± 3.7	230.0 ± 0.1	68.4 ± 1.6	1.61 ± 0.4

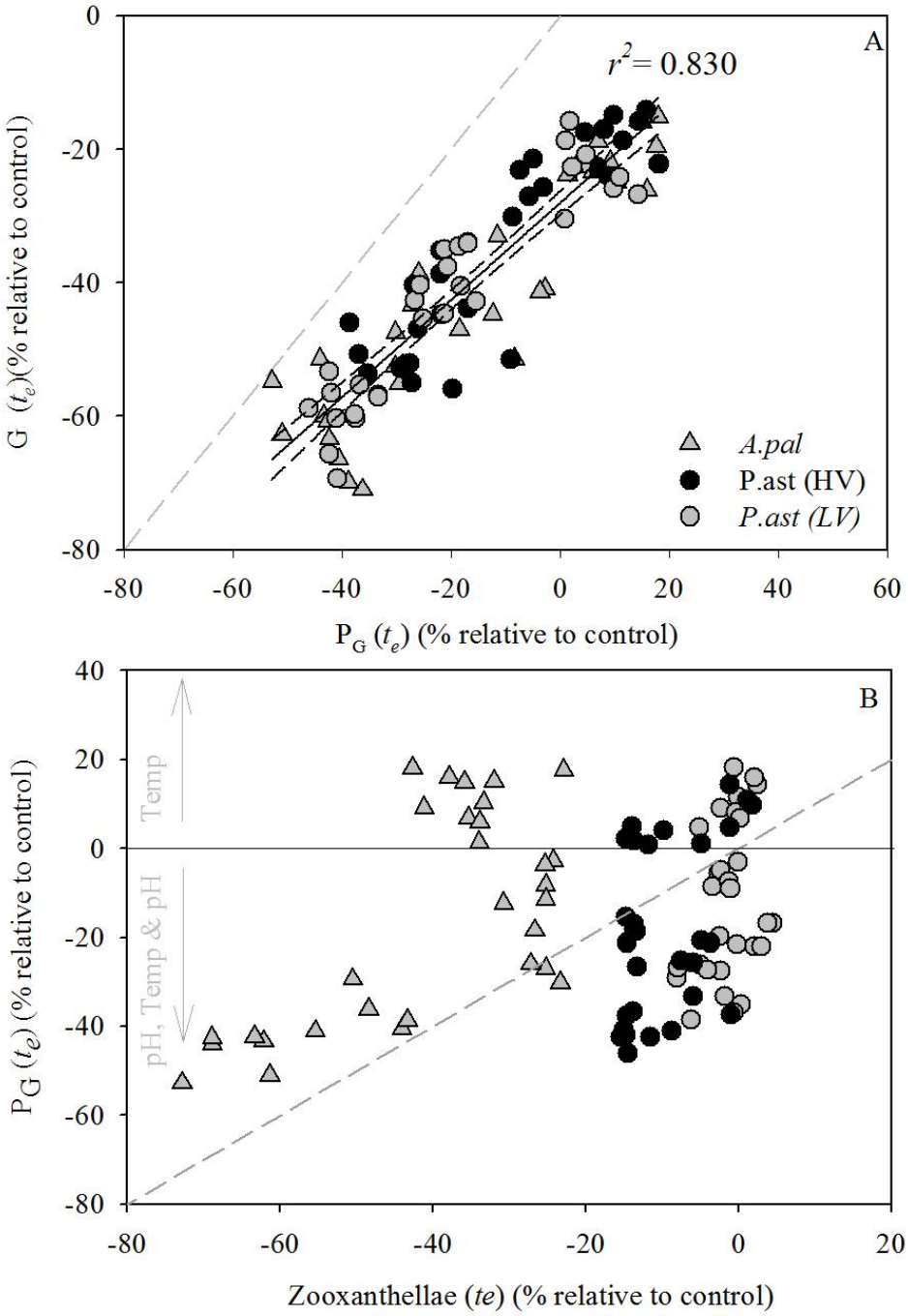


Figure 5.10| Gross productivity (P_G) versus calcification (G) and zooxanthellae percent change for *Acropora palmata* (outer-reef, low-variability site), *Porites astreoides* (seagrass, high-variability site) and *P. astreoides* (outer-reef, low-variability site) from Little Cayman, Cayman Islands, BWI. A) Percent change in P_G versus G and B) Percent change in P_G plotted against the percent change in zooxanthellae. Relative percent change (±SE) was detemined by averaging the experimental replicates per treatment (n= 5) and standardising by the control at the end of the experiment (t_e).

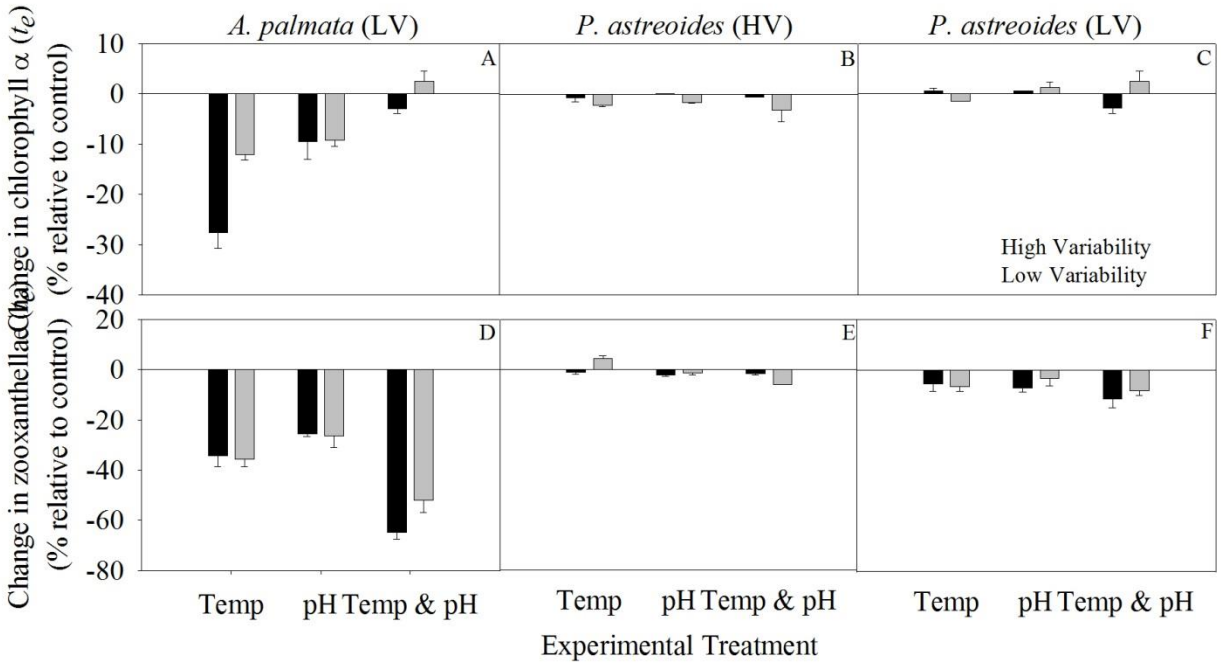


Figure 5.11| The relative percent change in chlorophyll α and zooxanthellae for *Acropora palmata* (outer-reef, low-variability site) (A, D), *Porites astreoides* (seagrass, high-variability site) (B, E) and *P. astreoides* (outer-reef, low-variability site) (C, F) relative to the controls, within the high-variability and low-variability experimental tanks.
Experimentation ran for five weeks, with a three week acclimitasation phase. Relative percent change (± SE) was detemined by averaging the experimental replicates per treatment (n= 5) and standardising by the control at the end of the experiment (t_e). Corals were from Little Cayman, Cayman Islands, BWI, with control conditions resprentative of present day in situ conditions for the seagrass and outer-reef, whilst experimental conditions best represent the temperature increases and pH decreases estimated under the IPCC A1B scenario.

5.4| Discussion

5.4.1| Species metabolic responses to enhanced temperature and low pH

The overall result of the study aligns with the body of literature suggesting that calcification rates will fall under lower seawater pH (Gattuso & Hansson, 2011; Hoegh-Guldberg, 2011; Kroeker *et al.,* 2013), with little-to-no upregulation in calcification from elevated temperature (Anthony *et al*., 2008). Upregulation of pH requires energy (Cohen & Holcomb, 2009) and these results suggest that the levels of photosynthesis sustained under low pH and elevated temperature, combined with any heterotrophy, were not able to meet the corals energy demands to maintain calcification at current day rates.

Within this study, both *A. palmata* and *P. astreoides* experience a large decrease in calcification despite differences in their life history traits and growth forms. McCulloch *et al*. (2012) reported different abilities of *Acropora spp.* and *Porites spp.* in sustaining calcification under future OA based on their abilities to up-regulate their internal pH. Within this study however both species shows a similar negative response, which may indicate a threshold level for which species are able to upregulate pH. Supporting this theory is previous work demonstrating a significant decrease in calcification (*ca.* 40 %) of *P. astreoides* along a natural lowering pH gradient in Yucatan (Crook *et al.,* 2013), which is also similar to decreases measured within laboratory studies (Albright & Langdon, 2011; De Putron *et al*., 2011). However, at Puerto Morelos, Mexico, *P. astreoides* have been documented to maintain net calcification in under saturated seawater (Crook *et al*., 2012), with other studies also documenting little-to-no negative impact on coral calcification (Carricart-Ganivet & Merino, 2001; Rodolfo-Metalpa *et al*., 2011; Comeau *et al.,* 2013). A diversity of responses have been recorded for coral species to lower pH and/or elevated temperature demonstrating the

complexities of interacting factors (SCBD, 2014) and the need to consider the coral as a holiobiont (Morrow *et al.,* 2012), e.g. the coral, algal *Symbiodinium*, and bacterial communities.

Findings within this study demonstrate the importance of species differences even at the *Symbiodinium* level. For *P. astreoides* changes in rates of gross photosynthesis over the experimental treatments were independent of the density of zooxanthellae. This suggests that the loss in photosynthesis occurred due to the cells themselves becoming less productive. *A. palmata* however, when exposed to the pH treatments, experienced a decrease in gross photosynthesis that corresponded with a reduction in zooxanthellae cell density and a decrease in chlorophyll α; thus the loss of cells explained the reduction in productivity. OA induced bleaching has been documented by Anthony *et al.* (2008) on both massive and branching corals. The cause of OA induced bleaching (depigmentation) remains debated, but could include direct impacts of acidosis (Leggat *et al.,* 1999), disruption to the carbon-concentration mechanisms (Kim *et al.,* 2004) or disruption to the photoprotective mechanism of corals due to the reduction of PGPase (Crawley *et al*., 2010).

5.4.2| Coral's native habitat influence on their metabolic response

All corals examined within this study were sourced from habitats where the mean pH is currently elevated (seagrass: 8.142 ± 0.03) or in accordance with global averages (outer-reef: 8.118 ± 0.01) (Manzello *et al.,* 2012; IPCC, 2015). Differences between native habitats were therefore based on the magnitude of daily pH and temperature oscillations, as well as the duration of time spent at particular carbonate chemistry conditions. Within this study, it did not appear that *P. astreoides* from the HV seagrass habitat was more adversely affected than the *P. astreoides* from the LV habitat to temperature and pH conditions predicted in 2100, over the study duration tested. There was actually a small enhanced ability in sustaining calcification rates relative to the

colonies from the more stable environment, however, further replication over a longer study duration would be necessary to assess any significance in this difference.

These findings suggest that environmental history of short exposure to less favourable (pH and/or temperature) conditions is not significant enough to promote an adaptive or acclamatory response evident over the short duration of this study. Alternatively, the differences in environmental history may be too small to affect coral physiology. The results do potentially highlight the importance of the duration of prior exposure to less favourable conditions. Guadayold *et al.* (2014) highlight that short-diel exposures to sub-optimal conditions may not be long enough for acclimatisation or species to prolonged events of unfavourable conditions. These findings are in accordance with work from the Florida Reef Tract where habitats exposed to large highly-dynamic diel and seasonal fluctuations in pH experienced no reduced effect to elevated pCO_2 conditions expected under OA (Okazaki *et al.*, 2012). Prior exposure to elevated temperature has been shown to enhance resilience to anomalous stressors, e.g. though changes in *Symbiodinium* clade (e.g. temperature, Baker *et al*., 2004; Keshavmurthy *et al*., 2013). However, recent work in the Mediterranean highlights the need for prolonged exposure to unfavourable conditions for thermal adaption to potentially take place (Rodolfo-metalpa *et al.*, 2014).

Another important point to consider is that *A. palmata* from the LV outer-reef site was able to maintain its calcification rates under current conditions experienced in the seagrass habitat. This is unsurprising as the seagrass had a high mean pH, however, it does indicate that *A. palmata* can tolerate large fluctuations in pH including extreme lows (< 7.8). It also demonstrates the importance of other factors (e.g. fecundity, available substrate) in determining the ability of corals to expand their niches into non-reef habitats (Sanford & Kelly, 2011).

5.4.3| The role of variability on the corals metabolic response

There was very little difference in the mean coral metabolism between the HV and LV experimental treatments, independent of coral species. Interestingly however, the HV pH and pH and temperature treatments induced a response of denser coral calcification. The HV conditions resulted in periods of extended low pH (at night) and consequently low Ω_{arg}. In abiogenically grown aragonite, low Ω_{arg} has resulted in shorter and wider crystals compared to longer thinner crystals in elevated Ω_{arg} (Cohen & Holcomb, 2009). Similarly, aragonite crystals of corals raised under low pH conditions were observed to have disordered, tightly packed bundles, with short, wide crystals (Cohen & Holcomb, 2009; Holcomb & Cohen, 2009) which would result in a higher skeletal density similar to what we observed within the HV treatment. Although the HV habitat under current conditions still has a large diel variability and low pH at night, it seems plausible that the duration of low pH under current conditions is not significant enough to induce changes in crystal formation as experienced under the experimental change of -0.3 pH units.

An important consideration from this study is the influence daily pH and temperature oscillations have on the duration of time habitats spend under certain carbonate conditions, as this will ultimately shape whether habitats sustain calcification above dissolution thresholds on a net daily basis. For aragonite the saturation state is one; however, some experiments have documented dissolution occurring from 1.3 units (Yamamoto *et al*., 2012). For Mg-calcite, the saturation state ($\Omega_{Mg\text{-}calc}$) has been experimentally measured at 3.0-3.2 units with carbonate sediment dissolving from 3.7-3.8 units (Yamamoto *et al*., 2012). If we consider the results of this study, both habitats had very similar mean $\Omega_{arg,}$ however, their daily exposure to over-or-under saturated conditions was very different. During the daytime, CO_2 removed by photosynthesis resulted in an increase in pH (and increase in Ω) and conversely at night CO_2 from respiration led to a decrease in pH (and decrease in Ω) (Silverman *et al*., 2009). TA was controlled within

the experiment only drifting in accordance with calcification or dissolution over each 3 h incubation. Consequently diel cycles in saturation states occurred.

Under current ambient seawater conditions we see that neither habitat experienced conditions over a diel-cycle close to the Ω_{arg}. In the HV habitat there were periods of time at night when the G-D for carbonate sediments was low enough that dissolution could supersede calcification. The result of this is a potential offsetting of changes in low pH because it increases the seawater buffering ability by reducing the depletion of TA (Anthony *et al.,* 2011b; Andersson *et al.,* 2013). For example, studies that have considered the buffering role of dissolution have shown a 9 % and 11 % unit offset of pH and Ω_{arg} respectively (Andersson *et al.,* 2013). However in this study there were periods of time when the habitat saturation state was lower than the $\Omega_{Mg\text{-}calc}$ which meant dissolution could occur for species utilising the more soluble polymorph of $CaCO_3$, e.g. foraminifera and coralline algae (Yamamoto *et al*., 2012).

Looking forward to 2100 however, a different story emerges based on the experimental treatments conducted. Under LV the whole system spent time under the carbonate sediment Ω and $\Omega_{Mg\text{-}calc,}$ but remained above the Ω_{arg}. For the HV habitat, some time was spent above the $\Omega_{Mg\text{-}calc}$; thus allowing net calcification to supersedes dissolution. However, during the nighttime period, respiration increased the ambient pCO_2 and created conditions that are in the range where aragonite dissolution has been documented to exceeding rates of calcification (Yamamoto *et al*., 2012). From these results it becomes apparent that habitats have a very different response to future OA based on the duration of time they spend at given carbonate chemistry conditions. Also, whether dissolution of carbonate sediment (Anthony *et al.,* 2011b; Andersson *et al.,* 2013) or enhanced daytime productivity of photoautrotrophs (Manzello *et al.,* 2012; Buapet *et al*.,

2013; Hendriks *et al.*, 2014) can off-set the influence of OA on inshore habitats remains to be seen.

5.4.4| The relative influence of temperature and pH on the metabolic activity of corals

Determining the relative influence of temperature and pH on coral metabolism has yielded a variety of experimental responses (SCBD, 2014). Within this study, reduced pH had a larger negative impact on photosynthesis and calcification rates than elevated temperature. An additive response was documented for pH and temperature stress on decreasing coral calcification. However, work on molluscs have documented a synergistic relationship on reducing calcification rates (Rodolfo-Metalpa *et al.,* 2011) a trend also observed on massive and branching coral species (Anthony *et al*., 2008). In some instances an antagonistic impact has been seen, with temperature off-setting some of the negative impacts of OA (Byrne & Przeslawski, 2013).

Low pH in this study resulted in a net decrease in calcification as observed across numerous laboratory and field studies (see Kroeker *et al*., 2010, 2013). Elevated temperature stimulated photosynthesis (as per Reynaud *et al.,* 2003); however, this elevation did not transpire into elevated calcification rates, with rates actually decreasing. Work by Anthony *et al.* (2008) found that at intermediate warming levels (equivalent to levels within this study) productivity increased for *Acropora sp*. but fell for *Porites sp.*, with both having an overall decrease in calcification rates. The cause of decreased calcification despite elevated productivity is not fully understood but it is plausible that the thermal windows that govern metabolic processes (e.g. inorganic carbon acquisition) are breached, resulting in a negative response (Anthony *et al*., 2008). As previously discussed, upregulation of the internal calcifying fluid is energetically

costly and thus, corals that are unable to maintain photosynthesis due perhaps to a narrower thermal tolerance window are likely to experience a decrease in calcification rates.

5.5| Key findings

5.5.1 Carbonate chemistry manipulation

- The method employed was able to recreate a temperature increase (2.2 ± 0.03 °C) and pH decrease of 0.3 units (± 0.02) superimposed on the habitats natural diel cycles.

5.5.2 Species metabolic responses to enhanced temperature and low pH

- Calcification rates fell for both *A. palmata* and *P. astreoides* with calcification rates closely coupled with net photosynthesis rates.
- For *A. palmata* OA induced bleaching (loss of zooxanthellae) corresponded with the loss in productivity; however, for *P. astreoides* the loss was associated with reduced efficiency of the zooxanthellae.

5.5.3 Coral's native habitat influence on their metabolic response

- Prior exposure to low pH and/or elevated temperature did not provide any adaption or acclimatisation for *P. astreoides* over the duration of this study.

5.5.4 The role of variability on the corals metabolic response

- Ambient variability did not influence the overall net effect on corals, with decreased calcification and productivity observed across experimental treatments.
- Oscillating conditions of habitats results in very different conditions experienced for the ambient corals which has the potential to influence species responses to future OA.
- Exposure to HV treatments resulted in denser corals, independent of species.

5.5.5 The relative influence of temperature and pH on the metabolic activity of corals

- pH had a greater negative influence on calcification than temperature.
- Temperature alone stimulated photosynthesis, but in combination with low pH reduced photosynthesis.
- Respiration remained unchanged across treatments.
- Elevated temperature and low pH had an additive response on reducing calcification.

Chapter 6| Discussion

The thesis set out to explore non-reef habitats that house corals and experienced variability in carbonate chemistry to: (i) understand the levels of variability corals are already exposed to within these systems (Chapters 3 & 4), (ii) understand which corals are found living in these non-reef habitats (Chapters 3 & 4), (iii) know the metabolic cost for coral species found living in non-reef habitats (Chapters 3 & 4), and (iv) understand whether corals living in non-reef habitats experience the same metabolic cost as corals from a more stable reef environment when subjected to pH and temperature stress predicted under future climate change (Chapter 5). Fundamental to addressing any of these thesis aims was initially establishing the appropriate sampling methods to capture accurate and precise carbonate chemistry and coral metabolic activity data (Chapter 2). Through addressing these aims, this chapter will explore the results from the thesis and assess whether the local chemistry of non-reef systems can act as a buffer against future ocean acidification (OA). The chapter will also consider the corals of non-reef habitats and what this means for the future of coral reefs. Finally the chapter will consider what upcoming OA research needs to cogitate.

6.1| Characterisation of non-reef habitats

Fundamental to research is obtaining accurate and precise data that allows characterisation of the system being assessed. Through work conducted in this thesis (see Chapter 2), a novel respirometery chamber: the "Flexi-Chamber" was created, which fills an existing knowledge gap by allowing *in situ* coral metabolic work to be conducted cost-effectively without the need for specimen removal. The testing and validation of the Flexi-Chamber compared to other respirometry vessels identified how variable chamber properties can be (e.g. light penetration).

Similarly, testing of the Flexi-Chamber identified the need for careful sensitivity analysis to determine the appropriate biomass-to-volume ratio and flushing time; a step often unreported and potentially unaccounted for in incubation studies (e.g. Okazaki *et al*., 2013). In the same way, few studies have provided appropriate testing and analysis of their vessels making it hard for the scientific community to interpret differences between experimental results (e.g. Levy *et al*., 2004; Anthony *et al.,* 2008; Comeau *et al.,* 2014). Within OA studies, experimental results have been highly variable, even for the same species, making it more important to have a full analysis conducted and reported of the experimental set-up.

In attempting to deconvolve the ability for non-reef systems to buffer OA the full suite of carbonate chemistry parameters needs to be known over both short- (i.e. diel) and long- (i.e. seasonal) term cycles. Whilst several studies have provided information on the latter by installing long-term instrumentation (e.g. the SeaFET, Price *et al*., 2012; Comeau *et al.,* 2014) and conducting regular (weekly or monthly) discrete water samples for TA (e.g. Manzello *et al.,* 2012; Price *et al*., 2012; Yates *et al*., 2014), no known studies have considered the short-term diel variability of all carbonate chemistry parameters, rather than just one (e.g. pH or pCO_2). As demonstrated in Chapter 3, non-reef habitats can spend more than 5 % of their daily time at a pH below 7.9, with Chapter 5 highlighting the differences in daily Ω of non-reef habitats. Consequently on a daily basis, corals can experience periods when calcification is energetically favourable, or costly. Arguably, it is this proportion of daily time that will determine how corals respond to future environmental change and is dependent on the diel variability of the system, which can only be characterised by high-resolution sampling of more than one carbonate chemistry parameter as the Ω ultimately need to be determined. Although it is noted that 24 h water sampling schedules require a large effort (time and manpower), results from this thesis

demonstrate the value of the data obtained and highlight the importance of accurately determining the natural carbonate chemistry variability at high-resolution (see Chapters 2-5).

6.2| Seagrass and mangrove non-reef habitats

Arguments have been put forward that OA is only an open-ocean syndrome and cannot be transposed onto coastal ecosystems, due to the large fluctuations in pCO_2 they inherently experience as a result of metabolic, physiochemical and local hydrography conditions (e.g. Durate *et al*., 2013). Results from this thesis support the view that the impacts of OA on coastal ecosystems must be considered separately to those on the open-ocean. Non-reef habitats adjacent to coral reefs have highly dynamic carbonate chemistry conditions. From the abiotic variables measured within this thesis, differences in carbonate chemistry appear to be the dominant driver in structuring coral biomass and growth between the non-reef habitats examined (see Chapters 3 & 4). Corals were found persisting within non-reef habitats across bioregion sites despite the innate variability of these systems, as demonstrated in Chapters 3 and 4, and by Yates *et al*., 2014. The heterogeneity of non-reef habitats relative to the outer-reef created an inshore-to-offshore gradient (see Chapters 3, 4 & Manzello *et al*., 2012). The magnitude of this gradient appears to be seasonal, influenced by the rainfall, temperature, light and salinity differences between wet and dry seasons (see Chapter 3 & Manzello *et al*., 2012; Hendriks *et al*., 2014). The direction of this gradient, i.e. whether pH is elevated or lowered relative to the open-ocean appears to be dependent on the habitat type.

The ability of seagrass habitats to elevate local seawater mean pH from the ambient background control has been well documented across regional locations (e.g. Schmalz and Swanson, 1969; Invers *et al.*, 1997; Semesi *et al.*, 2009a; Hofmann *et al.*, 2010; Manzello *et al.*,

2012; Hendriks *et al*., 2014), supporting the hypothesis that seagrass habitats play a buffering role for resident corals (Hofmann *et al.,* 2011; Manzello *et al.,* 2012; Hendriks *et al.,* 2014). A model formed from records of seagrass metabolism in the Indo-Pacific estimated that seagrass metabolism could enhance coral calcification downstream by 18 % (Unsworth *et al*., 2012). However, none of these prior studies have actually explored the metabolic activity of resident coral species in non-reef habitats. It is essential to know the metabolic cost for corals to persist in these environments, in order to ascertain if they can provide a buffering service for resident coral species that results in maintained and/or enhanced calcification. For example, if seagrass habitats do elevate local seawater pH, but other ambient conditions make it difficult for coral calcification (e.g. light, nutrient availability, temperature), any local buffering benefits would be dampened or restricted to downstream environments where other conditions maybe more favourable. However, downstream buffering has a greater risk of being lost due to the dilution by ocean currents; consequently it is important to study resident corals of non-reef habitats, which to-date have largely been understudied.

Within this thesis, seagrass habitats (and the back-reef habitat) experienced an overall elevation in mean pH relative to the adjacent outer-reef, but with periods of high and low pH, which resulted in some native corals sustaining calcification and some experiencing a reduction relative to the outer-reef controls (17.0 ± 6.1 %, see Chapters 3 & 4). Previous studies have demonstrated that under oscillating CO_2 rather than continuously elevated CO_2 both coral recruits (Dufault *et al*., 2012) and adult corals (*Acropora hyacinthus*) had elevated growth rates (Comeau *et al.,* 2014), demonstrating the importance of variability in influencing coral calcification. Biologically-mediated elevation of mean pH and consequently Ω_{arg} recorded in this thesis supports the potential of seagrass habitats to act as refugia from OA (Semesi *et al.,* 2009a,

b; Kleypas *et al*., 2011; Anthony *et al.*, 2011a; Manzello *et al.*, 2012). The low pH (around 7.8 pH units) for seagrass habitats at night resulted in chemical conditions where carbonate-sediment dissolution could occur (Yamamoto *et al*., 2012). The dissolution of carbonate-sediment that was found to be abundant in the seagrass habitat increases local TA and pH creating a self-buffered system (see Chapter 3, Andersson *et al.*, 2013).

In contrast, mangrove habitats were found to lower local mean pH and Ω_{arg} relative to the outer-reef, suggesting that they are unlikely to buffer the impacts of OA. Supporting this conclusion is the metabolic impairment of resident corals that resulted in large decreases in calcification rates relative to the outer-reef (70.0 ± 7.3 %, see Chapter 4). More suitable descriptions of the services they are providing include: (i) pre-conditioning of local corals to future seawater conditions and/or, (ii) naturally selecting for corals that can tolerate low pH. In both cases mangrove systems seem likely to support an important genetic store of tolerant corals. The role of mangrove habitats in pre-conditioning corals to a low pH environment expands on other ecological services they may provide as put forward by Yates *et al*. (2014) through elevating downstream TA as a result of carbonate-sediment dissolution (i.e. buffering potential similar to seagrass habitats). Within the mangrove systems studied in this thesis, the environmental conditions that would drive carbonate-dissolution and consequently elevate TA downstream were relatively rare (< 3 hours per day), suggesting that pre-conditioning corals to low pH, or natural selection of tolerant corals to future OA, offers a more appropriate description of the services mangroves can provide.

Fundamental to the services described for non-reef habitats is the heterogeneity in their physiochemical environment which ensures their conditions remain out of balance with the open-ocean. Within this thesis, the variability in carbonate chemistry conditions of seagrass habitats

appears to be tightly coupled with the local cover of photoautotrophs (see Chapter 3 & 4). Other studies have concluded similar findings through *in situ* experimentation (Manzello *et al.,* 2012; Shaw *et al*., 2013; Hendriks *et al.,* 2014), modelling (Unsworth *et al.,* 2012) and laboratory analysis (Semesi *et al*., 2009b; Anthony *et al*., 2011b). It is important to note however, that it is still debated within the scientific community whether top-down (i.e. photoautotrophs adjust the local carbonate chemistry) or bottom-up (i.e. chemical-physio differences promote photoautotroph communities which in turn influence the local conditions) drivers result in the heterogeneity of non-reef habitats. Results from this thesis suggest that seagrass habitats are under more internal control (top-down) than mangrove habitats, however, whether this balance shifts with changing external forces remains to be seen. Either way the large cover of photoautotrophs, such as seagrasses, utilise CO_2 in photosynthesis during daylight hours, removing CO_2 from seawater and consequently elevating pH and Ω_{arg} (Anthony *et al*., 2011b; Buapet *et al*., 2013). Peaks of elevated pH corresponded with the time of day and average PAR further supporting the hypothesis that local phototrophic activity is the primary influence on seawater carbonate chemistry of seagrass habitats during daylight hours (see Chapter 3). At night, respiration draws down the local seawater pH in the absence of photosynthesis (Hendriks *et al.,* 2014). The nightly draw-down of pH helps to create the characteristic diel swings in carbonate chemistry experienced in the seagrass habitats. TA in seagrass habitats was generally lower than the open-ocean demonstrating that calcification is occurring, however, the high cover of non-calcifying organisms resulted in seagrass habitats dominated by photosynthesis (CO_2 uptake) and respiration (CO_2 release) as demonstrated in all nA_T-nC_T plots (see Chater 3 & 4 and Manzello *et al.,* 2012; Hendriks *et al.,* 2014; Yates *et al*., 2014).

Mangrove habitats within this study had carbonate chemistry conditions in part influenced by the local benthic composition, but they also appeared to be largely affected by other biological processes such as decomposition (Lugo, 1974; Lovelock & Ellison 2007; Bouillion *et al*., 2008; Kristensen *et al.,* 2008). The mangrove habitats demonstrated a similar daily trend in pH as observed in seagrass habitats (i.e. a relative elevation in pH during daylight hours with a reduction at night, see Chapter 4), however, the magnitude of this variability was greatly reduced. The reduction in variability can be accounted for by the reduction in benthic photoautotrophs (of 80.5 %). However, the large overall decrease in mean pH of mangrove habitats is still unaccounted for. It seems likely that a combination of: (i) microbial respiration processes (Kristensen *et al.,* 2008; de Souza Rezende *et al*., 2013), (ii) mineralisation of organic matter (Hyde & Lee, 1997; Bouillion *et al*., 2008), and (iii) mangrove respiration which is dominant in the root network (Lovelock *et al*., 2006; Huxham *et al*., 2010), drive down local mean pH by the release of CO_2 into the water column (Shafer & Roberts, 2007). In addition, mangrove soils contain prokaryotes that conduct anaerobic respiration within the anoxic sediment. In some cases, mangrove soils that are high in pyrite can become oxidised, creating iron oxide and sulfate. The sulfate can react with water to produce sulphuric acid (Kristensen *et al.,* 2008). Collectively these processes can influence the local pH and DIC. Mangroves have long been reported to impact heavily upon the local carbon balance of tropical coastal ecosystems (Borges *et al*., 2005); however, their exact contribution is still debated due to difficulties in tracing carbon within this system (Bouillion *et al*., 2008).

It remains unclear how changes in climate will influence coastal ecosystems due to the multitude of drivers and their complex interactions that exists (Durate *et al.,* 2013). For example, relationships between species cannot easily be predicted or understood where they act

predominantly in a non-additive manor, due to synergistic or antagonistic relationships that can vary between response level (e.g. community versus population), or trophic guild (e.g. autotrophs versus heterotrophs) (Crain *et al.*, 2008). Fundamental to the climate service previsions described for seagrass and mangrove habitats is the maintenance of heterogeneity and non-equilibrium with the open-ocean. Although it is hard to predict how these complex ecosystems will respond, evaluation of current literature can provide some insight into the possible effects of climate change on seagrass and mangrove habitats and their connected ecosystems.

Seagrass habitats are currently under threat from coastal degradation (Durate *et al*., 2002; Boudouresque *et al.*, 2009), with seagrass habitats being lost worldwide (Waycott *et al*., 2009). Shoot densities that are fundamental to the productivity of seagrasses have also been reported to be decreasing in the Mediterranean (Marbà & Duarte, 2010). Increased storm activity predicted under future climate change may increase destruction of coastal systems (Short & Neckles, 1999). Any degradation of seagrass habitats reduces their ability to buffer marine calcifiers because the modification of seawater pH is directly linked to the abundance of seagrass habitats (Hendriks *et al*., 2014).

Changes in DIC are also predicted to directly influence marine plants (Short & Neckles 1999) due to the increased availability of CO_2 and HCO_3^- that can be utilised for photosynthesis (Koch, 1994; Beer & Koch, 1996; Buapet *et al*., 2013). Seagrasses are carbon limited (Hendriks *et al*., 2014), and therefore the increase in CO_2 could enhance photosynthetic rates (Durako, 1993) and increase the growth and biomass of seagrass beds (Zimmerman *et al.*, 1997). Studies that have explored naturally high CO_2 locations have provided compelling evidence that seagrasses will flourish (Hall-Spencer *et al.*, 2008; Fabricius *et al*., 2011; Durate *et al.*, 2013),

sustaining their ability to modulate pH (Durate *et al.*, 2013). Associations with other climate stressors however, could reduce photosynthetic rates despite the increased abundance of DIC. For example, elevated temperature has been documented to adversely affect the photosynthesis-to-respiration ratio of the seagrass species *Zostera marina L* (Marsh *et al.*, 1986). Increasing levels of UVB can also damage plant tissue (Short & Neckles, 1999). However, again the ability to predict habitat responses to environmental change are complicated as sea-level rise will result in deeper water, reducing the risk of UVB damage but also reducing the light availability needed for photosynthesis (Short & Neckles, 1999). It becomes apparent that the multitude of factors interacting to influence seagrass ecosystems make it hard to predict how they will ultimately respond to future environmental change (Hendriks *et al*., 2014). However, at the habitat level it seems likely that seagrasses will flourish given the increased availability of CO_2. It will then depend on the preservation of these habitats, and the availability of shallow inshore land for them to recruit to as sea-level changes, to ensure their ecosystem function and services.

Mangrove habitats are threatened by similar stressors that affect seagrass habitats including: habitat destruction (Feller *et al.,* 2010), physical disturbance (Valiela *et al.*, 2001), protein damage from elevated UVB levels (Lovelock & Ellison, 2007) and adverse effects on photosynthesis rates as species temperature thresholds are exceeded (Lovelock & Ellison, 2007). Temperature increases associated with climate change will also increase respiration rates of both mangroves and associated microbial communities. The predicted average increase in temperature of 2 ºC over the next century is estimated to increase plant and soil respiration by *ca*. 20 %, shifting photosynthesis-to-respiration ratios and influencing local DIC levels (Lovelock & Ellison, 2007). Importantly, temperature changes are predicted to be different across bioregions, with areas of the western pacific predicted to experience temperature increases up to 4 °C under

business-as-usual IPCC scenarios (IPCC, 2015). Consequently, regional differences will likely occur, whereby the stressors discussed will be enhanced within these hotter locations and non-reef habitats may have to alter their geographic distribution to survive.

Mangrove distribution could be impacted because they have water-dispersed propagules that may be influenced by changes in ocean currents (Lovelock & Ellison, 2007). The ability of mangroves to alter their geographic dispersal could be beneficial if it allows them to establish in more favourable environments, e.g. cooler regional locations. Mangrove habitats are also under threat from rising sea level because they may not be able to maintain their peat production enough to keep their leaves above water (Feller *et al*., 2010; Huxham *et al*., 2010). Although mangroves could potentially shift landwards, increasing urbanisation of coastal areas is limiting this option for many mangrove systems. Enzyme activity of mangrove microbial communities could also be affected by changes in pH, with Yamada and Suzumura (2010) reporting a decrease in the activity of some hydrolytic enzymes important in organic matter degradation. Reduced enzyme efficiency will influence carbon cycling and the control of local DIC levels. Like seagrass beds, mangroves may experience enhanced rates of photosynthesis under elevated CO_2 levels. However, the complexity of bio-geophysical conditions of mangrove habitats, combined with the lack of long-term studies investigating stressors on mangrove systems, make it hard to determine how this non-reef habitat will respond to future climate change. It seems plausible that mangrove systems will maintain their services as long as they have the capacity to shift their distribution in accordance with rising sea-levels and elevating temperatures. As with seagrasses, mangrove habitats need to be protected via regional management to help maintain their ecosystem services (Lovelock & Ellison, 2007; Feller *et al*., 2010).

An important consideration of non-reef habitats and coral reefs is that they are inter-connected systems with each other. Consequently, they should not be considered in isolation when evaluating their ability to sustain their ecosystem function and services under future climate change. Several studies have explored the importance of inter-connectivity of habitats across the whole reef continuum for fish ontogenetic shifts, e.g. from mangroves, to seagrasses and then to the main reef (e.g. Sheaves, 2005; Dorenbosch *et al.,* 2007; Unsworth *et al*., 2008). However, the connectivity of all of these habitats in terms of carbonate chemistry has largely been unexplored. Seagrasses have been proposed to buffer downstream coral reef systems (e.g. Anthony *et al.*, 2013); a clear example of their inter-connected relationship. However, how mangrove and seagrass habitats interact has received minimal-to-no attention. Seagrass habitats are typically found on the seaward side of mangroves, so it is possible that the low pH of mangrove habitats (and elevated pCO_2 and H^+) can actually enhance the productivity of seagrass habitats by stimulating photosynthesis. In turn, this may increase the buffering effect seagrasses can provide to resident corals and downstream coral reefs. Coral reefs are dominated by calcification which results in the by-product of CO_2 which may also feedback onto local seagrass habitats, stimulating photosynthesis during daylight hours. It is also possible that dead seagrass can wash inshore during incoming tides (Bouillion *et al.,* 2008), providing a source or organic matter for decomposition within the mangroves, which in turn facilitates the cycle of lowering local seawater pH within mangrove habitats. The described interactions are dependent on tidal cycles, residency time and time-of-day. Although untested it seems highly logical that inter-connected biogeochemical services exist in terms of carbonate chemistry across a reef continuum and is an area for further research (Figure 6.1).

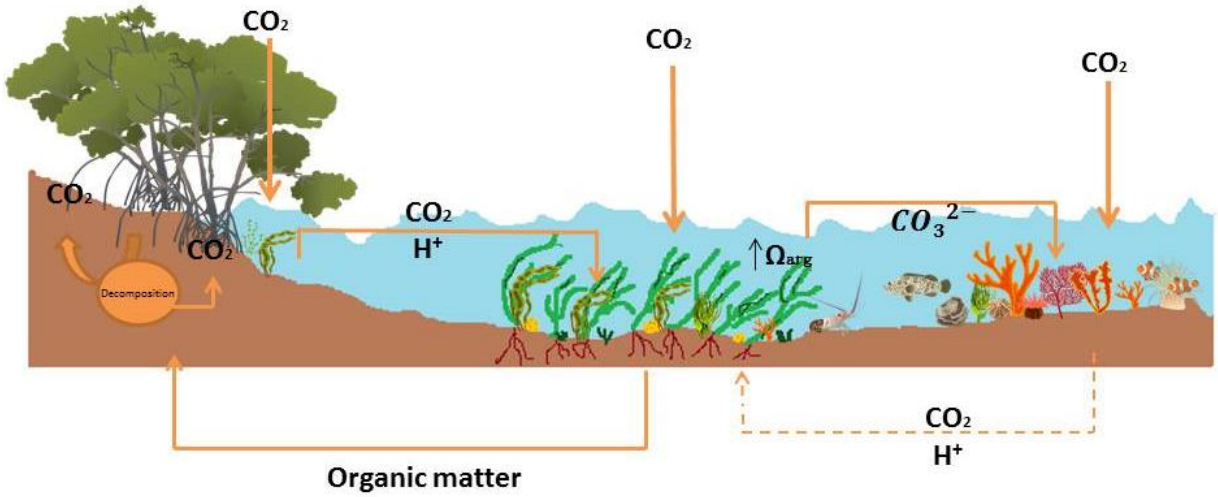


Figure 6.1| A simple example of how mangrove, seagrass and coral reef carbonate chemistry maybe connected. Mangrove habitats could supply CO_2 and H^+ to seagrass habitats, which can utilise this additional CO_2 during photosynthesis to raise local pH, $[CO_3^{2-}]$and aragonite saturation (Ω_{arg}) downstream on coral reefs. Coral reefs may provide CO_2 and H^+ back to the seagrass as a by-product of calcification: this process is indicated as a dashed line as there is currently no evidence for this, indicating the need for further research. Dead seagrass materials can wash inshore and supply organic matter to mangroves where decomposition is prevalent, releasing CO_2 and contributing to the characteristic low pH of the mangrove system. Local mangrove organic material can also be used in decomposition. CO_2 is also being supplied to all habitats from the atmosphere.

6.3| Corals of non-reef habitats

Results from this thesis significantly expand upon recent localised reports that a relatively large range of coral species can survive in highly variable non-reef habitats (see Chapter 3, 4 and Yates *et al*., 2014). The ability for corals to survive within these habitats demonstrates that they are either buffered (e.g. in seagrass habitats) or adapted and/or acclimatised to low pH conditions (e.g. in mangrove habitats). It is also possible that non-reef habitats naturally select for coral species that are able to tolerate their conditions. For example, the corals found in mangrove habitats may be the species that have an innate physiological plasticity to survive low pH

conditions due to their genotype. Consequently, investigating the coral populations found in marginal non-reef habitats, that already experience conditions predicted under future climate change, may provide evidence of what future coral communities will look like.

The non-reef habitats investigated in this thesis contained a range of coral species that were not restricted to encrusting or massive forms (Fabricius *et al.,* 2011; Yates *et al*., 2014) but also included architecturally complex genera (e.g. *Acropora* and *Pocillipora*, see Chapter 3 & 4). The corals documented in non-reef habitats had different life-history strategies, for example, corals fell into three of the four life history categories established by Darling *et al.* (2012) (Competitive, Weedy and Stress-tolerant). Differences in life history strategies may influence the abilities for coral species to survive (Sheppard *et al.,* 2010). Interestingly, about one third of corals in both the seagrass and mangrove habitats fell into the competitive life-history category of Darling *et al*. (2012), which would suggest that they are only dominant in ideal communities. Work from this thesis expands our knowledge on what conditions corals can persist under, and demonstrates that generalised classification of species tolerance cannot always predict the ability of corals to expand into highly-variable, often sub-optimal non-reef habitats.

Persistence of corals in the non-reef habitats demonstrates that either: (i) these corals have a larger fundamental niche than initially accounted for or, (ii) these coral species have expanded their fundamental niche into less optimal environments, compared to the realised niche of most corals that are determined due to biological pressures (e.g. competition). Quantifying the accurate scale of coral species fundamental niches is important in determining changes in species distribution and the potential threats they may face under changing environmental conditions (Wright *et al*., 2006). The magnitude of climate change threats will be unequal around the world, with sea surface temperature elevating most around the equator, whilst OA impacts will be

predominant around the poles (Meissner *et al*., 2012). Consequently it remains unclear how the geographic distribution of coral populations will be affected, because these two climate stressors are acting antagonistically on geographic dispersion (Meissner *et al*., 2012; Yara *et al*., 2012). Corals that have a larger fundamental niche may therefore have a greater chance of surviving changes in environmental conditions as they can survive across a broader range of habitats, thus reducing the risk of extinction if one habitat becomes uninhabitable under climate change.

Within this thesis the total number of coral species recorded in non-reef habitats was similar across regions, but these total values represented very different proportions of the overall numbers of coral species found within each bioregion location (see Chapter 4). Corals found in the non-reef habitats of the Indian and Pacific Ocean sites only represented 1-2 % of coral species found in the Indo-Pacific (7- 14 species of over 700). Conversely, corals in the Atlantic Ocean non-reef habitat sites represented 20-30 % of the total number of coral species currently documented in the Atlantic region (8-15 of up to 40 species, see Chapter 4). Differences between bioregions is an important observation as it raises questions over the ability of coral reefs in the Indo-Pacific to sustain their characteristic of high-biodiversity over times of wide-spread environmental change. Conversely, for the Atlantic region it is promising that a wide range of coral species were found in the non-reef habitats, as there is less functional redundancy in this low-biodiversity region. Consequently, any missing species could represent a whole functional group (Bellwood *et al*., 2004). For example, *Acropora palmata* is the only tall tabular coral in the Caribbean so its absence from a habitat would mean that a whole functional group was not represented. Whether the high proportion of total species of the Atlantic Ocean site that are found within non-reef habitats reflects the bioregions overall reduced species pool, past environmental histories, present-day ecological and/or environmental pressures, or is a feature of

regionally-specific evolutionary relationships remains unclear. Understanding which mechanisms are responsible for the regional differences is important as it will inform whether adaptive or acclamatory forces are responsible for corals to persist in these non-reef habitats, and ultimately, what time-scales are necessary for these mechanisms to come into effect, which in turn influences potential management strategies.

Importantly, the absence of a coral species from a non-reef habitat could be indicative of their tolerance to the local carbonate chemistry conditions, however, it is also likely to be the result of other biotic or abiotic conditions, e.g. fecundity or recruitment ability (Sanford & Kelly, 2011). Complex interactions between abiotic factors and the stage of a corals life-cycle may also be responsible, for example, studies have documented increased sensitivity of coral recruits and early life stages to low pH (Morita *et al*., 2009; Albright *et al*., 2010; Albright & Langdon, 2011). In this thesis, *A. palmata* was not found in the Atlantic Ocean seagrass habitat site and was also not found to settle as new recruits into this habitat (see Chapter 3). However, under experimental conditions, adult *A. palmata* was found to maintain productivity and calcification rates under carbonate chemistry and temperature conditions currently experienced in the seagrass habitat (see Chapter 5). Consequently, we see the complexities that exist in evaluating why certain corals are not located in non-reef habitats, as it is likely for *A. palmata* that supply side ecology limited recruitment (Sanford & Kelly, 2011), however it is also possible that the highly variable pH was unfavourable for recruitment (Albright & Langdon, 2011). Ultimately, if a species is absence from a non-reef system then it cannot experience the potential climatic services of that habitat. It is possible however, that coral species could be transplanted and raised within non-reef habitats, e.g. mangroves, if regular exposure to low pH does indeed result in adaption to these conditions.

6.3.1| Adaption and/or acclimatisation of corals

To-date few OA studies have investigated the ability of species to acclimatise and/or adapt to low pH environments (see however, Sunday *et al*., 2011; Lohbeck *et al.,* 2012; Foo *et al*., 2012). It has generally been thought that species have little chance of adapting to low pH as few natural gradients were thought to exist (Kelly & Hofmann, 2012). However, as demonstrated in this thesis, natural pH gradients exist at sites across bioregions where daily pH variations already exceeds mean global levels predicted over the next 60 years. Investigating these natural gradients to test for adaption and acclimation is a research priority and a clear area for progressive work from this thesis.

Initial exploration of phylogenetic trees revealed that the coral species present in non-reef habitats of this study were taxonomically diverse, occurring in both of the two major mitochondrially derived coral clades: (i) "robust" corals that typically consist of solid, heavy calcified skeletons, and (ii) "complex" corals that are less heavily calcified (Romano & Palumbi, 1996; Kitahara *et al.,* 2010). It is believed that these two clades diverged from each other early in the history of the Scleractinian group (Romano & Cairns, 2000) and consequently corals in these two clades have been exposed to alike past evolutionary conditions. Therefore, acclimation and/or genetic adaptation to local environmental conditions over more recent time scales likely explain why some coral species are able to colonise and survive in non-reef habitats (Kelly & Hofmann, 2012). Paleoecology analysis of coral reefs around Papua New Guinea also suggested that local environmental conditions are most influential in shaping coral communities (Pandolfi, 1996). Further exploration is needed of coral phylogenetic trees to fully deconvolved the role of past evolutionary history on coral recruitment into non-reef habitats, however, it appears that

local environmental conditions are key in driving adaption. The low mean pH of mangrove habitats and the high variability of pH in the seagrass habitats consequently provide environmental conditions that may drive adaption in resident corals. Non-reef habitats have the potential to give their resident corals a "head-start" to adapting to future climate change conditions due to the low and variable pH they already experience.

Increasingly we understand that adaption can occur over relatively short time frames (Carroll *et al.*, 2007), with epigenetic changes potentially imprinting past environmental history into a species DNA that has the potential to be passed onto future generations, speeding up rates of adaption (Chinnusarry *et al*., 2009; Klironomos *et al*., 2012). Importantly, short-term phenotypic plasticity can also help coral populations persist in environments long enough for genetic adaption to occur (Munday *et al.*, 2013). Phenotypic plasticity has already been documented to allow corals to persist across environmental gradients (Bongaerts *et al.*, 2011). Acclimation to low pH has been documented for the coccolithophore algae *Emiliania huxleys* (Lohbeck *et al*., 2012) as well as the cold water coral *Lophelia pertusa* (Form & Riebesell, 2012). However, Okazaki *et al.* (2013) reports that stress tolerant corals of Florida Bay were equally sensitive to future OA, despite frequent exposure to pCO_2 and temperature variability. In this case it appears that a species may have a maximum acclamatory ability that is not influenced by its environmental history. Results from this study support the notion that corals may have a maximum acclamatory ability, with *P. astresoides* exposed to life-long pH variability showing no enhanced tolerance to extreme low pH (7.6-7.7, see Chapter 5). Further research is needed to explore the maximum acclamatory potential of corals.

For tropical coral species to acclimatise, both the host and *Symbiodinium* must utilise available resources (Hennige *et al*., 2010). Within this thesis, the non-destructive protocol of the

Flexi-Chamber meant that *Symbiodinium* analysis was not possible. Different clades of *Symbiodinium* have shown enhanced tolerance to temperature stress (e.g. Baker *et al*., 2004). Although this is another understudied area in OA research, a study on free-living cells have shown a large shift in their *Symbiodinium clades* to elevated CO_2 (Brading *et al*., 2011), thus suggesting that the clade of *Symbiodinium* will likely affect how corals respond to future OA. The high energetic demand of calcification under low pH means that corals will rely even more on the energy produced by their *Symbiodinium,* supporting the hypothesis that corals capable of upregulating or changing their *Symbiodinium* clade under environmental stress (e.g. Baker *et al.,* 2004 with temperature), will be better able to acclimatise and/or adapt to changes in ocean pH. The coral host its-self may also be able to assist with adaption (Kelly & Hofmann, 2012). For example, the host can regulate protective compounds (e.g. Baird *et al.*, 2009) which could shield photosynthetic organelles helping to maintain rates of photosynthesis which are known to be tightly coupled with rates of calcification. Clearly the ability for corals to acclimatise and/or adapt to low pH conditions is dependent on the holobiont (i.e. genetic content from both the host and microbial communities) and the rate of environmental change.

6.3.2| Coral physiological responses – Results from this thesis corroborate the growing consensus that species-specific responses exist for corals to changes in pH (see Chapter 4, Kroeker *et al.,* 2013). A large range of physiological responses have been documented for corals exposed to low and more variable pH (Ries *et al*., 2009), which can be explained by: (i) the ability of coral species to modify H^+ concentrations within the calicoblastic fluid (McCulloch *et al*., 2012; Jokiel *et al.*, 2013), (ii) the potential for species to utilise HCO_3^- as well as CO_3^{2-} for calcification (Comeau *et al.,* 2012), and (iii) the response of additional and multiple interactive

stressors interacting with the pH effect (e.g. pH & temperature, Anthony *et al*., 2008). Physiological differences measured both in the laboratory (Chapter 5) and field (Chapter 4), between massive *Porites* and branching *Acropora,* to changing pH suggest that a corals ability to sustain calcification is tightly coupled to their ability to maintain photosynthesis. Previous studies have also demonstrated a close coupling of calcification and photosynthesis (Gatusso *et al*., 1999; Langdon & Atkinson, 2005), although their exact relationship remains unresolved (Allemand *et al.*, 2011).

The Ca^{2+}-ATPase pump that supplies Ca^{2+} to the calicoblastic fluid and removed H^{+} (Al-Horani *et al*., 2003; McCulloch *et al*., 2012) has advanced our understanding of how the two processes may interact. During daylight, the combined activity of metabolic respiration and photosynthesis result in an active carbon cycle that produces ATP. Elevation of ATP during light enhances processes that require ATP, such as calcification (Al-Horani *et al*., 2003). Photosynthesis and calcification may also be related by the direct modification of internal DIC (Schneider & Erez, 2006), with photosynthesis removing CO_2 and consequently raising intracellular pH and Ω towards more favourable conditions for calcification (Allemand *et al*., 1998). Alternatively, calcification could stimulate photosynthesis by supplying CO_2 via the by-product of $CaCO_3$ formation, thus ensuring that CO_2 levels are not depleted for photosynthesis (McConnaughey *et al.*, 2000).

The coupled relationship between photosynthesis and calcification observed within this thesis demonstrates that future susceptibilities of corals to changes in pH will largely depend on their abilities to sustain photosynthesis. Consequently, stressors that influence photosynthesis will greatly impact a corals response to changes in pH. Temperature, as demonstrated in the laboratory study (see Chapter 5) impacts photosynthesis and calcification rates. Interestingly

however, under the elevated temperature treatment, the relationship between photosynthesis and calcification was uncoupled, with no increase in calcification documented despite the rise in temperature. These results are broadly consistent with the experimental work of Anthony *et al.* (2008) on *Acropora* and *Porites spp.* exposed to future IPCC IV and VI scenarios. Again, the exact, mechanism for the uncoupling of photosynthesis and calcification is not clear, however, it seems likely that the thermal windows that govern metabolic processes (e.g. inorganic carbon acquisition) are breached, resulting in a negative response (Anthony *et al*., 2008). Thermal tolerance windows are species-specific (Coles & Jokiel, 1978) and would explain why some studies have observed an increase in calcification with increased temperature (e.g. Reynaud *et al.*, 2003), whilst other have observed no change at all (e.g. Langdon & Atkinson, 2005). In addition, the *Symbiodinium* associated with corals can have different thermal tolerances (Keshavmurthy *et al*., 2013) that would affect the susceptibility of the coral to increasing temperature.

Other abiotic variables will undoubtedly interact to determine how corals will respond to low pH and ultimately meet their daily metabolic needs. Within this thesis, rates of respiration were found to remain relatively stable despite the reduction in photosynthesis. It is possible that heterotrophic energy acquisition was upregulated to maintain the basal energetic demands lost from photosynthesis. Grottoli *et al*. (2006) reported that some coral species were able to increase heterotrophy after a bleaching event to maintain the corals basal metabolic needs. Heterotrophy has also been documented to offset the negative impacts of high pCO_2 on *Porites spp*. over a one month period (Edmunds 2011), with biomass-normalised rates of calcification actually increasing. An increase in light availability has also been shown to enhance calcification (e.g. Marubini *et al*., 2001; Suggett e*t al.*, 2013). The multitude of variables that influence corals make

it is hard to predict how coral species will ultimately respond to future changes in seawater pH. However, results from this thesis, combined with recent literature (McCulloch *et al*., 2012) suggest that maintenance of calcification under lower pH is an energetically costly process. Consequently, coral species will have to redistribute energy supplies to account for the additional energy to calcify under low pH. Corals will therefore be unable to distribute the same energy resources to other important biological processes (Cohen & Holcomb, 2009). The difference between species to tolerate environmental change will affect their own survival but will also affect the overall structure of coral reefs.

6.4| The future of coral reef ecosystems

The different susceptibility of corals to environmental change will likely impact the structure and biodiversity of coral reefs. Within this thesis, branching species appeared to be the most vulnerable to changes in pH, a phenomenon also predicted by McCulloch *et al.* (2012). Branching corals form the 3D architecture of coral reefs, providing habitat complexity utilised by reef fish and invertebrates (Bellwood *et al.*, 2004; Sheppard *et al.*, 2010). A loss of reef complexity could have dire consequences for many reef organisms, threatening reef biodiversity, ecosystem functioning and service provision (Alvarez-Filip *et al.*, 2009). Reef structure could also be influenced by changes in growth form under lower pH. The laboratory study (see Chapter 5) found higher coral density under the highly variable, low pH treatments. If corals are able to maintain calcification at low pH the crystal structure could be altered to shorter, less organised, and denser formations (Cohen & Holcomb, 2009), potentially resulting in smaller corals which would again alter the composition of coral reefs.

Changes in reef calcification under low pH are accompanied by the potential increase in the efficiency of marine plants to photosynthesise. The increased efficiency of marine plants can be beneficial in helping to regulate and buffer the local chemical conditions (Manzello *et al*., 2012; Hendriks *et al.*, 2014). However, there is also the risk of a phase-shift occurring from a coral-dominated to an algal-dominated reef system (Hughes, 1994; Pandolfi *et al*., 2005). Corals generally have slow growth rates and under lower pH it is likely that growth rates will fall even more (De'arth *et al*., 2009). Conversely, marine plants have relatively fast growth which could be enhanced under increased photosynthetic activity from elevated CO_2 levels (Zimmerman *et al.*, 1997). Similarly, other reef organisms may thrive, e.g. non-calcifying anthozoan (Suggett *et al.*, 2012), macro algae (Fabricious *et al*., 2011) and sponges (Be*ll et al*., 2013) which corals will have to compete against for space and other resources. Competitive interactions between species and taxa under OA conditions will be central in determining the composition of future reefs.

Successful survival of corals on the main reef, as well as in non-reef habitats will depend on their colonisation ability, both in terms of growth and recruitment (Hennige *et al.*, 2010). Currently, it is unclear whether the corals found in the non-reef habitats of this study are self-recruiting. All colonies were typically small (see Chapter 3 & 4), however, due to their altered growth within these habitats it cannot be assumed that they are not reproductively active (see Chapter 5). Further population genetics is necessary to determine the source of corals within non-reef habitats because this will ultimately dictate the climate services they can provide. If corals in the non-reef habitats originate from the main reef, then the buffering and/or pre-conditioning services they can provide are dependent on coral populations being sustained on the main reef. Conversely, if non-reef habitats are self-recruiting, then under changing environmental conditions they may be able to populate surrounding reef environments,

potentially with more resistant coral species that have adapted to tolerate a greater range of environmental conditions. Importantly, under OA calcification will become more energetically costly (Cohen & Holcomb, 2009), which will likely reduce the energy available for other important processes such as reproduction. Consequently, maintaining viable coral populations is antagonised by the challenge of meeting the additional energetic costs of maintaining basic physiological processes under OA.

Another important consideration for the future of coral reefs is how the abiotic structure of the reef will respond to changing pH. This thesis has focused on the ability of corals to sustain calcification; however, corals accrete their framework onto the dead skeletons of old coral colonies. Consequently, the integrity of the foundations of the reef may be compromised under low pH as there is no live tissue attempting to maintain the structure. Similarly, reef bioeroders appear to flourish under OA conditions (e.g. Fabricious *et al.*, 2011), worsening the threat of erosion. Reefs therefore could be at risk from a loss of physical stature (Kroeker *et al.*, 2013).

Results from this thesis significantly expands on the growing evidence that at low mean pH, as experienced in mangrove habitats, coral calcification is suppressed. Crook *et al.* (2013) demonstrated a 40 % reduction in calcification of *Porites astreoides* exposed to life-time low pH. If low pH conditions became the norm within classical reef settings, the functional role of reef building corals as the ecosystem architects that support system biodiversity and productivity is greatly threatened (Dove *et al.*, 2013). OA has the potential to impact all ecosystem services, including: (i) supporting services (e.g. processes that serve all other ecosystem provisions) such as primary production and nutrient cycling, (ii) provisioning services such as raw materials and food sources, (iii) regulating services, e.g. climate regulation, and (iv) cultural services, such as

education and recreation (SCBD, 2014). Importantly however, coral reefs are inter-connected systems that include habitats that have the potential to provide local climate regulation.

Results from this thesis highlight that non-reef habitats have the potential to provide important local climate management for coral reef ecosystems. The proximity of non-reef habitats in relation to coral reefs may either: (i) buffer corals, or (ii) provide a source of genetic tolerant corals that can help sustain reef populations. As demonstrated throughout the discussion, there are several areas that remain understudied which need to be addressed to fundamentally determine how these non-reef habitats can best be utilised to help manage the negative effects of future climate change. Only then will we be able to address whether strategies such as coral non-reef nurseries, targeted development of non-reef habitats by coral reefs, or out planting of mangrove corals onto the main reef, are viable management options. A major finding of this thesis is that a range of service provisions are provided by seagrass and mangrove non-reef habitats that have previously received little-to-no attention. The importance of non-reef habitats in carbon cycling, local climate management options, and as important natural laboratories for climate change research, significantly enhances their conservation value.

6.5| Considerations for ocean acidification research

As explored in this chapter, there are still significant knowledge gaps within OA research. A fundamental finding of this thesis is that scientists need to study the coral populations of non-reef habitats that have typically been under-explored by coral reef scientists as they are considered sub-optimal for coral growth (see however, Hennige *et al*., 2010; Yates *et al.,* 2014). However, as marine habitats are exposed to rapid changes in environmental conditions, it will arguable be these non-reef habitats that are better able to deal with change. The natural pH gradients non-reef

habitats provide, demonstrate that there are systems where corals are exposed to conditions that may allow adaption and/or acclimation to occur. Studies therefore need to consider the levels of natural pH variability corals are exposed to and how this compares to the scale of gene flow and a species life-span (Kelly & Hofmann, 2012). Future research needs to explore the capacity for corals in non-reef habitats to adapt and/or acclimatise by methods such as: (i) exploration of phylogenetic trees, (ii) laboratory quantification of standing genetics through breeding or selection experimentations, (iii) reciprocal transplant experiments, (iii) exploration of transcriptomic data, and (iv) quantitative genetics. The inter-connectivity of non-reef habitats based on their carbonate chemistry also needs further examination. We also need to determine the population genetics of non-reef habitats to determine if these habitats are self-recruiting. Further global analysis of non-reef habitats is necessary to determine the fundamental niches of coral species. The coral holobiont also needs to be further explored, as does the response of *Symbiodinium* to elevated CO_2.

Results from this thesis also demonstrate the importance of resolute method development, highlighting the need for future OA research to fully explore and report experimental set-ups, particularly for coral physiological studies. Findings from this thesis also demonstrate the importance of variability in carbonate chemistry conditions of habitats. Consideration of the amount of time habitats spend at specific pH conditions is imperative in determining how they will likely respond to future OA. Both the mean and variability in pH conditions are important in determining coral physiology, and future OA studies need to account for habitat variability. Finally, further work is needed to explore how acidification interacts with other environmental stressors and consequently how coral populations may shift their geographic location in the future.

6.6| Conclusions

Coral reefs are at threat from climate change, however, reefs are not isolated systems, they are interconnected with highly-dynamic non-reef habitats that may buffer against low pH or provide a source of pre-conditioned corals that are able to sustain growth under low pH conditions (see Chapters 3, 4 & 5). A range of coral species are found living in non-reef habitats that are already exposed to low and variable pH predicted under future climate change, demonstrating some level of tolerance in corals to low pH (see Chapter 3 & 4). The ability of non-reef habitats to potentially drive acclimatisation or promote adaption to suboptimal temperature and pH remains unclear, as does the maximum acclamatory potential of coral species (see Chapter 5). The environmental heterogeneity of both seagrass and mangrove systems is essential in maintaining different biogeochemical conditions that underpin the ecosystem services described. Appropriate experimental design is fundamental to encapsulating the environmental variability of a habitat, with both mean and variability in local conditions influencing the physiological response of local corals (see Chapters 2 & 3). Non-reef habitats have been under-studied by coral scientists and greater effort is needed to study coral populations in these systems. Data from this thesis contribute significantly to the efforts identifying options to manage or mitigate against the possible impacts of climate change stressors on one of the world's most important ecosystems (Salm *et al.*, 2006; Yates *et al*., 2014).

Appendices

Appendix 1| Procedure for standardising hydrochloric acid for alkalinity titration

To standardise hydrochloric acid the following procedure was used:

A. The standard solution of sodium carbonate was prepared

1. Dry 1.0g sodium carbonate (AR) in an oven 80 °C for 18 h
2. Cool in a desiccator, weigh out 0.8686 g sodium carbonate; add to a 1 L columetric flask, and dilute with deionised water (DIW) to the 1 L mark.

$$NA_2CO_3: \frac{0.8686}{(\frac{105.59}{2})} = 0.01639\ N \qquad [S1]$$

B. The Hydrochloric acid solution was prepared

1. Add 900 ml DIW to a 1 L flask, and then add 1.7 ml concentrated HCL (32 %) to the flask, and mix thoroughly: and then dilute with DIW to the 1 L mark.
2. Dilute HCL: (1.7 ml x 10.18 N)/1000 ml = 0.0173 N

C. The sodium carbonate solution was titrated with diluted HCL

1. Add 25 ml of sodium carbonate into a 1000 ml beaker,
2. Titrate with the diluted HCL solution (Using the Titrino titrator, Buckingham, UK)
3. Record the volume of acid (ml) taken to reach the second equivalence point
4. Calculate the accurate concentration if the diluted HCL:

Standardised Acid Concentration = (0.01639 N x 25 ml)/ Volume of HCL used (ml)

Appendix 2| The time taken to stabilise oxygen levels to respiration levels for *Siderastrea cf. stellata*

For five colonies of *Siderastrea cf. stellata.* the time taken for oxygen levels to drop to respiration rates was determined. A black opaque bag was covered over the respirometry chambers and the time taken for oxygen levels to level off before depletion was measured at five min increments. Oxygen was measured by drawing out 30 ml water samples and testing the oxygen levels using an oxygen probe (RDO probe (ORION RDO, model 087010MD; Fisher Scientific, USA). Tests were conducted in *ca.* 3 m of water in Salvador Brazil and all colonies were a similar size (surface area *ca.* 12 cm^2). Results showed that it took between *ca.* 30-70 min for oxygen levels to stabilise (Figure S.1).

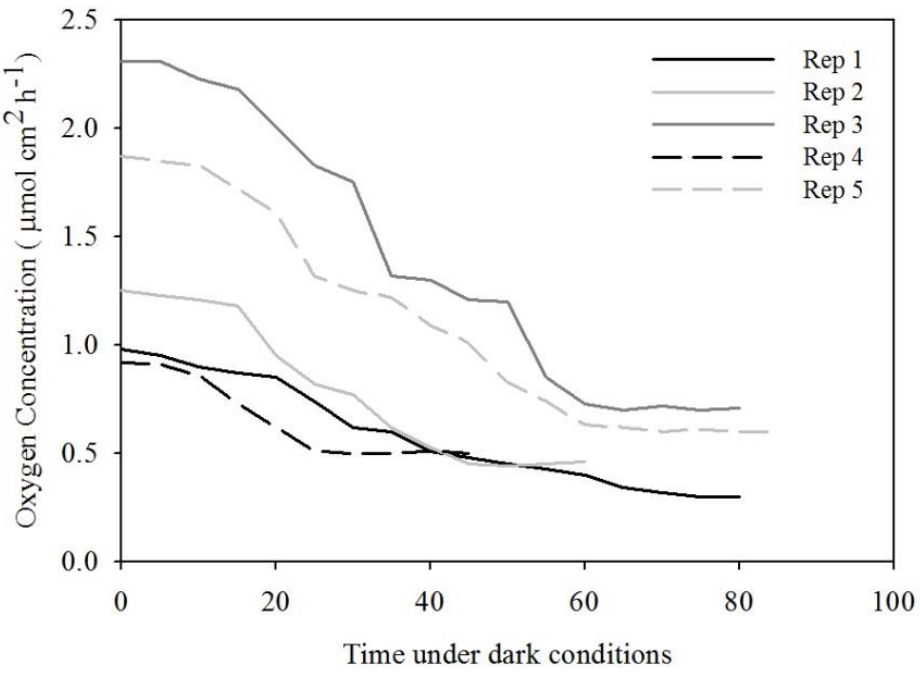


Figure S.1| The time it took for five colonies of *Siderastrea cf. stellata* oxygen concentrations to change from daytime to night-time rates. The colonies were tested in Salvador Brazil.

Appendix 3| CPCe point density

Summary – To ensure that the correct point density (number of points per image frame) was used to obtain robust estimates of percent cover, a power analysis was conducted (Pante & Dustane, 2012). Three tiles from the back-reef and three tiles from the seagrass habitats had their actual percent cover established. To determine the number of points needed on CPCe to provide an estimate of percent cover with 95 % confidence of the actual percent cover, 5, 10, 20, 40, 80, 160 and 320 points were initially overlaid on each image using CPCe. The estimated percent cover for each number of points was compared to the actual percent cover using the Chi-squared test (of association).

Results – For the back-reef habitat between 80 and 160 points were necessary in CPCe to get a significant Chi-squared test of association ($X^2 < 21.03$, $d.f. = 12$, $P = 0.05$) between the actual and observed percent covers. For the seagrass habitat between 10 and 20 points were necessary ($X^2 < 11.07$, $d.f = 5$, $P = 0.05$, Table S.1). Further analysis on the most diverse tiles of each habitat (Tile 2 for the back-reef and tile 3 for the seagrass) determined that 150 points for the back-reef (Figure S.2) and 60 points for the seagrass (Figure S.3) were optimal in estimating the percent cover with 95 % accuracy, whilst maintaining the species diversity (Table S.1). The precision of repeating the estimated percent cover three times was tested; however, the error was minimal ($e < 0.02$) once an optimum number of points (150) was used, making it preferable to reduce error from repeated sampling by just sampling each tile once.

Table S.1| CPCe point density summary.

Habitat	Replicate Tile	Number of points needed to get significant Chi-squared test of association result	Number of points needed to get the same observed and actual number of species
Back-reef	1	80	120
	2	160	160
	3	80	120
Seagrass	1	10	20
	2	10	40
	3	20	60

For each habitat and tile replicate, the number of points necessary in CPCe to obtain a significant Chi-squared test of association results (back-reef: $X^2 < 21.03$, $d.f. = 12$, $P = 0.05$; Seagrass: $X^2 < 11.07$, $d.f = 5$, $P = 0.05$) is shown, is the number of points needed to get the same observed and actual number of species.

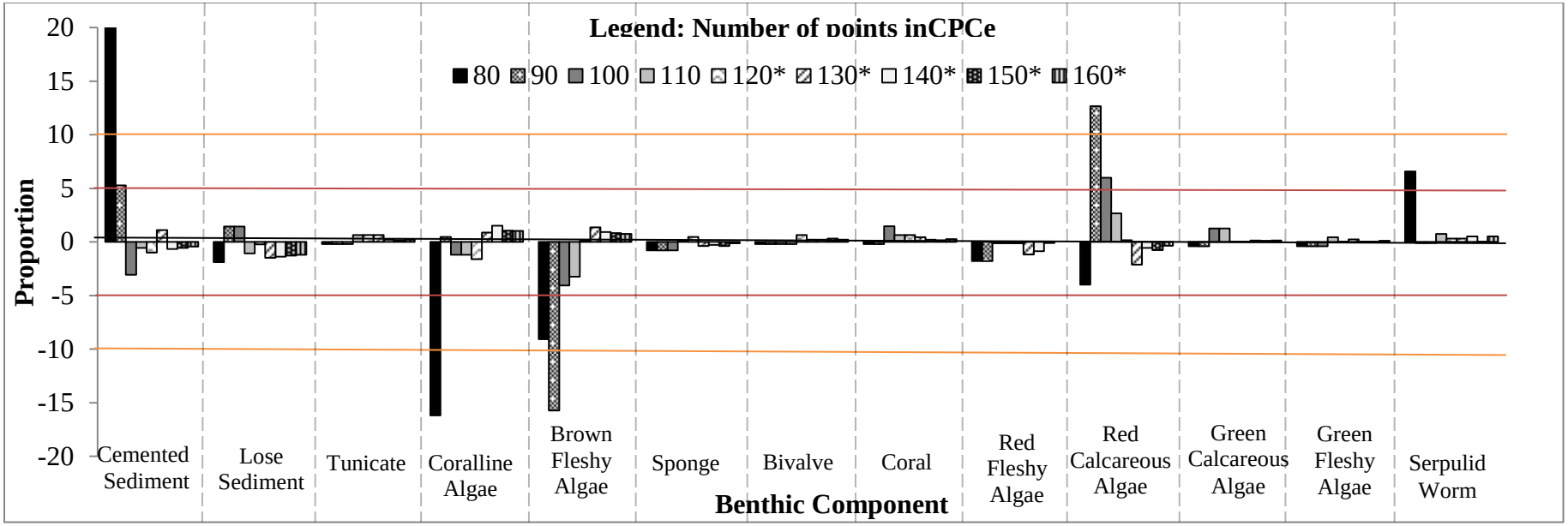


Figure S.2| The proportion of each benthic component for the back-reef tile two (the tile with the greatest diversity) that was above or below the actual percent cover using 80 up to 160 points in CPCe in 10 point increasing increments. * in the legend denotes a significant Chi-Squared test of association result (X^2< 21.023, *d.f.*= 12, *P*= 0.05).

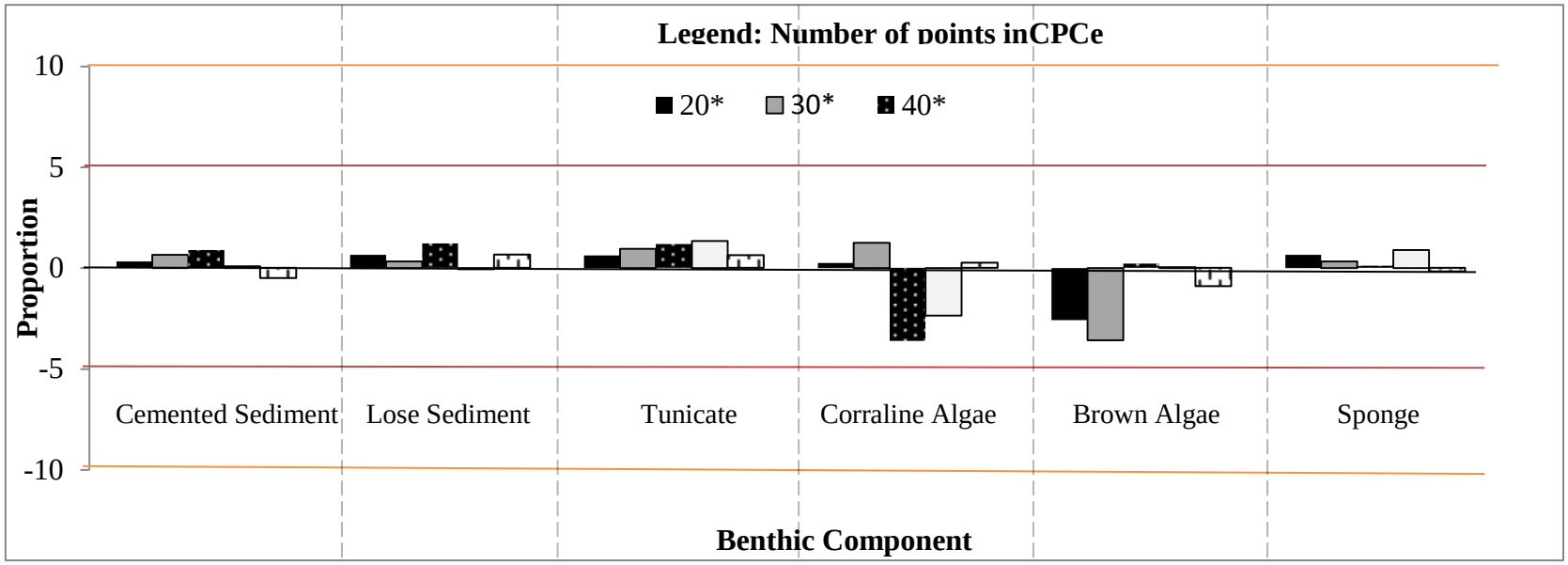


Figure S.3| The proportion of each benthic component for the seagrass tile three (the tile with the greatest diversity) that was above or below the actual percent cover using 20 up to 60 points in CPCe in 10 point increasing increments. * in the legend denotes a significant Chi-Squared test of association result ($X^2 < 11.07$, $d.f. = 5$, $P = 0.05$).

Appendix 4| Linear Mixed Effects (LME) models

The final model applied to compare each abiotic variable between habitats, and seasons was:

$$A_J = \alpha + \beta_1 * \text{Season}_J + \beta_2 * \text{Habitat}_J + \varepsilon_J \quad [S2]$$

Where A is the abiotic parameter being measured, J is the site, α is the intercept, β_1 is the slope of Season, β_2 is the effect of habitat for site J, and ε_J is the residual error.

The model input into R was: modelpH<-

lme(meanA~season*habitat,random=~1|site,data=ocean)

Similar model application was applied for lunar cycle comparisons:

$$A_J = \alpha + \beta_1 * \text{Season}_J + \text{lunarcycle}_J + \beta_2 * \text{Habitat}_J + \varepsilon_J \quad [S3]$$

Where A is the abiotic parameter being measured, *J* is the site, α is the intercept, β_1 is the slope of Season, β_2 is the effect of habitat for site *J*, and ε_J is the residual error.

The model input into R was:

modelpH<-lme(meanpH~season+lunarcycle*habitat,random=~1|site,data=ocean)

An adaption of this model was also used to examine the coral physiology data obtained. Mixed Effects (LME) models with coral species as a random effect, were applied as described to examine the effect of habitat on daily net photosynthesis, respiration and calcification.

Appendix 5| The seasonal and lunar cycle means and ranges in abiotic variables for each of the nine sites on Little Cayman, Cayman Islands, BWI.

Table S.2| Temporal ranges in abiotic variables for each of the nine sites on Little Cayman.

Abiotic Variable	Season	Lunar Cycle	Site and Habitat					
			Back-reef 1		Back-reef 2		Back-reef 3	
			Mean (±SE)	Range	Mean (±SE)	Range	Mean (±SE)	Range
pH	**Summer**	**Spring**	8.12 (0.01)	0.31	8.15 (0.01)	0.37	8.15 (0.01)	0.32
		Neap	8.16 (0.01)	0.27	8.16 (0.01)	0.35	8.14 (0.01)	0.30
	Winter	**Spring**	8.19 (0.01)	0.23	8.20 (0.01)	0.37	8.23 (0.01)	0.35
		Neap	8.20 (0.01)	0.22	8.19 (0.01)	0.21	8.20 (0.01)	0.18
pCO_2 (atm)	**Summer**	**Spring**	309 (9.97)	274	292 (8.87)	285	274 (7.57)	240
		Neap	281 (6.71)	219	272 (9.34)	262	273 (7.94)	235
	Winter	**Spring**	246 (6.11)	187	222 (7.43)	281	226 (6.54)	260
		Neap	206 (3.60)	139	213 (4.75)	153	199 (3.50)	106
Total Alkalinity ($\mu mol/kg^{-1}$)	**Summer**	**Spring**	2239 (7.03)	248	2260 (10.62)	353	2220 (5.98)	228
		Neap	2279 (9.36)	302	2257 (9.74)	299	2291 (8.07)	299
	Winter	**Spring**	2232 (1.66)	201	2235 (4.10)	151	2230 (5.10)	176
		Neap	2244 (3.86)	138	2247 (3.84)	101	2230 (5.68)	226
Aragonite Ω	**Summer**	**Spring**	4.2 (0.08)	2.5	4.3 (0.09)	3.5	4.4 (0.09)	3.0
		Neap	4.2 (0.07)	2.3	4.4 (0.10)	2.9	4.3 (0.08)	2.2
	Winter	**Spring**	4.9 (0.06)	2.0	5.3 (0.09)	3.1	5.2 (0.06)	2.7
		Neap	5.4 (0.06)	2.5	5.3 (0.06)	1.8	5.3 (0.06)	1.9
Temperature (°C)	**Summer**	**Spring**	29.6 (0.05)	2.1	30.1 (0.11)	3.4	29.8 (0.08)	2.8
		Neap	29.2 (0.11)	3.8	29.8 (0.11)	3.7	29.6 (0.08)	2.7
	Winter	**Spring**	27.4 (0.12)	3.2	27.9 (0.16)	5.0	27.7 (0.14)	4.8
		Neap	26.4 (0.09)	2.8	26.8 (0.09)	3.6	26.9 (0.08)	3.2
Salinity (ppm)	**Summer**	**Spring**	35.2 (0.03)	0.5	35.0 (0.04)	1.0	35.4 (0.03)	0.5
		Neap	35.2 (0.03)	0.5	35.2 (0.03)	1.0	35.2 (0.03)	0.5
	Winter	**Spring**	35.2 (0.03)	0.5	35.1 (0.03)	0.5	35.2 (0.03)	0.5
		Neap	35.2 (0.03)	0.5	35.2 (0.03)	1.0	35.1 (0.03)	1.0
Light ($\mu mol\ m^{-2}\ s^{-1}$)	**Summer**	**Spring**	304.1 (54.48)	1735.0	308.7 (54.16)	1326.8	328.2 (55.97)	1581.0
		Neap	350.8 (57.19)	1479.8	332.7 (61.08)	1530.9	303.8 (52.24)	1275.3
	Winter	**Spring**	319.1 (55.19)	1428.8	284.3 (48.92)	1345.7	339.9 (59.6)	1530.9
		Neap	324.8 (53.89)	1530.9	282.6 (51.25)	1479.0	319.4 (54.7)	1530.9
Water Movement ($cm^{-1}s^{-1}$)	**Summer**	**Spring**	18 (0.54)	19	16 (0.39)	13	16 (0.61)	20
		Neap	18 (0.46)	13	17 (0.40)	11	20 (0.54)	21
	Winter	**Spring**	13 (0.39)	12	15 (0.49)	13	14 (0.44)	12
		Neap	10 (0.31)	11	12 (0.30)	8	24 (0.42)	13

Abiotic Variable	Season	Lunar Cycle	Site and Habitat					
			Seagrass 1		Seagrass 2		Seagrass 3	
			Mean (±SE)	Range	Mean (±SE)	Range	Mean (±SE)	Range
pH	**Summer**	**Spring**	8.17 (0.02)	0.614	8.12 (0.02)	0.551	8.12 (0.02)	0.598
		Neap	8.13 (0.02)	0.576	8.12 (0.02)	0.59	8.12 (0.02)	0.541
	Winter	**Spring**	8.15 (0.02)	0.504	8.16 (0.02)	0.519	8.21 (0.01)	0.473
		Neap	8.19 (0.02)	0.506	8.20 (0.01)	0.409	8.22 (0.01)	0.48
pCO_2 (atm)	**Summer**	**Spring**	285 (20.08)	608	313 (14.31)	511	301 (15.32)	591
		Neap	279 (17.74)	570	300 (16.52)	585	280 (14.62)	507
	Winter	**Spring**	276 (12.62)	433	272 (14.55)	461	232 (9.88)	378
		Neap	243 (11.97)	459	240 (10.70)	329	220 (10.33)	403
Total Alkalinity ($\mu mol/Kg^{-1}$)	**Summer**	**Spring**	2264 (8.14)	351	2264 (11.28)	331	2241 (10.67)	304
		Neap	2250 (10.64)	302	2222 (6.52)	203	2246 (5.43)	179
	Winter	**Spring**	2251 (6.30)	277	2207 (4.68)	153	2219 (4.75)	226
		Neap	2242 (4.86)	176	2236 (3.39)	101	2234 (5.17)	176
Aragonite Ω	**Summer**	**Spring**	4.4 (0.15)	4.7	4.3 (0.12)	4.0	4.3 (0.13)	4.1
		Neap	4.5 (0.15)	4.6	4.2 (0.14)	4.7	4.2 (0.13)	4.1
	Winter	**Spring**	4.9 (0.13)	4.3	5.0 (0.13)	4.0	5.2 (0.11)	4.3
		Neap	5.1 (0.13)	4.1	5.3 (0.12)	3.5	5.4 (0.13)	4.2
Temperature (°C)	**Summer**	**Spring**	29.8 (0.07)	2.8	30.2 (0.12)	3.6	30.2 (0.11)	3.6
		Neap	29.2 (0.12)	4.1	29.9 (0.13)	4.4	29.8 (0.11)	3.5
	Winter	**Spring**	28.1 (0.20)	6.7	28.0 (0.18)	6.7	27.8 (0.15)	5.3
		Neap	26.9 (0.10)	4.3	26.9 (0.11)	4.3	26.9 (0.09)	3.0
Salinity (ppm)	**Summer**	**Spring**	35.2 (0.06)	2.0	35.0 (0.06)	2.0	35.0 (0.05)	2.0
		Neap	35.0 (0.07)	2.0	35.0 (0.06)	2.0	35.0 (0.07)	2.0
	Winter	**Spring**	35.3 (0.04)	0.5	35.4 (0.03)	1.0	35.3 (0.03)	1.0
		Neap	35.2 (0.03)	1.0	35.2 (0.03)	1.0	35.3 (0.03)	1.0
Light ($\mu mol\ m^{-2}\ s^{-1}$)	**Summer**	**Spring**	327.5 (54.43)	1224.7	352.6 (61.68)	1530.9	358.3 (63.16)	1632.9
		Neap	308.4 (53.08)	1275.7	329.7 (60.53)	1530.9	333.6 (56.44)	1567.2
	Winter	**Spring**	265.8 (43.53)	969.6	311.5 (53.03)	1377.8	260.2 (46.36)	1224.7
		Neap	252.0 (40.53)	918.5	307.1 (51.03)	1275.7	313.8 (51.98)	1256.5
Water Movement ($cm^{-1}s^{-1}$)	**Summer**	**Spring**	16 (0.65)	19	17 (0.57)	19	19.5 (0.42)	13
		Neap	16 (0.64)	21	19 (0.43)	11	17.6 (0.38)	12
	Winter	**Spring**	12 (0.41)	14	12 (0.30)	8	12.4 (0.33)	11
		Neap	13 (0.48)	15	15 (0.60)	18	14.1 (0.56)	18

Abiotic Variable	Season	Lunar Cycle	Site and Habitat					
			Outer reef 1		Outer reef 2		Outer reef 3	
			Mean (±SE)	Range	Mean (±SE)	Range	Mean (±SE)	Range
pH	**Summer**	**Spring**	8.12 (0.00)	0.006	8.11 (-0.00)	-0.00	8.12 (-0.00)	0.009
		Neap	8.11 (0.00)	0.003	8.11 (-0.00)	0.007	8.12 (-0.00)	0.007
	Winter	**Spring**	8.11 (0.00)	0.003	8.12 (-0.00)	0.007	8.11 (-0.00)	-0.00
		Neap	8.12 (0.00)	0.011	8.11 (-0.00)	-0.00	8.12 (-0.00)	-0.00
***p*CO_2**	**Summer**	**Spring**	317 (1.20)	21	321 (1.23)	26	321 (0.67)	12
(atm)		**Neap**	319 (0.91)	17	319 (0.33)	6	320 (0.89)	21
	Winter	**Spring**	324 (0.41)	9	314 (0.91)	16	317 (0.74)	17
		Neap	317 (1.32)	24	327 (0.92)	18	320 (0.45)	11
Total Alkalinity	**Summer**	**Spring**	2357 (6.70)	110	2377 (7.59)	162	2404 (4.11)	90
(µmol/Kg^{-1})		**Neap**	2365 (5.65)	100	2391 (0.39)	9	2375 (4.44)	97
	Winter	**Spring**	2418 (2.50)	50	2343 (4.00)	70	2343 (4.37)	90
		Neap	2355 (6.18)	99	2420 (5.33)	106	2394 (2.86)	60
Aragonite Ω	**Summer**	**Spring**	3.5 (0.01)	0.19	3.5 (0.01)	0.30	3.6 (0.01)	0.25
		Neap	3.5 (0.01)	0.22	3.6 (-0.00)	0.05	3.5 (0.01)	0.15
	Winter	**Spring**	3.6 (0.01)	0.11	3.5 (0.01)	0.10	3.4 (0.01)	0.18
		Neap	3.5 (0.01)	0.16	3.6 (0.01)	0.20	3.6 (0.01)	0.12
Temperature	**Summer**	**Spring**	28.6 (0.01)	0.2	28.4 (0.01)	0.1	38.5 (0.01)	0.3
(°C)		**Neap**	28.5 (0.01)	0.3	28.5 (0.01)	0.3	28.5 (0.01)	0.3
	Winter	**Spring**	27.5 (0.01)	0.2	27.6 (0.01)	0.2	27.4 (0.01)	0.1
		Neap	27.5 (0.02)	0.3	27.4 (0.01)	0.3	27.4 (0.02)	0.3
Salinity	**Summer**	**Spring**	35.5 (-0.00)	-0.00	35.5 (-0.00)	-0.00	35.5 (-0.00)	-0.00
(ppm)		**Neap**	35.5 (-0.00)	-0.00	35.5 (-0.00)	-0.00	35.5 (-0.00)	-0.00
	Winter	**Spring**	35.5 (-0.00)	-0.00	35.5 (-0.00)	-0.00	35.5 (-0.00)	-0.00
		Neap	35.5 (-0.00)	-0.00	35.5 (-0.00)	-0.00	35.5 (-0.00)	-0.00

Appendix 6| Carbonate chemistry statistical results Little Cayman, Cayman Islands, Atlantic Ocean.

Table S.3| Intra-habitat site comparison of mean and coefficient of variation (Cv) of carbonate chemistry parameters (pH, pCO_2, total alkalinity, Ω_{arg}): 2-Way ANOVA with post hoc Tukey-Kramer test. Only significant intra-habitat differences at 0.05 % significance are shown, all other results were non-significant.

Variable	ANOVA	Comparison Group	Post hoc *P* -value
pH Cv	$F_{2,11}$ = 5.84 *P* = 0.005**	SG1 Vs SG3	0.05*
pCO_2 Cv	$F_{2,11}$ = 8.15 *P* = 0.0005***	SG1 Vs SG3	0.005**

*** indicates a significant statistical test with $P < 0.001$, ** indicates a significant statistical test with $P < 0.01$, * indicates a significant statistical test with $P < 0.05$. SG = Seagrass, BR = Back Reef.

Table S.4| Carbonate chemistry LME model results.

A| Model parameters to estimate the inshore to offshore gradient (∆) between the lagoon non-reef sites and the outer reef sites as a function of habitat for each season. Study site was modelled as a random effect.

Model Term	Estimate	SE	*t*-value	*P*-value
Model 1: ∆ pH				
Intercept=Summer:Back reef	0.0409	0.0084	4.8710	0.0001
Season=Winter	0.0700	0.0078	9.0331	0.0001
Habitat=Seagrass	-0.0245	0.0078	-3.1551	0.0016
Intercept=Summer-Seagrass	0.0164	0.0084	1.9578	0.0500
Season=Winter	0.0694	0.0078	8.9570	0.0001
Habitat=Back reef	0.0245	0.0078	3.1550	0.0016
Intercept=Winter:Back reef	0.1109	0.0084	13.2113	0.0001
Season=Summer	-0.0700	0.0078	-9.0330	0.0001
Habitat= Seagrass	-0.0250	0.0078	-3.2309	0.0013
Intercept= Winter:Seagrass	0.0859	0.0084	10.2282	0.0001
Season=Summer	-0.0694	0.0078	-8.9570	0.0001
Habitat=Back reef	0.0250	0.0078	3.2309	0.0013
Model 2: ∆ npCO_2				
Intercept=Summer:Back reef	-41.3736	7.9123	-5.2290	0.0001
Season=Winter	-42.3521	6.5778	-6.4387	0.0001
Habitat=Seagrass	34.3960	6.5778	5.2291	0.0001
Intercept=Summer-Seagrass	-6.9776	7.9123	-0.8819	0.3780

Season=Winter	-46.4457	6.5778	-7.0610	0.0001
Habitat=Back reef	-34.3960	6.5778	-5.2291	0.0001
Intercept=Winter:Back reef	-83.7257	7.9123	-10.5817	0.0001
Season=Summer	42.3521	6.5778	6.4387	0.0001
Habitat= Seagrass	30.3024	6.5778	4.6068	0.0001
Intercept= Winter:Seagrass	-53.4233	7.9123	-6.7519	0.0001
Season=Summer	46.4457	6.5778	7.0610	0.0001
Habitat=Back reef	-30.3024	6.5778	-4.6068	0.0001
Model 3: Δ ntotal alkalinity				
Intercept=Summer:Back reef	-156.9896	4.7441	-33.0912	0.0001
Season=Winter	100.7381	6.7092	15.0149	0.0001
Habitat=Seagrass	-18.2020	6.7092	-2.7130	0.0067
Intercept=Summer-Seagrass	-175.1915	4.7441	-36.9280	0.0001
Season=Winter	115.8809	6.7092	17.2719	0.0001
Habitat=Back reef	18.2020	6.7092	2.7130	0.0067
Intercept=Winter:Back reef	-56.2514	4.7441	-11.8570	0.0001
Season=Summer	-100.7381	6.7092	-15.0149	0.0001
Habitat= Seagrass	-3.0592	6.7092	-0.4560	0.6485
Intercept= Winter:Seagrass	-59.3106	4.7441	-12.5019	0.0001
Season=Summer	-115.8809	6.7092	-17.2719	0.0001
Habitat=Back reef	3.0592	6.7092	0.4560	0.6485
Model 4: Δ Ω_{arg}				
Intercept=Summer:Back reef	0.0076	0.0475	0.1610	0.8722
Season=Winter	0.8984	0.0623	14.4127	0.0001
Habitat=Seagrass	0.0043	0.0623	0.0684	0.9455
Intercept=Summer-Seagrass	0.0120	0.0475	0.2508	0.8020
Season=Winter	0.7900	0.0623	12.6735	0.0001
Habitat=Back reef	-0.0042	0.0623	-0.0684	0.9455
Intercept=Winter:Back reef	0.9060	0.0475	19.0883	0.0001
Season=Summer	-0.8984	0.0623	-14.4127	0.0001
Habitat= Seagrass	-0.1041	0.0623	-1.6708	0.0949
Intercept= Winter:Seagrass	0.8019	0.0475	16.8941	0.0001
Season=Summer	-0.7900	0.0623	-12.6735	0.0001
Habitat=Back reef	0.1041	0.0623	1.6708	0.0949

B| Model parameters to estimate the Coefficient of Variation (Cv) between sites of pH, pCO_2, total alkalinity and Ω_{arg} as a function of habitat for each season. Study site was modelled as a random effect.				
Model Term	**Estimate**	**SE**	***t*-value**	***P*-value**
Model 5: Cv pH				
Intercept=Summer:Back reef	0.0109	0.0005	20.3437	0.0001
Season=Winter	-0.0031	0.0007	-4.3958	0.0001
Habitat=Outer reef	-0.0106	0.0007	-15.0052	0.0001
Habitat=Seagrass	0.0077	0.0007	10.8456	0.0001
Intercept=Summer:Outer reef	0.0003	0.0005	0.6208	0.5398
Season=Winter	-0.0001	0.0007	-0.0884	0.9302
Habitat=Back reef	0.0106	0.0007	15.0052	0.0001
Habitat=Seagrass	0.0183	0.0007	25.8508	0.0001
Intercept=Summer:Seagrass	0.0186	0.0005	34.5993	0.0001
Season=Winter	-0.0031	0.0007	-4.4212	0.0001
Habitat=Back reef	-0.0077	0.0007	-10.8456	0.0010
Habitat=Outer reef	-0.0183	0.0007	-25.8508	0.0001
Intercept=Winter: Back reef	0.0078	0.0005	14.5658	0.0001
Season=Summer	0.0031	0.0007	4.3958	0.0001
Habitat=Outer reef	-0.0076	0.0007	-10.6977	0.0001
Habitat=Seagrass	0.0076	0.0007	10.8202	0.0010
Intercept=Winter: Outer reef	0.0003	0.0005	0.5046	0.6178
Season=Summer	0.0001	0.0007	0.0884	0.9302
Habitat=Back reef	0.0076	0.0007	10.6977	0.0010
Habitat=Seagrass	0.0152	0.0007	21.5180	0.0001
Intercept=Winter: Seagrass	0.0155	0.0005	28.7881	0.0001
Season=Summer	0.0031	0.0007	4.4212	0.0001
Habitat=Back reef	-0.0077	0.0007	-10.8202	0.0070
Habitat=Outer reef	-0.0152	0.0007	-21.5180	0.0001
Model 6: Cv pCO_2				
Intercept=Summer:Back reef	0.2510	0.0138	18.1715	0.0001
Season=Winter	-0.0463	0.0190	-2.4361	0.0215
Habitat=Outer reef	-0.2279	0.0190	-11.9833	0.0001
Habitat=Seagrass	0.1836	0.0190	9.6556	0.0001
Intercept=Summer:Outer reef	0.0231	0.0138	1.6754	0.1050
Season=Winter	-0.0021	0.0190	-0.1080	0.9147
Habitat=Back reef	0.2279	0.0190	11.9833	0.0001
Habitat=Seagrass	0.4115	0.0190	21.6389	0.0001
Intercept=Summer:Seagrass	0.4346	0.0138	31.4633	0.0001
Season=Winter	-0.0350	0.0190	-1.8414	0.0462
Habitat=Back reef	-0.1836	0.0190	-9.6556	0.0001
Habitat=Outer reef	-0.4115	0.0190	-21.6389	0.0001
Intercept=Winter: Back reef	0.2047	0.0138	14.8180	0.0001
Season=Summer	0.0463	0.0190	2.4361	0.0215
Habitat=Outer reef	-0.1836	0.0190	-9.6553	0.0001
Habitat=Seagrass	0.1949	0.0190	10.2503	0.0010

Intercept=Winter: Outer reef	0.0211	0.0138	1.5267	0.1381
Season=Summer	0.0021	0.0190	0.1080	0.9147
Habitat=Back reef	0.1836	0.0190	9.6553	0.0001
Habitat=Seagrass	0.3785	0.0190	19.9055	0.0001
Intercept=Winter: Seagrass	0.3996	0.0138	28.9284	0.0001
Season=Summer	0.0350	0.0190	1.8414	0.0462
Habitat=Back reef	-0.1949	0.0190	-10.2503	0.0001
Habitat=Outer reef	-0.3785	0.0190	-19.9055	0.0001
Model 7: Cv Total Alkalinity				
Intercept=Summer:Back reef	0.0318	0.0027	11.9226	0.0001
Season=Winter	-0.0141	0.0038	-3.7582	0.0008
Habitat=Outer reef	-0.0145	0.0038	-3.8654	0.0006
Habitat=Seagrass	0.0013	0.0038	0.3566	0.7241
Intercept=Summer:Outer reef	0.0172	0.0027	6.4561	0.0001
Season=Winter	-0.0022	0.0038	-0.5792	0.5671
Habitat=Back reef	0.0146	0.0038	3.8654	0.0006
Habitat=Seagrass	0.0159	0.0038	4.2220	0.0002
Intercept=Summer:Seagrass	0.0331	0.0027	12.4269	0.0001
Season=Winter	-0.0147	0.0038	-3.8879	0.0006
Habitat=Back reef	-0.0013	0.0038	-0.3566	0.7241
Habitat=Outer reef	-0.0159	0.0038	-4.2220	0.0002
Intercept=Winter: Back reef	0.0176	0.0027	6.6078	0.0001
Season=Summer	0.0142	0.0038	3.7582	0.0008
Habitat=Outer reef	-0.0026	0.0038	-0.6865	0.4980
Habitat=Seagrass	0.0009	0.0038	0.2268	0.8222
Intercept=Winter: Outer reef	0.0150	0.0027	5.6369	0.0001
Season=Summer	0.0022	0.0038	0.5792	0.5671
Habitat=Back reef	0.0026	0.0038	0.6865	0.4980
Habitat=Seagrass	0.0034	0.0038	0.9133	0.3689
Intercept=Winter: Seagrass	0.0185	0.0027	6.9285	0.0001
Season=Summer	0.0147	0.0038	3.8879	0.0006
Habitat=Back reef	-0.0009	0.0038	-0.2268	0.8222
Habitat=Outer reef	-0.0034	0.0038	-0.9133	0.3689
Model 8: Cv Ω_{arg}				
Intercept=Summer:Back reef	0.1678	0.0063	26.5358	0.0001
Season=Winter	-0.0622	0.0084	-7.4234	0.0001
Habitat=Outer reef	-0.1498	0.0084	-17.8824	0.0001
Habitat=Seagrass	0.1000	0.0084	11.9415	0.0010
Intercept=Summer:Outer reef	0.0179	0.0063	2.8385	0.0083
Season=Winter	-0.0043	0.0084	-0.5112	0.6133
Habitat=Back reef	0.1500	0.0084	17.8824	0.0001
Habitat=Seagrass	0.2498	0.0084	29.8239	0.0001
Intercept=Summer:Seagrass	0.2677	0.0063	42.3603	0.0001

Season=Winter	-0.0607	0.0084	-7.2509	0.0001
Habitat=Back reef	-0.1000	0.0084	-11.9415	0.0060
Habitat=Outer reef	-0.2498	0.0084	-29.8239	0.0001
Intercept=Winter: Back reef	0.1055	0.0063	16.6985	0.0001
Season=Summer	0.0622	0.0084	7.4234	0.0001
Habitat=Outer reef	-0.0919	0.0084	-10.9701	0.0001
Habitat=Seagrass	0.1015	0.0084	12.1141	0.0001
Intercept=Winter: Outer reef	0.0137	0.0063	2.1611	0.0394
Season=Summer	0.0043	0.0084	0.5111	0.6133
Habitat=Back reef	0.0919	0.0084	10.9701	0.0001
Habitat=Seagrass	0.1933	0.0084	23.0842	0.0001
Intercept=Winter: Seagrass	0.2070	0.0063	32.7517	0.0001
Season=Summer	0.0607	0.0084	7.2509	0.0001
Habitat=Back reef	0.1015	0.0084	-12.1141	0.0001
Habitat=Outer reef	0.1933	0.0084	-4.7657	0.0001

C| Model parameters to estimate the mean between sites of pH, pCO_2, total alkalinity and Ω_{arg} as a function of habitat for each season. Study site was modelled as a random effect.

Model Term	Estimate	SE	*t*-value	*P*-value
Model 9: mean pH				
Intercept=Summer:Back reef	8.1533	0.0084	971.2630	0.0001
Season=Winter	0.0733	0.0101	7.2711	0.0001
Habitat=Outer reef	-0.0317	0.0101	-3.1398	0.0040
Habitat=Seagrass	-0.0433	0.0101	-4.2965	0.0002
Intercept=Summer:Outer reef	8.1217	0.0084	967.4745	0.0001
Season=Winter	0.0017	0.0101	0.1653	0.8699
Habitat=Back reef	0.0317	0.0101	3.1398	0.0040
Habitat=Seagrass	-0.0117	0.0101	-1.1568	0.0471
Intercept=Summer:Seagrass	8.1100	0.0084	966.1039	0.0001
Season=Winter	0.0783	0.0101	7.7668	0.0001
Habitat=Back reef	0.0433	0.0101	4.2965	0.0002
Habitat=Outer reef	0.0117	0.0101	1.1568	0.0471
Intercept=Winter: Back reef	8.2267	0.0084	979.9983	0.0001
Season=Summer	-0.0733	0.0101	-7.2711	0.0001
Habitat=Outer reef	-0.1033	0.0101	-10.2456	0.0001
Habitat=Seagrass	-0.038	0.0101	-3.8008	0.0007
Intercept=Winter: Outer reef	8.1233	0.0084	967.6878	0.0001
Season=Summer	-0.0017	0.0101	-0.1653	0.8699
Habitat=Back reef	0.0103	0.0101	10.2456	0.0001
Habitat=Seagrass	0.0650	0.0101	6.4448	0.0001
Intercept=Winter: Seagrass	8.1883	0.0084	975.4309	0.0001
Season=Summer	-0.0783	0.0101	-7.7668	0.0001

Habitat=Back reef	0.0383	0.0101	3.8008	0.0007
Habitat=Outer reef	-0.0650	0.0101	-6.4448	0.0001
Model 10: mean pCO_2				
Intercept=Summer:Back reef	283.5617	6.7778	41.8374	0.0001
Season=Winter	-64.9067	7.8937	-8.2226	0.0001
Habitat=Outer reef	35.6800	7.8937	4.5201	0.0001
Habitat=Seagrass	36.6800	7.8937	4.6161	0.0001
Intercept=Summer:Outer reef	319.2417	6.7778	47.1018	0.0001
Season=Winter	0.9300	7.8937	0.1178	0.9071
Habitat=Back reef	-35.6800	7.8937	-4.5201	0.0001
Habitat=Seagrass	0.7583	7.8937	0.0961	0.0442
Intercept=Summer:Seagrass	320.0000	6.7778	47.2139	0.0001
Season=Winter	-72.8333	7.8937	-9.2267	0.0001
Habitat=Back reef	-36.4383	7.8937	-4.6161	0.0010
Habitat=Outer reef	-0.7583	7.8937	-0.0961	0.0442
Intercept=Winter: Back reef	218.6550	6.7778	32.2609	0.0001
Season=Summer	64.9067	7.8937	8.2226	0.0001
Habitat=Outer reef	101.5167	7.8937	12.8604	0.0001
Habitat=Seagrass	28.5117	7.8937	3.6119	0.0012
Intercept=Winter: Outer reef	320.1717	6.7778	47.2392	0.0001
Season=Summer	-0.9300	7.8937	-0.1178	0.9071
Habitat=Back reef	-101.5167	7.8937	-12.8604	0.0001
Habitat=Seagrass	-73.0050	7.8937	-9.2485	0.0010
Intercept=Winter: Seagrass	247.1667	6.7778	36.4678	0.0001
Season=Summer	72.8333	7.8937	9.2267	0.0001
Habitat=Back reef	-28.5117	7.8937	-3.6119	0.0012
Habitat=Outer reef	73.0050	7.8937	9.2485	0.0001
Model 11: mean Total Alkalinity				
Intercept=Summer:Back reef	2257.6350	8.9987	250.8857	0.0001
Season=Winter	-21.2733	12.7260	-1.6716	0.1057
Habitat=Outer reef	120.5317	12.7260	9.4713	0.0001
Habitat=Seagrass	-9.8017	12.7260	-0.7702	0.4476
Intercept=Summer:Outer reef	2378.1667	8.9987	264.2801	0.0001
Season=Winter	1.8333	12.7260	0.1441	0.8865
Habitat=Back reef	-120.5317	12.7260	-9.4713	0.0001
Habitat=Seagrass	-130.3333	12.7260	-10.2415	0.0001
Intercept=Summer:Seagrass	2247.8333	8.9987	249.7965	0.0001
Season=Winter	-16.3333	12.7260	-1.2835	0.2099
Habitat=Back reef	9.8017	12.7260	0.7702	0.4476
Habitat=Outer reef	130.3333	12.7260	10.2415	0.0001
Intercept=Winter: Back reef	2236.3617	8.9987	248.5216	0.0001
Season=Summer	21.2733	12.7260	1.6716	0.1057
Habitat=Outer reef	143.6383	12.7260	11.2870	0.0001
Habitat=Seagrass	-4.8617	12.7260	-0.3820	0.7053

Intercept=Winter: Outer reef	2380.0000	8.9987	264.4838	0.0001
Season=Summer	-1.8333	12.7260	-0.1441	0.8865
Habitat=Back reef	-143.6383	12.7260	-11.2870	0.0001
Habitat=Seagrass	-148.5000	12.7260	-11.6690	0.0001
Intercept=Winter: Seagrass	2231.5000	8.9987	247.9814	0.0001
Season=Summer	16.3333	12.7260	1.2835	0.2099
Habitat=Back reef	4.8617	12.7260	0.3820	0.7053
Habitat=Outer reef	148.5000	12.7260	11.6690	0.0001
Model 12: mean Ω_{arg}				
Intercept=Summer:Back reef	4.3069	0.1006	42.8300	0.0001
Season=Winter	0.9476	0.1422	6.6633	0.0001
Habitat=Outer reef	0.2183	0.1422	1.5348	0.0360
Habitat=Seagrass	0.1223	0.1422	0.8590	0.0477
Intercept=Summer:Outer reef	4.5252	0.1006	45.0006	0.0001
Season=Winter	-0.0030	0.1422	-0.0212	0.9833
Habitat=Back reef	-0.2183	0.1422	-1.5349	0.0360
Habitat=Seagrass	-0.0961	0.1422	-0.6759	0.0447
Intercept=Summer:Seagrass	4.5252	0.1006	45.0056	0.0001
Season=Winter	-0.0030	0.1422	-0.0212	0.9833
Habitat=Back reef	-0.2183	0.1422	-1.5348	0.0477
Habitat=Outer reef	-0.0961	0.1422	-0.6759	0.1360
Intercept=Winter: Back reef	5.2545	0.1006	52.2532	0.0001
Season=Summer	-0.9476	0.1422	-6.6633	0.0001
Habitat=Outer reef	-0.7323	0.1422	-5.1496	0.0001
Habitat=Seagrass	-0.4316	0.1422	-3.0351	0.0051
Intercept=Winter:Outer reef	4.5221	0.1006	44.9707	0.0001
Season=Summer	0.0030	0.1422	0.0212	0.9833
Habitat=Back reef	0.7323	0.1422	5.1496	0.0001
Habitat=Seagrass	0.3007	0.1422	2.1145	0.0435
Intercept=Winter:Seagrass	4.8228	0.1006	47.9610	0.0001
Season=Summer	-0.3938	0.1422	-2.7692	0.0099
Habitat=Back reef	0.4316	0.1422	3.0351	0.0051
Habitat=Outer reef	-0.3007	0.1422	-2.1145	0.0435

D| Model parameters to estimate the Coefficient of Variance (Cv) between sites of pH, pCO_2, total alkalinity and Ω_{arg} as a function of habitat for each lunar tidal cycle and season. Study site was modelled as a random effect. From the model, results for lunar tidal cycle for each habitat are shown.

Model Term	Estimate	SE	*t*-value	*P*-value
Model 13: ΔpH				
Intercept=Neap:Back reef	0.0094	0.0010	9.4498	0.0001
Lunar Tidal Cycle=Spring	-0.0001	0.0009	-0.1183	0.9061
Intercept=Neap:Outer reef	0.0007	0.0010	0.6761	0.5006
Lunar Tidal Cycle=Spring	0.0002	0.0009	0.1865	0.8525

Intercept=Neap:Seagrass	0.0184	0.0010	18.6383	0.0001
Lunar Tidal Cycle=Spring	-0.0019	0.0009	-2.0248	0.0456
Model 14: ΔpCO_2				
Intercept=Neap:Back reef	0.2577	0.0315	8.1857	0.0001
Lunar Tidal Cycle=Spring	-0.0323	0.0315	-1.0268	0.3071
Intercept=Neap:Outer reef	0.0466	0.0315	1.4800	0.1421
Lunar Tidal Cycle=Spring	-0.0154	0.0315	-1.1228	0.6257
Intercept=Neap:Seagrass	0.5067	0.0315	16.0979	0.0001
Lunar Tidal Cycle=Spring	-0.1056	0.0315	-3.3543	0.0011
Model 15: Δ Total alkalinity				
Intercept=Neap:Back reef	0.0273	0.0032	8.4261	0.0001
Lunar Tidal Cycle=Spring	-0.0002	0.0028	-0.0749	0.9405
Intercept=Neap:Outer reef	0.0211	0.0032	6.5146	0.0001
Lunar Tidal Cycle=Spring	-0.0000	0.0028	-0.0117	0.9907
Intercept=Neap:Seagrass	0.0314	0.0032	9.7131	0.0001
Lunar Tidal Cycle=Spring	-0.0007	0.0028	-0.2390	0.8116
Model 16: Δ Ω_{arg}				
Intercept=Neap:Back reef	0.1362	0.0131	10.4236	0.0001
Lunar Tidal Cycle=Spring	-0.0011	0.0125	-0.0875	0.3905
Intercept=Neap:Outer reef	0.0183	0.0131	1.4009	0.1644
Lunar Tidal Cycle=Spring	0.0092	0.0125	0.7386	0.4619
Intercept=Neap:Seagrass	0.2515	0.0131	19.2432	0.0001
Lunar Tidal Cycle=Spring	-0.0207	0.0125	-1.6580	0.1005

E| Model parameters to estimate the mean between sites of pH, pCO_2, total alkalinity and Ω_{arg} as a function of habitat for each lunar tidal cycle and season. Study site was modelled as a random effect. From the model, results for lunar tidal cycle for each habitat are shown.

Model Term	Estimate	SE	*t*-value	*P*-value
Model 17: mean pH				
Intercept=Neap:Back reef	8.1904	0.0141	581.5556	0.0001
Lunar Tidal Cycle=Spring	-0.0287	0.0137	-2.0915	0.0391
Intercept=Neap:Outer reef	8.1038	0.0141	575.4062	0.0001
Lunar Tidal Cycle=Spring	-0.0030	0.0137	-0.2175	0.8283
Intercept=Neap:Seagrass	8.1645	0.141	579.7139	0.0001
Lunar Tidal Cycle=Spring	-0.0264	0.0137	-1.9189	0.0579
Model 18: mean pCO_2				
Intercept=Neap:Back reef	259.4031	12.2144	21.2373	0.0001
Lunar Tidal Cycle=Spring	23.9431	11.7524	2.0037	0.0479
Intercept=Neap:Outer reef	339.2763	12.2144	27.7765	0.0001

Lunar Tidal Cycle=Spring	1.2367	11.7524	0.1052	0.9164
Intercept=Neap:Seagrass	292.3756	12.2144	23.9368	0.0001
Lunar Tidal Cycle=Spring	22.6797	11.7524	1.9298	0.0567
Model 19: mean Total alkalinity				
Intercept=Neap:Back reef	2270.1467	12.2429	185.4259	0.0001
Lunar Tidal Cycle=Spring	-26.1384	12.2429	-2.1350	0.0353
Intercept=Neap:Outer reef	2396.2482	12.2429	195.7258	0.0001
Lunar Tidal Cycle=Spring	-14.3740	12.2429	-1.1741	0.2432
Intercept=Neap:Seagrass	2250.4127	12.2429	183.8140	0.0001
Lunar Tidal Cycle=Spring	-1.7462	12.2429	-0.1426	0.8869
Model 20: mean Ω_{arg}				
Intercept=Neap:Back reef	4.6386	0.1255	36.9496	0.0001
Lunar Tidal Cycle=Spring	-0.1752	0.1255	-1.3958	0.1659
Intercept=Neap:Outer reef	4.3338	0.1255	34.5222	0.0001
Lunar Tidal Cycle=Spring	-0.0816	0.1255	-0.6503	0.5170
Intercept=Neap:Seagrass	4.5921	0.1255	-1.0634	0.0001
Lunar Tidal Cycle=Spring	-0.1821	0.1255	-1.4508	0.1500

Appendix 7| Abiotic stats, Little Cayman, Cayman Islands, Atlantic Ocean.

Table S.5 | Abiotic variables (temperature, light, salinity and water velocity) LME model results.

A| Model parameters to estimate the Coefficient of Variation (Cv) between sites of temperature, light, salinity and water velocity as a function of habitat for each season. Study site was modelled as a random effect.

Model Term	Estimate	SE	*t*-value	*P*-value
Model 1: Cv Temperature				
Intercept=Summer:Back reef	0.0232	0.0018	13.2336	0.0001
Season=Winter	0.0054	0.0022	2.4905	0.0144
Habitat=Outer reef	-0.0200	0.0022	-9.2396	0.0001
Habitat=Seagrass	0.0059	0.0022	2.7241	0.8722
Intercept= Summer:Outer reef	0.0032	0.0018	1.8248	0.7100
Season=Winter	-0.0001	0.0022	-0.0595	0.9527
Habitat=Seagrass	0.0200	0.0022	9.2396	0.0001
Intercept:Summer:Seagrass	0.0291	0.0018	16.5972	0.0001
Season:Winter	0.0052	0.0022	2.4191	0.0174
Intercept=Winter:Back reef	0.0286	0.0018	16.3088	0.0001
Habitat=Outer reef	-0.0256	0.0022	-11.7895	0.0001
Habitat=Seagrass	0.0057	0.0022	2.6528	0.0093
Intercept= Winter:Outer reef	0.0031	0.0018	1.7514	0.0829
Habitat=Seagrass	0.0313	0.0022	14.4423	0.0001
Model 2: Cv Light				
Intercept=Summer:Back reef	1.4924	0.0221	67.6216	0.0001
Season:Winter	-0.0003	0.0291	-0.0089	0.9929
Habitat=Seagrass	0.0004	0.0291	0.0141	0.8611
Intercept=Summer:Seagrass	1.4928	0.0221	67.6401	0.0001
Season=Winter	-0.0075	0.0291	-0.2573	0.7978
Intercept=Winter:Back reef	1.4922	0.0221	67.6099	0.0001
Habitat:Seagrass	-0.0068	0.0291	-0.2343	0.8155
Model 3: Cv Salinity				
Intercept=Summer:Back reef	0.0072	0.0028	25.964	0.0001
Season=Winter	-0.0004	0.0004	-0.8883	0.3820
Habitat=Outer reef	-0.0072	0.0004	-18.278	0.0010
Habitat=Seagrass	0.0096	0.0004	24.2664	0.0010
Intercept= Summer:Outer reef	0.0003	0.0028	0.1156	0.9088
Season=Winter	-0.0001	0.0004	-0.0727	0.9426
Habitat=Seagrass	0.0168	0.0004	42.5443	0.0010
Intercept:Summer:Seagrass	0.0168	0.0028	60.2821	0.0001
Season:Winter	-0.0087	0.0004	-21.9920	0.0001
Intercept=Winter:Back reef	0.0072	0.0003	25.9643	0.0001
Habitat=Outer reef	-0.0072	0.0004	-18.2778	0.0001

Habitat=Seagrass	0.0096	0.0004	20.1190	0.0010
Intercept= Winter:Outer reef	0.0001	0.0003	0.0128	0.9899
Model 4: Cv Water Velocity				
Intercept=Summer:Back reef	0.2216	0.0188	11.7896	0.0001
Season:Winter	0.0368	0.0240	1.5370	0.1291
Habitat=Seagrass	-0.0039	0.0240	-0.1628	0.8712
Intercept=Summer:Seagrass	0.1985	0.0188	10.5618	0.0001
Season=Winter	0.0191	0.0240	0.8009	0.4261
Intercept=Winter:Back reef	0.1848	0.0188	9.8313	0.0001
Habitat:Seagrass	0.0137	0.0240	0.5734	0.5684

B| Model parameters to estimate the mean between sites of temperature, light, salinity and water velocity as a function of habitat for each season. Study site was modelled as a random effect.

Model Term	Estimate	SE	*t*-value	*P*-value
Model 5: mean Temperature				
Intercept=Summer:Back reef	29.6948	0.1415	209.8489	0.0001
Season=Winter	-2.4907	0.1931	-12.8965	0.0001
Habitat=Outer reef	-1.2114	0.1931	-6.2726	0.0001
Habitat=Seagrass	0.1592	0.1931	0.8241	0.1232
Intercept= Summer:Outer reef	28.4833	0.1415	201.2879	0.0001
Season=Winter	-0.0337	0.1931	-0.1747	0.8616
Habitat=Seagrass	1.3706	0.1931	7.0967	0.0001
Intercept:Summer:Seagrass	29.8539	0.1415	210.9737	0.0001
Season:Winter	-2.3948	0.1931	-12.3998	0.0001
Intercept=Winter:Back reef	27.2040	0.1415	192.2472	0.0001
Habitat=Outer reef	1.2456	0.1931	6.4492	0.0001
Habitat=Seagrass	0.2551	0.1931	1.3208	0.1896
Intercept= Winter:Outer reef	28.4496	0.1415	201.0493	0.0001
Habitat=Seagrass	-0.9905	0.1931	-5.1284	0.0001
Model 6: mean Light				
Intercept=Summer:Back reef	321.3705	8.2520	38.9447	0.0001
Season:Winter	-9.6979	11.6700	-0.8310	0.4090
Habitat=Seagrass	13.6489	11.6700	1.1696	0.2464
Intercept=Summer:Seagrass	335.0194	8.2520	40.5987	0.0001
Season=Winter	-49.9503	11.6700	-4.2802	0.0001
Intercept=Winter:Back reef	311.6727	8.2520	37.7695	0.0001
Habitat:Seagrass	-26.6036	11.6700	-2.2797	0.2590
Model 7: mean Salinity				
Intercept=Summer:Back reef	35.1697	0.2430	144.3421	0.0001
Season=Winter	-0.0400	0.0344	-1.1639	0.2543
Habitat=Outer reef	-0.1950	0.0344	-5.6740	0.0001
Habitat=Seagrass	-0.1767	0.0344	-5.1405	0.0001

Intercept= Summer:Outer reef	35.0016	0.2430	144.3179	0.0001
Season=Winter	-0.0017	0.0344	-0.0485	0.9617
Habitat=Seagrass	0.0184	0.0344	0.5353	0.0010
Intercept:Summer:Seagrass	35.0200	0.0243	144.0723	0.0001
Season:Winter	0.2650	0.0344	7.7108	0.0001
Intercept=Winter:Back reef	35.1567	0.0243	146.6961	0.0001
Habitat=Outer reef	-0.1567	0.0344	-4.5586	0.0001
Habitat=Seagrass	0.1283	0.0344	3.7342	0.0009
Intercept= Winter:Outer reef	35.0000	0.0243	144.2493	0.0001
Habitat=Seagrass	0.2850	0.0344	8.2928	0.0010
Model 8: mean Water Velocity				
Intercept=Summer:Back reef	13.0231	0.7367	17.6784	0.0001
Season:Winter	4.8819	0.9096	5.3674	0.0001
Habitat=Seagrass	0.1227	0.9096	0.1349	0.8931
Intercept=Summer:Seagrass	13.1458	0.7367	17.8450	0.0001
Season=Winter	4.3171	0.9096	4.7464	0.0001
Intercept=Winter:Back reef	17.9051	0.7367	24.3054	0.0001
Habitat:Seagrass	-0.4421	0.9096	-0.4861	0.6285

C| Model parameters to estimate the Coefficient of Variance (Cv) between sites of temperature, light, salinity and water velocity as a function of habitat for each lunar cycle and season. Study site was modelled as a random effect. From the model, results for lunar cycle for each habitat are shown.

Model Term	**Estimate**	**SE**	***t*-value**	***P*-value**
Model 9: Cv Temperature				
Intercept=Neap:Back reef	0.0259	0.0018	14.2845	0.0001
Lunar Cycle=Spring	0.0001	0.0023	0.04159	0.9669
Intercept=Neap:Outer reef	0.0038	0.0018	2.1197	0.0365
Lunar Cycle=Spring	-0.0014	0.0023	-0.6167	0.5389
Intercept=Neap:Seagrass	0.0304	0.0018	16.8320	0.0001
Lunar Cycle=Spring	0.0025	0.0023	1.1060	0.2714
Model 10: Cv Light				
Intercept=Neap:Back reef	319.0138	9.3139	34.2515	0.0001
Lunar Cycle=Spring	-4.9845	13.1718	-0.3784	0.7063
Intercept=Neap:Seagrass	307.4276	9.3139	33.0075	0.0001
Lunar Cycle=Spring	5.2332	13.1718	0.3973	0.7063
Model 10: Cv Salinity				
Intercept=Neap:Back reef	0.0071	0.0016	6.1313	0.0001
Lunar Cycle=Spring	-0.0001	0.0016	-0.0140	0.9889
Intercept=Neap:Outer reef	0.0003	0.0016	0.0261	0.9793
Lunar Cycle=Spring	-0.0001	0.0016	-0.0151	0.9880

Intercept=Neap:Seagrass	0.0123	0.0016	10.6860	0.0001
Lunar Cycle=Spring	-0.0003	0.0016	0.1717	0.8649
Model 11: Cv Water Velocity				
Intercept=Neap:Back reef	15.2269	0.8814	17.2753	0.0001
Lunar Cycle=Spring	0.4745	1.2049	0.3938	0.6950
Intercept=Neap:Seagrass	15.8102	0.8814	17.9371	0.0001
Lunar Cycle=Spring	-1.0116	1.2049	-0.8395	0.4042

D| Model parameters to estimate the mean between sites of temperature, light, salinity and water velocity as a function of habitat for each lunar cycle and season. Study site was modelled as a random effect. From the model, results for lunar cycle for each habitat are shown.

Model Term	**Estimate**	**SE**	***t*-value**	***P*-value**
Model 12: mean Temperature				
Intercept=Neap:Back reef	28.1361	0.2696	104.3593	0.0001
Lunar Cycle=Spring	0.6267	0.3813	1.6436	0.1034
Intercept=Neap:Outer reef	28.4452	0.2696	105.5060	0.0001
Lunar Cycle=Spring	0.0425	0.3813	0.1115	0.9115
Intercept=Neap:Seagrass	28.2841	0.2696	104.9085	0.0001
Lunar Cycle=Spring	0.7448	0.3813	1.9534	0.0536
Model 13: mean Light				
Intercept=Neap:Back reef	319.0138	9.3139	34.2515	0.0001
Lunar Cycle=Spring	-4.9845	13.1718	-0.3784	0.7063
Intercept=Neap:Seagrass	307.4276	9.3139	33.0075	0.0001
Lunar Cycle=Spring	5.2332	13.1718	0.3973	0.6924
Model 14: mean Salinity				
Intercept=Neap:Back reef	35.1600	0.0417	844.0201	0.0001
Lunar Cycle=Spring	0.0334	0.0589	0.5658	0.5760
Intercept=Neap:Outer reef	35.0017	0.0417	840.2193	0.0001
Lunar Cycle=Spring	-0.0017	0.0589	-30.0283	0.9776
Intercept=Neap:Seagrass	35.1750	0.0417	844.3802	0.0001
Lunar Cycle=Spring	-0.0450	0.0589	-0.7638	0.4514
Model 15: mean Water Velocity				
Intercept=Neap:Back reef	15.2269	0.8814	17.2753	0.0001
Lunar Cycle=Spring	0.4745	1.2049	0.3938	0.6950
Intercept=Neap:Seagrass	15.8102	0.8814	17.9371	0.0001
Lunar Cycle=Spring	-1.0116	1.2049	-0.8395	0.4042

Appendix 8| Statistical results for bioregion site comparison of environmental variables

Table S.6| Mean and coefficient of variation (C_V) comparison of environmental variables: carbonate chemistry (pH, total alkalinity and Ωarg), temperature, light, nitrates and salinity among habitats: 2-Way ANOVA with post hoc Tukey-Kramer test.

Environmental Variable	ANOVA	Post hoc *P*-values					
		SG Vs OR	SG Vs BR	SG Vs M	OR Vs BR	OR Vs M	BR Vs M
pH $_{CV}$	$F_{3,36}$ = 103.07 *P*< 0.0005***	*P* < 0.05*	*P* < 0.05*	*P* < 0.05*	*P* < 0.005**	*P* < 0 .001***	N/S
pH mean (Total scale)	$F_{3,36}$ = 62.94 *P* < 0.0005***	*P* < 0.0001***	N/S	*P* < 0.005**	*P* < 0.05*	*P* < 0 .0005***	*P* < 0.001***
Total alkalinity C_V	$F_{3,36}$ = 18.06 *P* < 0.0005***	*P* < 0.005***	N/S	*P* < 0.02*	N/S	*P* < 0 .006**	N/S
Total alkalinity mean (µmol Kg/SW)	$F_{3,36}$ = 172.35 *P* < 0.0005***	*P* < 0.0005***	N/S	*P* < 0.005**	*P* < 0.005***	*P* < 0.005**	*P* < 0.001***
Ω_{arg} C_V	$F_{3,36}$ = 87.51 *P* < 0.0005***	*P* < 0.0005***	*P* < 0.0004***	*P* < 0.02*	*P* < 0.005**	*P* < 0.0005***	*P* < 0.05*
Ω_{arg} mean	$F_{3,36}$ = 60.31 *P* < 0.0005***	*P* < 0.015*	N/S	*P* < 0.0005***	*P* < 0.0001***	*P* < 0.0005***	*P* < 0.0005***
Temperature C_V	$F_{3,5}$ = 0.56 *P* = N/S	*P* < 0.05*	N/S	N/S	N/S	*P* < 0.05*	N/S
Temperature mean (°C)	$F_{3,36}$ = 1.48 *P* = N/S	N/S	N/S	N/S	N/S	N/S	N/S
Light C_V	$F_{3,5}$ = 0.81 *P* = N/S	N/S	N/S	N/S	N/S	N/S	N/S
Light DLI	$F_{3,36}$ = 0.24 *P* = N/S	N/S	N/S	N/S	N/S	N/S	N/S
Salinity C_V	$F_{3,5}$ = 1.51 *P* = N/S	N/S	N/S	N/S	N/S	N/S	N/S
Salinity mean (ppm)	$F_{3,36}$ = 32.44 *P* < 0.0005***	N/S	N/S	*P* < 0.0005***	N/S	*P* < 0.005**	*P*< 0.0004***
Nitrates C_V	$F_{3,5}$ = 0.10 *P* = N/S	N/S	N/S	N/S	N/S	N/S	N/S
Nitrates mean (µM)	$F_{3,36}$ = 68.51 *P* < 0.0005***	*P* < 0.005**	N/S	N/S	*P* < 0.05*	*P* < 0 .0005***	N/S

N/S indicates a non-significant statistical difference with *P* > 0.05, * indicates a significant statistical test with *P* < 0.001, ** indicates a significant statistical test with *P* < 0.01, * indicates a significant statistical test with *P* < 0.05. SG = Seagrass, OR = Outer-reef, BR = Back-reef and M = Mangrove.**

Appendix 9| Exploration of changes in calcification associated with mean or Cv of pH, temperature and light.

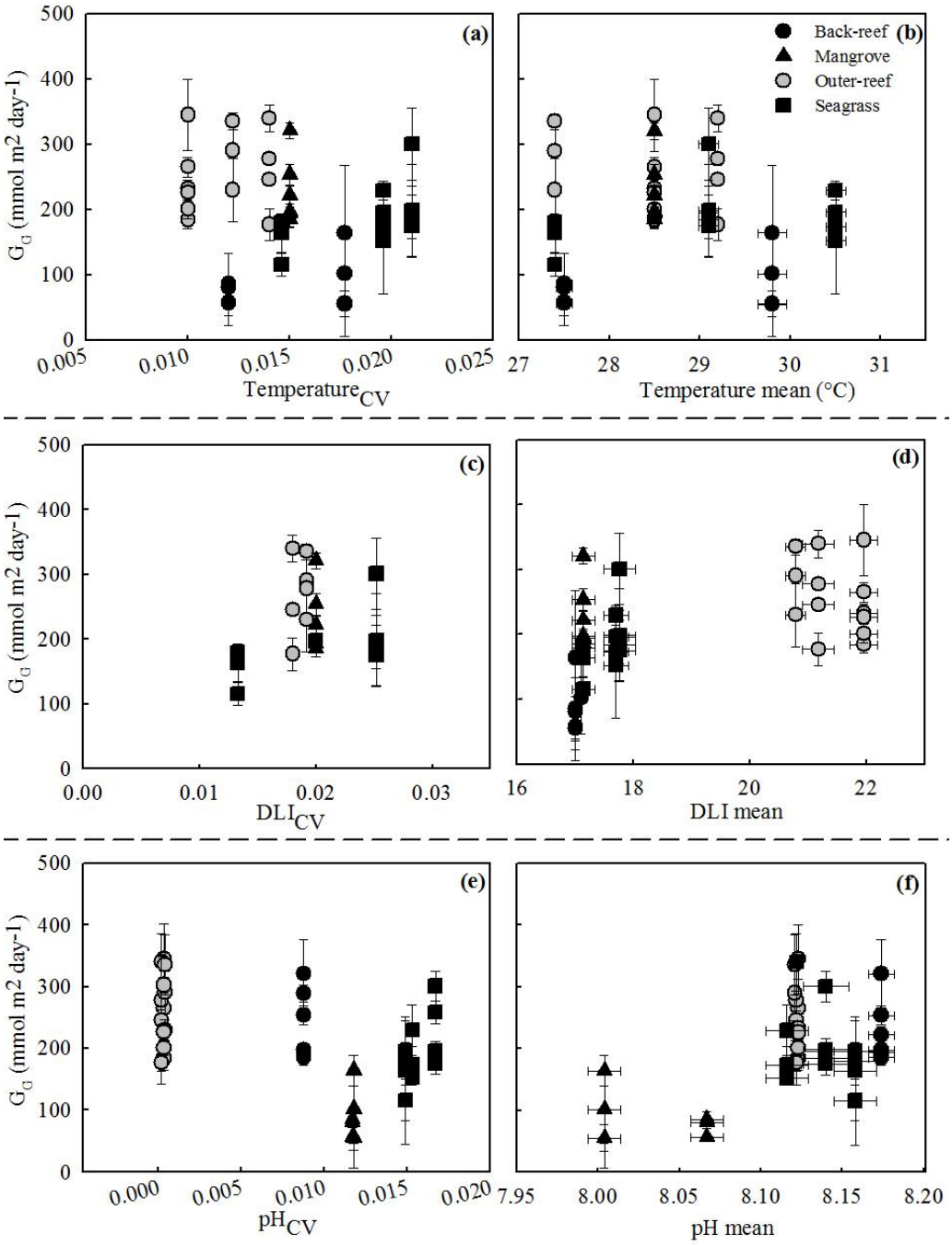


Figure S.4| Gross calcification in relation to temperature (a & b), light (c & d) and pH (e & f). All data plotted are mean values ± standard error (SE, *n*=5), except for pH_{CV} (see main text) for the dominant coral species examined across non-reef habitats (seagrass, back-reef and mangrove) and outer-reef habitat for all bioregion sites. Regression is shown with 95 % confidence interval.

Appendix 10| Statistical results coral metabolic activity, bioregion site comparison.

Table S.7| Model parameters to estimate daily net Photosynthesis (P) and Respiration (R) as a function of habitat (back-reef, outer reef control, seagrass, mangrove). Bioregion (Indian, Pacific and Atlantic Ocean sites) was modelled as a random effect.

Metabolic Parameter	Model terms	Estimate	SE	*t*-value	*P*-value
P	Intercept (Back-reef)	33.4788	3.5655	9.40	0.0001
	Habitat: Outer-reef	8.3802	4.0844	2.05	0.0408
	Habitat: Mangrove	-5.1939	4.8633	-1.07	0.0257
	Habitat: Seagrass	-9.5026	4.1681	-2.28	0.0314
	Intercept: (Seagrass)	23.9762	2.6463	9.06	0.0001
	Habitat: Outer-reef	17.8828	3.2096	5.57	0.0001
	Habitat: Mangrove	4.3087	3.8931	1.11	0.0278
	Intercept: (Mangrove)	28.2849	3.4687	8.15	0.0001
	Habitat: Outer-reef	13.5741	3.8553	3.52	0.0017
R	Intercept (Back-reef)	37.4524	2.6506	14.13	0.0001
	Habitat: Outer-reef	0.3681	2.5423	0.15	N/S
	Habitat: Mangrove	-0.1021	3.1653	-0.03	N/S
	Habitat: Seagrass	3.7217	2.6167	1.42	N/S
	Intercept: (Seagrass)	41.1742	2.1925	18.78	0.0001
	Habitat: Outer-reef	-3.3536	1.9395	-1.72	N/S
	Habitat: Mangrove	-3.8238	2.3961	-1.60	N/S
	Intercept: (Mangrove)	37.3504	2.6578	14.05	0.0010
	Habitat: Outer-reef	0.4702	2.3859	0.20	N/S

Appendix 11| Non-linear models used to analyse the lab manipulation data

The results were analysed using an ANOVA in R software (R 237 Development Core Team, 2011), and by multi-model selection framework

ANOVA Linear models – Three ANOVA analyses were performed, main effects, second order interactions, and with third order interactions. As the experiment could not be fully factorial it was necessary to eliminate species-habitation interaction terms from the ANOVA. In R this was written as:

$$v \sim 1 + s + h + v + T + pH \quad \text{[S4]}$$

$$v \sim 1 + (s + h + v + T + pH)^2 - s:h \quad \text{[S5]}$$

$$v \sim 1 + (s + h + v + T + pH)^3 - s:h:(1 + v + T + pH) \quad \text{[S6]}$$

Non-linear models – A series of non-linear models were applied (NL1-NL6, see Table S.8). The model used had the following structure:

$$V = V_0 \times \left[1 + a_s s + a_h h\right] \times \begin{bmatrix} 1 + T \left(b_T + b_{T,v} v + b_{T,h} h\right) \\ + pH \left(c_{pH} + c_{pH,v} v + c_{T,h} h\right) \\ + T \cdot pH \left(d_{T,pH} + d_{T,pH,v} v + d_{T,pH,h} h\right) \end{bmatrix} \quad \text{[S7]}$$

Table S.8| The parameters in the non-linear models (NL1-NL6).

Variable	NL1	NL2	NL4	NL6
V_0	•	•	•	•
a_s	•	•	•	•
a_h	•	•	•	•
b_T	•	•	•	•
$b_{T,v}$			•	•
$b_{T,h}$				•
c_{pH}	•	•	•	•
$c_{pH,v}$			•	•
$c_{pH,h}$				•
$d_{pH,T}$		•	•	•
$d_{pH,T,v}$			•	•
$d_{pH,T,h}$				•

Further details:
NL1 – control conditions depend on species & habitat stress terms for *T* and *pH*

NL2 – control conditions depend on species & habitat stress terms for *T*, *pH* and the interaction between *T*&*pH*

NL4 – control conditions depend on species & habitat stress terms for *T*, *pH* and the interaction between *T*&*pH* and each stress term depends on variability

NL6 – control conditions depend on species & habitat stress terms for *T*, *pH* and the interaction between *T*&*pH* and each stress term depends on variability and habitat

T is temperature, *pH* is the pH treatment, *h* is the habitat where the colonies originated, *v* is the level of variability the corals were exposed to within the treatment (e.g. high or low variability conditions representative of the seagrass or outer-reef respectively).

Linear versus non-linear model comparison – By expanding the non-linear model as a power series and collecting like terms, the parameters of the non-linear can be related to the parameters of the linear model. For example, expanding equation 2 and collecting terms that involve only $h \times T$ yields:

$$V_0 \left(a_h b_T + b_{T,h} \right) T \times h. \qquad \text{[S8]}$$

The term evaluates to 10.17, which falls into the confidence interval of the habitat interaction term in the linear model AN3 (Table S.9; 12.48 [3.8, 21.1]). This demonstrates how the non-linear is able to organise the terms differently. In this case, the temperature habitat interaction in a linear mode can be separated into two parts: one from differences in the 'base-line' due to habitat ($a_h b_T$) and one from differences in the stress response due to habitat ($b_{T,h}$).

Table S.9| Resolution of linear (AN3) and non-linear (NL6) model outputs.

				Maximum likihood			
		NL6 expansion		AN3			
	Variable			**Est**	**CI lower**	**CI upper**	**sig**
1st order	(Intercept)	299.10	Y	297.19	291.1	303.3	*
	spc	19.44	Y	23.11	17.0	29.2	*
	hab	-1.20	Y	-2.85	-9.0	6.9	
	var			-0.01	-5.9	0.0	
	temp	24.83	Y	31.03	22.4	39.7	*
	pH	-51.74	Y	-50.66	-59.3	-42.0	*
	spc:var			2.07	-5.0	7.4	
	spc:temp	1.61	N	-9.81	-18.5	-1.1	*
2nd order	spc:pH	-3.36	Y	-4.70	-13.4	11.3	
	hab:var			-0.23	-5.5	0.6	
	hab:temp	10.07	Y	8.92	0.3	17.6	*
	hab:pH	11.57	Y	12.48	3.8	21.1	*
	var:temp	10.17	Y	12.02	4.1	19.9	*
	var:pH	-18.84	Y	-24.64	-32.6	-16.7	*
	temp:pH	-79.26	Y	-86.38	-98.6	-74.1	*
	spc:var:temp	0.66	Y	-4.19	-10.3	1.9	
	spc:var:pH	-1.22	N	7.44	1.3	13.6	*
3rd order	spc:temp:pH	-5.43	N	8.28	-4.0	20.5	
	hab:var:temp	-0.04	Y	2.85	-6.9	9.0	
	hab:var:pH	0.08	Y	-5.04	-11.2	1.1	
	hab:temp:pH	1.83	Y	-4.58	-16.8	11.0	
	var:temp:pH	11.07	Y	12.57	2.6	22.6	*

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