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Ecosystem calcification and production in two Great Barrier Reef coral reefs: methodological challenges and environmental drivers

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Southern Cross University

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Ecosystem calcification and production in two Great Barrier Reef coral reefs: Methodological challenges and environmental driver

Ashly McMahon

Thesis prepared as fulfillment of the requirements for the Doctor of Philosophy (PhD) by Research

Southern Cross University

2018

Supervisors:
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Declaration

I, Ashly McMahon, certify that the work presented in the thesis, to the best of my knowledge and belief, is original, except as acknowledged in the text, and that the material has not been submitted, either in whole or part, for a degree at this or any other university.

I acknowledge that I have read and understood the University’s rules and requirements relating to the awarding of my Philosophy Doctorate (PhD) and to my thesis. I certify that I have complied with these

Date 20/12/2018
(Signed and dated)
Abstract

Coral reefs are some of the most important ecosystems on the planet, but subject to a number of local and global threats. Constraining the drivers and rates of coral reef metabolism can give a deeper understanding of how climate change and eutrophication may alter coral reef functioning. This thesis aims to improve our understanding about coral reef calcification and productivity drivers, including major research gaps on the importance of diel cycles, influence of \( \Omega_{\text{aragonite}} \), nutrient dynamics, and bleaching. My first data chapter traces natural nutrient additions through bird guano into the reef at a coral cay (Heron Island), and the nutrient implications to net ecosystem calcification (NEC) and net ecosystem production (NEP). A minimum addition of 2.1 mmol m\(^{-2}\) d\(^{-1}\) of nitrate was found to enter the lagoon through tidal pumping of groundwater source using a radon mass balance model. An independent approach measuring the changes in nutrient additions during slack water isolation periods on the reef implied additions of 5.4 mmol m\(^{-2}\) d\(^{-1}\) of nitrate for the same period. The groundwater-derived nutrient addition had a clear influence on daytime NEC and NEP. My second data chapter demonstrates the use of an automated system for measuring carbonate chemistry dynamics and the related metabolism. The system measured total alkalinity (30 minute intervals), pH (10 minute intervals) and the partial pressure of carbon dioxide (1 minute intervals). High frequency sampling allowed for detailed data analysis of the optimum sampling interval to best capture NEC and NEP. Differences of up to 12 and 30% were revealed for NEC and NEP respectively depending on time steps used for calculations. High resolution data also allowed for data integration approaches to be examined with variation of 2 to 7% for NEC and 1 to 3% for NEP. NEC was found to be positively correlated with PAR and NO\(_x\). My third data chapter investigated differences between Eulerian...
and Lagrangian approaches to estimate NEP and NEC, and the influence of a mass bleaching event on metabolic rates at Lizard Island. Differences in methods resulted in varying NEC and NEP rates between 31.6 to 137.7 mmol m\(^{-2}\) d\(^{-1}\) (NEC) and -253.1 to 50.5 mmol m\(^{-2}\) d\(^{-1}\) (NEP). Using the most robust assumptions (paired end members and 2 current meters) rates of 44.3 (NEC) and -4.5 (NEP) mmol m\(^{-2}\) d\(^{-1}\) were determined for the system. Matching my assumptions to best replicate previous work, I observed a decline in NEC of ~45% from 2008/9 to 2016 which is consistent with a coral cover decline from 8.3/7.1% to 2.6% following a bleaching event in 2015. Overall, this thesis investigates the drivers of coral reef ecosystem metabolism and the ability of the different approaches and analytical methods to quantify changes being experienced. It showed that subtle differences in analytical methods, sampling approaches and data interpretation techniques can cause important variation when comparing various studies. Comparisons among different investigations required to build long-term datasets can only be made after reconciling these differences.
Statement of Contribution


I performed fieldwork, analysed the data and wrote the original manuscript. My co-author supported design, data analysis, editing, fieldwork and materials


I co-designed the project, performed fieldwork, analysed the data and wrote the original manuscript. My co-authors supported design, editing, field work and materials


I co-designed the project, performed fieldwork, analysed the data and wrote the original manuscript. My co-authors supported design, editing, field work and materials

Principal Supervisor name: Professor Isaac Santos

Signature and Date: 10/08/2018
During my PhD, I also contributed to field work, data analysis and editing of the following publications:


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Chapter 1

General Introduction
Coral reefs are the most diverse ecosystem on the planet (Hoegh-Guldberg et al., 2007; Pandolfi et al., 2011). They offer coastal protection, building materials, various fisheries, new chemical compounds, and tourism industries to neighbouring communities, supporting more than 450 million people from 109 countries (Moberg and Folke, 1999). These services are already being impacted by physical, biological and chemical processes such as storms, bleaching, eutrophication, crown of thorns starfish outbreaks and ocean acidification (Andersson and Mackenzie, 2012). With the current decline in ecosystem health, reef communities will become less diverse and resilient, with corals becoming rare and reef structures that can’t be maintained by declining calcification rates (Hoegh-Guldberg et al., 2007). In the future, ocean acidification (OA) and global warming are likely to be the leading causes of reef degradation (Hoegh-Guldberg et al., 2007).

1.1 Ocean Acidification and Climate Change

Atmospheric CO₂ reacts with the surface of seawater to form carbonic acid represented by the equation in figure 1.1. Anthropogenic activities raising atmospheric CO₂ are resulting in ocean acidification (OA) by elevating this CO₂ dissociation. Carbonic acid reduces the availability of carbonate for calcification through the disassociation of bicarbonate and the removal of carbonate from the water column by free protons as shown in Figure 1.1 (Hoegh Guldberg et al., 2007).
Figure 1.1. Chemical changes of CO$_2$ entering the ocean and removing carbonate impacting the rates that corals can calcify (Hoegh-Guldberg et al., 2007)

Dissolution of the reef structure occurs when the aragonite saturation state ($\Omega_{\text{aragonite}}$) of the surrounding water is under-saturated, resulting in a breakdown of the coral’s CaCO$_3$ skeleton (Andersson and Mackenzie, 2012). The $\Omega_{\text{aragonite}}$ is the thermodynamic potential of CaCO$_3$ to form or dissolve. Aragonite is more soluble than calcite and is, therefore, more readily available for coral skeleton growth. Dissolution diminishes coral skeleton density and thereby the ability of the reef structure to absorb wave energy and storm damage, making reefs more susceptible to degradation (Hoegh-Guldberg et al., 2007). The long-term decline in pH associated with OA and rising seawater temperatures from ocean warming are increasing the vulnerability of coral reefs.
The global rise in oceanic CO₂ concentrations and related ocean acidification may be enhanced in coral reefs relative to the open ocean due to local biogeochemical processes, such as eutrophication (Cyronak et al., 2014). Overlapping long and short term global and local drivers of coral reef pH are expected to drive coral degradation and decrease resilience to OA. The pH level in lagoonal coral reef systems typically have large diel variation that encompasses the range of seasonal change (Gray et al., 2012). At Heron Island in the Great Barrier Reef (GBR), pH varies up to 0.75 units in a day, surpassing the IPCC predicted decline by the end of 2100 (Santos et al., 2011). High temporal resolution measurements of coral reef metabolism need to be obtained to quantify changes of this magnitude.

Ice cores from the Antarctic dating back 740,000 years indicate that without anthropogenic inputs, the global climate would have remained stable well into the future (Augustin et al., 2004). In the previous 4.5 Myr, a maximum atmospheric CO₂ concentration of 390 ppm and a minimum of 280 ppm was found from long term ice cores with temperatures at peak CO₂ higher than at the current value of ~ 400. (Pagani et al., 2009). These periods of increased CO₂ and temperature did not lower the \( \Omega_{\text{aragonite}} \) as they currently do due to higher concentrations of calcium and/or total alkalinity (TA) (Gattuso and Hansson, 2011). Achieving these higher levels calcium and TA in oceanic waters required to compensate for the rapid increase in atmospheric CO₂ would take millennia of rock weathering (Kump et al., 2009). Historically speaking, there are no previous cases over a geological scale that parallel the rate or magnitude of current changes taking place due to the speed of the CO₂ increase and the ability of the organisms to adapt and endure those changes (Hönisch et al., 2012). Changes in \( p\text{CO}_2 \), temperature, and \( \Omega_{\text{aragonite}} \) are currently being experienced quicker than organisms can adapt (Hoegh-Guldberg et al., 2007).
Currently, the rate of CaCO₃ dissolution via ocean acidification will not buffer the negative effects of increasing CO₂ on marine calcifiers on a large scale (Andersson and Mackenzie, 2012). Small-scale experiments on sediment dissolution in coral reefs indicate that dissolution rates of CaCO₃ had a net increase in alkalinity into the water column of between 5.1 – 8.8 mmol m⁻² d⁻¹ allowing for partial buffering of shallow coral reef ecosystems on a localised scale (Cyronak et al., 2013). Inputs of alkalinity from adjacent tropical ecosystems such as mangroves may also locally buffer coral reefs against ocean acidification (Sippo et al., 2016). As coral reefs degrade, macroalgae are expected to colonise, which draw down CO₂, potentially offsetting some of the effects of OA at a local scale within reefs with long residence times (Anthony et al., 2011). Short-term buffering from CaCO₃ dissolution from sediments, external inputs, and/or higher macroalgae/seagrass cover increasing the Ωaragonite around certain reefs may allow for higher survival of calcifiers until longer-term mechanisms such as seafloor carbonate neutralisation and terrestrial carbon neutralisation influence the water chemistry significantly. Global anthropogenic CO₂ inputs are likely to overwhelm these local buffers over the long term.

Several previous studies used seawater Ωaragonite to predict when coral reefs would shift from a state of net accretion of CaCO₃ to a state of net dissolution. The calcium carbonate saturation state (Ωaragonite) is determined by the product of the concentrations of [Ca²⁺] and [CO₃²⁻] divided by the stoichiometric solubility product (K*sp) (Andersson et al., 2003). Ωaragonite has been predicted to be less than 3 for 50% – 70% of the time when CO₂ = 500 ppm and 2.5 when CO₂ = 655 ppm allowing for a projection using metabolism data from a reef to determine the tipping point from net accretion to net dissolution (Gray et al., 2012). The exact value of CO₂ in the atmosphere for the tipping point is most likely site specific and dependent on the organisms making up that reef structure. CO₂ concentrations of 480 ppm (Hoegh-Guldberg et al.,
(2007) to 560-840 ppm (Guinotte and Fabry, 2008) have been put forth as tipping points from a state of net calcification to net dissolution. Calcification will still occur after the reef’s tipping point has been reached, though rates will be lower than the rates of dissolution, leading to a decline of the reef structure (Shamberger et al., 2011).

1.2 Coral Bleaching

The recent massive bleaching event on the Great Barrier Reef illustrates some of the challenges faced by coral reefs (Hughes et al., 2017b). Climate change is resulting in increasing frequency and severity of coral bleaching events (Hughes et al., 2017a). The response of a specific reef to bleaching is dependent on the cause, duration, and severity of the event. For instance, if a reef bleached due to eutrophication, higher levels of organic productivity are likely to occur due to excess nutrients available for uptake by marine algae in comparison to an oligotrophic reef that bleaches due to higher prolonged temperatures (Kayanne et al., 2005). In the recent GBR bleaching event, there was no difference in bleaching severity across different water qualities or marine park protection status, highlighting that even reefs under maximum marine park protection are also threatened by climate change. Reefs that have suffered prior bleaching showed no difference in their susceptibility to repeated bleaching, indicating either that there is no build-up of resistance to bleaching from prior events or that the resistance threshold was exceeded during this event (Hughes et al., 2017b).

1.3 Coral Reef Metabolism

Net ecosystem calcification (NEC) describes the balance between sources of calcium carbonate (biogenic calcification and inorganic calcium carbonate precipitation) and sinks (dissolution) while net ecosystem production (NEP) is the balance between photosynthesis and
respiration in a coral reef. Primary production is the generation of new organic materials by the process of photosynthesis and productivity is the flux of nutrients from the surrounding environment into living organisms for the generation of biomass. The metabolic processes of NEC and NEP impact water chemistry with changes in NEP influencing $\Omega_{\text{aragonite}}$ via respiration and production releasing or up taking CO$_2$ (Shaw et al., 2012). This change in CO$_2$ alters the pH of the water and thereby $\Omega_{\text{aragonite}}$ on time scales of hours to seasons. $\Omega_{\text{aragonite}}$ is reduced by declining pH and elevated from increasing temperatures, with pH being a larger driver than temperature (Gattuso et al., 1999). Night time coral reef dissolution is already evident in many systems due to respiration driving down the pH past the solubility point of different CaCO$_3$ minerals. The exact makeup of the calcifiers skeletal body changes the solubility of the structure. Organisms that produce high Mg-calcite dissolve more readily than those with aragonite (Yamamoto et al., 2012). Dissolution also occurs via bioerosion from organisms such as chitons, sea urchins, fish, and boring microflora, either increasing dissolution directly or indirectly by exposing a more readily dissolved material to the water column (Andersson and Mackenzie, 2012). Calcareous algae may be especially susceptible to OA with their external skeleton, whereas corals have a calicoblastic layer protecting their skeletal structure (Gattuso et al., 1999). This layer can potentially modify seawater chemical properties and help to partially buffer seawater pH (Gattuso et al., 1999).

Coral reef metabolic rates vary significantly around the world (Table 1.1). To assess ecosystem-scale metabolism, three main approaches are used: slack water, Lagrangian, and Eulerian methods. These three approaches rely on quantifying changes in the carbonate chemistry of seawater overlying benthic communities. The slack water approach utilises the natural low-tide isolation occurring in some reef flats and lagoons to constrain the water body,
allowing for the determination of seawater carbonate chemistry changes. This approach can only be performed in areas where isolation occurs for long enough to observe the changes in the water chemistry such as Heron Island (McMahon et al., 2013), One Tree Island (Silverman et al., 2012) and Lady Elliot Island (Shaw et al., 2012) in the Great Barrier Reef. The Lagrangian method tracks a parcel of water across the reef flat utilising drifters (or another form of water current measurement) and compares the difference from the initial water sample to the end point water sample. This method relies on a consistent current that is unmixed across the reef flat such as at One Tree Island (Shaw et al., 2014). The Eulerian approach is similar to the Langragian approach in utilising a consistent current across a reef flat. However, the Eulerian approach tracks all samples to the same point on the reef with the current speeds recorded to determine the time it takes to travel from the oceanic endmember (starting point) to the point of interest. This approach can be used in most places that have consistent currents such as Lizard Island (Silverman et al., 2014). Differences in metabolic rates estimated among methods may be significant so care must be used in comparing studies (Shaw et al., 2014).

Understanding the metabolism and predicting what may happen to coral reefs in the future often relies on relationships between NEC and the seawater $\Omega_{\text{aragonite}}$. With this approach, the effects of rising atmospheric $\text{CO}_2$ on seawater pH can be used to determine when NEC will become negative (i.e., net dissolution) at a given $\Omega_{\text{aragonite}}$. Recently, there is debate over if this is the best method for forecasting the future of a reef due to the concurrent changes in NEP and NEC affecting the seawater $\Omega_{\text{aragonite}}$ (Cyronak et al., 2018; Cyronak et al., 2015). Comparison of TA–DIC slope regressions reflects the balance of NEP and NEC indicating the state of coral reef health (Cyronak et al., 2018). This method gives a baseline for future studies to investigate potential differences in reef composition. By utilising reef metabolism data, insight into the
The trophic balance between calcifying organisms and non-calcifying organisms on a reef can be obtained (Silverman et al., 2012).

Table 1.1: NEC rates of worldwide metabolism studies

<table>
<thead>
<tr>
<th>Location/Reference</th>
<th>Method/Environment/Other Remark</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biosphere 2 (modern conditions)</td>
<td></td>
<td>Location</td>
</tr>
</tbody>
</table>
1.4 Nutrient Dynamics

In addition to $\Omega_{\text{aragonite}}$, nutrient concentrations are also considered a major driver of NEC and NEP in coral reefs (Atkinson et al., 1994). Nutrient concentrations in coral reefs are often very low, creating oligotrophic conditions and effective recycling of nutrients to maintain high productivity (Duarte and Cebrian, 1996). Reefs that are limited by nutrients have limited growth, effectively reducing metabolism relative to its potential (Koop et al., 2001). Coral reef nutrient studies have focused mainly on sections of coastline that are heavily impacted with anthropogenic derived nutrient additions via riverine pathways (Fabricius, 2005). Coral reefs in pristine condition are often isolated from anthropogenic inputs (Dubinsky and Stambler, 1996). While a series of nutrient addition experiments have been performed (Koop et al., 2001), natural nutrient additions to coral reefs have largely been unstudied and little information is available on how nutrient dynamics in coral reef may influence key metabolic processes such as NEC and NEP. Nutrient additions, when not in excess, lead to increases in coral’s zooxanthellae density, protein, and Chl$\alpha$ levels and a masking of the effects of decreasing $\Omega_{\text{aragonite}}$ and NEC (Chauvin et al., 2011).

1.5 Future Management

Future management of coral reefs needs to take into account the complex effects that climate change and pollution have on the structure and function of coral reefs. Without proper reef management, nearly all coral reef ecosystem services are expected to decline (Rogers et al., 2015). The rezoning of the GBR in 2004 offered some benefits to fisheries but were largely ineffectual against large-scale impacts such as climate change (Hughes et al., 2015). Current reef management uses historical data to set goals, halt declines and restore baseline conditions.
Looking at the state of the ecosystem and the services it provides as a function of future conditions may be a better management tool for impacts that are likely to become more severe with a changing climate (Rogers et al., 2015).

1.6 Thesis Aims, Hypothesis, and Structure

Climate change, ocean acidification and nutrient additions are significant threats to coral reefs and little research has been done in situ to assess the effects these stressors have on NEC and NEP. Episodic events such as cyclones and severe bleaching may also have major effects on coral reef metabolism. These events are likely to increase in frequency with rising temperatures and climate change. The overall aim was to investigate whether coral reef NEC and NEP was influenced by diel cycles, $\Omega_{\text{aragonite}}$, nutrient dynamics, and bleaching. A further aim was to evaluate if disparity in methods/analytical approaches can add unintentional bias. The next three data chapters make up the bulk of this thesis and focus on these specific issues:

1. Does bird derived guano drive NEC and NEP in lagoons on Heron Island?

Manipulative experiments have shown that nutrient additions modify coral reef metabolic rates (Koop et al., 2001), but little is known about how natural nutrient fluctuations may alter NEC and NEP. In this chapter, I quantify natural localised nutrient addition into a coral reef derived from bird guano and groundwater pathways. I then used the detailed nutrient observations to investigate how nitrogen inputs may enhance NEC and NEP. This chapter has been published in Journal of Geophysical Research – Oceans.

2. Can we improve interpretation of coral reef NEC and NEP drivers by using new, automated techniques that allow high resolution observations?
Quantifying NEC and NEP is time consuming and often results in small datasets. In this chapter, I demonstrate how high-resolution, automated seawater carbon chemistry observations can improve accuracy on metabolic studies while reducing labour, and how it can provide additional insight into drivers of NEC and NEP. I automated a VINDTA 3C to simultaneously measure alkalinity and dissolved inorganic carbon to quantify NEC and NEP at short intervals, along with other automated sensors to continuously measure the carbon parameters of a coral reef lagoon to establish rates of metabolic processes. This chapter has been published in Estuarine, Coastal and Shelf Science.

3. How does climate change and significant bleaching events impact NEC and NEP in coral reefs?

Few in situ studies have been done on the post-impact effects of climate change and significant disturbances on coral reef NEC and NEP (Kayanne et al., 2005). In this chapter, I hypothesize that substantial disturbances due to climatic changes will significantly alter the metabolic rates of coral reefs. I rely on historical pre-bleaching data and new post-bleaching data to determine the metabolic changes in a coral reef after a significant disturbance from severe bleaching on NEC and NEP drivers. This chapter was conducted at Lizard Island, in the northern GBR, one of the most impacted sites during the 2015 and 2016 mass bleaching event.
Chapter 2

Nitrogen enrichment and speciation in a coral reef lagoon driven by groundwater inputs of bird guano

Abstract - While the influence of river inputs on coral reef biogeochemistry has been investigated, there is limited information on submarine groundwater discharge (SGD) nutrient fluxes. Here, we reveal significant saline groundwater-derived nutrient inputs from bird guano entering a coral reef lagoon off Heron Island in the Great Barrier Reef, Australia. We used multiple experimental approaches including groundwater sampling, beach face transects, and detailed time series observations to assess the dynamics and speciation of groundwater nutrients as they travel across the island and discharge into the coral reef lagoon. Nitrogen speciation shifted from nitrate-dominated groundwater (>90% of total dissolved nitrogen) to a coral reef lagoon dominated by dissolved organic nitrogen (DON; ~86%). There was a minimum input of nitrate of 2.1 mmol m$^{-2}$ d$^{-1}$ into the lagoon from tidally-driven submarine groundwater discharge estimated from a radon mass balance model. An independent approach based on the enrichment of dissolved nutrients during isolation at low tide implied nitrate fluxes of 5.4 mmol m$^{-2}$ d$^{-1}$. A correlation was observed between nitrate and daytime net ecosystem production and calcification. We suggest that groundwater nutrients derived from bird guano may offer a significant addition to oligotrophic coral reef lagoons and drive ecosystem productivity and the coastal carbon cycle near Heron Island. The large input of groundwater nutrients in Heron Island may serve as a natural ecological analogue to other coral reefs subject to large nutrient inputs from anthropogenic sources.
2.1. Introduction

Coral reefs support industries such as fisheries, building materials and tourism while providing protection for coastal communities [Moberg and Folke, 1999]. A changing climate, land use practices and increasing CO₂ are having detrimental impacts on coral reefs around the world [Hoegh-Guldberg et al., 2007]. Understanding nutrient sources and dynamics within coral reefs is important for proper management and understanding reef resilience under climate change [Rogers et al., 2015]. Coral reefs are considered oligotrophic systems that rely on very tight internal recycling for supply of nutrients required for growth. As a result, productivity in most reefs is limited by low dissolved nutrient concentrations [Koop et al., 2001]. With increasing anthropogenic nutrient inputs, coral reef CaCO₃ frameworks may be gradually replaced by algae [Amato et al., 2016; Lapointe, 1997]. Therefore, nutrient additions to coral reefs may exacerbate the effects of increasing atmospheric CO₂ and ocean acidification driving lower calcification rates [Hoegh-Guldberg et al., 2007].

Excessive inputs of nutrients from sources such as raw and treated sewage and fertilizers can cause eutrophication which stimulates algal production that outcompetes corals and may favor some coral species while damaging others [Dubinsky and Stambler, 1996]. Pristine reefs gather external nutrients from surrounding areas through oceanic currents, along with river and groundwater inputs and atmospheric deposition [Hernández-Terrones et al., 2011; Lowe and Falter, 2015]. While extensive research has been done on the influence of terrestrial nutrient runoff and river inputs on reefs [Brodie et al., 2016; Fabricius, 2005] as well as oceanic inputs [Falter et al., 2016; Lowe and Falter, 2015], there is limited information on groundwater-derived nutrient inputs into coral reefs.
Submarine groundwater discharge (SGD) can supply large loads of nutrients into the coastal ocean and coral reefs [Moore, 2006]. Pioneer work suggested a relationship between eutrophication in Caribbean coral reefs and inputs of nitrogen-enriched SGD [D'Elia et al., 1981; Lewis, 1987]. A number of recent investigations further revealed significant fresh groundwater inputs in coral reef systems with adjacent anthropogenic forcing such as the Yucatan peninsula in Mexico [Null et al., 2014], Shiraho Reef in Okinawa, Japan (Blanco et al., 2011), Hawaiian coastal reefs [Bishop et al., 2017; Swarzenski et al., 2017] and Raratonga Lagoon, Cook Islands [Tait et al., 2014]. Groundwater is often enriched in dissolved nitrogen relative to phosphorus and as a result has high N/P ratios [Reading et al., 2017; Santos et al., 2013; Slomp and Van Cappellen, 2004]. Since coral reef waters are often N-limited [Koop et al., 2001], groundwater inputs with high N/P can drive the ecology and metabolism of coral reefs [Lewis, 1987].

Using naturally occurring tracers such as radon ($^{222}$Rn) has become more commonplace to detect SGD inputs into surface waters. Radon is part of the uranium-238 decay chain that is present in most sediments. With a short half-life of 3.8 days, radon is an ideal tracer for SGD into surface waters [Burnett et al., 2001]. Radon can be released to surface water via both fresh groundwater discharge or seawater recirculation in sediments. In tidal beach environments, seawater enters the sediments at high tide, mixes with the groundwater and acquires a $^{222}$Rn signal. At low tide, this recirculated seawater then flushes back out into the coastal ocean via tidal pumping [Li et al., 1999]. Water that is in contact with sediment for several hours will often acquire a detectable $^{222}$Rn signal allowing for tidal pumping to be quantified. $^{222}$Rn concentrations are often measured using commercially available continuous automated monitors [Burnett et al., 2001; Dulaiova et al., 2010] that are ideal for observations over tidal time scales. In many cases, radon-derived SGD may be related primarily to seawater recirculation in
sediments rather than fresh SGD [Santos et al., 2010]. Seawater recirculation in sediments is
driven by mechanisms that include wave setup, bio irrigation, wave pumping, tidal pumping, and
convection [Santos et al., 2012b]. A large influx of seawater recirculation in sediments dilutes
fresh groundwater but can still release groundwater tracers such as radon as well as dissolved
nutrients [Slomp and Van Cappellen, 2004].

Santos et al. [2010] reported 24-h radon time series observations and nutrient
concentrations in groundwater samples from Heron Island. In this paper, we build on this
previous investigation by reporting a much larger dataset including nutrient time series
observations in both surface water and groundwaters and assessing whether those nutrients drive
a biogeochemical response at the ecosystem scale in terms of coral reef photosynthesis and
calcification. Overall, the work by Santos et al. [2010] focused on a bottom up approach (i.e.,
estimated fluxes from the seabed), while the current work focuses on top down evidence (i.e., a
response of SGD in the water column). Here, we hypothesize that dissolved nutrient dynamics in
a coral reef lagoon (Heron Island, Great Barrier Reef) is driven by the input of tidally-driven
recirculated groundwater from the adjacent island. Because of large bird populations on the small
island, birds are a major source of nutrients to the shallow groundwater [Schmidt et al., 2004].
Our objectives are to (1) use radon to estimate tidally-driven groundwater-derived nutrient inputs
to the reef lagoon, (2) use an independent approach based on nutrient variability in the lagoon to
validate the radon approach, and (3) assess whether groundwater-derived nutrients contribute to
local photosynthesis and calcification rates.
2.2. Material and Methods

2.2.1. Study site. The Great Barrier Reef (GBR) in Australia is a UNESCO world heritage site protected by the Great Barrier Reef Marine Park Authority (GBRMPA). The GBR stretches for over 3000 km along Australia’s northeastern coastline and has thousands of small coral cay islands populated by birds. Within the southern section lies Heron Island, a coral cay approximately 800-m long by 300-m wide surrounded by a 27 km² shallow coral reef lagoon. The island rises a maximum of 3.6 m above sea level. Previous research [McMahon et al., 2013] calculated net ecosystem calcification (NEC) and net ecosystem production (NEP) for the Heron Island lagoon using a tidal isolation approach. The surrounding lagoon isolates from oceanic waters during low tides. The lagoon level is higher than the open ocean effectively preventing any mixing between lagoon and oceanic water for 2-4 hours during each low tide.

The Island soils and surrounding lagoon sediments have a median grain size of 800 µm with high permeability ($\sim 10^{-10}$ m²) and porosities ($\sim$45%) [Santos et al., 2010; Wild et al., 2004]. Groundwater entry into the lagoon is visible at low tide at the beach face where seepage forms erosive channels running into the lagoon. The Island has a resort and research station on its western side. All other areas are a national park supporting large populations of noddy turns (up to 40,000 nests) and shearwaters (up to 30,000 nests), along with lower numbers of various other birds [Hemson, 2015]. These birds are known to drive extremely high concentrations of nitrate in shallow island groundwaters [Santos et al., 2010; Schmidt et al., 2004], but it remains unclear whether and how those nutrients return to the ocean via groundwater pathways.
2.2.2. Field sampling. Sampling was carried out on two occasions between the 7th of October and the 21st of October 2011, and from 4th April to 1st May 2012. Several experiments were performed during these two periods: (1) bores were sampled across the island under bird nesting areas to determine the composition of groundwater influenced by bird droppings; (2) tidal time series observations were made in two wells located near the high tide mark to assess the underground mixing of island groundwater and lagoon water over tidal cycles; (3) beach transects were sampled from small groundwater erosive channels that are observed on the beach at low tide across the lagoon to characterize the composition of groundwater seeping into the lagoon; (4) a lagoon low tide time series was conducted to assess the accumulation of groundwater-derived nutrients into the lagoon, and whether these nutrients drive the metabolism of the lagoon over spring-neap cycles; and (5) finally, detailed 24 hour time series observations were made to develop a radon mass balance model to estimate groundwater-derived nutrient fluxes into the lagoon.

Established groundwater bores [Chen and Krol, 1997] were used for assessing nutrient concentrations in island groundwater. While all bores available were sampled once to determine the spatial variability of nutrients, two bores were selected for 24-hour time series observations to assess mixing between lagoon and island groundwater on tidal time scales. All bores were first purged at least 3 times their water volume before samples were taken using a peristaltic pump. Samples for DO, pH and temperature were taken immediately with calibrated Hach probes. Nutrients and radon samples were taken and stored for analyses back in the laboratory.

Beach transects were sampled at low tide along a transect starting at the exit point of groundwater flow channels out into the lagoon. Sampling was conducted during both 2011 and 2012 trips during day and nighttime low tides. Sampling started at -0.5 m from the low tide water
line (i.e., at the exit point of a seepage channel) and progressed to 0.5 m, 1 m, 2 m, 4 m, 8.5 m and 12 m from low tide mark into the lagoon encompassing different stages of groundwater mixing with the lagoon water. Samples were measured using a Hach luminescent dissolved oxygen (DO) probe, Metrohm Electrode Plus pH probe, and water samples were collected for nutrients (NO₃, NH₄, PO₄, TDN, and TDP). Radon could not be collected because only small sample volumes were available.

Low tide sampling and 24-hour time series observations were carried out during each trip. The sampling site was approximately 10 m off the beach in front of the Heron Island Research Station. For low tide sampling, samples were collected approximately 1-2 hours each side of each low tide while natural lagoon isolation occurred preventing mixing of oceanic water which simplifies the interpretation of results. Net ecosystem calcification (NEC) and net ecosystem production (NEP) rates were previously published in McMahon et al. [2013]. NEC was calculated from low tide time series data using the alkalinity anomaly technique [Chisholm and Gattuso, 1991]. NEP was calculated using the change in DIC while taking into account NEC using the low tide time series data [Silverman et al., 2012]. Lagoon time series observations (1 hour time steps) covering at least 24 hours were made during each trip to assess surface waters while mixing occurred with oceanic water. The 24 h time series sampling location was the same as the low tide time series [McMahon et al., 2013].

Radon was measured using a Rad7 (Durridge) radon detector. The Rad7 was connected to a showerhead style gas equilibrium device (GED) that extracts the gas from a continuous water stream. The air stream passes through a moisture absorbing compound (Drierite), and back to the Rad7 before being returned to the GED [Burnett et al., 2001]. All nutrient samples were filtered with 0.45 µm cellulose acetate disposable filters and frozen until
analysis. Nutrient samples for NH₄, NOₓ, PO₄, TDN, and TDP were analyzed using a Lachat Flow Injection Analysis unit with uncertainties better than 5% for inorganic nutrients and about 15% for total dissolved nutrients as described in detail elsewhere [Eyre and Ferguson, 2005]. The system was calibrated for low nutrient levels typical of coral reef ecosystems. Dilutions were used for groundwater samples. DON and DOP were estimated as the difference between total dissolved nutrients and inorganic nutrients. NOₓ represents the sum of nitrate and nitrite and is assumed to represent mostly nitrate.

2.2.3. Estimating groundwater discharge rates. A non-steady-state ²²²Rn mass balance was used to estimate groundwater discharge rates (in units of cm³ water m⁻² of lagoon bed per day or just cm d⁻¹) described in detail elsewhere [Burnett and Dulaiova, 2003]. The same radon model and related assumptions have been applied to Heron Island initially by Santos et al. [2010] and later by Cyronak et al. [2014] and O’Reilly et al. [2015] in a greenhouse gas context. In short, the model estimates SGD fluxes (in 1-hour time steps) by assuming that temporal variability in the ²²²Rn inventories is balanced by sources (groundwater, ²²⁶Ra decay and diffusion) and sinks (atmospheric evasion, decay and mixing with low radon offshore water). The SGD flux (cm d⁻¹) was then estimated by dividing the unaccounted for ²²²Rn fluxes by the groundwater endmember concentrations. SGD-derived nutrient fluxes were estimated using the radon-derived SGD flux and the average nutrient concentrations in groundwater. Several assumptions to estimate the nutrient groundwater endmember were used to offer a possible range in groundwater-derived fluxes (see discussion). Uncertainties for each approach were estimated using the uncertainties in the radon-derived SGD rates (~40% as reported in Cyronak et al., [2014]) and the natural variability of nutrient concentrations in each assumed endmember. The
uncertainties of final fluxes were then estimated using error propagation analysis [Sadat-Noori et al., 2016 and references therein].

2.3 Results

2.3.1. Groundwater spatial survey.

A groundwater survey in Heron Island indicated high concentrations of nutrients within the Island. NO$_x$ reached 2173 µmol L$^{-1}$ while PO$_4$ reached a maximum of 6.7 µmol L$^{-1}$ (Table 2.1). Average values of 1539 µmol L$^{-1}$ NOx were obtained for all groundwater samples, with negligible concentrations of NH$_4$ and DON. The DIN:DIP ratios ranged from 186:1 to 971:1 with an average value of 608:1, while TDN:TDP ranged from 174:1 to 1018:1 with an average value of 650:1. Strong spatial variation was present implying localized sources and higher concentrations in the brackish waters just below the shallow freshwater lens (likely recent bird dropping inputs).

2.3.2. Groundwater time series.

A 24-h groundwater time series was conducted at two locations (Figure 2.1). The 2011 bore was located at the high tide mark, and showed strong tidal trends of all parameters with NO$_x$ showing a near 4 fold increase and the ratio of DIN:DIP changing from 27:1 to 80:1 during the tidal cycle (Figure 2.2). The peak in NO$_x$ concentrations was observed about 3 hours after low tide. The 2012 groundwater time series was obtained from a bore located 80 m from the high tide mark. This bore showed similar tidal trends as the 2011 sampling with NO$_x$ and DIN:DIP showing 3-fold increases during the tidal cycle. These results demonstrate active tidally-driven underground mixing between island groundwater and the coral reef lagoon.
Table 2.1. Groundwater nutrient concentrations and N:P ratios in Heron Island. Sample locations are shown in Figure 2.1. All nutrients are in units of µmol L\(^{-1}\). *GW 8 and 9 are averages of time series wells (Figure 2.2) and were excluded from overall groundwater averages.

<table>
<thead>
<tr>
<th></th>
<th>Salinity</th>
<th>NO(_x)</th>
<th>NH(_x)</th>
<th>DON</th>
<th>PO(_x)</th>
<th>DOP</th>
<th>TDN</th>
<th>TDP</th>
<th>DIN:DIP</th>
<th>TDN:TDP</th>
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<tr>
<td>GW1</td>
<td>27</td>
<td>1594</td>
<td>0</td>
<td>0</td>
<td>2.6</td>
<td>0</td>
<td>1594.0</td>
<td>2.6</td>
<td>601.9</td>
<td>598.2</td>
</tr>
<tr>
<td>GW2</td>
<td>27.7</td>
<td>1416.7</td>
<td>2.7</td>
<td>11.5</td>
<td>1.5</td>
<td>0</td>
<td>1430.9</td>
<td>1.5</td>
<td>941.4</td>
<td>952.4</td>
</tr>
<tr>
<td>GW3</td>
<td>12</td>
<td>1488.4</td>
<td>1.5</td>
<td>4.0</td>
<td>0</td>
<td>0</td>
<td>1489.8</td>
<td>4.0</td>
<td>370.6</td>
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<td>GW4</td>
<td>26.5</td>
<td>1419.5</td>
<td>19.8</td>
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<td>0</td>
<td>0</td>
<td>1439.3</td>
<td>2.3</td>
<td>628.5</td>
<td>714.1</td>
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<td>0</td>
<td>1433.2</td>
<td>2.9</td>
<td>478.7</td>
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<td>2.2</td>
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<td>21.7</td>
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<td>828.1</td>
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<td>GW8*</td>
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<td>40.4</td>
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<td>0.9</td>
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<td>GW9*</td>
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<td>1265.0</td>
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<td>Average</td>
<td>19.3</td>
<td>1539.6</td>
<td>0.3</td>
<td>13.4</td>
<td>3.0</td>
<td>0.1</td>
<td>1536.7</td>
<td>2.9</td>
<td>608.0</td>
<td>649.8</td>
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<td>Standard Deviation</td>
<td>2.8</td>
<td>118.3</td>
<td>0.3</td>
<td>4.8</td>
<td>0.5</td>
<td>0.1</td>
<td>117.2</td>
<td>0.6</td>
<td>88.4</td>
<td>97.2</td>
</tr>
</tbody>
</table>

2.3.3. Beach transect.

A transect off the beach out into the lagoon showed that the effect of groundwater dilution as it mixes with nearshore lagoon waters reaches up to 8 m from shore (Figure 2.3). The trends clearly show that NO\(_x\) and PO\(_x\) are being supplied to the lagoon via groundwater seeps along the beach face. Both DON and DOP showed higher concentrations within the lagoon itself than in the groundwater source. There were differences in N:P ratios depending on whether we use TDN:TDP or DIN:DIP ratios. TDN:TDP started from 14:1-18:1 before reaching lagoon levels of 10:1-12:1. DIN:DIP showed higher groundwater levels of 15:1 to 22:1 before mixing to lagoon levels of approximately 5:1. Day and night sampling showed little variation with the exception of DON during 2011. Similar levels of nutrients were found during the 2011 and 2012 surveys implying that tidally-driven groundwater discharge having a small seasonal variability.
**Figure 2.1.** Study site located on the east coast of Australia (A) at the northwestern side of the lagoon (B) with sampling sites located throughout the Island (C). Black circle indicates low tide sampling and lagoon time series samples. Blue circles indicate groundwater bores used for groundwater time series. Yellow circles indicate groundwater bores used for spatial survey.
Figure 2.2. Groundwater time series conducted in 2011 (left) and 2012 (right) showing tidally driven groundwater concentrations of nutrients and N:P ratios. The 2011 bore was located at the high tide mark, while the 2012 bore was located approximately 80 m inland from high tide mark (see Figure 2.1).
Figure 2.3. Beach transect during low tide away from a beach groundwater seepage channel. The first sample (-0.5 m) was collected from a seepage channel, the second sample (0 m) was collected at the low tide mark where the seepage channel first meets the lagoon, and the subsequent samples were collected in the lagoon away from the beach. Nutrient concentrations show gradual mixing of seeping groundwater with lagoon water.
2.3.4. Low tide time series.

About 1 month of sampling lagoon waters during each low tide showed the effects of spring-neap tidal cycles (Figure 2.4). NO$_x$ had a near 2-fold increase during a period of greater tidal ranges with PO$_4$ showing a similar pattern but to a smaller extent. This increase coincided with an increase in the $^{222}$Rn signal being detected at the sampling site. Nutrient levels remained low for the entire sampling periods with an average of 0.8 µmol L$^{-1}$ for NO$_x$, 0.3 µmol L$^{-1}$ for PO$_4$, 0.9 µmol L$^{-1}$ for NH$_4$ and an average DIN:DIP of 5.5:1. Observations of DIC and alkalinity during the same experiment were used to estimate net ecosystem calcification (NEC) and net ecosystem production (NEP) as reported in McMahon et al. (2013).

2.3.5. 24-hour lagoon time series.

Detailed (i.e., 1-hour time steps) time series observations were made in the lagoon for about 25 hours during both trips (Figure 2.5). $^{222}$Rn showed a strong tidal trend occurring with higher $^{222}$Rn values during low tide and oceanic water dilution starting at mid tides. NO$_x$ showed a two-fold increase during a tidal cycle which tracked a similar two fold increase in $^{222}$Rn. DIN:DIP showed an increase from an average of 3.6:1 to an average of 7.5:1 while TDN:TDP showed an increase from 7.5:1 to 10:1. Slight variations between the two years was observed for all other parameters. A positive correlation was observed between $^{222}$Rn and NO$_x$ during the 2011 observations (Figure 2.6) with a higher $^{222}$Rn signal corresponding to a higher NO$_x$ concentration. Similarly, DIN:DIP and TDN:TDP corresponded in a comparable pattern showing higher ratios at higher $^{222}$Rn signals. In 2012, the correlations between $^{222}$Rn and nutrient concentrations in the lagoon were usually not significant (Figure 2.6).
Figure 2.4. Month-long low tide time series observations in the lagoon while isolated from oceanic waters. Radon data (from Cyronak et al., 2014) was only obtained for a 15-day period during low tide sampling. A 2-fold variation in NO\textsubscript{x} and PO\textsubscript{4} and a 4-fold change in NH\textsubscript{4} and DIN:DIP were observed. Observations of dissolved inorganic carbon and alkalinity during the same experiment are reported in McMahon et al. (2013) to estimate net ecosystem productivity and calcification.
Figure 2.5. Time series of lagoon water during 2011 (black circles) and 2012 (white squares). Low tide has enhanced $^{222}$Rn which in turn drives higher NO$_x$, NH$_4$, DIN:DIP and TDN:TDP. A 3-point smoothing was applied to the dataset. Vertical shading indicates nighttime. The 2011
time series lasted 27 hours and started at 12:00 on the 09/10/2011, while the 2012 time series lasted 28 hours starting at 14:00 11/10/2012.

2.3.6. Groundwater-derived fluxes into the lagoon. Groundwater-derived nutrient fluxes into the lagoon were calculated by multiplying the average radon-derived SGD rates of 26.4 cm/d [Cyronak et al., 2014] by the groundwater nutrient endmember concentration (Table 2.2). We used three contrasting groundwater endmember assumptions to provide a potential range in groundwater-derived fluxes: (1) The average in concentrations from island groundwater wells excluding two beach face wells with low concentrations (i.e., the bore time series wells 8 and 9 in Table 2.1). This provides a maximum endmember concentration as these samples overlook any nutrient transformations along the groundwater transport pathway; (2) An average of concentrations in beach groundwater wells (i.e., wells 8 and 9) located near the high tide mark. This endmember represents data from a location that is clearly exchanging with the lagoon; and (3) an average of concentrations in seeping groundwater collected from a seepage channel, i.e., average from all samples from first sample point -0.5 m in Figure 2.3. This final approach would represent the lowest possible endmember concentration because the samples were clearly mixed with lagoon water and overlooks any high concentration seepage that may be occurring beyond the beach face. The results of groundwater flux estimates using these assumptions are summarized in Table 2.2. As expected, large differences in estimated fluxes were observed using the three different endmember assumptions with a 3- to 100-fold variability in nutrient fluxes depending on the groundwater endmember chosen.
Table 2.2. SGD-derived nutrient fluxes into the Heron Island lagoon assuming SGD rates of 26.4±10.3 cm/day as established from a radon mass balance (Cyronak et al., 2014) and three different groundwater endmember assumptions (see text). Uncertainties are reported using error propagation analysis.

<table>
<thead>
<tr>
<th>Island Groundwater (µmol L⁻¹)</th>
<th>High tide mark bore (µmol L⁻¹)</th>
<th>Beach seep (µmol L⁻¹)</th>
<th>SGD Flux Island Groundwater (mmol m⁻² d⁻¹)</th>
<th>SGD Flux High tide mark bore (mmol m⁻² d⁻¹)</th>
<th>SGD Flux Beach seep (mmol m⁻² d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOx 1536±118</td>
<td>40.4±22.2</td>
<td>8.1±0.8</td>
<td>405.5±161.2</td>
<td>10.7±7.2</td>
<td>2.1±0.9</td>
</tr>
<tr>
<td>NH₄ 0.3±0.3</td>
<td>0.0±0.1</td>
<td>0.6±0.2</td>
<td>0.1±0.1</td>
<td>0.0±0.0</td>
<td>0.2±0.1</td>
</tr>
<tr>
<td>DON 13.4±4.8</td>
<td>5.3±1.8</td>
<td>6.5±1.8</td>
<td>3.5±1.9</td>
<td>1.4±0.7</td>
<td>1.7±0.8</td>
</tr>
<tr>
<td>PO₄ 3.0±0.5</td>
<td>0.9±0.1</td>
<td>0.5±0.1</td>
<td>0.8±0.3</td>
<td>0.2±0.1</td>
<td>0.1±0.1</td>
</tr>
<tr>
<td>DOP 0.1±0.1</td>
<td>0.3±0.1</td>
<td>0.5±0.2</td>
<td>0.0±0.0</td>
<td>0.1±0.0</td>
<td>0.1±0.1</td>
</tr>
<tr>
<td>TDN 1539±117</td>
<td>44.4±20.8</td>
<td>15.2±2.7</td>
<td>406.7±161.5</td>
<td>11.7±7.1</td>
<td>4.0±1.7</td>
</tr>
<tr>
<td>TDP 2.9±0.6</td>
<td>1.2±0.1</td>
<td>1.0±0.1</td>
<td>0.8±0.3</td>
<td>0.3±0.1</td>
<td>0.3±0.1</td>
</tr>
</tbody>
</table>

2.4. Discussion

2.4.1. Groundwater-derived nutrient fluxes. Groundwater, which is often overlooked as a significant source of nutrients within coral reefs systems, has been shown to be a source of nutrients from large land masses in previous studies. For example, volcanic oceanic islands have been considered SGD hotspots [Johnson et al., 2008; Kim et al., 2013; Moosdorf et al., 2015]. Small sedimentary islands with coarse sediments such as Heron Island can also have high SGD rates and be significantly influenced by migratory birds that use them for nesting. During the shearwater breeding season going from October to April, foraging is carried out by nesting parents up to 1100 km away from Heron Island bringing nutrients in the form of guano back to the island [McDuie et al., 2015]. The seabirds deposit about 1000 kg N ha⁻¹ year⁻¹ on Heron Island which can then make its way into groundwater and eventually to the lagoon through SGD.
This nitrogen deposition rate is higher than some of the most fertilized crops on Earth [Schmidt et al., 2010].

Figure 2.6. $^{222}$Rn concentrations versus nutrients during lagoon time series observations for 2011 (left) and 2012 (right).
Preliminary groundwater observations in Heron Island showed extremely high nutrient concentrations similar to the ones described here [Santos et al., 2010]. The spatial differences between the sampled groundwater bores can be partly attributed to Heron Island’s groundwater structure. Heron Island has a thin fresh groundwater lens overlying saline groundwater [Chen and Krol, 1997]. The fresh groundwater floats on saline groundwater and does not effectively exchange with the lagoon. As a result, SGD off Heron Island is dominated by tidally-driven recirculated seawater [Santos et al., 2010]. The fast seawater infiltration and the short residence time within the Island are apparent from dynamic $^{222}\text{Rn}$ and nutrient concentrations in samples from the high tide mark well (Figure 2.2) revealing mixing of seawater with groundwater over tidal time scales (Figures 2.2 and 2.7). Similar mixing in nearshore groundwater has been shown in the highly permeable karstic Yucatan Peninsula in Mexico [Null et al., 2014].

Initial estimates by Santos et al. [2010] at Heron Island revealed nitrate fluxes of $>7.9$ mmol m$^{-2}$ d$^{-1}$ using a non-steady state radon mass balance with a SGD rates of $>40$ cm/d. Using our lower radon-derived SGD rate of 26.4 cm/d, SGD-derived nitrate fluxes varied from 2.1 mmol m$^{-2}$ d$^{-1}$ (using beach groundwater seep as the endmember) to 406.5 mmol m$^{-2}$ d$^{-1}$ (using inland groundwater bores as the endmember) depending on endmember assumptions (Table 2.2). Since the beach face is clearly the exit point of island groundwater, we assume that the beach face endmember best represents groundwater-derived nutrient fluxes entering the lagoon after any transformations within the island. Choosing an endmember at the exit point of groundwater has been suggested by recent investigators [Rocha et al., 2016; Sadat-Noori et al., 2016] as an approach that provides the minimum possible SGD and prevents a potentially incorrect interpretation that SGD is important. Using this assumption, our estimated SGD fluxes (DIN $=2.1$ mmol m$^{-2}$ d$^{-1}$) from the small Heron Island coral cay are similar to those found in the Gulf of
Figure 2.7. Groundwater nutrient concentrations versus $^{222}$Rn. Black circles indicate groundwater bore time series data at the high tide mark. Grey circles indicate groundwater bores located throughout the Island.
Aqaba (2.1 - 2.4 mmol m\(^{-2}\) d\(^{-1}\)) and lower than those found in Kona Hawaii (116 - 220 mmol/m\(^2\)/d), West Maui (53 mmol m\(^{-2}\)/d\(^{-1}\)), Florida (33 mmol m\(^{-2}\) d\(^{-1}\)) and Mauritius (543 mmol m\(^{-2}\) d\(^{-1}\)) [Paytan et al., 2006]. In all these cases, groundwater offered a significant source of nutrients into the surrounding coral reef.

Similarly, high inputs of nutrients into coral reefs have been found on Shiraho Reef in Japan [Blanco et al., 2011] where inputs from SGD can increase nitrate concentrations to 20-fold greater than baseline oceanic conditions, leading to changes in the Chl-a concentrations by a factor of 4. These inputs can help to sustain coral reefs and may be an often overlooked input of new nutrients. With N fixation estimated at approximately 0.02 mmol m\(^{-2}\) d\(^{-1}\) [Dubinsky, 1990] by coral reefs, SGD inputs may deliver larger quantities of N into nearshore waters and possibly neighboring reefs/oceanic waters. Changes in the terrestrial environment can lead to changes in the amount of nutrients sourced from bird guano. For example, in Palmyra Atoll, pristine bird habitat had 26.5 times more N input than modified palm forests owing to native birds not utilizing modified areas [McCauley et al., 2012]. This was suggested to drive lower phytoplankton and zooplankton concentrations in surrounding waters. Most SGD studies to date have looked at systems neighboring anthropogenic sources with high nutrient concentrations in fresh groundwater [Lewis, 1987; Bishop et al., 2017; Swarzenski et al., 2017]. This study shows that natural islands that have little to no anthropogenic inputs of N and P can still supply nutrients via saline SGD to the surrounding reefs.

The radon-derived nutrient fluxes shown in Table 2.2 can be independently checked by assessing the temporal changes in nutrient concentrations in the lagoon at low tide (Figure 2.4). By utilizing low tide data only when mixing with oceanic waters is negligible [McMahon et al., 2013], the slope of nutrient inventories (i.e., molarity per m\(^2\) of lagoon bottom) versus time
reveals nutrient fluxes into the lagoon in units comparable to the estimated SGD rates. While there must be usage of nutrients in the lagoon, we found that nitrate always builds up in the lagoon following the low tide isolation. This likely occurs because nutrient uptake by corals is minimized at sluggish conditions [Falter et al., 2004; Hearn et al., 2001] such as low tide. The average rate of NO₃ accumulation was 5.4 mmol m⁻² d⁻¹ for the 47 low tides investigated. Similarly, PO₄ and NH₄ increased at an average of 1.2 mmol m⁻² d⁻¹ and 1.7 mmol m⁻² d⁻¹ during isolation, respectively. These fluxes estimated from lagoon water temporal changes at low tide fluxes are on the same order of magnitude as our minimum groundwater-derived nutrient fluxes estimated from the independent radon mass balance model. For example, assuming the unmixed beach face represents the groundwater endmember, results in SGD NO₃ fluxes of 2.1 mmol m⁻² d⁻¹ which is about 40% of the low tide time series approach. Considering the uncertainties in groundwater endmember, and the wide range of SGD-derived fluxes depending on the assumptions made (Table 2.2), these independent approaches provide a reasonable agreement. Therefore, the top down (i.e., water column time series) and bottom up (i.e., radon derived SGD and groundwater endmember) approaches demonstrate our dataset is internally consistent and provide confidence in our suggestion that SGD is a significant source of dissolved nitrogen to the coral reef lagoon.

2.4.2. N:P ratios and nutrient speciation. When considering the impact of the groundwater associated nutrients into the lagoon, the ability of the organisms to uptake nutrients should be considered. The ratios and speciation of nutrients are important to determine which nutrient is the limiting factor within coral reefs and coastal waters. Redfield [1958] put forth that the rate at which nitrogen and phosphate is taken up is 16 parts nitrogen to 1 part phosphate (16:1). While
this ratio may be higher (30:1) within coral reefs [D’Elia and Wiebe, 1990], nutrient ratios are important to understand the biological effects in any coastal ecosystem. It is generally thought that nitrogen is the most limiting nutrient source in coral reefs [D’Elia and Wiebe, 1990], allowing for the high ratios of N:P in groundwater to have a greater effect in coral reef biogeochemistry. As a result, nitrogen entering coral reefs will often increase growth until a negative threshold is reached [Koop et al., 2001].

Within groundwater sources, the ratio of N:P varies greatly. For example, in Florida Bay, there was a maximum N:P of 140:1 in groundwaters [Fourqurean et al., 1997] and in Barbados groundwater discharge was enriched in nitrate relative to phosphate due to nitrogen fertilizers [Lewis, 1987]. Heron Island surface waters have low ratios with DIN:DIP <10 and TDN:TDP ranging from 7 to 15 indicating that nitrogen limitation within the lagoon is likely a major restriction on productivity. The groundwater within the island showed very high ratios >186:1 for DIN:DIP and >174:1 for TDN:TDP. These high N:P ratios occur due to extremely high NOx in the middle of the island where birds concentrate [Hemson, 2015]. This ratio is lowered as groundwater mixes with seawater under the beach. At approximately 50 m onshore from the beach face the groundwater DIN:DIP has an average of 100:1 (well 7), which is further reduced to 50:1 at the high tide mark (Figure 2.2). DIN:DIP ratios further decrease to approximately 20:1 when groundwater reaches the beach face seep. The lowering of the N:P ratios as groundwater travels towards the beach likely may result not only from mixing, but also high denitrification rates. While we cannot estimate groundwater denitrification rates with the data available, Heron Island lagoon carbonate sediments have some of the highest reported denitrification rates in coastal systems worldwide [Eyre et al., 2013; Kessler et al., 2014; Santos et al., 2012].
Figure 2.8. Nutrients as drivers of Net Ecosystem Production (NEP) and Net Ecosystem Calcification (NEC) showing slight correlations to NO\textsubscript{x} levels within surface waters. Grey circles indicate daytime and black circles indicate night time. Only daytime samples were used for
correlations due to production and calcification only being active during daylight hours. NEC and NEP values originally reported in McMahon et al. (2013).

The nitrogen speciation also shifted as water travels from the groundwater bores to the lagoon (Figure 2.9). Initially, NO₃ dominates the nitrogen pool. As groundwater mixes with the lagoon, DON becomes more significant. In the lagoon, DON accounted for 86% of the TDN pool. A similar shift in phosphorus speciation was not clearly observed likely because groundwater was not as enriched in phosphate as it was enriched in NO₃ (Table 2.1). Island groundwater was on average ~1000-fold more enriched than the lagoon in NO₃, and only ~6-fold more enriched in DIP. As a result, while a groundwater phosphate signal was detected off the island, the gradient in nitrate concentrations was much steeper during the beach transect sampling (Figure 2.3). Therefore, it seems that phosphorus as retained in the island at higher proportions than nitrogen as expected for groundwater systems [Slomp and Van Cappellen, 2004]. The observed shift in nitrogen speciation shows interaction between oceanic water entering the lagoon and biological processes occurring within the sediments [Santos et al., 2012a]. In coral reefs, nutrient additions from oceanic sources are also present, with high levels of DON traced to oceanic supply during high tides [Thibodeau et al., 2013]. DON can be internally produced from algae and corals within the lagoon [Haas and Wild, 2010; Tanaka et al., 2011a; Tanaka et al., 2011b]. The availability of DON within coral reef systems to be up taken is unknown but with suggestions that it is not as readily available to organisms [Knapp et al., 2005]. Therefore, the preferential input of more bioavailable NO₃ than DON further supports the suggestion that groundwater discharge can be a major driver of coral reef productivity.
Figure 2.9. A conceptual model summarizing the main findings of this investigation, including tidally-driven groundwater-derived nitrate fluxes into the coral reef lagoon, changing nutrient speciation along the groundwater-lagoon transect, and the shift in TDN and N:P ratios along the transect.
2.4.3. Implications. High dissolved nutrient concentrations have been shown to limit coral reef growth [Koop et al., 2001]. SGD inputs have been hypothesized to sustain productivity on coral reefs [Lewis, 1987], but this has not been investigated in detail. Tidal pumping within beach sediments has already been demonstrated at Heron Island with $^{222}$Rn and groundwater level observations apparently influencing pH and dissolved oxygen [Santos et al., 2011] as well as dissolved carbon dioxide, methane and nitrous oxide in the coral reef lagoon [Cyronak et al., 2014; O'Reilly et al., 2015]. We examined the correlation between daytime NEP and NEC and nutrient concentrations (Figure 2.8) and found a positive correlation between NEC and NO$_x$ (p=0.01) during the day when photosynthesis is possible. If we exclude the first 3 hours of “daytime” (when photosynthesis is often slow), then NEC also has a significant correlation to NH$_4$ (p=0.03). While light is often the major control on coral reef NEC and NEP, dissolved nutrients are also well known to drive coral productivity [Atkinson et al., 2001; Falter et al., 2012; ]. Our observations imply that the groundwater-derived nitrate is having a detectable influence on NEP and NEC, driving higher rates of NEP and NEC through nutrient additions into the lagoon.

Cultural eutrophication poses a serious threat to coral reefs throughout the world. Increases in dissolved nutrients above their long term averages can cause coral reef systems to become dominated by algae due to over stimulation of nutrient fueled growth [Dubinsky and Stambler, 1996] even if nutrient addition does not cause direct lethal damage [Koop et al., 2001]. A threshold value for high nutrient levels has not been agreed upon since corals from different geographic locations have different responses to varying degrees of nutrient enrichment. Additionally, high levels of nutrients may affect one species negatively, while others tolerate
these levels [Koop et al., 2001]. This may lead to some coral reefs becoming less diverse in the future, even if large scale eutrophication does not occur.

The natural groundwater-derived nutrient inputs to the Heron Island coral reef lagoon have likely occurred since early in the island formation. The dissolved nutrient inputs do not accumulate in the lagoon as implied from the 24-h time series observations. Mixing with oceanic waters at high tide dilutes nitrate and DON to concentrations that approach zero (Figure 2.5). The input of groundwater derived-nitrogen is likely to be a historical process in Heron Island and several other coral islands densely populated by birds. Based on the observed correlations between dissolved nitrate and coral ecosystem calcification and photosynthesis (Figure 2.8), we suggest groundwater-derived nitrate partially drives the relatively higher calcification and productivity rates observed off Heron Island than observed at many other coral reefs (McMahon et al., 2013). Systems such as Heron Island with nutrient inputs apparently dominated by groundwater may offer a natural analogue to study human impacted systems to gain a better understanding of the issues they face. Estimating groundwater nutrient inputs should allow for best management practices to be determined [Young et al., 2008]. Current reef management looks at the past to determine future baselines. However, assessing essential reef services and what measures need to be implemented to keep ecosystem functions has recently been proposed as a better management tool [Rogers et al., 2015]. With nutrients released at low tide likely to be retained by the lagoon, it is important to have a proper understanding of the impacts changes in SGD nutrient sources may have [Santos et al., 2010]. To properly evaluate the effects, the groundwater source needs to be evaluated with respect to its flux of nutrients and implications to the broader ecosystem.
2.5. Conclusions

Multiple lines of evidence covering different spatial and temporal scales revealed that saline, recirculated groundwater is a major nutrient source that increases productivity of nearshore waters off Heron Island in the Great Barrier Reef. The nutrient source is clearly related to bird guano that is being brought to the island through bird migration and reproduction. Therefore, coral reefs surrounding islands with large bird populations can be partially sustained by groundwater-derived nutrient inputs. This scenario is different than fringing coral reef systems on volcanic islands where fresh groundwater was suggested to be a major source of nitrogen. Groundwater nutrients appeared to be up taken within approximately 20 m from the island. Due to the isolation of the lagoon at low tide, the reef lagoon apparently can utilize nutrient additions prior to mixing with oceanic waters. There was a transformation of nitrogen speciation from the nitrate-dominated central section of the island to the DON-dominated lagoon as well as a decrease in N:P ratios within the lagoon. Most previous nutrient studies conducted on coral reef ecosystems have assessed areas with large anthropogenic sources nearby. More isolated reefs that have large bird populations may also have large additions of nutrients supporting the coral reef ecosystem around them.
Chapter 3

Determining coral reef calcification and production using automated alkalinity, pH and $pCO_2$ measurements at high temporal resolution

3.1 Abstract - We investigated coral reef carbonate chemistry dynamics and metabolic rates using an automated system that measured total alkalinity (TA, 30 min intervals), pH on the total scale (pHT, 10 min intervals) and the partial pressure of carbon dioxide (pCO₂, 1 min intervals) over two weeks at Heron Island (Great Barrier Reef, Australia). The calculation of pHT (using the pCO₂ and TA pair) and pCO₂ (using the pH and TA pair) had similar values to the measured pHT and pCO₂ values. In contrast, calculated TA from the pCO₂-pH pair showed a large discrepancy with measured TA (average difference between measured and calculated TA = 52 µmol kg⁻¹). High frequency sampling allowed for detailed analysis of the observations and an assessment of optimum sampling intervals required to characterise the net ecosystem calcification (NEC) and production (NEP) using a slack water approach. Depending on the sampling interval (30 min to 2 hour time steps) used for calculations, the estimated daily NEC and NEP could differ by 12% and 30%, respectively. Abrupt changes in both NEC and NEP were observed at dawn and dusk, with positive NEC during these periods despite negative NEP. Integrating NEC and NEP over a full diel cycle using 1 or 2 hour integration time steps resulted in small differences of 2 to 7% for NEC and 1 to 3% for NEP. A diel hysteresis pattern rather than a simple linear relationship was observed between the aragonite saturation state (Ωₐₐ) and NEC. The observed hysteresis supports recent studies suggesting that short-term observations of seawater Ωₐₐ may not be a good predictor of long-term changes in NEC due to ocean acidification. The slope of the DIC to TA relationship was slightly higher (0.33) in 2014 than in an earlier study in 2012 (0.30). The automated, high frequency sampling approach employed here can deliver high precision data and can be used at other coral reef research stations to reveal long-term changes in NEC and NEP potentially driven by ocean acidification, eutrophication or other local changes.
3.2 Introduction

Coral reefs support large biodiversity and provide important services for humans such as sustenance, recreation and coastal protection (Moberg and Folke, 1999). Due to their calcium carbonate (CaCO₃) framework, coral reefs are thought to be highly susceptible to the deleterious effects of rising seawater carbon dioxide (CO₂) concentrations and associated changes in carbonate chemistry, termed ocean acidification (OA) (Hoegh-Guldberg et al., 2007; Kleypas et al., 1999). OA is the result of anthropogenic CO₂ entering seawater from the atmosphere resulting in a decrease in carbonate ion (CO₃²⁻) concentration and increase in H⁺ concentration, thereby lowering the pH and aragonite saturation state (Ωₘ). Since the beginning of the Industrial Revolution, global ocean pH has decreased by about 0.1 units, and a further decline of about 0.14 to 0.35 units is projected by the end of this century (IPCC, 2007).

With the anticipated lowering of oceanic pH and Ωₘ, coral reefs are expected to transition from a state of net accretion towards a state of net dissolution (Andersson and Gledhill, 2013; Eyre et al., 2014; Silverman et al., 2009). Both in situ (Albright et al., 2018; McMahon et al., 2013; Shamberger et al., 2011; Shaw et al., 2015; Silverman et al., 2012) and laboratory mesocosm experiments (Jokiel et al., 2008; Langdon et al., 2003; Langdon et al., 2000) have documented decreased rates of net calcification in coral reef environments at higher CO₂ concentrations, low pH, and low Ωₘ. Along the Florida Reef tract (USA), reefs have already been found to be net dissolving during winter, with one reef already net dissolving over the entire year (Muehllehner et al., 2016). Increased dissolution can also influence the structure of the reef since dissolution of CaCO₃ can cause increased vulnerabilities to severe weather and other similar events (Hoegh-Guldberg et al., 2007). A decline of up to 40% in net ecosystem calcification (NEC) at future atmospheric CO₂ levels of 560 ppm has been estimated as a
baseline of the changes expected (Kleypas and Langdon, 2006). In order to assess future impacts of ocean acidification on coral reef health, it is important to quantify present coral reef metabolic rates (i.e., photosynthesis and respiration, and calcification and CaCO\textsubscript{3} dissolution).

Quantifying coral reef metabolic rates is challenging. Studies on the metabolism of coral reefs often rely on observations of dissolved inorganic carbon (DIC) and total alkalinity (TA) concentrations in ambient seawater to tease apart the processes of net ecosystem production and calcification (Anthony et al., 2011). There are two main approaches by which metabolic rates in coral reefs are typically estimated at the ecosystem scale, both relying on sampling a combination of seawater pH, DIC, TA and/or $\rho$CO\textsubscript{2} to constrain the seawater carbonate system. The flow respirometry approach analyses changes in carbonate chemistry as seawater travels over a reef and thus requires accurate assessment of water flow and direction which can be difficult to characterise in heterogeneous coral reefs (Falter et al., 2012; Shamberger et al., 2011). The slack water method utilises the natural isolation of a coral reef lagoon at low tide when sea level falls below the reef crest, but requires minimal water flow over the reef (Lowe and Falter, 2015). The latter method obviously relies on a specific reef topography but several studies to date have successfully employed this approach (McMahon et al., 2013; Shaw et al., 2012; Silverman et al., 2012). Whereas the flow respirometry approach ideally measures carbonate chemistry changes over a small spatial scale, the slack water approach requires resolving temporal variability in great detail.

Because the seawater chemistry of coral reefs is often highly dynamic in space and time (Falter et al., 2012; Ohde and van Woesik, 1999; Shamberger et al., 2014; Shaw et al., 2012; Silverman et al., 2012), high resolution sampling may be essential to quantify metabolic rates when using both the flow respirometry and slack water approaches. Most previous NEC studies
relied on labour intensive sampling and analysis. For example, McMahon et al. (2013) required approximately 4 hours of discrete sampling every low tide over 47 tidal cycles to obtain daily integrated NEC and NEP rates. High precision, high resolution automated pH and \( pCO_2 \) observations can now be made routinely (Byrne, 2014), and significant progress in alkalinity automation has been made (Briggs et al., 2017; Li et al., 2013; Liu et al., 2015; Roche and Millero, 1998; Watanabe et al., 2004). Here, we made a simple modification to a high precision instrument that allows continuous, automated alkalinity observations at ~30 min time steps. We demonstrate the combined use of automated alkalinity, pH and \( pCO_2 \) observations to provide for a more intricate interpretation of coral reef metabolic rates. While all the data reported here are original, we compare our quasi-continuous, high-precision, automated observations over 14 consecutive days to previous observations based on discrete sampling and manual analysis (McMahon et al., 2013). Both studies relied on sampling at low tide at the same site (Heron Island, Great Barrier Reef).

### 3.3 Material and Methods

#### 3.3.1 Experimental approach

Field measurements were undertaken at the Heron Island Research Station approximately 80 km offshore from Gladstone (Queensland Australia) to assess whether automated high resolution, continuous observations may provide greater insights into coral reef metabolism than lower frequency manual sampling. Overall, our experiment produced automated, continuous observations of alkalinity (30 min time intervals), pH on the total scale, \((pH_r); 10\) minute time
intervals), \( pCO_2 \) (1 min time intervals), and salinity, temperature, and dissolved oxygen (10 min time intervals) for a total of 14 days from 14/04/2014 to 28/04/2014.

Heron Island is a coral cay in the Great Barrier Reef, Australia. Samples were taken directly off the southern side of Heron Island within the reef flat and lagoon that covers 26.4 km² and has an average depth of about 1.7 m. Approximate coral coverage of the lagoon is 6.9 km² with the remaining 19.5 km² covered by coarse CaCO₃ sand (Wild et al., 2004). The lagoon becomes isolated at low tide allowing for calculation of coral reef metabolism (i.e. NEC and NEP) using the slack water approach. The sample site (23°27’S, 151°55E) was located 10 m off the beach at low tide adjacent to the Heron Island Research Station. This same location was used in a number of previous studies focusing on carbon, greenhouse gas, and nutrient cycling (Cyronak et al., 2014; McMahon and Santos, 2017; McMahon et al., 2013; O'Reilly et al., 2015; Santos et al., 2010) though all the data reported here are original. Specifically, we build on previous work (McMahon et al., 2013) when discrete samples were taken manually approximately 1-2 hours on either side of low tide at 30-minute intervals. Because discrete sampling and analysis are labour intensive, McMahon et al. (2013) sampled only around low tide and could not cover full diel cycles. The automated approach reported here allows for a much more detailed dataset to be obtained with less effort. Weather conditions during the sampling period were typical of the location with an average air temperature of 24.5 °C (24.0 °C in 2012), wind speed of 24 km/h (33.5 km/h in 2012) and average wind direction of 121° (124° in 2012).

To allow for automated sampling, water was pumped from the lagoon site to a laboratory at the research station. Forty-eight meters of 25 mm flexible polyethylene hose was fitted to an under-water pump, which then supplied filtered water through a 200 μm membrane prior to splitting off into 3 feed lines to different instruments to measure seawater carbonate chemistry; a
Sami2 pH meter, a VINDTA 3C for total alkalinity (TA) and a showerhead gas equilibration device (GED) connected to a Picarro gas analyser for \( p\text{CO}_2 \) (Figure 3.1). All pump connections were tested for air leaks prior to the start of the experiment. The pump flow rate was 15.2 L/min with a residence time in the hose of approximately 2 minutes. At the seawater intake within the lagoon, a calibrated Hydrolab DSX5 multiprobe logged dissolved oxygen (DO) (±0.2mg/L), temperature (±0.1 °C), salinity (± 0.5%) and photosynthetically active radiation (PAR) at 10-minute intervals. A CTD data logger (Van Essen) was placed within the GED in the laboratory to measure pressure and temperature.

**Figure 3.1.** Experimental setup used. Hydrolab DSX5 was at pump site location in reef lagoon. Water was pumped from the same location through a 200 micron filter and split into a GED, VINDTA 3C, Sami2 pH and excess waste water. A closed air loop from the GED went to the Picarro CO\(_2\) analyser through Drierite to reduce moisture.
The Sami2 pH logger uses a colorimetric method with the indicator meta-cresol purple (Martz et al., 2003). Temperature was measured both within the Sami2 pH and at the seawater intake, while salinity was measured at the intake. The Sami2-pH was set up in an overflowing bucket at the exit of the piping to measure pH at 10 minute intervals. All instruments were calibrated before and after the experiment, except for the Sami2 pH that was calibrated by the manufacturer to an accuracy of ±0.003 units (total pH scale). Temperature changes during water transport from the intake to the laboratory were calculated using the temperature difference from the Hydrolab DSX5 at the lagoon site and CTD logger in the laboratory. Maximum temperature deviations of 1.4°C and an average of 0.2°C were observed which is relatively small compared to the natural diel variability of 6.7°C.

A VINDTA 3C (Versatile INstrument for the Determination of Total inorganic carbon and titration Alkalinity) drew sample aliquots directly from the feed line. The DIC portion of the VINDTA 3C was not operating, so only alkalinity was determined during this deployment. Total alkalinity (TA) was measured by Gran titration using 0.1M HCl in a 100 ml chamber, kept at a constant temperature (25 ºC) via a temperature-controlled water bath circulating water through an external water jacket around the outside of the chamber. Reverse osmosis (i.e., TA-free) water was used to flush the tubing and chamber first, followed by flushing with the new sample between runs. Automation of the VINDTA3C was achieved with a purpose-built script using AutoIT v3 activating the VINDTA every 30 minutes for a specified protocol. Certified reference materials (CRM) batch 136 (Dickson 2010) along with in-house alkalinity standards were run daily with standard deviations of 2.3 µmol kg⁻¹ and 3.8 µmol kg⁻¹, respectively for nominal concentrations of 2246.7 µmol kg⁻¹ and 2103.0 µmol kg⁻¹, respectively, and used to correct
sample measurements assuming a linear drift. Acid molarity was standardised daily using at least 3 CRM measurements.

The third feed line supplied seawater to a showerhead style gas equilibration device (General Oceanics) at a flow rate of about 3 litres per minute. The headspace of the GED was connected in a closed loop through a desiccant column (magnesium perchlorate) to an automated, high precision cavity ring down spectrometer (CRDS, Picarro G2201-i) measuring CO₂ concentrations (Maher et al., 2013). The calibration was checked before and after deployment by running reference gasses (CO₂ 306 ppm and 2017 ppm), with no drift detected. This setup has an equilibration time of about 10 minutes (Santos et al., 2012; Webb et al., 2016). pCO₂ in seawater was calculated from the measured dry CO₂ mol fraction, corrected for water vapour pressure and temperature differences between the water inlet point and the GED, using standard equations (Pierrot et al., 2009; Weiss, 1974).

3.3.2 Calculations

Coral reef metabolic rates were estimated based on temporal changes in carbonate chemistry parameters using different time intervals. Net Ecosystem Calcification (NEC; mmol C m⁻² h⁻¹) was calculated per Kinsey (1978):

\[
NEC = -0.5\Delta TAd\rho/\Delta t
\]

(1)

where \(\Delta TA\) is the change in total alkalinity between sampling intervals (mmol t⁻³), \(d\) is the water depth (m), \(\rho\) is the in-situ density of the seawater (t m⁻³) and \(\Delta t\) is the time interval between sample intervals (h). Net ecosystem production (NEP; mmol C m⁻² h⁻¹) was calculated using
changes in DIC and correcting for additional changes associated with NEC and air-water CO₂ gas exchange as:

\[ \text{NEP} = \frac{\Delta \text{DIC}}{\Delta t} - \text{NEC} + F_{\text{CO}_2}/24 \]  

(2)

where \( \Delta \text{DIC} \) is the change in DIC (mmol t⁻³) over the time period t (h) and \( F_{\text{CO}_2} \) is the average sea-air flux of CO₂ over the respective time period calculated according to:

\[ F_{\text{CO}_2} \text{ (mmol m}^{-2} \text{ d}^{-1}) = k \alpha (p_{\text{CO}_2}\text{(water)} - p_{\text{CO}_2}\text{(air)}) \]  

(3)

where \( k \) is the gas transfer velocity for CO₂ (Raymond and Cole, 2001) and \( \alpha \) is the solubility coefficient of CO₂ calculated as a function of temperature and salinity (Weiss, 1974). \( p_{\text{CO}_2}\text{(air)} \) was set to 400 μatm. DIC was calculated using the program CO2SYS from measured pH₆, total alkalinity, temperature and salinity. Previously, measured DIC and calculated DIC showed excellent correlation (\( r^2 = 0.974 \)) (Cyronak et al., 2013). Stoichiometric equilibrium constants for carbonic acid were those of Mehrbach et al. (1973) as refitted by Dickson and Millero (1987), whereas that for bisulphate ion was calculated according to Dickson (1990). In situ temperature and salinity corrections were applied to the data. As the Sami2 pH does all calculations for a fixed salinity of 35 (pH₃₅), pH was recalculated for in situ salinities according to (Dickson et al., 2007):

\[ pK2 = 1245.69/T + 3.8275 + 0.00211(35-S) \]  

(4)

\[ \text{pH}_{\text{in situ salinity}} = pK2_{\text{in situ salinity}} + \text{pH}_{\text{salinity 35}} - pK_{\text{salinity 35}} \]  

(5)

where \( pk2 \) is the dissociation constant of protonated m-cresol purple, \( T \) is the recorded SAMI cell temperature in Kelvin and \( S \) is the salinity of the measurement. Temperature and salinity used to derive the pH and \( p_{\text{CO}_2} \) were obtained at the lagoon water intake. Water depths were also
obtained from the lagoon. The lagoon depth at low tide was nearly constant. An average depth over the observation time was used in all calculations as previously done (McMahon et al., 2013).

3.4. Results and Discussion

3.4.1 Data integrity

Our automated time series revealed high temporal variability in alkalinity, pHₚ, and pCO₂ during the observations (Figure 3.2), demonstrating the importance of continuous observations to characterize the dynamics of carbonate chemistry in coral reefs. Overall, the 3 measured parameters followed expected diel cycles with pCO₂ and alkalinity dropping and pH increasing during the day as a result of calcification and photosynthesis (McMahon et al., 2013; Santos et al., 2011; Silverman et al., 2012). The overall observed range in alkalinity (2204.2 to 2418.7), pHₚ (7.85 to 8.43) and pCO₂ (170 to 657) were comparable to previous studies in Heron Island (Albright et al., 2015; McMahon et al., 2013) and nearby coral reef lagoons (Shaw et al., 2012; Silverman et al., 2012) using lower frequency observations.
Figure 3.2. Calculated TA (from pH and $p$CO$_2$), pH (from TA and $p$CO$_2$) and $p$CO$_2$ (from TA and pH) (circles) against measured values (crosses).
Calculation of all four carbonate chemistry parameters (DIC, pH$_T$, pCO$_2$, TA) can be undertaken using 2 out of the 4 measurable parameters. To gain insight into the analytical performance of the system, we compared measured to calculated values using the other two carbonate chemistry parameters as also done previously (Cyronak et al., 2013; Hata et al., 2004; Millero et al., 1993). Calculated pCO$_2$ from the measured pH$_T$-TA pair was strongly correlated to measured pCO$_2$ ($r^2 = 0.97$, slope $1.04 \pm 0.009$) and measured pH$_T$ was strongly correlated to the pH$_T$ calculated from the measured pCO$_2$-TA pair ($r^2 = 0.998$, slope $1.009 \pm 0.002$) (see also Figure 3.2). Additionally, plotting calculated values against measured values with the resulting $r^2$ of 0.998, showed that the instrument was stable for the duration of the study. However, calculation of TA from the pH-pCO$_2$ pair yielded pronounced deviation from measured data. Whereas measured TA had a standard deviation of 35 µmol/kg of all samples during the study duration, the non-ideal pairing of pH and pCO$_2$ resulted in a standard deviation of calculated TA of approximately 84 µmol kg$^{-1}$ and overall there was an average offset between measured and calculated TA of 52 µmol kg$^{-1}$. All parameters showed no change in the calibration offset during the deployment indicating instrument stability.

The limitations of the pH-pCO$_2$ pair are well known (Gray et al., 2011), and are related to propagated analytical uncertainties of the pH$_T$-pCO$_2$ pair. Following Dickson (2010) and using the average concentrations observed in the lagoon (pH$_T$ 8.1, pCO$_2$ 400 µatm and TA 2300 µmol kg$^{-1}$) and the estimated analytical uncertainties of each instrument (0.003, 2 µatm, and 2.3 µmol kg$^{-1}$ respectively), the limitation of the pH-pCO$_2$ pair to calculate alkalinity as well as DIC is readily demonstrated. Using the pH-TA pair to calculate pCO$_2$ results in an uncertainty of 3.3 µatm, which is equivalent to 0.8% of the average pCO$_2$ observed in the lagoon and 1.6-fold greater than the analytical uncertainty of observations. Using the pCO$_2$-TA pair to calculate pH$_T$
results in an uncertainty of 0.002 unit which is better than the analytical uncertainty of the Sami2 pH instrument used here. Finally, using the $pCO_2$–pH pair to calculate TA results in an uncertainty of 35.1 $\mu$mol kg$^{-1}$, which is equivalent to 1.5% of the average TA observed in the lagoon, and 15-fold greater than the analytical uncertainty of TA measured with our VINDTA 3C system ($= \pm 2.3 \, \mu$mol kg$^{-1}$). Furthermore, pH and $pCO_2$ measurements are highly temperature dependent and have to be adjusted to in situ temperatures when measured in the laboratory. The reliance on different temperature measurements adds further potential errors which are compounded. For instance, a temperature uncertainty of 0.2 $^\circ$C increases the analytical uncertainties to 4.7 $\mu$atm for $pCO_2$ and 0.004 units for pH (1.17% and 0.04% of average concentrations for $pCO_2$ and pH, respectively). We highlight, however, that while our $pCO_2$ and pH measurements are not ideal to calculate NEC and NEP, earlier work successfully used a combination of $pCO_2$ and pH to provide reasonable estimates of DIC and TA to calculate NEC and NEP in a coral reef in Japan (Hata et al., 2004).

Measurement accuracy is extremely important when calculating carbonate chemistry parameters. In highly dynamic systems such as coral reefs, sampling of individual parameters needs to be synchronized as best as possible to ensure an accurate set of carbonate system parameters from which to calculate the remaining ones. This can be problematic when using instruments with time lags in their response. For instance, while the Sami pH instrument takes an immediate discrete sample when activated, the $pCO_2$ analyser requires that the water and air in the equilibrator reach equilibrium which usually takes about 10 minutes (Santos et al., 2012; Webb et al., 2016), and the measured $pCO_2$ is actually a function of the in-situ changes over the proceeding 10 minutes. Hence, in dynamic systems, such as coral reefs, equilibrium is likely never completely achieved. This may be especially problematic during isolation at low tide when
rapid changes in concentrations occur due to a smaller water volume (Cyronak et al., 2014). The poor relationship between measured TA and calculated TA using the $pCO_2$-pH pair may be due, in part, to time lags in the CO$_2$ response. This is consistent with previous findings (e.g., Gray et al., 2011) and demonstrates the importance of over-determining the carbonate chemistry in dynamic coral reefs with more than two parameters, and to include DIC or TA. Reliable instruments for measuring pH and $pCO_2$ are commercially available as automated units, but, ideally, all carbonate parameters should be measured. In cases where only 2 parameters can be measured, we suggest the $pCO_2$-TA or pH-TA pair, or potentially a DIC-TA or DIC-pH pair that could not be measured here.

3.4.2 Calculating NEC and NEP using different sampling intervals

High temporal resolution automated measurements allowed us to calculate NEC and NEP using different time intervals when employing the slack water method at low tide. NEC and NEP were calculated using equations 1, 2 and 3 at different time steps: (1) From the start to endpoint samples for the lagoon isolation at low tide (Silverman et al., 2012), (2) by 1 hour time intervals within the lagoon isolation at low tide (McMahon et al., 2013), and (3) a sample-to-sample approach (30 minute intervals) during isolation at low tide. This resulted in 25 NEC and NEP estimates for the first approach, 64 for the second approach and 175 estimates for the third approach. Without high resolution temporal observations, short term dynamics may be missed especially during transition times such as dawn and dusk. The NEC and NEP values estimated from the 3 different approaches varied (Table 3.1). Overall, daily NEC differences were 6% between approach 1 and 3, 5% between approach 2 and 3 and 12% between approach 1 and 2. NEP showed a maximum variation of 23% for daytime and 12% for night time values when
comparing the different approaches. These results show that some differences arise when
different sampling intervals are utilised, due to non-linear changes in NEP and NEC throughout
the slack tide isolation period.

Table 3.1. Average NEC and NEP values for daytime, night-time and total day using 3 different
sampling intervals (mmol m\(^{-2}\) h\(^{-1}\)). Approach 1, start of isolation to end of isolation (usually 2-3
hours), Approach 2, 1 hour sample intervals within tidal isolation, and Approach 3, 30 minute
intervals within tidal isolation.

<table>
<thead>
<tr>
<th></th>
<th>Approach 1</th>
<th>Approach 2</th>
<th>Approach 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEC Day</td>
<td>12.34</td>
<td>14.15</td>
<td>13.12</td>
</tr>
<tr>
<td>NEC Night</td>
<td>0.46</td>
<td>0.13</td>
<td>0.45</td>
</tr>
<tr>
<td>NEC Total</td>
<td>6.40</td>
<td>7.14</td>
<td>6.79</td>
</tr>
<tr>
<td>NEP Day</td>
<td>59.35</td>
<td>73.03</td>
<td>67.17</td>
</tr>
<tr>
<td>NEP Total</td>
<td>18.76</td>
<td>24.35</td>
<td>19.64</td>
</tr>
</tbody>
</table>

This variation can be broken down into several parts. Approach 3 appears to capture extra
sensitivity during the night, especially from 02:00 to 06:00 when both NEC and NEP turn
positive on a few occasions (Figure 3.3), as previously observed at this study site (McMahon et
al., 2013) and in mesocosm experiments (Jokiel et al., 2014). It is difficult to explain positive
NEP at night time. We suspect that these positive values may be due to undetected advection of
different water mass even during the slack water period. Such change can easily be missed if
longer sampling intervals are used (e.g. approach 1, full isolation lasting up to 4 hours). The full
isolation approach appears to have the greatest variation in comparison to the other two
approaches. This is most likely due to the longer integration time with the full isolation approach, which clearly may miss some of the short-term temporal variability in metabolism. Therefore, some of the NEC and NEP variations among previous studies may be due to artefacts caused by different sampling intervals covering different time scales. Obviously, high resolution time series observations in multiple coral reefs would be ideal for comparative studies. In the absence of such data, the variability reported in Table 3.1 resulting from using different time steps when making calculations provides guidance for comparisons.
3.4.3 Integrating NEC and NEP over a diel cycle

In order to obtain representative daily NEP and NEC rates for a coral reef, individual estimates need to be integrated over a diel cycle (McMahon et al., 2013). Integrating NEP and NEC poses many challenges when using time series observations. First, slack waters only occur
over a few hours each day. Second, low tide times are variable requiring the sampling of multiple consecutive low tides to obtain diurnal integrated NEC and NEP rates. Finally, night-time sampling may be logistically challenging, potentially creating a sampling bias against respiration and dissolution if fewer samples are collected at night. In addition, changes in climatic drivers (e.g. light, temperature, and rainfall) over the several weeks of sampling required to constrain hourly NEC and NEP rates may confound the interpretation of data. Commonly, 1 hour time steps sampled at different times of the day over several days to weeks are used in field studies (McMahon et al., 2013; Shamberger et al., 2011). Here, our high-resolution sampling allowed us to assess whether different sampling time steps influence the final integrated daytime, night-time and diel NEC and NEP. We estimated integrated NEC and NEP using 1-h and 2-h time steps. Whereas the shorter time steps allow for a greater detail and resolution, the longer time step smooths the data and presumably reduces artefacts associated with analytical uncertainties. For this calculation, the diurnal integration started at 00:00 and encompassed either 1 or 2 hours depending on the approach.

The integrated NEC and NEP values varied between time steps and as a function of the way in which data were processed (Tables 2 and 3). The apparent differences between sampling interval when integrating NEC and NEP were mainly evident during the night (up to 58.7% and 11.9% for NEC and NEP respectively). Therefore, the sampling frequency chosen may influence final estimates of metabolic rates and should be considered in future studies. Studies utilising a low number of samples or samples not evenly distributed throughout a 24-hour period may lead to gaps in daily integrated NEC and NEP. Logistical issues or assumed non-variability of night samples can lead to a small number of samples being taken. Our observations show that this
assumption does not hold true for this system, demonstrating the importance of high resolution observations during the entire day when estimating integrated NEC and NEP in coral reefs.

**Table 3.2.** Average 24-h integrated NEC rates using 1 hour and 2 hour integration intervals and 3 different approaches (mmol m$^{-2}$ h$^{-1}$). Approach 1, Start of isolation to end of isolation, Approach 2, 1 hour sample intervals within tidal isolation, and Approach 3, 30 minute intervals within tidal isolation.

<table>
<thead>
<tr>
<th>NEC</th>
<th>Approach 1</th>
<th>Approach 2</th>
<th>Approach 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daytime (1 hour)</td>
<td>12.34</td>
<td>14.15</td>
<td>13.12</td>
</tr>
<tr>
<td>Daytime (2 hour)</td>
<td>12.59</td>
<td>13.08</td>
<td>13.43</td>
</tr>
<tr>
<td>Night-time (1 hour)</td>
<td>0.46</td>
<td>0.13</td>
<td>0.45</td>
</tr>
<tr>
<td>Night-time (2 hour)</td>
<td>0.72</td>
<td>0.15</td>
<td>0.46</td>
</tr>
<tr>
<td>Daily (1 hour)</td>
<td>6.40</td>
<td>7.14</td>
<td>6.78</td>
</tr>
<tr>
<td>Daily (2 hour)</td>
<td>6.66</td>
<td>6.62</td>
<td>6.95</td>
</tr>
</tbody>
</table>
Table 3.3. Average 24-h integrated NEP rates using 1 hour and 2 hour integration intervals and 3 different approaches (mmol m\(^{-2}\) h\(^{-1}\)). Approach 1, Start of isolation to end of isolation, Approach 2, 1 hour sample intervals within tidal isolation, and Approach 3, 30 minute intervals within tidal isolation.

<table>
<thead>
<tr>
<th></th>
<th>Approach 1</th>
<th>Approach 2</th>
<th>Approach 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daytime (1 hour)</td>
<td>59.35</td>
<td>73.03</td>
<td>67.17</td>
</tr>
<tr>
<td>Daytime (2 hour)</td>
<td>57.94</td>
<td>73.02</td>
<td>63.33</td>
</tr>
<tr>
<td>Night-time (1 hour)</td>
<td>-21.83</td>
<td>-24.34</td>
<td>-21.81</td>
</tr>
<tr>
<td>Night-time (2 hour)</td>
<td>-19.23</td>
<td>-24.90</td>
<td>-23.68</td>
</tr>
<tr>
<td>Daily (1 hour)</td>
<td>18.76</td>
<td>24.35</td>
<td>19.64</td>
</tr>
<tr>
<td>Daily (2 hour)</td>
<td>19.36</td>
<td>24.06</td>
<td>19.83</td>
</tr>
</tbody>
</table>

3.4.4 Can NEC and NEP be estimated during the high tide slack water?

The slack water sampling approach that utilises the lagoons natural isolation at low tide has become a common experimental approach for estimating coral reef lagoon metabolic rates (McMahon et al., 2013; Shaw et al., 2015; Silverman et al., 2012). Our high-resolution observations allow assessing whether slack water measurements during high tides could be also used. From visual observations, we assume that slack water (no or negligible currents) at high tide lasted for 1 hour both sides of the high tide. We thus use a 2-hour interval to estimate NEC and NEP at high tide. A comparison of NEC and NEP calculations using low and high tide data revealed a significant difference between the low tide and the high tide approaches (Figure 3.4). Perhaps most importantly, the temporal variability of the high tide estimates are inconsistent and do not reflect any expected patterns of reef metabolism as displayed by the conventional low tide slack water approach.
**Figure 3.4.** A comparison of high tide to low tide NEC and NEP using the slack water approach. Black squares represent approach 1, black circles represent approach 2, and black triangles represent approach 3. Grey circles represent low tide data (2 hour time steps) for comparison purposes.
Differences between high and low tide methods were usually greater than 100% for both NEC and NEP. Twenty four hour integrated NEC values also showed a difference greater than 100% from NEC values calculated using low tide data. Previously, slack water metabolic studies assumed vertical homogeneity in carbonate chemistry at shallow sites, which was demonstrated in a location (<1-m deep) in the Great Barrier Reef (Shaw et al., 2012). High tide sampling may create some vertical variability (Lowe and Falter, 2015) and pockets of unique carbonate chemistry that are not representative of the overall reef. Furthermore, the larger water volumes at high tide lead to smaller concentration changes associated with NEC and NEP, leading to greater uncertainty at high tide. Overall, utilising the slack water at high tide does not give comparable data to the isolated low tide slack water approach at Heron Island and should be avoided or used with care.

3.4.5 DIC versus TA

Andersson and Gledhill (2013) suggested that the slope of DIC to TA may offer a better understanding of the reef’s health. The relationship between DIC and TA relates to the ratio of calcifying organisms (e.g., corals) to photosynthetic active organisms (e.g., micro and macro-algae) allowing for an understanding of the reef composition and dominant metabolic pathways (Watanabe et al., 2006). This understanding could then be used as a baseline for assessing future changes to community composition of a given coral reef following ocean acidification or local impacts.

The DIC to TA ratio (utilising the same sampling and integration time steps) at Heron Island has shifted from 0.295 to 0.333 between 2012 and 2014 (Figure 3.5). Both ratios are
within observed ratios for reefs within the GBR (Cyronak et al., 2018; McMahon et al., 2013; Shaw et al., 2012; Silverman et al., 2012). The ratio indicates that the reef was more dominated by NEP in 2012 than 2014. NEP influences the surrounding water by raising pH and consequently $\Omega_{ar}$, helping to offset some ocean acidification at the local level (Andersson and Gledhill, 2013). The shift in the DIC to TA ratios is consistent with changes in estimated NEC and NEP rates. Both NEC and NEP were higher in 2014 than in 2012 (McMahon et al., 2013), with NEC rising from 2.4 to 7.1 and NEP rising from 5.5 to 24.3 mmol m$^{-2}$ h$^{-1}$ (Figure 3.6). Therefore, NEP was about 3 times higher than NEC in 2012, and 4 times higher in 2014. It is unclear whether the shift in DIC to TA ratios and the relative increase in NEP represent a short term interannual variability or the continuation of a long-term shift towards an organic dominated reef. Long term studies are required to address these issues.

### 3.4.6 Hysteresis between NEC and $\Omega_{ar}$

NEC had previously been predicted as a linear function of $\Omega_{ar}$ (Ohde and van Woesik, 1999; Shamberger et al., 2011). A previous study at this site (McMahon et al., 2013) and in mesocosm experiments (Jokiel et al., 2014) and other in situ locations (Shaw et al., 2015) have shown that the NEC correlation to $\Omega_{ar}$ had a hysteresis pattern over a 24 hour period (Figure 3.7), which may prevent the application of a linear model to predict long term NEC as a function of diel changes in $\Omega_{ar}$. The relationship between NEC and $\Omega_{ar}$ appears more complicated than first expressed, with the influence of changes in one section (i.e. 06:00 – 12:00) of the daily cycle possibly impacting the linear model in different ways than previously expected. The rate of NEP relative to that of NEC determines the daily variation of $\Omega_{ar}$ due to the water chemistry alterations of photosynthesis and respiration (NEP). $\Omega_{ar}$ levels will increase in systems with high photosynthesis relative to calcification through the increased uptake of CO$_2$ (Jokiel et al., 2014).
Figure 3.5 shows the relative changes in $\Omega_{ar}$ when rates of TA and DIC shift due to a reduction or increase in NEC or NEP. Changes in NEC will shift TA values horizontally along the x axis and vertically along the y axis, while changes in NEP will only change DIC vertically along the y axis. Comparison to our previous sampling (McMahon et al., 2013) at the same location shows a difference in $\Omega_{ar}$. The $\Omega_{ar}$ reached a minimum of 2.5 during the current study with 2012 $\Omega_{ar}$ levels dropping to 1.9. Maximum $\Omega_{ar}$ showed a similar increase from 4.4 in 2012 to 4.7 in 2014. Overall, 2014 had higher $\Omega_{ar}$ than 2012 during the peak production period between 14:00 – 16:00. The apparent difference in $\Omega_{ar}$ levels may be indicative of the effects of rising NEP has on the localized water column during isolation. Jokiel et al. (2014) showed that, in mesocosm experiments, $\Omega_{ar}$ was uncoupled with NEC, and therefore the relationship between DIC and TA may offer a better understanding of the way in which the reef is functioning.
Figure 3.5. Relationship between TA and DIC. Black circles represent 2014 samples and grey circles represent 2012 observations (McMahon et al. 2013). Background colour contour indicates $\Omega_{ar}$. 
**Figure 3.6.** Relationship of NEP and NEC. Black icon indicates new data (2014) while grey indicates previous dataset (2012).
Figure 3.7. Comparison of hysteresis between current sampling period (2014) and in 2012 (McMahon et al. 2013). Black icon indicates current sampling while grey indicates 2012 sampling. Square indicates time range between 00:00 and 06:00, circle indicates time period between 06:00 and 14:00, upright triangle indicates time period between 14:00 – 18:00 and upside down triangle indicates time period between 18:00 -24:00. Arrows show hysteresis in $\Omega_{ar}$ levels during a diel cycle travelling from middle bottom (00:00) to left quadrant (06:00) to upper middle (14:00) to middle right (18:00) and back to middle bottom (00:00).
Some previous metabolism studies focus on $\Omega_{ar}$ effects on NEC to predict variations of the current metabolic rates using short term carbonate chemistry observations in the field or in mesocosm experiments (Falter et al., 2012; McMahon et al., 2013; Shaw et al., 2012; Silverman et al., 2012). Corals modify their ambient seawater chemistry, and NEP seems to influence both $\Omega_{ar}$ and NEC, making it difficult to disentangle the multiple drivers of coral NEC and to determine the external influences on internal processes (Andersson and Gledhill, 2013; Cyronak et al., 2015). Indeed, recent mesocosm experiments suggested that NEP has a major influence on $\Omega_{ar}$ with photosynthesis shifting the equilibrium of the carbonate system (Jokiel et al., 2014). Further research is needed to understand the future changes in the linkages between $\Omega_{ar}$, NEC and NEP in coral reefs. We suggest that continuous, long-term time-series observations of the carbonate system would be suitable to disentangle those processes.

### 3.5 Conclusions

Automated instruments measuring the carbonate parameters of pH, $pCO_2$ and TA with high precision in Heron Island’s lagoon permitted for the estimation of the reef’s metabolic rates. The coupling of instruments allowed for over-determination of the carbonate system required for metabolic studies. Calculated data allowed for assessment of internal consistency, ensuring accuracy was maintained during the sampling period. If calculated values are to be used within highly variable systems such as coral reefs, DIC or TA need to be measured to ensure accurate data. A simple modification of the VINDTA 3C enabled automated TA observations that allows smaller teams to perform accurate, high resolution observations during both daytime and night-
time hours at research stations or from research vessels. This approach could help to evaluate anthropogenic changes associated with coral reefs and provide a better understanding of annual water chemistry changes that are poorly understood.

The high frequency sampling regime permitted for comparison between NEC and NEP estimates integrated over different time intervals. Notable differences were found when using different sampling intervals. Whereas these differences may be small relative to expected seasonal and spatial variability, they should be considered when possible. Hysteresis in the NEC versus $\Omega_{sr}$ relationship was apparent at Heron Island, supporting recent suggestions that short-term variability in $\Omega_{sr}$ should not be used to infer long-term changes in NEC. Differences in both the pattern of hysteresis and the ratio of DIC to TA were evident when compared to a prior study at the same location in 2012 (McMahon et al., 2013). It remains unclear whether this difference was due to short-term effects, seasonality or indicative of longer term shifts in the ratio between NEC and NEP. Long-term observations should provide better understanding of NEC and NEP drivers and are urgently needed.
Chapter 4

Coral reef calcification and production after the 2016 bleaching event at Lizard Island (Great Barrier Reef)

McMahon, A., Santos, I. R., Maher, D. T., Schulz, K. G., Scott, A, Silverman, J, and Davis, K, L. Coral reef calcification and production after the 2016 bleaching event at Lizard Island (Great Barrier Reef). (Submitted)
Abstract

Severe bleaching events have affected the Great Barrier Reef (GBR) causing mass losses of hard coral cover. Here, we use flow respirometry approaches to assess coral reef net ecosystem calcification (NEC) and net ecosystem production (NEP) following the 2015/2016 bleaching event at Lizard Island in the northern GBR, a heavily impacted area. Previous studies conducted in 2008 and 2009 (Silverman et al. 2014) were used as pre-impact data. Lagrangian and Eularian approaches provided varied results. Estimated NEC (31.6 to 137.7 mmol m$^{-2}$ day$^{-1}$) and NEP (-876.7 to 50.5 mmol m$^{-2}$ day$^{-1}$) rates in 2016 were highly sensitive to assumptions about seawater transit times and the oceanic endmember concentrations. Using the most robust assumptions (i.e., Eulerian flow and paired endmembers) resulted in NEC and NEP rates of 44.3 and -4.5 mmol m$^{-2}$ day$^{-1}$ respectively. Replicating an earlier approach resulted in post-bleaching NEC in 2016 of 32.2 mmol m$^{-2}$ day$^{-1}$, 40 – 46% lower than pre-bleaching estimates in 2008 and 2009 (61 and 54 mmol m$^{-2}$ day$^{-1}$). The slopes of a total alkalinity vs. dissolved inorganic carbon (TA – DIC) plot decreased from ~ 0.3 in 2008 and 2009 to 0.1 in 2016, indicating elevated organic production and a shift in community function. Changes in NEC relative to the previous study were not driven by changing $\Omega_{\text{aragonite}}$. Coral cover shifted from 8.3% and 7.1% in 2008/9 to 2.9% in 2016. We demonstrate a clear decrease in coral reef NEC following bleaching and highlight that subtle assumptions/methodological differences may create bias in the interpretation of results. Therefore, comparing coral reef metabolism datasets and predicting long-term coral reef calcification based on existing short-term datasets needs to be done with care.
4.1. Introduction

Higher frequencies of severe storm, thermal stress and ocean acidification associated with global climate change are threatening the viability of corals and their ability to maintain the CaCO₃ framework of coral reefs (Grahem et al., 2015; Silverman et al., 2014; Woolsey et al., 2012), which provides critical habitat for a diverse range of marine organisms (Hoegh-Guldberg et al., 2007). Unprecedented prolonged thermal stress events in 2016 caused large-scale coral bleaching on Australia’s Great Barrier Reef (GBR) with complete species die off in some sections (Hughes et al., 2017b). The most severe impact occurred in the northern ~ 1000 km of the GBR with < 9% of 1,156 coral reefs surveyed remaining unaffected (Hughes et al., 2017b). Observations of severely bleached (i.e., > 60% of corals bleached) reefs were estimated to be four times greater than previous bleaching events in 1998 and 2002, indicating that this event was the most severe on record in the GBR (Hughes et al. 2017b). An average rise in temperature of 0.9 °C triggered this event (Van Hooidonk et al. 2016) and even under a conservative CO₂ emissions scenario, warming trends between 2010 and the end of the 21st century are expected to be between 0.3 and 0.7 °C in coral reefs, which is comparable to the increase that has been observed in the past century (Hughes et al. 2017). This further warming is predicted to cause severe bleaching in the majority of reefs globally by 2050 (Van Hooidonk et al. 2016).

Coral reefs can undergo complete trophic phase shifts from coral to algae dominated if multiple local (e.g., reduced water quality, over-fishing, etc.) or global (e.g., climate change) stressors interactively influence the area (Van de Leemput et al. 2016). The resulting reduction in ecosystem services provided by coral reefs, such as shoreline protection, fisheries and tourism, may significantly impact coastal communities that rely on them (Moberg and Folke, 1999). Once reefs shift from coral to algal dominated, their ability to return to their original state is unlikely.
(Van de Leemput et al. 2016). The 2016 bleaching event resulted in significant impacts to the northern GBR with complete species die off on large portions of the reef (Hughes et al. 2017b). Reductions in chronic stressors and the concurrent maintenance of key ecological processes driven by different functional marine herbivore groups are both required for recovery to occur (Graham et al. 2013). Recovery of affected reefs may occur over a period of decades provided that future severe bleaching and weather events do not further impact these areas during this time, which is unlikely (Hughes et al. 2017).

Investigations on the effects of coral bleaching have primarily focused on changes in community composition and coral cover (Hughes et al., 2017, and references therein). Coral reef metabolism studies show the current state of the reef and how it functions in terms of its chemical interaction with the surrounding water. While metabolic rates are expected to increase with higher sea surface temperatures (SST) (Andersson and Mackenzie 2012), very little information is available on how bleaching may drive net ecosystem metabolism in coral reefs and currently available reports vary. Kayanne et al. [2005] investigated ecosystem metabolism of reefs in Japan and Palau before and after bleaching. They found lower rates of net ecosystem production (NEP) at the two reefs after bleaching and contrasting responses of net ecosystem calcification (NEC), where estimated rates were unaltered in one reef but reduced in the other. Disparities between the few published studies complicate the ability to predict future reef metabolic states.

Assessing NEC and NEP in coral reefs often relies on measuring temporal changes in the total alkalinity (TA) and dissolved inorganic carbon (DIC) of reef water as well as estimating residence or transit times (Shaw et al. 2014). Recent metabolic studies in coral reefs have employed a variety of metrics to assess reef states including absolute metabolic rates,
comparisons of NEC to the aragonite saturation state of seawater (\(\Omega_{ar}\)), and ratios of TA to DIC (Shamberger et al. 2011, Shaw et al. 2015). \(\Omega_{ar}\) is projected to drop 0.9 to 1.4 units from the pre-industrial period to the end of the present century due to anthropogenic emissions of CO\(_2\) to the atmosphere and the resulting ocean acidification (Gattuso, 2011). Previous field studies have reported a positive correlation between NEC and \(\Omega_{ar}\) and this trend has been used to predict when a reef will change from a state of net accretion to net dissolution (Shamberger et al. 2011, Shaw et al. 2012, etc.), though concerns have been raised about the validity of such projections (Cyronak et al. 2015). The ratio of TA to DIC can provide insights into reef metabolism and the relative contributions of algae and coral in altering seawater carbonate chemistry (Andersson and Gledhill 2013, Cyronak et al. 2015).

The focus of this report is on NEC and NEP at Lizard Island, northern GBR, which has recently been severely impacted by multiple cyclones (cyclone Ita in 2014 and Cyclone Nathan in 2015) and the 2015/2016 mass coral bleaching event. We discuss methodological challenges to estimate NEC and NEP in coral reefs using seawater chemistry, compare post-bleaching observations to pre-bleaching observations made during 2008/2009 using a similar technique on the same reef flat at the same time of the year. (Silverman et al. 2014), and discuss possible changes in reef metabolism brought about by the bleaching event.

4.2. Methods

Fieldwork occurred from 10 – 22 September 2016 on Lizard Island, GBR. Lizard Island is located 240 km north of Cairns and approximately 30 kilometres offshore in the middle of the GBR lagoon. The Lizard Island group refers to the main granite island (~ 7 km\(^2\) in area) and 3
smaller nearby islands. The island contains a small resort, a scientific research station, and a campsite scattered around the perimeter, with the majority of the island left uninhabited. All islands in the group are national parks and the surrounding waters are protected as part of the Great Barrier Reef Marine Park. To gain insights into the potential changes in reef metabolism by the 2015/2016 bleaching and two cyclones, previous work by Silverman et al. (2014) in 2008 and 2009 were used as a comparative baseline of coral reef metabolism at the site. In accordance with the previous study, our observations were made on the southern reef flat between South Island and Bird Island (-14.6983, 145.4605), extending approximately 300 m from the reef crest towards Lizard Island (Figure 4.1).

To investigate post-disturbance coral reef metabolic rates and test the comparability of estimates provided by different methodologies, several approaches were used to estimate NEC and NEP from changes in the carbonate chemistry of seawater flowing over the reef flat. The first approach employed the Lagrangian technique which follows changes in the chemistry of parcel of water as it flows over the reef, similar to the approach used by Barnes et al. (1976) during the LIMER expedition. A floating equipment package was deployed for 30-minute float intervals over a 12-day period, consisting of a Manta-2 multiprobe measuring water temperature (±0.1°C), Dissolved Oxygen (±1%), Salinity (±1%) and pH (±0.02 units) every 5 minutes, a Holux GPS tracker measuring at 30 second intervals, and a drogue to track the water column movement and ensure correct direction and speed. In the second approach, two current meters (SonTek Argonaut-XR) were deployed, one at the reef crest where transects began (approximately 0.5 – 2.5 m deep), and the other 250 m downstream near the reef flat where transects finished (approximately 1.0 – 3.0 m deep). Current meters were set to measure water
depth, speed and direction at 10-minute intervals to estimate travel times and provide comparison values to the drogue speed.

Figure 4.1. Study site map showing floating transect paths. Black lines denote transects used for study while red lines denote transects that were omitted from final analysis. The 2008 and 2009 data are available from the same reef but located about 300 m SW near South Island. (Image adapted from Google earth)
Filtered (0.45 µm cellulose acetate) water samples were taken at the beginning and end of each transect to measure dissolved inorganic carbon (DIC), total alkalinity (TA), nitrate + nitrite (NO₃⁻), ammonia (NH₄⁺), orthophosphate (PO₄³⁻), total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP). DIC was measured using an AIRICA automated CO₂ analyser running sets of four replicates with an average of the three closest replicates used for final concentration calculations. A Dickson’s certified reference material was run every 10 samples to ensure no drift during measurements with precision better than 2 µmol kg⁻¹ (Dickson, 2010). TA was measured using a Metrohm Tritrando auto-titration instrument with 0.01 M HCl using the Gran titration method. Standards (in–house, referenced against Dickson’s certified reference material) were run every 10 samples with no drift detected. Duplicates were run for each sample with the average variation < 2 µmol kg⁻¹. Dissolved nutrient samples were analysed by a Lachat Flow Injection Analysis Unit (± 5%) (Eyre and Ferguson 2005).

Net Ecosystem Calcification (NEC) was estimated as follows:

$$NEC = -0.5 \frac{\Delta TA_{dp}}{\Delta t}$$

where \( d \) is the average water depth (m) across the sampled profile using measurements integrated from the deployed current meter depth sensors, \( \Delta t \) is the transit time between samples and \( \Delta TA \) is the change in TA (µmol kg⁻¹) during the transit time, and \( \rho \) is the density of seawater (kg L⁻¹).

Net Ecosystem Production (NEP) was estimated as follows:

$$NEP = \frac{-\Delta DIC_{dp}}{\Delta t} - G_{net} - F_{CO₂}$$

where \( \Delta DIC \) is the change in DIC across the reef flat for the transit time (\( \Delta t \)). \( F_{CO₂} \) is the air-sea flux of CO₂ as described by [Wanninkhof, 1992]. \( F_{CO₂} \) was calculated using the CO₂Calc
program (Pierrot et al. 2006) using wind speed measured in the Lizard Island lagoon (Australian Institute of Marine Science buoy) and the gas transfer velocity parameterization of Wanninkhof (1992). Calculation of $\Omega_{ar}$ was done with CO2SYS using the constants for K1, K2 from Mehrbach et al. (1973) refit by Dickson and Millero (1987) and KHSO$_4$ from Dickson (1990). Calculations used *in situ* water temperature and salinity with the carbonate parameters of TA and DIC. Daily integrations of NEC and NEP was done using a 1-hour integration average of all calculated values. A total of 40 sample pairs were obtained. However, 6 pairs were omitted from the analysis due to their significant diversion from the direction of the cross-reef transect (see red lines in Figure 4.1).

Because of excellent precision for TA and DIC analysis, uncertainties in the NEC and NEP estimates are expected to result primarily from uncertainties in transit times across the reef between the upstream and downstream stations. To offer a possible range in NEC and NEP rates and allow comparisons to previous work, we estimated seawater residence times using Lagrangian and Eulerian approaches. The Lagrangian approach assumes that the transit time is equal to the drogue travel time (30 min). The Eulerian approach relies on the current meters to estimate seawater transit times and the flow-continuity assumption. The bottom-deployed current meters provide vertical profiles of current velocities that are integrated to determine water column current speed, generating transit times that can be different than the drogue travel time. Because two current meters were deployed, the Eulerian approach was further classified into four approaches: (1) The EU1 approach relied on the current meter data at the reef crest only; (2) the EU2a approach relied on the current meter data on the reef flat only, (3) the EU3 approach relied on an average of data from both current meters; and (4) the EU2b approach relied on the reef flat current meter only and an average TA and DIC concentration at the reef crest obtained during
daytime only. The EU2b assumptions represent the closest match in the methodology of earlier work (Silverman et al. 2014), allowing for more reliable pre- and post-disturbance comparisons.

Benthic cover surveys were conducted to estimate the percentage of coral and algae present at the study site. Benthic cover was assessed in the 250 × 100 m study site using 96 randomly placed 1 × 1 m quadrats photographed using a Canon 7D camera with a Tokina 10-17 mm lens and nauticam 7D housing parallel to the dominant flow. Benthic categories underlying 20 random points per quadrat were identified. Categories included: branching coral, non-branching coral, soft coral, calcifying algae, non-calcifying algae, rubble, sand, consolidated reefal substrate, and other live coral.

4.3. Results

TA and DIC concentrations on the reef flat had a consistent diel cycle revealing an uptake of both TA and DIC during the day consistent with calcification and photosynthesis (Figure 4.2). Overall, TA and DIC changes in 2016 occurred over a narrower range in comparison to 2008 and 2009 measurements (Figure 4.2). Nutrient concentrations during the sampling period were consistently low (average NO$_x$ = 0.04 µmol L$^{-1}$, NH$_4$ = 0.44 µmol L$^{-1}$, PO$_4$ = 0.16 µmol L$^{-1}$, DON = 8.89 µmol L$^{-1}$, DOP = 0.10 µmol L$^{-1}$, TDN = 9.40 µmol L$^{-1}$, and TDP = 0.26 µmol L$^{-1}$), with no measurable nutrient uptake/addition over the reef flat. Nutrient concentrations were not correlated with changes in TA or DIC.
Figure 4.2. TA and DIC of samples taken from the reef crest (black circles), reef flat (white circles) during this sampling period and reef crest (grey triangles) and reef flat (white triangles) during the 2008 and 2009 sampling periods.

The general current direction over the reef flat was consistent over the study period, except for one day (20 September 2016) when winds abated and current direction ran parallel to the reef crest (as indicated by the red lines in Figure 4.1). NEC and NEP estimates from this day were excluded from the dataset to ensure that estimates provided reflect the metabolism over the
same portion of the reef flat. The general current direction was similar to surveys in 2008/2009 and 1975/1976 (Barnes et al., 1976; Kinsey, 1977; Silverman et al., 2014). Current speeds measured on the reef flat were slightly higher than those measured in previous studies (Table 4.1), perhaps due to stronger southerly winds. Current speeds measured on the reef crest were higher than the downstream measurements, most likely due to shallower water at the upstream station and the cumulative effect on the friction on the water as it flowed over the reef flat (Figure 4.3).

The different approaches to estimate water transit times resulted in different NEC and NEP rates (Table 4.1). NEC and NEP rates calculated using the Lagrangian approach were the highest of all estimates due to the relatively faster drogue travel time (Figure 4.3). The Eulerian approaches that utilised different current meter arrangements also varied considerably.
Figure 4.3: Current speeds estimated using the drogue transects compared to corresponding average water column speed derived from the Sontek current profiler measurements made on the reef crest and reef flat throughout the period of this study. CM1 line is reef crest current meter and CM2 line is reef flat current meter. Grey dashed line shows 1:1 ratio.

Daytime NEC displayed the expected trend, increasing during the morning hours and decreasing from its noontime peak value until sundown (Figure 4.4 using approach EU2b) and had an overall positive correlation with PAR (Figure 4.5). In comparison to the 2008 and 2009 studies, peak NEC in this study occurred nearly 2 hours earlier (ca. 11:00 am) and was ~ 30% lower (Figure 4 using approach EU2b). Low to negative (CaCO₃ dissolution) NEC values were observed during the night with positive NEC starting at sunrise. The average $\Omega_{ar}$ was lower than in 2008 and 2009 studies. Daytime $\Omega_{ar}$ did not increase to similar maximum levels (3.9 vs. 4.7), while night time levels were similarly low (Figure 4.4).
NEP was highly different between the two studies, with lower average daytime positive NEP (CO₂ uptake, \textit{i.e.}, decreased net production), while during the night time NEP was significantly more negative (CO₂ evolution, \textit{i.e.}, increased respiration) than in the 2008 and 2009 study. Night-time maximum respiration was higher in 2016 than in 2008 with daytime maximum NEP rates increasing from 1356 to 1905 mmol C m⁻² d⁻¹. Live coral cover in 2016 was 2.91% consisting of 0.3% branching hard coral, 2.6% non-branching hard coral and 0.1% soft coral (Figure 4.6). Algal cover consisted of 0.8% calcifying and 16.2% non-calcifying.

\textbf{Table 4.1:} NEC and NEP estimates (mmol m⁻² d⁻¹) based on changes in carbon chemistry and different approaches to estimate seawater transit times. Eularian (EU1, EU2a, EU2b, EU3) transit times are corrected for flow direction.

<table>
<thead>
<tr>
<th></th>
<th>Lagrangian (Drogue Transit Times)</th>
<th>EU1 (Reef Crest Current Meter)</th>
<th>EU2a (Reef Flat Current Meter)</th>
<th>EU2b (Reef Flat Current Meter and Daytime Oceanic Endmember)</th>
<th>EU3 (2 Current Meters)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current speed avg (cm/s)</td>
<td>10.8 ± 4</td>
<td>4.2 ± 1.4</td>
<td>4.6 ± 1.7</td>
<td>4.6 ± 1.7</td>
<td>4.4 ± 2.4</td>
</tr>
<tr>
<td>Transit time (hours)</td>
<td>0.5 ± 0</td>
<td>2.2 ± 1.7</td>
<td>1.3 ± 0.5</td>
<td>1.3 ± 0.5</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>Total NEC</td>
<td>137.7±14.3</td>
<td>31.6±3.3</td>
<td>56.9±5.9</td>
<td>32.2±3.3</td>
<td>44.3±4.6</td>
</tr>
<tr>
<td>Total NEP</td>
<td>50.5±11.5</td>
<td>-17.2±3.9</td>
<td>8.3±1.9</td>
<td>-876.7±198.9</td>
<td>-4.5±4.5</td>
</tr>
<tr>
<td>Daytime NEC</td>
<td>313.7</td>
<td>73.0</td>
<td>124.3</td>
<td>138.0</td>
<td>98.7</td>
</tr>
<tr>
<td>Daytime NEP</td>
<td>1044.9</td>
<td>293.3</td>
<td>443.1</td>
<td>144.0</td>
<td>368.2</td>
</tr>
<tr>
<td>Night-time NEC</td>
<td>-38.3</td>
<td>-9.8</td>
<td>-10.5</td>
<td>-73.6</td>
<td>-10.1</td>
</tr>
<tr>
<td>Night-time NEP</td>
<td>-943.9</td>
<td>-327.8</td>
<td>-426.4</td>
<td>-1897.8</td>
<td>-377.1</td>
</tr>
</tbody>
</table>
Figure 4.4. Diel cycle measurements of NEC, $\Omega_{\text{arag}}$ and NEP calculated using the EU2b approach (reef flat current speed and constant reef crest TA and DIC) during this study and the studies conducted by Silverman et al (2014) on the South Island reef flat. Shaded grey areas indicate the nighttime period between sunset and sunrise.
Figure 4.5. Correlation of diel variation in NEC and NEP, calculated using the EU2b approach, with corresponding values of photosynthetic active radiation (PAR) using the EU2b approach (average ocean TA and reef flat current meter). Black circles denote night-time values (PAR=0) and grey circles denote daytime values.
Figure 4.6.

Relative coverage distribution of different benthic types in the reef flat area where the community metabolism measurements were conducted during this study.

4.4. Discussion

We first discuss some of the challenges in estimating NEC and NEP in coral reefs. This analysis highlights uncertainties that may emerge from different methodologies to estimate seawater transit times and assumptions (as often not measured) about initial DIC and TA.
concentrations. We then discuss differences in NEC and NEP using the post-bleaching 2016 dataset compared to data from 2008/2009.

4.4.1 Hidden assumptions and methodological challenges

Comparisons between NEC and NEP datasets need to be cautiously performed to ensure any observed differences in rates are not biased by methodological differences. Variabilities in data collection (Shaw et al. 2014) and interpretation approaches (McMahon et al. 2018) may result in different NEC and NEP estimates within the same site and potentially alter interpretations of spatial and temporal trends. Here, we highlight two often overlooked sources of uncertainty that may influence NEC and NEP data interpretation: (1) estimates of seawater transit times and (2) assumptions about the oceanic TA and DIC concentrations.

Both Lagrangian and Eulerian approaches were utilized to estimate seawater transit times. The Lagrangian approach resulted in a diurnal average NEC much greater than the three different Eulerian approaches (Table 4.1). This variation is due to the different transit time estimates between methods. The Lagrangian approach following a drifter for 30 minutes resulted in an average current speed of $10.8 \pm 4.0$ cm s\(^{-1}\), while Eulerian approaches EU1, EU2 (a and b), and EU3 had an average of $4.2 \pm 1.4$, $4.6 \pm 1.7$, and $4.4 \pm 2.4$ cm s\(^{-1}\), respectively (Table 4.1). Current speed variation was primarily observed at the reef crest site where current speeds peaked at a depth of approximately 0.6 m (~33 cm s\(^{-1}\)) and declined as water became deeper over the reef crest. Subsurface drogues can produce faster velocity estimates due to waves near the surface causing current (Booth 1981) and surfing on waves in shallow water, explaining the enhanced NEC and NEP rates estimated from the Lagrangian approach. This highlights that speed derived from drogue data should be used with caution when estimating coral reef
community metabolic rates, and that the NEC rates via our Lagrangian approach are overestimated.

Both the Eulerian or Lagrangian approaches require oceanic (or reef crest) TA and DIC concentrations to estimate ecosystem metabolism. Because sampling oceanic waters adjacent to coral reefs is often logistically more difficult than sampling within reef flats, oceanic endmember sample sizes are typically smaller. For example, the Lizard Island reef crest is quite difficult and dangerous to access due to waves breaking and very shallow water in the wave trough that can result in grounding of vessels. As a result, only daytime TA and DIC data are available during the 2008 and 2009 NEC estimates (Silverman et al. 2014). Here, we paired reef crest and reef flat samples to prevent uncertainties due to potential variability in the oceanic endmember. In this case, using individual pairs of reef crest/flat samples resulted in higher NEC rates than when using only daytime averages as done previously. The reef crest waters at Lizard Island had a detectable diel trend in TA ranging from 2282 during the day to 2302 µmol L\(^{-1}\) at night during this study. Utilising an average daytime TA from oceanic samples as the initial value for all reef flat observations lowered the daily NEC by 24.7 mmol m\(^{-2}\) day\(^{-1}\) for the EU2b approach compared to the EU2a. As a result, the often-employed assumption that reef crest TA and DIC concentrations remain constant may lower estimates by nearly 50% of the total NEC rate calculated for this reef than when using individual pairs of TA and DIC observations. Hence, such assumptions can have a major impact on estimates of NEC in coral reefs and comparisons between datasets need to be made carefully. We suggest that it may be important to sample TA and DIC on the reef crest during the night to capture the full diel variation and avoid the need to assume constant reef crest TA and DIC. While we believe the EU3 approach (\textit{i.e.}, relying on two
current meters and paired samples) offers the most reasonable NEC and NEP estimates for Lizard Island, we will use the EU2b approach to compare NEC and NEP to earlier datasets.

### 4.4.2 Comparing 2016 to 2008 and 2009 using the EU2b approach

The EU2b approach is modelled after methodologies and associated assumptions used by Silverman et al. (2014) to estimate NEC and NEP. The approach relies on transit times estimated using one current meter on the reef flat and the average of the daytime TA and DIC at the reef crest (see reef crest end member values in Figure 4.2). Both 2008/2009 and 2016 studies utilised a flow respirometry approach to measure reef ecosystem metabolism and samples were collected within ~300 m on the same reef flat. Both studies assume negligible mixing of the water parcel during travel. Variability within the results are unlikely to be driven by cross reef heterogeneity in community composition and cover. Both 2016 and 2008/2009 observations were all performed in September with nearly identical water temperatures and nutrient levels (Table 4.2).

Since the EU2b approach matches earlier assumptions, comparisons between pre- and post-bleaching rates of ecosystem metabolism can be attempted. EU2b NEC rates in 2016 were 47% and 40% lower than in 2008 and 2009, respectively, with a contraction in the daytime range and an expansion in the nighttime range of calcification estimates (Table 4.2). When integrated over a diel cycle, NEC was $61 \pm 12 \text{ mmol m}^{-2} \text{ day}^{-1}$ and $54 \pm 13 \text{ mmol m}^{-2} \text{ day}^{-1}$ in 2008 and 2009, respectively, while in 2016 NEC was $32 \pm 3.3 \text{ mmol m}^{-2} \text{ day}^{-1}$. This encompasses a difference in daytime minimum/maximum range of $16 – 472 \text{ mmol m}^{-2} \text{ day}^{-1}$ and $47 – 439 \text{ mmol m}^{-2} \text{ day}^{-1}$ for 2008 and 2009, respectively and $58 – 330 \text{ mmol m}^{-2} \text{ day}^{-1}$ in 2016. Night time minimum/maximum rates were $-19 – 11 \text{ mmol m}^{-2} \text{ day}^{-1}$ and $-57 – 38 \text{ mmol m}^{-2} \text{ day}^{-1}$ in 2008.
and 2009 and \(-425 – 102\) mmol m\(^{-2}\) day\(^{-1}\) during 2016. In comparison to \textit{Barnes et al.} [1976] (83 – 105 mmol m\(^{-2}\) day\(^{-1}\) overall), the rate of NEC in 2016 decreased by 61– 69%.

NEP decreased in comparison to the 2008 study from a daytime minimum/maximum range of \(-385 – 1356\) mmol m\(^{-2}\) day\(^{-1}\) and night time minimum/maximum range of \(-376 – 82\) mmol m\(^{-2}\) day\(^{-1}\) with an overall average of 10 mmol m\(^{-2}\) day\(^{-1}\) (unpublished data) in 2008 to a daytime range of \(-2041 – 1905\) mmol m\(^{-2}\) day\(^{-1}\) and a night-time range of \(-3221 - -618\) mmol m\(^{-2}\) day\(^{-1}\) with an overall average NEP of \(-876 ± 199\) mmol m\(^{-2}\) day\(^{-1}\). Silverman et al. (2014) used an average open ocean TA (2278.1 ± 5.3 and 2269.3 ± 15.4 µmol kg\(^{-1}\) in 2008 and 2009, respectively) and DIC (1955.1 ± 2.2 µmol kg\(^{-1}\) obtained in 2008 only) based on 7 and 9 daytime samples in 2008 and 2009, respectively. If we use daytime averages (34 samples) of TA (2290.5 ± 3.9 µmol kg\(^{-1}\)) and DIC (1982.2 ± 22.5 µmol kg\(^{-1}\)) to match Silverman et al. (2014) (approach EU2b in Table 4.1), the final NEC rates would be 27% lower than the most reasonable approach (EU3) integrating flow speeds over the transect.
Table 4.2: A comparison of 2016 observations to earlier studies. Table updated from Silverman et al. (2014).

<table>
<thead>
<tr>
<th></th>
<th>1975/76</th>
<th>2008</th>
<th>2009</th>
<th>2016 (EU2b approach)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atmospheric pCO₂ (ppm)</td>
<td>331</td>
<td>386</td>
<td>387</td>
<td>400</td>
</tr>
<tr>
<td>Diel average Temperature (°C)</td>
<td>26.7</td>
<td>24.9±0.48</td>
<td>26.5±0.63</td>
<td>26.2±0.73</td>
</tr>
<tr>
<td>Reef water diel average Ω&lt;sub&gt;arag&lt;/sub&gt; with pCO₂ at corresponding atmospheric equilibrium</td>
<td>4.33</td>
<td>3.76</td>
<td>3.93</td>
<td>3.49</td>
</tr>
<tr>
<td>Diel average reef water Ω&lt;sub&gt;arag&lt;/sub&gt;</td>
<td>3.65</td>
<td>3.45</td>
<td>3.39</td>
<td></td>
</tr>
<tr>
<td>Wind Speed (m/s)</td>
<td>-</td>
<td>7.4±1.4</td>
<td>8.2±2.1</td>
<td>7.1±1.6</td>
</tr>
<tr>
<td>Wind Direction</td>
<td>-</td>
<td>163±10</td>
<td>151±8</td>
<td>123±11</td>
</tr>
<tr>
<td>Current Direction</td>
<td>-</td>
<td>311±22</td>
<td>332±51</td>
<td>304±12</td>
</tr>
<tr>
<td>Current Speed (Average) (cm/s)</td>
<td>-</td>
<td>0-8.5 (2.9±1.2)</td>
<td>0-13.9 (3.6 ± 2.1)</td>
<td>1.7-7.1 (4.6 ± 1.4)</td>
</tr>
<tr>
<td>Ocean TA (Average) (µmol kg⁻¹)</td>
<td></td>
<td>2278±5.3</td>
<td>2269±15.4</td>
<td>2291±5.3</td>
</tr>
<tr>
<td>Ω&lt;sub&gt;arag&lt;/sub&gt; slope</td>
<td>-</td>
<td>253±35</td>
<td>286±67</td>
<td>338±49</td>
</tr>
<tr>
<td>Ω&lt;sub&gt;arag&lt;/sub&gt; when NEC=0</td>
<td>-</td>
<td>3.4</td>
<td>3.23</td>
<td>3.05</td>
</tr>
<tr>
<td>DIC:TA slope</td>
<td>-</td>
<td>0.29±0.04</td>
<td>0.35±0.06</td>
<td>0.10±0.02</td>
</tr>
<tr>
<td>Daytime NEC range (mmol m² d⁻¹)</td>
<td>-</td>
<td>16-472</td>
<td>47-439</td>
<td>60-330</td>
</tr>
<tr>
<td>Night-time NEC range (mmol m² d⁻¹)</td>
<td>-</td>
<td>-19-11</td>
<td>-57-38</td>
<td>-425-103</td>
</tr>
<tr>
<td>Diurnal average NEC (mmol m² d⁻¹)</td>
<td>83-105</td>
<td>61±12</td>
<td>54±13</td>
<td>32±3.3</td>
</tr>
<tr>
<td>Daytime NEP range (mmol m² d⁻¹)</td>
<td>-</td>
<td>-385-1356</td>
<td>-</td>
<td>-2041-1905</td>
</tr>
<tr>
<td>Night-time NEP range (mmol m² d⁻¹)</td>
<td>-</td>
<td>-376 - -82</td>
<td>-</td>
<td>-3221 - -618</td>
</tr>
<tr>
<td>Diurnal average NEP (mmol m² d⁻¹)</td>
<td>-</td>
<td>10</td>
<td>-</td>
<td>-876.7±199</td>
</tr>
</tbody>
</table>

There are two inherent differences between our EU2b estimates and the 2008 and 2009 dataset: (1) duration of observations, and (2) tidal conditions. During the 2008 and 2009 studies, sampling occurred over 2 – 3 days near neap tides when currents flowing over the reef where minimal. During 2016, sampling was carried out over a 12-day period encompassing both spring and neap tides with varying water flow during the sampling period. During the 2008 and 2009 study periods, current speeds were between 0 – 8.5 cm s⁻¹ (average 2.9 ± 1.2 cm s⁻¹) and 0 – 13.9 cm s⁻¹ (average 3.6 ± 2.1 cm s⁻¹), respectively (Silverman et al. 2014). During 2016, current
speeds across the sampling site ranged from 1.3 – 7.1, with an elevated average of 4.6 ± 1.4 cm s\(^{-1}\). It is unclear whether the discrepancies in current speeds are due to different waves or wind action, or simply a natural variability related to sampling over two weeks in 2016, and over 2 – 3 days in both 2008 and 2009 encompassing different tidal ranges. Elevated water flow over the substratum is well known to increase metabolic rates by facilitating the uptake of solutes from seawater as revealed from mesocosm (Atkinson et al. 1994, Langdon and Atkinson 2005), chamber (Cyronak et al. 2013), flume (Comeau et al. 2014) and in situ (Shaw et al. 2014) investigations. For example, flume experiments showed a significant correlation between flow rate and NEC during daytime and nighttime and suggested that flow may at least partially mitigate the effects of ocean acidification in coral reefs (Comeau et al. 2014). With faster currents, NEC rates should have increased from 2008 and 2009 estimates, indicating that our interpreted bleaching impact may be underestimated.

During and after coral bleaching occurs, NEC rates can remain stable as other organisms such as coralline algae and calcifying macroalgae *Halimeda* spp. increase in cover in response to increased space (Kayanne et al. 2005). In a comparison of two different reefs (Shiriho Reef in Japan and Ngerdiluches Barrier Reef in Palau) influenced by the same bleaching event in 2000, two different responses were recorded. In Shiriho, coral cover decreased from 7.1% to 5.8% in 4 months due to bleaching and recovered to 6.7% after 1 year and maintained a steady NEC during the event. In Palau, both NEP and NEC decreased with NEP showing a larger reduction, driving a change in the ratio of NEP/NEC (Kayanne et al. 2005). In a recent mesocosm experiment, coral densities of 40% and 80% showed no difference in NEC and NEP in Bermuda, while a repetition of the same densities in situ had a linear correlation between coral density and NEC and NEP (Page et al. 2017).
Coral cover at Lizard Island decreased following the cyclones and bleaching event from an estimated 8.3% and 7.1% in 2008 and 2009 (Silverman et al. 2014), similar to the coral covers presented by earlier metabolic studies at this site (Barnes et al. 1976, Kinsey 1979, Pichon and Morrissey 1981, Chisholm and Barnes 1998), to 2.6% in 2016. However, higher coral cover does not always directly relate to higher NEC and NEP [Page et al., 2017]. Previously, benthic community sampling of the reef flat has shown that composition was relatively homogenous along the southern reef crest and flat between South Island and Bird Island (Kinsey 1979, Silverman et al. 2014). We highlight, however, that methods of evaluation between studies varied and comparisons should be made with care. Silverman et al. (2014) analysed satellite imagery to evaluate the reflectance and associated that with the benthic type to determine benthic cover, whereas our study used photo quadrats taken across the sampling area to assess benthic cover.

The NEC rates at Lizard Island have decreased consistently with the reduction in coral cover from previous studies (Pichon and Morrissey 1981, Silverman et al. 2014). Calcification rates vary among different species of coral and surrounding water conditions allowing for some differences in coral cover percentage and the corresponding NEC rates. Shaw et al. (2016) predicted there would be a 53% decline in calcification by the end of the century without changes to benthic composition, with a further decline in calcification of 5.7% for each 10% decline in calcifier coverage due to ocean acidification. Calcifying algae can also play a significant role in NEC with uptake rates measured on Lizard Island up to 9.6 mmol CaCO₃ m⁻² h⁻¹ (Chisholm 2000). While calcifying algae coverage was only determined to be 0.8% of the benthos during this study, the method of coral cover evaluation (top-down photography) may overlook coralline algae located under rocks and coral skeletons. Previous metabolism studies at
Lizard Island did not estimate the cover of calcifying algae to provide means for a direct comparison.

### 4.4.3 Relationships between NEC and $\Omega_{\text{aragonite}}$ and TAC:DIC ratios

The relationship between NEC and $\Omega_{\text{ar}}$ has been utilised to predict when a reef will shift from a state of net accretion to a state of net dissolution (Shamberger et al. 2011, Shaw et al. 2015). A change in the slope of the NEC versus $\Omega_{\text{ar}}$ relationship was observed when comparing 2008 and 2009 and 2016 observations (Figure 4.7). The slope increased from 253 ± 35 and 286 ± 67 in 2008 and 2009 respectively to 338 ± 49 in 2016 (Table 4.2). A significant difference in slopes was found between 2008 and 2016 ($p = 0.0004$) while 2009 and 2016 showed no significant difference ($p = 0.096$). $\Omega_{\text{ar}}$ was lower in 2016 than in previous studies with a concurrent reduction in NEC rates. This is due to the offshore seawater chemistry (see table 4.2) and a larger sample number throughout both the day and night capturing the daily average in more detail. The $\Omega_{\text{ar}}$ point at which NEC = 0 was reduced from 3.30 in 2008 and 2009 to 3.05 in 2016. We also observed an overall reduction of $\Omega_{\text{ar}}$ with values of 3.2 – 4.8 and 2.9 – 4.4 in 2008 and 2009, respectively, to 3.0 – 3.9 in 2016. Changes in water chemistry differentially reduced $\Omega_{\text{ar}}$ and NEC, highlighting that $\Omega_{\text{ar}}$ may not necessarily be a sole long-term predictor of NEC as discussed elsewhere (Andersson and Gledhill 2013, McMahon et al. 2013, Cyronak et al. 2018), especially when stressors such as bleaching are involved. NEC is directly or indirectly affected by a number of parameters including PAR, seawater CO$_2$ chemistry, temperature, nutrient availability, and hydrology (e.g. Kleypas et al. 2001, Silverman et al. 2007, Falter et al. 2012, McMahon and Santos 2017), while $\Omega_{\text{ar}}$ is a function of the concentrations of TA and DIC as well as temperature and salinity (Andersson and Gledhill 2013).
The relative change in NEC calculated according to the equation developed by Silverman et al. (2009) for coral reef NEC as a function of the diel average temperature and $\Omega_{\text{ar}}$ (Table 2) is 21% lower in 2016 compared to the 2008 and 2009 studies. Thus, the overall proportional contribution of the change in $\Omega_{\text{ar}}$ between the studies seems to be comparable to the decrease in live coral cover. This discrepancy can be settled by taking into account the nearly 7-fold increase in night time dissolution (NEC < 0) in 2016 compared to the 2008 and 2009 studies.

The slope of the TA – DIC relationship on the reef can indicate the relative proportions of calcification/dissolution and photosynthesis/respiration, and whether the reef is a net sink or source of CO$_2$ to the atmosphere (Suzuki and Kawahata 2003). Photosynthesis lowers DIC, while calcification lowers TA and DIC. An increase in a TA:DIC slope relative to a baseline value indicates a greater contribution of calcification/dissolution to the observed changes in reef water carbonate chemistry, while a reduced slope indicates a greater contribution of community photosynthesis/respiration. These variations have been used to assess the status of coral reef communities with respect to the balance between benthic heterotrophic (corals) and autotrophic (macro-algae) populations [Suzuki and Kawahata, 2003]. Thus, a reef that has undergone a trophic phase shift in favour of macro-algae would display a decreased TA:DIC slope relative to its baseline value and vice versa.
Figure 4.7. Comparison of NEC and $\Omega_{ar}$ values measured during this study and the 2008 and 2009 studies using the EU2b approach. NEC goes from calcification to dissolution (NEC<0) at $\Omega_{ar}$ 3.4, 3.23 and 3.05 for 2008, 2009 and 2016 studies, respectively.

The TA–DIC slope at Lizard Island fell from 0.29 ± 0.04 and 0.35 ± 0.06 in 2008 and 2009, respectively, to 0.10 ± 0.02 in 2016, further indicating a decline in calcification on the reef (Figure 4.8). The 2-fold increase in nighttime NEP and 7-fold increase in nighttime dissolution in 2016 compared to 2008 and 2009 seem to play an important role in determining the change in the TA:DIC slope.
Figure 4.8: Comparison of DIC and TA measurements that were made during the 2008 and 2009 and 2016 studies on the South Island reef flat on field of constant $\Omega_{ar}$ contours. DIC and TA values were normalized to a constant salinity of 35. $\Omega_{ar}$ contours were calculated at constant temperature of 25° C and salinity of 35. All TA and DIC measurements were run using certified reference materials to ensure proper calibration and comparison to previous sampling is accurate.
The shift towards a more organically-driven metabolism is expected for disturbed coral reefs. When examined in a mesocosm with identical coral species (Pocillipora damicornis and Montipora capitate) and rhodoliths (i.e., crustose coralline algae covering 20 – 30% of the tanks) under various climate change treatments, the TA:DIC stayed relatively constant despite the relations between NEC and ambient $\Omega_{ar}$ showing the relative dominance of organically driven processes in determining the $\Omega_{ar}$ in coral reefs (Andersson et al. 2009). The most comprehensive understanding of reef processes and health state occurs when both TA:DIC and NEC vs. $\Omega_{ar}$ analyses are considered (Suzuki and Kawahata 2003). Combining TA:DIC and NEC vs. $\Omega_{ar}$ reveals that Lizard Island has undergone a change in both the absolute rates and ratio of NEC and NEP by shifts in reef community structure that were influenced by the recent thermal stress and bleaching events on the reef.

4.4.4 Comparisons to other coral reefs

Estimates of coral reef metabolism are highly variable on both local and global scales. Analytical methods and seasonality often differ between studies, making comparisons over space and time difficult. NEC varies considerably around the globe from -22 mmol m$^{-2}$ d$^{-1}$ in Bermuda to 331 mmol m$^{-2}$ d$^{-1}$ in Kanohe Bay, Hawaii (see Shaw et al. 2014 for a summary). Lizard Island diel average NEC in 2016 (NEC = 44.3 mmol m$^{-2}$ d$^{-1}$) using the most reasonable assumptions (i.e., approach EU3) were within the broad range observed in other study sites in the GBR. Diel average NEC in the Southern GBR ranges from 33 mmol m$^{-2}$ d$^{-1}$ (One Tree Island; Shaw et al. 2014), 58 mmol m$^{-2}$ d$^{-1}$ (Heron Island; McMahon et al. 2013), 60 mmol m$^{-2}$ d$^{-1}$ (Lady Elliot Island; Shaw et al. 2012) 74 ± 24 mmol m$^{-2}$ d$^{-1}$ (One Tree Island; Silverman et al. 2012). The southern GBR reefs (One Tree Island, Heron Island, and Lady Elliot Island) are all coral cays
isolated from oceanic water during their low tide cycles, therefore the slack water method was utilized to estimate NEC at these sites, which may greatly differ from the flow respirometry methods used at Lizard Island. Coral ecosystem metabolism at One Tree Island was investigated using both flow respirometry (Kinsey 1977, Shaw et al. 2014) and slack water at low tide (Shaw et al. 2015). Results revealed 2-3-fold greater rates of NEP and NEC using flow respirometry than the slack water method (Shaw et al. 2015). Differences in methodologies may be attributed to secondary influences of flow on coral cover. However, regardless of the mechanisms of inherent variability between flow and slack methodologies, comparisons between studies should be made with caution and should only be made when the methods employed are identical.

4.5. Summary and Conclusions

My observations of coral reef metabolism at Lizard Island build on previous research and provide opportunities for longer-term datasets to be developed. While our results imply a reduction in coral reef calcification in response to bleaching and cyclones, the short-term datasets available may not allow for a straightforward assessment of long-term changes in NEC and NEP. The 44% decrease in NEC rates from 2008 and 2009 to 2016 (using EU2b approach) coincides with a significant 66% decline in coral coverage from ~ 7.7% to 2.6% and only a modest decline in $\Omega_{ar}$. We highlight that any comparisons made between studies need to be done with caution to best replicate previous studies. Whilst the rates provided from EU2b can be used to compare the 2008 and 2009 and 2016 datasets, we believe the EU3 approach with a NEC rate of 44 mmol m$^{-2}$ d$^{-1}$ produces the most reliable post-bleaching estimate. Furthermore, this study presents the importance of water flow monitoring as the floating drogue was overestimating current speed.
due to wind drift and wave surfing by a factor of at least two, potentially significantly affecting estimated metabolic rates. Night time dissolution (NEC < 0) and NEP increased substantially in 2016 compared to 2008 and 2009. These changes in metabolic rates are strongly reflected changes in the TA:DIC slopes. Overall, this study provides estimates of reef-scale responses of coral ecosystems to severe bleaching. Additional research is required to assess further degradation or recovery time-scales. As climate change continues to threaten coral reefs, understanding the response and recovery of these ecosystems to stressor events may provide essential information to coastal managers and stakeholders.
Chapter 5

Summary and Conclusions
The aims of this thesis were to evaluate the approaches often used for determining coral reef metabolic studies and obtain insights into their drivers. This thesis hypothesized that subtle methodological differences in both data collection and assumptions could cause variances in interpretation. Literature gaps surrounding how the aragonite saturation state, net ecosystem production and natural nutrient additions drive coral reef calcification were also evaluated. Better understanding of these factors builds knowledge and allows greater understanding of coral reefs.

Chapter 2 investigated the influence of naturally derived nutrients into a coral reef lagoon metabolism. Overall, natural nitrogen enrichment enhanced net ecosystem metabolic rates during the day, but had no effect at night. Groundwater was tracked across the island to the coral reef flat in a transect involving multiple sampling approaches. Groundwater sampling on the island revealed high nutrient levels (TDN = 1536 µmol L⁻¹) that were diluted with tidal flushing causing an addition to the lagoon. Groundwater seepage faces along the beach were sampled with minimum nitrate additions of 2.1 mmol m⁻² d⁻¹ being discharged using estimations from a radon mass balance model. An independent approach utilizing the enrichment of nitrate during the isolation of the lagoon at low tide implied fluxes of 5.4 mmol m⁻² d⁻¹. Groundwater nutrient speciation was also tracked from the island to the reef flat. Island groundwater was predominately (> 90%) nitrate changing to a dissolved organic nitrogen (DON) dominated system (DON ~ 86%) in the reef flat. Nutrients entering the lagoon during the low tide period when tidal pumping of groundwater was most dominant coincided with isolation of the lagoon. During isolation, nutrient additions by SGD remain within the constraints of the reef crest allowing for longer periods of utilization by metabolic processes. Nutrient additions and daytime NEC were found to be correlated suggesting that nutrient additions from naturally-derived bird guano helps drive metabolic rates in a coral reef’s otherwise oligotrophic environment.
In chapter 3, I used a new, automated sampling method to enable more detailed investigation into carbon dynamics on coral reefs. Sampling intervals were shortened to 30 minutes between sampling, allowing for a comparison of different sampling intervals to estimate NEC and NEP rates. Sampling interval steps measured at 30 minutes, 1 hour and 2 hour intervals showed a variation of up to 12 and 30% for NEC and NEP respectively. Variation mainly occurred at times where metabolic rates changed most rapidly (dawn, dusk, and peak daytime production/calcification) where the change in rates occurred quickly and could be easily misinterpreted with larger times between samples. Different integration of data approaches were also tested showing 2 to 7% variation for NEC and 1 to 3% for NEP. Calculation of TA, DIC, pH, and $pCO_2$ were performed to check data integrity and performance of calculated variables. pH and $pCO_2$ had similar values with TA (calculated from pH and $pCO_2$) having a large discrepancy (average difference TA = 60 $\mu$mol kg$^{-1}$). This chapter showed that automated sampling systems can give a better understanding of the reefs metabolism and the variation caused by different sampling intervals and analysis.

In chapter 4, the effects of the massive bleaching event in 2015/2016 were assessed to determine the changes on the metabolic performance of Lizard Island coral reefs. Lagrangian and Eularian flow respirometry methods were compared to determine variation caused by assumptions of oceanic endmember values and transit times over the reef flat. Previous research by Silverman et al., (2014) was used as pre-impact data to assess changes brought about by bleaching. A Eularian method utilizing the average daytime oceanic end member and only the reef flat current meter was used to best replicate previous research. Post impact NEC was 32.2 mmol m$^{-2}$ day$^{-1}$, showing a decrease of 47% and 40% to 2008/2009 respectively. Coral coverage over the sampling site reduced from 8.3 and 7.1% in 2008 and 2009, respectively, to 2.6% in
Comparison of TA – DIC slopes showed a reduction from ~ 0.3 in 2008 and 2009 to 0.1 in 2016, showing a shift to a more organic metabolism-dominated ecosystem. Variation among approaches had a maximum difference of nearly 50% for NEC. Using the most reasonable assumptions, paired endmembers and an average of the reef crest and reef flat current meter transit times, NEC was 44.3 mmol m\(^{-2}\) day\(^{-1}\) and NEP was -4.5 mmol m\(^{-2}\) day\(^{-1}\). Variation among sampling methods revealed major differences and comparison of datasets needs to be made with care to ensure the best comparison can be made to remove accidental bias.

Comparing slack water and flow respirometry methods on the same site produces higher NEC and NEP rates using the flow respirometry approach (Shaw et al., 2014). Chapter 4 showed that even within the flow respirometry method, variation occurs between Lagrangian and Eulerian approaches, indicating that method variation between studies may account for larger, unaccounted for errors when comparing various sites sampled using different methodologies. This, combined with differences in sampling intervals and data integration approaches from Chapter 3, could possibly account for up to 348% variation between Lagrangian and Eulerian (Lagrangian to EU1) or 93% variation among different Eulerian approaches (EU1 to EU2a) for NEC.

The examined drivers of coral reef NEC had both positive and negative influences coral reef metabolism. With coral reefs relying on efficient nutrient cycling (Glud et al., 2008), additions of nutrients in oligotrophic reef waters become important drivers of coral reef metabolism. Nutrients inputs through SGD, mainly in the form of NO\(_x\) were shown to be taken up within a close radius to the area where they enter and are retained due to tidal pooling of water. PAR likewise was shown to be positively correlated with NEC, reflecting the coral’s zooxanthellae utilizing sunlight to produce energy. While these two drivers increased NEC rates,
the mass bleaching event reduced both the coral cover by ~66% and NEC rates by 40 – 47%. Small, useable, nutrient additions into healthy reefs can stimulate NEC and NEP, whereas nutrient additions into areas with severe coral bleaching may induce a trophic shift and become dominated by NEP (Kayanne et al., 2005). With widespread bleaching occurring over the northern 1000 km of the GBR, trophic shifts may become permanent due to complete loss of taxa (Hughes et al., 2017). With climate change effects increasing, positive and negative drivers of NEC need to be evaluated in conjunction with each other for better understanding of how the ecosystem will react in the future.

Management of coral reefs is of vital importance to sustain their key ecological processes. Without management practices implemented, nearly all coral reef ecosystem services showed decline from fishing practices and climate change (Roger et al., 2015). Determining the ratio of NEP to NEC can indicate the current trophic phase of the reef (Silverman et al., 2012). These previous studies have enabled science-based management of localized chronic drivers of reef degradation such as fishing pressure and water quality to be managed with appropriate strategies for allowing key ecological species (e.g., different functional groups of herbivores) to balance algal takeover of coral reefs (Graham et al., 2013). With coral reefs in mainly oligotrophic waters, understanding and quantifying nutrient inputs from localized land sources will enable better understanding of how reefs will react to climate change with higher nutrients exacerbating the effects of global warming (Hough-Guldberg et al., 2007; Young et al., 2008). Understanding the full range of nutrient inputs from islands, such as the data provided for Heron Island in this thesis, facilitates a more accurate assessment of the effects that climate change may have on them. Knowledge of potential nutrient inputs will help management plans to be enacted to help mitigate future coral reef degradation due to climate change and bleaching events.
Future work needs to be undertaken to further assess differences between both reefs and methods to enable better understanding of coral reefs and the future impacts from climate change and other pressures. Due to the nature of water parcel tracking and the effects of weather on drogues (Hata et al., 2004), sampling under different weather conditions can reveal when tracking water parcels by drogues don’t give an accurate representation of current speed and direction. Method evaluation needs to be undertaken to determine at which times the results derived from different methods vary, including but not limited to weather conditions (e.g., wind, swell, etc.), seasonal conditions and flow conditions. Current speeds and water depth need to be measured at a minimum at the start and finish of each transect to properly determine flow rates and water volume transiting the reef. A standardized way in which studies measure current speeds and depth between sites would enable a more reliable global comparison of reef condition and enable better management by determining which reefs are under stress in certain regions. Detailed seasonal metabolic observations would also benefit the scientific community to better understand the range of change in NEC and NEP that happens on a yearly timescale for each method. The automated system designed and deployed in chapter 3 would enable detailed seasonal variations with reduced labor costs enabling easier long-term field investigations to be conducted. Greater understanding of seasonal changes in NEC and NEP and the associated drivers of coral reef metabolism throughout the year will help unravel the differences seen between locations and bring a greater understanding of their function. Overall, this thesis showed that subtle differences in analytical methods, sampling approaches and data interpretation techniques can cause important variation with implications for direct comparison of data from various studies. Comparisons among different investigations required to build long-term datasets can only be made after reconciling these differences.
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Appendix

Appendix 1. Statement of Contribution Chapter 2 Signatures

I performed fieldwork, analyzed the data and wrote the original manuscript. My co-author supported design, data analysis, editing, field work and materials

Ashly McMahon

I, Isaac R Santos agree that the above descriptions of the contributions of authors to these publications are accurate and correct

Isaac Santos
Appendix 2. Statement of Contribution Chapter 3 Signatures

I co-designed the project, performed fieldwork, analyzed the data and wrote the original manuscript. My co-authors supported design, editing, field work and materials

Ashly McMahon

I, **Isaac R Santos** agree that the above descriptions of the contributions of authors to these publications are accurate and correct

Isaac Santos

I, **Tyler Cyronak** agree that the above descriptions of the contributions of authors to these publications are accurate and correct

Tyler Cyronak

I, **Kai Schulz** agree that the above descriptions of the contributions of authors to these publications are accurate and correct

Kai Schulz
I, Damien Maher agree that the above descriptions of the contributions of authors to these publications are accurate and correct.