Coral-algae metabolism and diurnal changes in the CO$_2$-carbonate system of bulk sea water

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ABSTRACT

Precise measurements were conducted in continuous flow seawater mesocosms located in full sunlight that compared metabolic response of coral, coral-macroalgae and macroalgae systems over a diurnal cycle. Irradiance controlled net photosynthesis ($P_{\text{net}}$), which in turn drove net calcification ($G_{\text{net}}$), and altered pH. $P_{\text{net}}$ exerted the dominant control on [CO$_3^{2-}$] and aragonite saturation state ($\Omega_{\text{arag}}$) over the diel cycle. Dark calcification rate decreased after sunset, reaching zero near midnight followed by an increasing rate that peaked at 03:00 h. Changes in $\Omega_{\text{arag}}$ and pH lagged behind $G_{\text{net}}$ throughout the daily cycle by two or more hours. The flux rate $P_{\text{net}}$ was the primary driver of calcification. Daytime coral metabolism rapidly removes dissolved inorganic carbon (DIC) from the bulk seawater and photosynthesis provides the energy that drives $G_{\text{net}}$ while increasing the bulk water pH. These relationships result in a correlation between $G_{\text{net}}$ and $\Omega_{\text{arag}}$, with $\Omega_{\text{arag}}$ as the dependent variable. High rates of H$^+$ efflux continued for several hours following mid-day peak $G_{\text{net}}$ suggesting that corals have difficulty in shedding waste protons as described by the Proton Flux Hypothesis. DIC flux (uptake) followed $P_{\text{net}}$ and $G_{\text{net}}$ and dropped off rapidly following peak $P_{\text{net}}$ and peak $G_{\text{net}}$ indicating that corals can cope more effectively with the problem of limited DIC supply compared to the problem of eliminating H$^+$. Over a 24 h period the plot of total alkalinity ($A_T$) versus DIC as well as the plot of $G_{\text{net}}$ versus $\Omega_{\text{arag}}$ revealed a circular hysteresis pattern over the diel cycle in the coral and coral-algae mesocosms, but not the macroalgae mesocosm. Presence of macroalgae did not change $G_{\text{net}}$ of the corals, but altered the relationship between $\Omega_{\text{arag}}$ and $G_{\text{net}}$. Predictive models of how future global changes will effect coral growth that are based on oceanic $\Omega_{\text{arag}}$ must include the influence of future localized $P_{\text{net}}$ on $G_{\text{net}}$ and changes in rate of reef carbonate dissolution. The correlation between $\Omega_{\text{arag}}$ and $G_{\text{net}}$ over the diel cycle is simply the response of the CO$_2$-carbonate system to increased pH as photosynthesis shifts the equilibria and increases the [CO$_3^{2-}$] relative to the other DIC components of [HCO$_3^-$] and [CO$_2$]. Therefore $\Omega_{\text{arag}}$ closely tracked pH as an effect of changes in $P_{\text{net}}$, which also drove changes in $G_{\text{net}}$. Measurements of DIC flux and H$^+$ flux are far more useful than concentrations in describing coral metabolism dynamics. Coral reefs are systems that exist in constant disequilibrium with the water column.
INTRODUCTION
Recent field experiments have identified the need for accurate metabolic measurements on coral reefs at short time intervals in order to detect subtle aspects such as phase lags between the concentrations and flux rates of major metabolic parameters over the diurnal cycle (e.g., Shamberger et al., 2011; McMahon et al., 2013). There is a need to test the assumption that night calcification is very low and constant and that calcification is limited by inorganic carbon concentration in the form of \( \text{CO}_3^{2-} \). Finely detailed diurnal metabolic measurements are difficult to obtain in field investigations. For example, Falter et al. (2012) based their extensive study at Nigaloo Reef, NW Australia on a total of 13 summer measurements of calcification taken at various times over 12 days between 08:00 and 18:00 with one night measurement at 21:00. The following winter a total of 11 more measurements were made over 4 days with no night measurements. These data are valuable, but do not provide the diurnal resolution that is needed to fully describe coral and coral reef metabolism. Thus we undertook a laboratory investigation that would provide such data.

BACKGROUND INFORMATION
The term \( \Omega_{\text{arag}} \) is defined as:

\[
\Omega_{\text{arag}} = \frac{[\text{Ca}^{2+}] [\text{CO}_3^{2-}]}{K_{\text{sp}}}
\]  

(1)

where \( K_{\text{sp}} \) is the solubility constant of aragonite. The \([\text{Ca}^{2+}]\) in normal present-day oceanic seawater is essentially constant at 10.3 mmol kg\(^{-1}\) SW, normalized to salinity. Likewise, \( K_{\text{sp}} \) is a constant (at a given temperature, pressure, and salinity), so in shallow oceanic waters \( \Omega_{\text{arag}} \) is directly proportional to \([\text{CO}_3^{2-}]\). Changes in seawater pH shift the equilibria between the various forms of dissolved inorganic carbon (DIC) as follows:

\[
\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}^+ + \text{HCO}_3^- \leftrightarrow 2\text{H}^+ + \text{CO}_3^{2-}.
\]

(2)

Calcification inevitably produces an excess of \( \text{H}^+ \) and thus reduces total alkalinity \( (A_T) \) by two moles for every mole of \( \text{CaCO}_3 \) precipitated (Kinsey, 1978; Smith & Kinsey, 1978). The correct equations for calcification are as follows:

\[
\text{Ca}^{2+} + (\text{CO}_2 + \text{H}_2\text{O}) \leftrightarrow \text{CaCO}_3 + 2\text{H}^+
\]

(3)

\[
\downarrow
\]

\[
\text{Ca}^{2+} + (\text{H}^+ + \text{HCO}_3^-) \leftrightarrow \text{CaCO}_3 + 2\text{H}^+
\]

(4)

\[
\downarrow
\]

\[
\text{Ca}^{2+} + (2\text{H}^+ + \text{CO}_3^{2-}) \leftrightarrow \text{CaCO}_3 + 2\text{H}^+
\]

(5)

Equations 3–5 are written in two dimensions with a red arrow showing the relationship between the carbonate species (in parentheses) that shift with the changes in \([\text{H}^+]\).
described as Eq. (2). Dissolution is the reverse of the calcification reaction. Net calcification ($G_{\text{net}}$) is the sum of calcification (positive flux) and dissolution (negative flux). When the equations are written correctly in this manner the importance of protons becomes apparent with two moles of $\text{H}^+$ produced for every mole of $\text{CaCO}_3$ precipitated regardless of which form of dissolved inorganic carbon (DIC) is involved.

The following equations describe photosynthetic carbohydrate formation from the various available $\text{CO}_2$ species:

$$ (\text{CO}_2 + \text{H}_2\text{O}) \Leftrightarrow \text{CH}_2\text{O} + \text{O}_2 \quad (6) $$

$$ (\text{H}^+ + \text{HCO}_3^-) \Leftrightarrow \text{CH}_2\text{O} + \text{O}_2 \quad (7) $$

$$ (2\text{H}^+ + \text{CO}_3^{2-}) \Leftrightarrow \text{CH}_2\text{O} + \text{O}_2. \quad (8) $$

The photosynthesis equations are also written in two dimensions with the red arrows showing changes in distribution of species that occurs (Eq. (2)) with shifts in pH. Note that photosynthesis increases pH (lowers $[\text{H}^+]$) while the reverse reaction of respiration decreases pH (increases $[\text{H}^+]$). Net photosynthesis ($P_{\text{net}}$) is the sum of photosynthesis (positive flux) and respiration (negative flux). Unlike calcification-dissolution, photosynthesis-respiration does not alter total alkalinity ($A_T$).

In sum, photosynthesis and calcification both lower the seawater DIC, while respiration and $\text{CaCO}_3$ dissolution raise DIC. Only the precipitation or dissolution of $\text{CaCO}_3$ significantly alters $A_T$. Consequently, changes in $[A_T]$ can be used to calculate calcification and dissolution rates ($G_{\text{net}}$), and is widely used in this regard. Photosynthesis and respiration can radically alter $[\text{H}^+]$ and thus can alter relative concentration of $\text{CO}_3^{2-}$, $\text{HCO}_3^-$ and $\text{CO}_2$. Coral calcification is a biological process that is heavily influenced by the associated processes of photosynthesis and respiration ($P_{\text{net}}$) that modify pH. Protons can be considered a waste product of calcification Eqs. (3)–(5) and $\text{O}_2$ a waste product of photosynthesis Eqs. (6)–(8).

**METHODS AND MATERIALS**

A mesocosm experiment was undertaken in order to precisely measure the changes in bulk sea water chemistry and material flux caused by coral and algae metabolism over a diurnal cycle. The experiment was conducted in the flow-through mesocosm system at the Hawaii Institute of Marine Biology, Kaneohe Bay, Oahu, Hawaii. The mesocosm system has been described previously in detail (*Jokiel et al., 2008*, *Andersson et al., 2009*; *Jokiel, Bahr & Rodgers, in press*). The fiberglass mesocosm tanks were located in full sunlight and supplied with flowing seawater pumped from approximately 2 m depth at the edge of the Coconut Island coral reef. Each mesocosm received a flow of approximately 7.5 to 8.5 l min$^{-1}$ resulting in a turnover rate of approximately 1 h. Solar input at the site
Table 1 Mesocosm volume and biomass of the coral *Montipora capitata* and the macroalga *Gracillaria salicornia*.

| Mesocosm                     | Biomass                                      | Tank dimensions in cm                  |
|------------------------------|----------------------------------------------|----------------------------------------|
|                              |                                              | Length | Width | Water depth | Volume (l) |
| Coral only                   | 7,555 g buoyant weight coral                 | 117    | 117   | 38          | 520        |
| Coral plus Algae             | 7,555 g buoyant weight coral, and 3,151 g of *Gracillaria salicornia* | 117    | 117   | 38          | 520        |
| Algae                        | 3,151 g of *Gracillaria salicornia*          | 117    | 117   | 35          | 479        |

was monitored with a LiCor Brand Quantameter (Li-Cor Inc., Lincoln, NE, USA), which measured photosynthetically active radiance (PAR) between 400 nm and 700 nm.

Three continuous flow mesocosms were used for this experiment. The first mesocosm ("Coral only") was loaded with 7.6 kg buoyant weight of the reef coral *Montipora capitata* for close to 100% coverage of the bottom (Table 1). This buoyant weight translates into 11.5 kg dry skeletal weight (*Jokiel, Maragos & Franzisket, 1978*). The second mesocosm ("Coral plus Algae") contained the same weight of live coral plus 3.1 kg wet weight of the macroalgae *Gracillaria salicornia*. The third mesocosm ("Algae only") was loaded with the same weight of the macroalgae. A small biomass of calcifying organisms was present on the macroalgae in the form of epiphytes that were not removed. Dead skeletal material and sediment were excluded from all three mesocosms to reduce the complicating effect of decalcification of carbonates and related processes on $G_{net}$ (*Murillo, Jokiel & Atkinson, 2014*). The organisms in the mesocosms were allowed to acclimate to mesocosm conditions for one week prior to the experiment.

This experimental design allows the three treatments to be run simultaneously which eliminates the between-treatment variance due to solar irradiance, temperature and other factors that would occur if they were run at different times. The functioning of each community under identical conditions serves as a control and as a contrast to the other two communities. Response of these communities to the diurnal irradiance cycle and variation in other factors is non-linear, so the data were analyzed by graphical comparison and integrated response of 24 h $P_{net}$ and $G_{net}$.

The seawater inflow enters at the bottom in the center of each mesocosm (see *Smith et al., 1977* for details on the mesocosm system) at a vertical angle, which ensures a uniform and well-mixed system. Maintaining seawater inflow of reef water at constant rates insured that natural fluctuations in seawater chemistry observed on the adjacent reef during the diel cycle was preserved during the experiment. Water chemistry was sampled hourly from 06:00 on 24 April to 07:00 on 25 April 2012 at the inlet and outlet and the flow rate recorded. During the experiment the flow rate was precisely measured every hour for each mesocosm and inlet-outlet chemistry was determined. Temperature and salinity were measured with a YSI Brand Model 30 salinity–conductivity–temperature meter ($\pm 0.1$ °C; $\pm 0.1$ ppt). Dissolved oxygen (DO) was measured with a YSI Brand Model 57 Dissolved Oxygen Meter ($\pm 0.2$ mg l$^{-1}$), and pH$_{NBS}$ with an Accumet AP72...
pH/mV/temperature meter verified spectrophotometrically using m-cresol purple dye according to SOP 7 (Dickson, Sabine & Christian, 2007). $A_T$ was measured using a Titrino Model 877 titrator system. Alkalinity samples were equilibrated to 25 °C and run within an hour of being taken. Accuracy and precision of the titrations was confirmed with certified reference materials (CRM Batch 129) from the Dickson Laboratory, Scripps Institution of Oceanography which verified that our measurements of $A_T$ were accurate to within 0.18% of the Dickson CRM value. All carbonate parameters were calculated using the program CO2SYS (Pierrot, Lewis & Wallace, 2006) and stoichiometric dissociation constants defined by Mehrbach et al. (1973) and refit by Dickson & Millero (1987).

The mesocosms are well mixed systems and can be represented by a simple box model Anderson et al. (2009). Changes in total alkalinity ($A_T$) are attributed to calcification or carbonate dissolution Murillo, Jokiel & Atkinson (2014). The net calcification ($G_{net}$) can be easily calculated from Eq. (9) by measuring the amount of material in the inflowing ($F_{in}$) and outflowing ($F_{out}$) seawater, and the change per unit time in the mesocosm seawater between consecutive sampling times. For $G_{net}$ the resulting value is divided by 2 because two moles of $A_T$ are produced for every mole of CaCO$_3$ precipitated (calcification) or removed (dissolution) as shown by Eqs. (3)–(5).

$$G_{net} = \frac{F_{in} A_T - F_{out} A_T - \frac{dA_T}{dt}}{2}. \tag{9}$$

$P_{net}$ was measured in a similar manner using O$_2$ concentration, with one mole of carbon being produced for every mole of O$_2$ produced as shown by Eqs. (6)–(8). Likewise DIC and H$^+$ were calculated using Eqs. (11) and (12).

$$P_{net} = F_{in} O_2 - F_{out} O_2 - \frac{dO_2}{dt}. \tag{10}$$

$$\text{DIC}_{net} = F_{in} \text{DIC} - F_{out} \text{DIC} - \frac{d\text{DIC}}{dt}. \tag{11}$$

$$\text{H}^+_{net} = F_{in} \text{H}^+ - F_{out} \text{H}^+ - \frac{d\text{H}^+}{dt}. \tag{12}$$

**RESULTS AND DISCUSSION**

All of the measurements taken during this experiment are shown in Table 2. Calcification over the 24 h period (Fig. 1) shows the diurnal pattern related to irradiance, light enhanced calcification and dark calcification. Values for $G_{net}$ are high due to the large biomass of live coral, high solar irradiance in the shallow mesocosms and absence of sediment or dead carbonate skeleton which are subject to dissolution. $G_{net}$ in the “Corals only” and “Corals plus Algae” treatments track each other closely. Light saturation of calcification did not occur up to the maximum irradiance which exceeded 1,500 µmole photons m$^{-2}$ s$^{-1}$. This value is many times higher than that supplied by the artificial light typically used is most laboratory studies of coral calcification. Calcification rate is very low in the “Algae only” treatment due to low biomass of calcifying organisms, which are made up of various calcifying epiphytes. Low-levels of dark calcification occur at
Table 2: Measurements made during the mesocosm experiment of April 24–25, 2012.

| Time | Irradiance \( \mu \text{ mole photons m}^{-2} \text{ h}^{-1} \) | Salinity o/oo | Inlet—all mesocosms | Outlet Algae only mesocosm |
|------|----------------------------------------------------------|----------------|---------------------|-----------------------------|
|      | pH | Temp \( ^\circ\text{C} \) | \( O_2 \text{ In mg l}^{-1} \) | Flowliters min\(^{-1} \) | pH | Temp \( ^\circ\text{C} \) | \( O_2 \text{ Out mg l}^{-1} \) | \( G_{\text{net}} \) | \( P_{\text{net}} \) | Mean \( \Omega_{\text{arag}} \) |
| 6:00 | 0  | 34.8 | 7.95 | 2196.5 | 23.1 | 6.0 | 7.50 | 7.93 | 2196.0 | 23.0 | 6.0 |
| 7:00 | 56 | 34.9 | 7.95 | 2198.3 | 23.5 | 6.0 | 7.50 | 7.93 | 2196.7 | 23.2 | 6.1 | 0.3 | 22 | 2.49 |
| 8:00 | 222 | 34.8 | 8.03 | 2199.0 | 23.5 | 6.0 | 7.50 | 8.01 | 2193.5 | 23.3 | 6.4 | 1.0 | 127 | 2.70 |
| 9:00 | 464 | 34.9 | 8.03 | 2185.0 | 23.5 | 6.3 | 7.50 | 8.02 | 2190.7 | 23.4 | 7.1 | 0.0 | 340 | 2.93 |
| 10:00 | 854 | 34.8 | 8.06 | 2200.3 | 23.3 | 6.9 | 7.33 | 8.06 | 2182.9 | 23.6 | 8.0 | 1.6 | 539 | 3.09 |
| 11:00 | 1619 | 34.8 | 8.06 | 2206.2 | 23.9 | 6.6 | 7.50 | 8.11 | 2198.5 | 23.6 | 8.2 | 3.4 | 746 | 3.41 |
| 12:00 | 1705 | 34.8 | 8.09 | 2197.7 | 23.8 | 6.7 | 7.67 | 8.14 | 2199.6 | 24.4 | 7.8 | 0.8 | 756 | 3.70 |
| 13:00 | 1468 | 34.8 | 8.09 | 2200.1 | 24.0 | 6.6 | 7.50 | 8.16 | 2195.7 | 24.4 | 7.5 | 0.3 | 546 | 3.86 |
| 14:00 | 1702 | 34.8 | 8.10 | 2202.2 | 24.0 | 6.7 | 7.67 | 8.16 | 2194.7 | 24.6 | 7.8 | 1.6 | 534 | 3.93 |
| 15:00 | 1001 | 34.9 | 8.08 | 2194.6 | 24.1 | 6.7 | 7.83 | 8.15 | 2196.6 | 24.2 | 7.6 | 0.7 | 504 | 3.89 |
| 16:00 | 739 | 34.9 | 8.09 | 2208.4 | 24.1 | 6.6 | 7.67 | 8.15 | 2199.3 | 24.1 | 7.5 | 1.0 | 488 | 3.83 |
| 17:00 | 429 | 34.9 | 8.08 | 2208.1 | 24.0 | 6.4 | 7.76 | 8.12 | 2209.3 | 23.9 | 6.9 | 1.1 | 379 | 3.72 |
| 18:00 | 159 | 34.8 | 8.08 | 2210.7 | 23.9 | 6.3 | 7.67 | 8.08 | 2211.4 | 23.9 | 6.3 | -0.3 | 127 | 3.49 |
| 19:00 | 32 | 34.9 | 8.07 | 2199.0 | 23.9 | 6.3 | 7.50 | 8.08 | 2203.3 | 23.5 | 6.2 | -0.7 | -50 | 3.34 |
| 20:00 | 0 | 34.9 | 8.07 | 2184.7 | 23.8 | 6.3 | 7.67 | 8.07 | 2189.4 | 23.5 | 6.0 | -1.2 | -135 | 3.28 |
| 21:00 | 0 | 34.9 | 8.07 | 2206.4 | 23.8 | 6.3 | 7.67 | 8.05 | 2203.0 | 23.5 | 6.0 | -0.2 | -179 | 3.18 |
| 22:00 | 0 | 34.9 | 8.04 | 2180.8 | 23.5 | 6.1 | 7.67 | 8.04 | 2199.1 | 23.3 | 6.0 | -2.1 | -147 | 3.10 |
| 23:00 | 0 | 34.9 | 8.01 | 2192.5 | 23.4 | 6.1 | 7.67 | 8.03 | 2199.4 | 23.3 | 5.9 | -3.5 | -87 | 3.05 |
| 0:00 | 0 | 34.9 | 8.06 | 2201.4 | 23.6 | 6.3 | 7.67 | 8.02 | 2199.2 | 23.3 | 6.0 | -0.6 | -124 | 2.99 |
| 1:00 | 0 | 35.0 | 8.04 | 2201.4 | 23.4 | 6.4 | 7.67 | 8.04 | 2201.2 | 23.2 | 5.8 | 0.3 | -252 | 3.02 |
| 2:00 | 0 | 34.9 | 8.09 | 2214.8 | 23.7 | 6.3 | 7.67 | 8.06 | 2207.7 | 23.3 | 5.6 | 1.0 | -344 | 3.13 |
| 3:00 | 0 | 34.9 | 8.06 | 2214.3 | 23.6 | 6.1 | 7.50 | 8.05 | 2207.0 | 23.1 | 5.8 | 2.0 | -280 | 3.16 |
| 4:00 | 0 | 34.9 | 8.02 | 2209.2 | 23.3 | 6.2 | 7.50 | 8.04 | 2209.3 | 23.1 | 5.9 | 1.0 | -169 | 3.10 |
| 5:00 | 0 | 34.9 | 8.10 | 2216.6 | 23.6 | 6.3 | 7.50 | 8.06 | 2211.6 | 23.4 | 5.8 | 0.7 | -212 | 3.14 |
| 6:00 | 0 | 34.9 | 8.09 | 2214.3 | 23.5 | 6.1 | 7.50 | 8.07 | 2209.6 | 23.3 | 5.8 | 1.3 | -216 | 3.23 |

(continued on next page)
Table 2 (continued)

| Time   | pH 7.50 | Temp °C | G_out | P_out | Mean Ωarag | pH 7.50 | Temp °C | G_out | P_out | Mean Ωarag |
|--------|---------|---------|-------|-------|------------|---------|---------|-------|-------|------------|
| Flow liters min⁻¹ | 7.50    | 7.89    | 2190.8 | 23.0  | 5.6        | 8.33    | 7.93    | 2181.6 | 23.0  | 5.9        |
| 6:00   | 7.50    | 7.92    | 2179.7 | 23.1  | 5.8        | 3.3     | 264     | 2.36  | 8.33  | 7.89    | 2179.1  | 23.1  | 6.0        |
| 7:00   | 7.50    | 7.97    | 2174.0 | 23.3  | 6.3        | 6.1     | 67      | 2.55  | 8.00  | 7.96    | 2171.9  | 23.2  | 6.3        |
| 8:00   | 7.33    | 8.03    | 2165.2 | 23.4  | 7.5        | 6.2     | 361     | 2.83  | 8.17  | 7.99    | 2170.1  | 23.3  | 7.2        |
| 9:00   | 7.50    | 8.08    | 2156.9 | 23.5  | 8.9        | 8.5     | 883     | 3.12  | 8.33  | 8.04    | 2154.7  | 23.8  | 8.0        |
| 10:00  | 7.33    | 8.16    | 2165.7 | 24.5  | 8.4        | 10.2    | 983     | 3.80  | 8.00  | 8.12    | 2162.6  | 24.5  | 7.9        |
| 11:00  | 7.67    | 8.17    | 2162.9 | 24.4  | 8.1        | 9.3     | 853     | 3.89  | 8.33  | 8.13    | 2157.9  | 24.4  | 7.5        |
| 12:00  | 7.67    | 8.17    | 2159.3 | 24.6  | 8.4        | 10.8    | 845     | 3.93  | 8.33  | 8.12    | 2159.0  | 24.6  | 7.7        |
| 13:00  | 8.00    | 8.16    | 2164.4 | 24.3  | 8.1        | 9.5     | 768     | 3.89  | 8.67  | 8.10    | 2158.6  | 24.3  | 7.5        |
| 14:00  | 8.33    | 8.17    | 2174.3 | 24.0  | 7.8        | 9.0     | 644     | 3.77  | 8.00  | 8.09    | 2169.1  | 24.0  | 7.5        |
| 15:00  | 8.78    | 8.14    | 2183.0 | 23.9  | 7.0        | 8.3     | 472     | 3.61  | 8.00  | 8.09    | 2176.9  | 23.9  | 6.7        |
| 16:00  | 8.33    | 8.10    | 2194.5 | 23.6  | 6.2        | 5.6     | 61      | 3.43  | 8.33  | 8.06    | 2190.2  | 23.6  | 6.3        |
| 17:00  | 8.33    | 8.10    | 2190.3 | 23.6  | 5.9        | 3.4     | 277     | 3.21  | 8.17  | 8.05    | 2187.2  | 23.5  | 6.0        |
| 18:00  | 8.33    | 8.03    | 2172.5 | 23.2  | 5.9        | 2.9     | 366     | 3.04  | 8.17  | 8.04    | 2177.7  | 23.3  | 6.0        |
| 19:00  | 8.33    | 8.03    | 2192.6 | 23.2  | 5.6        | 3.6     | 450     | 2.99  | 8.17  | 8.04    | 2192.5  | 23.2  | 5.8        |
| 20:00  | 8.33    | 8.03    | 2189.4 | 23.2  | 5.6        | 0.7     | 484     | 3.00  | 7.83  | 8.03    | 2185.6  | 23.3  | 5.7        |
| 21:00  | 8.33    | 8.01    | 2190.4 | 23.1  | 5.6        | -0.9    | 410     | 2.94  | 8.17  | 8.02    | 2189.5  | 23.2  | 5.7        |
| 22:00  | 8.33    | 8.03    | 2188.3 | 23.2  | 5.6        | 2.0     | 446     | 2.84  | 8.33  | 8.00    | 2188.6  | 23.2  | 5.8        |
| 23:00  | 8.33    | 8.01    | 2190.2 | 23.2  | 5.4        | 3.3     | 621     | 2.84  | 8.17  | 8.01    | 2190.2  | 23.2  | 5.6        |
| 0:00   | 7.67    | 8.03    | 2196.5 | 23.3  | 5.2        | 4.0     | 691     | 2.96  | 8.33  | 8.03    | 2194.1  | 23.2  | 5.4        |
| 1:00   | 7.33    | 8.01    | 2200.2 | 23.1  | 5.4        | 3.6     | 556     | 2.98  | 8.17  | 8.02    | 2199.3  | 23.1  | 5.7        |
| 2:00   | 7.67    | 8.03    | 2200.5 | 23.4  | 5.4        | 3.4     | 578     | 2.99  | 8.17  | 8.05    | 2199.1  | 23.2  | 5.6        |
| 3:00   | 7.50    | 8.03    | 2200.3 | 23.3  | 5.4        | 4.0     | 538     | 3.00  | 8.33  | 8.04    | 2199.1  | 23.2  | 5.7        |
night. There is a drop in calcification to zero around midnight with a dark calcification rate peak at approximately 03:00 h.

The relationship between $G_{\text{net}}$ and $\Omega_{\text{arag}}$

The linear regression of $G_{\text{net}}$ plotted as a function of $\Omega_{\text{arag}}$ (Fig. 2) has become a widespread method of describing coral and coral reef calcification. A significant statistical relationship is obtained, with substantial variance that is generally assumed to be largely sampling error, or the result of other factors which influence $G_{\text{net}}$. However, the explanation appears be more complex, as will be discussed in the following sections.

Diel hysteresis, phase lags and night calcification patterns

McMahon et al. (2013) quantified $G_{\text{net}}$ in a healthy coral reef lagoon in the Great Barrier Reef during different times of day. Their observations revealed a diel hysteresis pattern in the $G_{\text{net}}$ versus $\Omega_{\text{arag}}$ relationship. This phenomenon can be demonstrated by labeling the points in Fig. 2 with the hour of day as shown in Fig. 3. The diel pattern moves from the lower left quadrant early in the day toward the upper right through mid-day and then back to the lower center during the night before returning to the lower left quadrant at first light. The pattern is nearly identical for the “Corals only” mesocosm (Fig. 3A) and the “Corals plus Algae” (Fig. 3B), which tracked each other closely (Fig. 1). The “Algae only” mesocosm did not show this pattern. The linear regression for the $G_{\text{net}}$ vs. $\Omega_{\text{arag}}$ data for the mesocosm with coral (Fig. 2) accounted for part of the variance ($R^2 = 0.40$). A linear regression does not adequately describe the variance resulting from the diel pattern.
Figure 2 Net calcification rate ($G_{net}$) plotted as a function of $\Omega_{arag}$ for (A) “Coral only” mesocosm, (B) “Coral and Algae” mesocosm and (C) “Algae only” mesocosm.

Cyronak et al. (2013) used chambers to measure in situ benthic solute fluxes at three different advection rates at Heron Island lagoon, Australia and observed a strong diurnal hysteresis pattern similar to that in Fig. 3. They suggested that diel hysteresis is caused by the diurnal interaction between photosynthesis and respiration. The data did not follow a trend consistent with the $\Omega_{arag}$ of the water column being the main driver of carbonate precipitation and dissolution. Instead, carbonate precipitation and dissolution in these sediment communities is linearly correlated to the rates of photosynthesis and respiration ($P_{net}$) occurring over the same time period.

Phase shifts

Evaluation of phase relations for the various parameters listed in Table 2 can be facilitated by scaling each variable on a 0 to 1 scale. The normalized value of $a_i$ for variable $A$ in the $i$th row was calculated using the equation:

$$\text{Normalized value } (a_i) = \frac{a_i - A_{\text{min}}}{A_{\text{max}} - A_{\text{min}}}$$  \hspace{1cm} (13)

where $A_{\text{min}}$ is the minimum value for variable $A$ and $A_{\text{max}}$ is the maximum value for variable. Figure 4 summarizes the results for the variables most often considered in the literature (pH, $\Omega_{arag}$, $P_{net}$ and $G_{net}$).
Figure 3  Net calcification rate versus $\Omega_{\text{arag}}$. Net calcification rate versus $\Omega_{\text{arag}}$ with each point labeled with hour of the day revealing the clockwise coral reef diel hysteresis pattern for: (A) "Coral only" mesocosm, (B) "Coral plus Algae" mesocosm and the (C) "Algae only" mesocosm, which showed no pattern.

Figure 4 shows that peak pH and $\Omega_{\text{arag}}$ lag behind $G_{\text{net}}$ throughout the daily cycle by two or more hours. The figure also shows that peak $G_{\text{net}}$ follows $P_{\text{net}}$ during daylight photosynthetic hours with a reverse during the nighttime hours. Shamberger et al. (2011) reported that $\Omega_{\text{arag}}$ lags behind $G_{\text{net}}$ on the reefs of Kaneohe Bay, Hawaii. McMahon et al. (2013) reported that peak $G_{\text{net}}$ rates occurred 2–3 h before the $\Omega_{\text{arag}}$ maximum on a healthy coral reef on the Great Barrier Reef. Thus $\Omega_{\text{arag}}$ (along with closely correlated $[\text{CO}_3^{2-}]$ and $[\text{H}^+]$ ratio) is not the primary driver of coral calcification over a diurnal cycle. The paradigm that $\Omega_{\text{arag}}$ correlates with $G_{\text{net}}$ on a global scale must be tempered with the caveat that other processes have a much greater influence on calcification on smaller spatial and temporal scales. The data presented above show that diurnal irradiance drives $P_{\text{net}}$, which in turn drives $G_{\text{net}}$, and which alters pH, which controls $[\text{CO}_3^{2-}]$ and $\Omega_{\text{arag}}$ as well as other variables based on concentration such as the ratio of $[\text{DIC}]$ to $[\text{H}^+]$. A better understanding of this hierarchy will resolve many of the contradictions in the literature on coral reef calcification.

**Night calcification**

Laboratory studies show that coral calcification continues in darkness, but at a lower rate than observed in light enhanced calcification (Schneider & Erez, 2006). Night calcification rates have generally been assumed to be low and constant at night, although this assump-
tion has largely gone untested. Figures 1 and 4 show decreasing dark calcification following sunset, reaching zero near midnight followed by an increasing rate of dark calcification and an increase in respiration (Figs. 5C and 5D) that rises to a peak at 03:00 well before dawn. This pattern has occurred consistently in our mesocosm experiments, with the same pattern observed in 30 separate mesocosm runs with different communities under various conditions as well as in flume studies at our site (Murillo, Jokiel & Atkinson, 2014). Barnes & Crossland (1980) used time-lapse photography to measure diurnal growth in the staghorn coral Acropora acuminata and found that night-time extension rate was similar to or greater than day-time extension. They suggested that, “symbiotic association permits rapid growth because the coral can invest in flimsy scaffolding at night with the certainty that bricks and mortar will be available in the morning”. Wooldridge (2013) has proposed a new model for “dark” coral calcification, whereby O$_2$-limitation of aerobic respiration during the night initiates a homeostatic host response that forms the skeletal organic matrix. The matrix formed at night subsequently allows rapid growth of the aragonite fibers during the “light-enhanced” period of calcification, when abundant energy derived from photosynthesis is available. Perhaps the mid-night calcification minimum observed in Figs. 1 and 4 at 00:00 reflects this period of organic matrix formation that precedes the 03:00 night calcification peak.
Figure 5  Diurnal changes in seawater chemistry in the three mesocosms for: (A) total alkalinity $A_T$, (B) aragonite saturation state $\Omega_{\text{arg}}$, (C) pH and (D) dissolved oxygen.

**Diurnal changes in concentration of $A_T$, pH, $\Omega_{\text{arg}}$ and DO**

The variables of $A_T$, pH, $\Omega_{\text{arg}}$ DIC, and DO are concentrations while $P_{\text{net}}$ and $G_{\text{net}}$ are flux rates. Care must be taken when comparing concentrations to flux rates because flux rate can be high when concentration is high or low. Or flux rate can be low when concentration is high or low. Figure 4 shows patterns that are difficult to interpret because the figure mixes flux rates with concentrations. This issue will be discussed and resolved later in this discussion, but first we will compare differences in concentrations of key variables over the diurnal cycle (Fig. 5).

Figure 5 reveals several important patterns:

1. Alkalinity in the “Algae only” mesocosm remained high during the entire diurnal cycle. In contrast, the mesocosms containing corals showed lower $A_T$ (Fig. 5A) caused by rapid calcification. $A_T$ reduction by the corals was greatest during the daylight hours when $G_{\text{net}}$ was high (Fig. 1) with the difference diminishing during nighttime hours.
2. The two mesocosms with algae maintained a higher $\Omega_{\text{arag}}$ throughout the mid-day portion of the diurnal cycle (Fig. 5B) which can be attributed to higher pH resulting from rapid rates of algae photosynthesis and coral photosynthesis (Eqs. (6)–(8)), with a less pronounced difference during the rest of the cycle.

3. The extreme difference in $\Omega_{\text{arag}}$ between the “Corals only” and the “Corals plus Algae” mesocosms (Fig. 5B) did not produce a corresponding difference in $G_{\text{net}}$ between the two mesocosms (Fig. 1), which demonstrates that $\Omega_{\text{arag}}$ is uncoupled from $G_{\text{net}}$ and explains differences encountered when comparing the $\Omega_{\text{arag}}$ versus $G_{\text{net}}$ relationship in different systems with different diurnal $P_{\text{net}}$ regimes.

4. Night-time pH and $\Omega_{\text{arag}}$ values (Figs. 5B and 5C) show less variability than $G_{\text{net}}$ (Fig. 1).

5. The high biomass in the “Coral plus Algae” mesocosm (Fig. 5D) resulted in the highest $O_2$ values during daylight hours (due to photosynthesis) and the lowest $O_2$ during the night (due to respiration). The “Algae only” treatment had the second highest daytime level $O_2$ due to algal photosynthesis and relatively high levels of $O_2$ at night.

6. The 03:00 calcification peak observed in Fig. 1 is shown by both a decrease in $O_2$ concentration and a drop in pH due to accelerated respiration. DO (Fig. 5D) and the pH (Fig. 5C) are measured independently and both show this effect to corroborate the observation.

Plotting DIC versus $A_T$ (Fig. 6) demonstrates the major influence of $P_{\text{net}}$ on seawater $\Omega_{\text{arag}}$. Calcification and dissolution shift $A_T$ values horizontally along the abscissa in Fig. 6 and influence DIC values vertically along the ordinate. However, photosynthesis and respiration change DIC along the ordinate without changing $A_T$. The observed hysteresis
pattern results from $P_{\text{net}}$ driving $G_{\text{net}}$ and increasing pH. A linear relationship accounting for only half of the variance ($R^2 \approx 0.5$) between DIC and $A_T$ was observed for the two rapidly calcifying mesocosms containing corals. This relationship does not hold for the low-calcification “Algae only” mesocosm.

As pointed out by *McMahon et al. (2013)*, connecting the points on a graph of $A_T$ vs. DIC reveals a circular hysteresis pattern over the diel cycle as shown for the $G_{\text{net}}$ versus $\Omega_{\text{arag}}$ plot (Fig. 6). $G_{\text{net}}$ can account for changes in both the $A_T$ and DIC concentrations. However, $P_{\text{net}}$ can only account for changes DIC concentration. Therefore, $\Omega_{\text{arag}}$ is a function of the changes in carbonate chemistry due to both $P_{\text{net}}$ and $G_{\text{net}}$, and any changes in DIC concentration relative to $A_T$ will result in different influences on $\Omega_{\text{arag}}$. For example, in systems with high organic production relative to calcification (Coral plus Algae mesocosm), $\Omega_{\text{arag}}$ will increase during daylight due to high pH caused by high uptake of $\text{CO}_2$ used for photosynthesis (Fig. 5D). Conversely, in systems with low organic production relative to calcification (Coral only mesocosm), $\Omega_{\text{arag}}$ will decrease due to the uptake of $A_T$. Any decrease in $G_{\text{net}}$ associated with an increase in $P_{\text{net}}$ will increase $\Omega_{\text{arag}}$ and change the way that $G_{\text{net}}$ responds to OA. Therefore, any prediction of future global changes on coral reef $G_{\text{net}}$ based on oceanic seawater $\Omega_{\text{arag}}$ must also take into account the influence of future localized $P_{\text{net}}$ on $G_{\text{net}}$ and $\Omega_{\text{arag}}$ as well as changes in carbonate dissolution of reef carbonates as described by *Murillo, Jokiel & Atkinson (2014)* for each reef location.

Comparisons between the hourly and daily $G_{\text{net}}$ and $P_{\text{net}}$ values (Table 3) show a similar calcification rate for both the “Coral only” mesocosm and the “Coral plus Algae” mesocosm in spite of the differences in DO, pH, $A_T$ (Fig. 5) and $P_{\text{net}}$. However, daily $P_{\text{net}}$ for the “Coral plus Algae” mesocosm was only one third of the $P_{\text{net}}$ of the “Coral only” mesocosm. Hourly production was much higher in the “Corals plus Algae” mesocosm during the daylight hours, but production was consumed by extremely high respiration during nighttime hours. The “Algae only” mesocosm showed very low daily $G_{\text{net}}$ and extremely high daily $P_{\text{net}}$.

*Anthony, Kleypas & Gattuso (2011)* proposed a model that areas dominated by algal beds draw CO$_2$ down and elevate $\Omega_{\text{arag}}$, potentially offsetting ocean acidification impacts at the local scale. Their model is based on the paradigm that $G_{\text{net}}$ is controlled by $\Omega_{\text{arag}}$. Results suggested that a shift from coral to algal abundance under ocean acidification can lead to improved conditions for calcification (i.e. increased $\Omega_{\text{arag}}$) in downstream habitats and that alga beds can provide a significant mechanism for buffering ocean acidification impacts at the scale of habitat to reef. However, this conclusion is at odds with the measured values shown in Table 3 and Fig. 5. $G_{\text{net}}$ in the “Corals plus Algae” treatment was the same as the “Coral Only” treatment even though the $\Omega_{\text{arag}}$ was much higher. In addition, the presence of the algae caused a precipitous drop in $P_{\text{net}}$. The flaw in their model appears to be the assumption that $G_{\text{net}}$ is controlled by $\Omega_{\text{arag}}$. Algal photosynthesis increases pH which shifts the equilibrium to higher $[\text{CO}_3^{2-}]$ and thus higher $\Omega_{\text{arag}}$. There is a direct correlation between $G_{\text{net}}$ and $\Omega_{\text{arag}}$ for a specific reef community, but not a cause and effect relationship.
### Table 3  Hourly and daily $G_{\text{net}}$ and $P_{\text{net}}$ values for the three mesocosms.

| Time of day | Solar irradiance ($\mu$mol photons m$^{-2}$ s$^{-1}$) | $G_{\text{net}}$ (mmol m$^{-2}$ h$^{-1}$) | $P_{\text{net}}$ (mmol m$^{-2}$ h$^{-1}$) |
|-------------|-----------------------------------------------------|----------------------------------------|----------------------------------------|
|             |                                                     | Corals only | Corals plus Algae | Algae only | Corals only | Corals plus Algae | Algae only |
| 07:00       | 56.3                                                | 4.6         | 3.3               | 0.3        | −59         | −264           | 22         |
| 08:00       | 221.6                                               | 6.5         | 6.1               | 1.0        | 65          | −67            | 127        |
| 09:00       | 464.6                                               | 5.8         | 6.2               | 0.0        | 327         | 361            | 340        |
| 10:00       | 854.0                                               | 8.1         | 8.5               | 1.6        | 553         | 883            | 539        |
| 11:00       | 1619.0                                              | 11.5        | 11.6              | 3.4        | 650         | 956            | 746        |
| 12:00       | 1705.0                                              | 10.3        | 10.2              | 0.8        | 685         | 983            | 756        |
| 13:00       | 1468.0                                              | 10.4        | 9.3               | 0.3        | 582         | 853            | 546        |
| 14:00       | 1702.0                                              | 11.5        | 10.8              | 1.6        | 521         | 845            | 534        |
| 15:00       | 1001.0                                              | 10.2        | 9.5               | 0.7        | 466         | 768            | 504        |
| 16:00       | 739.0                                                | 10.5        | 9.0               | 1.1        | 477         | 644            | 488        |
| 17:00       | 429.0                                                | 9.9         | 8.3               | −0.3       | 71          | 61             | 127        |
| 18:00       | 159.0                                                | 6.9         | 5.6               | −1.2       | −183        | −366           | −135       |
| 19:00       | 31.7                                                 | 4.4         | 3.4               | −0.7       | −123        | −277           | −50        |
| 20:00       | 0.0                                                  | 2.6         | 2.9               | −1.2       | −183        | −366           | −135       |
| 21:00       | 0.0                                                  | 2.9         | 3.6               | −0.2       | −247        | −450           | −179       |
| 22:00       | 0.0                                                  | 1.3         | 0.7               | −2.1       | −292        | −484           | −147       |
| 23:00       | 0.0                                                  | −0.2        | −0.9              | −3.5       | −221        | −410           | −87        |
| 24:00       | 0.0                                                  | 2.1         | 2.0               | −0.6       | −242        | −446           | −124       |
| Daily       |                                                     | 144         | 133               | 10         | 1226        | 427            | 2913       |

**Diurnal changes in material flux ($P_{\text{net}}, G_{\text{net}}, H^+ \text{ flux and DIC flux}$)**

$G_{\text{net}}$ and $P_{\text{net}}$ are measures of material flux. DO, pH and DIC are measures of concentration. The preceding discussion and numerous publications often compare concentrations of one material to flux rate of another material or vice versa. Much more can be learned by plotting DIC flux and $H^+$ flux rather than [DIC], [H$^+$] or pH in relation to $P_{\text{net}}$ and $G_{\text{net}}$. DIC flux and $H^+$ flux were calculated using the box model and graphed on a 0 to 1 scale in the same manner as in Fig. 4 with the result presented as Fig. 7. This figure illustrates the dynamic geochemical and physiological relationships involved in coral and coral reef metabolism.

DIC flux (uptake) in the highly calcifying mesocosms containing coral (Fig. 7) increases with increasing $P_{\text{net}}$ from 06:00 until mid-day peak $P_{\text{net}}$ and then decreases rapidly as $P_{\text{net}}$ decreases with decreasing irradiance. Furla et al. (2000) demonstrated the presence of a DIC pool within coral tissues. The size of this pool was dependent on the lighting...
Figure 7  Plot of normalized data for $P_{net}$, $G_{net}$, inverse DIC flux and $H^+$ flux for the experiment.

conditions, since it increased 39-fold after 3 h of illumination. If we apply this observation to the data shown in Fig. 7, it appears that the DIC pool had increased by mid-day, so rate of DIC uptake dropped rapidly as irradiance and photosynthesis declined. However, note that the high dissipation rates of $H^+$ continued for 2–3 h following the peak rates of $P_{net}$ and $G_{net}$ as the corals rid themselves of the backlog of $H^+$ generated by rapid calcification. Thus the lag of pH behind the peak flux rates of $P_{net}$ and $G_{net}$ (Figs. 4A and 4B) represents a disequilibrium that results from the lag in proton efflux from the corals. The correlation between $\Omega_{arag}$ and $G_{net}$ is simply the response of the CO$_2$-carbonate system to pH as [H$^+$] shifts the equilibria and redistributes the [CO$_3^{2-}$] relative to the other DIC components of [HCO$_3^-$] and [CO$_2$] (Eqs. (3)–(5)). Therefore $\Omega_{arag}$ closely tracks pH whereas $G_{net}$ tracks $P_{net}$ more closely. Changes in $\Omega_{arag}$ are a consequence of changes in $P_{net}$ and $G_{net}$, rather than a driver of $G_{net}$. Hence the $\Omega_{arag}$ peak and the pH peak lag behind the $P_{net}$ and $G_{net}$ peaks (Figs. 4A and 4B) due to lag in proton efflux seen in Fig. 7. This observation demonstrates the importance of understanding the difference between $H^+$ concentration and $H^+$ flux.

During the night the $H^+$ flux rate is very responsive to changes in $G_{net}$ in the “Algae only” and “Coral plus Algae” due to large changes in respiration (Fig. 7). The fluctuations of proton flux at night in the “Coral only” mesocosm are dampened considerably compared to the “Algae only” treatment. The “Coral plus Algae” mesocosm shows an intermediate response. Perhaps the coral skeleton acts as a buffer in a manner similar
Figure 8 The flux rate of calcification-dissolution ($G_{\text{net}}$) plotted against the concentrations of important variables of the CO$_2$-carbonate system for the “Corals only” mesocosm.

Back to the basics

The preceding sections have established the importance of using flux rates rather than concentrations when we are describing a dynamic metabolic system such as a coral or coral reef. Most of the previous research in this area has focused on the relationship between $G_{\text{net}}$, [CO$_3^{2-}$] (or its surrogate $\Omega_{\text{arag}}$), [HCO$_3^-$], and [H$^+$] expressed as pH. Plotting these variables in exemplary Fig. 8 is very informative and sheds light on results of previous studies.

A coral must uptake inorganic carbon in order to maintain photosynthesis and calcification. As a result [DIC] will decrease no matter which carbonate species (HCO$_3^-$, CO$_3^{2-}$ or CO$_2$) is taken up by the coral (Eqs. (3)–(5)). Thus we see a decline in [DIC] at high rates of $G_{\text{net}}$ (Fig. 8). [HCO$_3^-$], which has been identified as the preferred substrate for photosynthesis and calcification ([Weis, Smith & Muscatine, 1989; Furla et al., 2000; Roleda, Boyd & Hurd, 2012]) closely tracks [DIC] during daylight hours. In contrast, [CO$_3^{2-}$] lags behind $G_{\text{net}}$ and closely tracks pH during the day as shown for $\Omega_{\text{arag}}$ in Fig. 4. If [CO$_3^{2-}$] (or its surrogate $\Omega_{\text{arag}}$) drives calcification, then how do we explain the lag behind $G_{\text{net}}$? And if [CO$_3^{2-}$] is limiting, how do we explain the fact that [CO$_3^{2-}$] is increasing rather than decreasing as the coral calcifies rapidly and takes up inorganic carbon? [CO$_3^{2-}$] increases because of the increase in pH caused by rapid photosynthesis, which shifts the equilibrium between [HCO$_3^-$] and [CO$_3^{2-}$]. Thus, $P_{\text{net}}$ is the driver of changes in $G_{\text{net}}$ and [CO$_3^{2-}$] (Eqs. (2)–(5)). A basic physiological interpretation of the patterns shown in Fig. 8 is that daytime coral metabolism rapidly removes DIC (primarily in the form of HCO$_3^-$) while photosynthesis provides the energy that drives $G_{\text{net}}$ (Fig. 4). Higher pH resulting from rapid photosynthesis pushes the equilibria toward higher [CO$_3^{2-}$]. This scenario results in a correlation between $G_{\text{net}}$ and $\Omega_{\text{arag}}$, with $\Omega_{\text{arag}}$ as the dependent variable.

to that proposed by Suzuki, Nakamori & Kayanne (1995). The macroalgae lack the large skeletal carbonate buffer of reef corals.
During the night $[\text{HCO}_3^-]$, $[\text{DIC}]$, $[\text{CO}_3^{2-}]$ and pH mirror changes in $G_{\text{net}}$. However, note that $[\text{HCO}_3^-]$ diverges from $[\text{DIC}]$ and $[\text{CO}_3^{2-}]$ diverges from pH in darkness. The night divergence can be attributed to respiration causing a decrease in pH. The decreasing pH shifts the equilibria so that $[\text{CO}_3^{2-}]$ is converted to $[\text{HCO}_3^-]$, thereby changing the offset between the points. This phenomenon is also reflected in the pattern of diurnal hysteresis show in Fig. 3.

CONCLUSIONS AND RECOMMENDATIONS

Correlations do not establish cause and effect

Linear regression using $\Omega_{\text{arag}}$ as the independent variable may be useful as a first approximation, but is a poor descriptor of calcification dynamics on coral reefs. Much of the existing data on coral calcification was developed in static or low turnover incubation experiments under typical laboratory low irradiance artificial light sources on a 12 h light, 12 h dark cycle (Jokiel, Bahr & Rodgers, in press). This regime results in an unrealistic simulation of the actual diurnal cycle that occurs on coral reefs. The standard protocol has been to compare linear regressions between or among treatments. Linear regression provides a very limited description of the actual relationship between the key factors controlling organic and inorganic processes on coral reefs, which are more adequately described by data presentations such as that in Table 3 or Fig. 7. The linear regression approach does not fully embrace natural diurnal calcification patterns and phase lags because these processes are non-linear. The linear regression approach can lead to the assumption that $\Omega_{\text{arag}}$ is the independent variable driving the calcification reaction. Nevertheless, correlations at a single location or in a single experiment can result where all other factors are held constant because the two quantities are related to some extent. Use of $\Omega_{\text{arag}}$ as an independent variable to compare spatial and temporal variation in $G_{\text{net}}$ is known to create difficulties (Shamberger et al., 2011; Falter et al., 2012).

Numerous field and laboratory studies have demonstrated a positive correlation between $G_{\text{net}}$ and $\Omega_{\text{arag}}$ for corals and coral reefs Erez et al. (2011). Well-developed reefs occur within a narrow geographic range characterized by open ocean $\Omega_{\text{arag}} > 3.3$ (Kleypas et al., 1999), which could mean that coral communities have limited capacity to adapt to future levels of anthropomorphic ocean acidification (OA) projected for the 21st century. Recent reports suggest that healthy coral reefs could cease to exist within this time frame as OA continues and oceanic $\Omega_{\text{arag}}$ decreases (Hoegh-Guldberg et al., 2007; Silverman et al., 2009). However, there are inconsistencies in the relationship (slope and x-intercept) between $G_{\text{net}}$ as a function of $\Omega_{\text{arag}}$ on various reefs throughout the world (Shamberger et al., 2011). For example, Kaneohe Bay, Oahu, Hawaii contains rich coral reefs that show extremely high rates of $G_{\text{net}}$ while living at low $\Omega_{\text{arag}}$ levels (mean $\Omega_{\text{arag}} = 2.85$) (Shamberger et al., 2011). Shamberger et al. (2014) report the existence of highly diverse, coral-dominated reef communities at the Rock Islands of Palau that are living at low saturation states ($\Omega_{\text{arag}} = 1.9–2.5$). These values approach those projected for the tropical western Pacific open ocean by 2100 under future OA modeling scenarios. Identification of biological and environmental factors that enable these communities to persist at low $\Omega_{\text{arag}}$
could provide important insights into the future of coral reefs under increasing OA. So how do we account for the paradox of rich coral reefs growing at low $\Omega_{\text{arag}}$? Previous work has been based on the assumption that $G_{\text{net}}$ is controlled by or related directly to $\Omega_{\text{arag}}$. The present investigation indicates that $P_{\text{net}}$ rather than $\Omega_{\text{arag}}$ drives $G_{\text{net}}$ and that calcification rate is further limited by proton flux, with pH and $A_T$ playing the major role in controlling calcification. $\Omega_{\text{arag}}$ on coral reefs is simply a dependent variable being controlled largely by changes in pH due to photosynthesis. A correlation will exist at a given site, but will not be consistent between different coral reef communities.

The $[\text{DIC}]/[H^+]$ ratio correlates with $\Omega_{\text{arag}}$ in describing $G_{\text{net}}$ and is useful from a physiological point of view because it involves pH and all of the inorganic carbon species (Jokiel, 2011a; Jokiel, 2013). However, the $[\text{DIC}]/[H^+]$ ratio is simply another variable based on concentration (along with pH, $\Omega_{\text{arag}}$, $\text{CO}_2^-$, etc.) that shows a correlation with $G_{\text{net}}$. Nevertheless, the $[\text{DIC}]/[H^+]$ ratio can be important in describing $G_{\text{net}}$ in situations where $\Omega_{\text{arag}}$ is decoupled from $[H^+]$ as occurs in the paleo-ocean over time scales greater than 10,000 years (Hönisch et al., 2012). A calcifying organism must uptake DIC in order to continue the calcification reaction and must rid itself of the waste protons. So $G_{\text{net}}$ correlates directly to $[\text{DIC}]$ and inversely to $[H^+]$.

Results of this investigation (Fig. 7) demonstrate the difficulty that corals encounter in shedding waste protons generated during calcification. High rates of $H^+$ flux continued for several hours following peak $G_{\text{net}}$. An important conclusion of this work is that measurements of DIC flux and $H^+$ flux are far more useful in describing coral metabolism dynamics than [DIC] and [H+] (Fig. 8). Likewise, $\Omega_{\text{arag}}$ is not a very useful variable in that it simply tracks pH (Fig. 4). This pattern becomes clear when one considers that $[\text{CO}_3^-]$ (and hence $\Omega_{\text{arag}}$) shifts with changing $[H^+]$ as described in Eqs. (2)–(5). DIC flux follows $P_{\text{net}}$ and $G_{\text{net}}$ and decreases rapidly following peak $P_{\text{net}}$ and peak $G_{\text{net}}$ indicating that corals can cope more effectively with the problem of DIC supply compared to the problem of eliminating $H^+$.

**Future research directions**

The time lag between $G_{\text{net}}$ and $\Omega_{\text{arag}}$ reported previously in field studies (Shamberger et al., 2011; Cyronak et al., 2013; McMahon et al., 2013) provides evidence that diffusion and advection of materials between the coral and the water column involves time delays. One reason is that corals convert inorganic carbon to organic carbon, translocate the organic carbon to distal calcification sites, store organic carbon as lipid, and can eventually convert stored organic carbon back to inorganic carbon (Jokiel, 2011b), creating numerous possible phase lags for metabolic materials. The second reason for the time lag is that rapidly calcifying systems have difficulty dissipating waste protons as shown by continued rapid proton efflux for hours after peak calcification (Fig. 7). What other mechanisms can account for the phase lag? Boundary layers (BL) can slow the exchange of metabolic materials between the coral and the water column. The results of Cyronak et al. (2013) revealed that stirring had a net stimulatory effect on $A_T$ flux and on the diurnal cycle.
of hysteresis. Boundary layers slow exchange of metabolic materials, so this is an area of investigation that can provide an explanation.

Three hydrodynamic boundary layers have previously been defined and measured (Shashar, Cohen & Loya, 1993; Shashar et al., 1996). The Diffusion Boundary Layer (DBL) is only a few mm thick and in contact with the coral epidermis. The Momentum Boundary Layer (MBL) controls water movement in the proximity of the sessile organisms and is thicker by an order of magnitude than the DBL. The Benthic Boundary Layer (BBL), which controls the interactions of the reef with the surrounding sea water, was typically found to be more than 1 m thick and characterized by a roughness height of 31 cm and a shear velocity of 0.42 cm s$^{-1}$ in the studies.

The DBL is a thin layer of stagnant seawater adjacent to the coral produced by frictional drag. This quiescent layer influences the flux of material between the benthic surface and the water column. The transport of Ca$^{2+}$, CO$_2$, CO$_3^{2-}$, HCO$_3^-$, O$_2$, nutrients and H$^+$ through the DBL is limited by the physical processes of diffusion and advection (Jokiel, 1978; Lesser et al., 1994; Kaandorp et al., 2005; Kaandorp, Filatov & Chindapol, 2011). Kühl et al. (1995) found that zooxanthellae photosynthesis resulted in a build-up of O$_2$ in the photosynthetic tissue of up to 250% saturation and a tissue pH of up to 8.6 (i.e., 0.7 pH units above the pH value of the overlying seawater). In darkness the O$_2$ within the coral tissue was depleted by respiration to near anoxic (<2% air saturation) conditions, with tissue pH of 7.3–7.4. O$_2$ and pH profiles demonstrated the presence of a 200–300 µm thick DBL that separated the coral tissue from the overlying flowing seawater. Various models invoke boundary layer controls on coral metabolism. Kaandorp et al. (2005) and Kaandorp, Filatov & Chindapol (2011) addressed DBL limitation of DIC influx while Jokiel (2011a), Jokiel (2011b) and Jokiel (2013) challenged the paradigm that calcification is limited by CO$_2^{−}$ supply on the reactant side of the calcification equation. Rather, he argued that rate of dissipation of H$^+$ on the product side due to boundary layer conditions can be the actual limiting factor.

Boundary layer limitation of photosynthesis provides an analog to boundary layer limitation of calcification. Photosynthetic rate can be limited by rate of waste O$_2$ dissipation through the boundary layer rather than being limited by supply of reactant CO$_2$. By analogy, calcification can be limited by rate of removal of waste protons rather than by availability of inorganic carbon. The importance of water motion in reducing boundary layer thickness and thereby increasing oxygen flux between the photosynthetic organisms and the water column has been demonstrated (Mass et al., 2010). By analogy, increased water motion can decrease boundary layer thickness and thereby increase removal of protons from the coral.

Studies of reef metabolism beginning with the classic work of Odum & Odum (1955) at Enewetak Reef flat and followed by others (Shamberger et al., 2011; Falter et al., 2012) were conducted in shallow water reef flats within the BBL in situations where unidirectional currents allowed calculation of flux rates. Substantial boundary layers occur over all reefs. For example, Price et al. (2012) investigated a range of sites from exposed coastal situations to lagoons and found that ambient variability in pH was substantial
and oscillated over a diurnal cycle with diel fluctuations in pH exceeding 0.2. Daily pH maxima were identified as an important control on calcification. Net accretion among sites was positively related to the magnitude and duration of pH above the climatological seasonal low, despite myriad other ecological (e.g., local supply, species interactions, etc.) and physical oceanographic (e.g., temperature, current magnitude and direction, wave strength, latitudinal gradients, etc.) drivers. In general, accretion rates were higher at sites that experienced a greater number of hours at high pH values each day. Where daily pH within the BBL failed to exceed pelagic climatological seasonal lows, net accretion was slower and fleshy, non-calcifying benthic organisms dominated space. Thus, key aspects of coral reef ecosystem structure and function are clearly related to natural diurnal variability in pH, which is driven primarily by photosynthesis and respiration as \( P_{\text{net}} \).

**The master variables**

The practice of calculating and comparing linear regressions of \( G_{\text{net}} \) vs. \( \Omega_{\text{arag}} \) to obtain a first approximation of calcification rates under different conditions is fraught with problems but probably will continue because it is ingrained in science and is convenient to use. The correlation of a primary biological response (\( G_{\text{net}} \)) to a primary physical chemistry measurement (\( \Omega_{\text{arag}} \)) is attractive, especially in modeling the possible future changes on coral reefs. Unfortunately, the physical chemistry concept of \( \Omega_{\text{arag}} \) has no basic physiological meaning in describing \( G_{\text{net}} \) other than a correlation with the \([\text{DIC}]:[\text{H}^+]\) ratio (Jokiel, 2013) as well as with other factors such as pH. There is no consistent relationship between \( \Omega_{\text{arag}} \) and \( G_{\text{net}} \) when comparing reefs throughout the world (Shamberger et al., 2011). Coral reefs are systems in constant disequilibrium with the water column. So we must take care not to be led astray in our thinking about the variables that actually drive and control coral and coral reef metabolism and bulk water chemistry. The correlation between \( G_{\text{net}} \) and other factors is a result of \( P_{\text{net}} \) driving both \( G_{\text{net}} \) and \( \Omega_{\text{arag}} \) (McMahon et al., 2013). The observed phenomenon of diurnal hysteresis and diurnal phase lag show the importance of measuring flux rates and emphasizes the challenge in predicting the future effects of OA on coral reefs. The method of using linear extrapolations of \( \Omega_{\text{arag}} \) to determine threshold levels that will shift coral reefs from net calcifying systems to a net dissolving state has been questioned (McMahon et al., 2013). Perhaps predicted changes in \( \Omega_{\text{arag}} \) in the open ocean can be used to calculate changes on reefs if we assume that the baseline on the reefs will change in concert with ocean values and that all other processes such as \( P_{\text{net}} \) and carbonate dissolution will not be influenced by OA. An explanation for the many paradoxes of coral calcification discussed herein has been presented as the “Two Compartment Proton Flux Model of Coral Metabolism” (Jokiel, 2011b). This model is focused on localized gradients that influence coral metabolism with a focus on proton flux, carbon pools and translocation of fixed carbon. A major feature of the model is the presence of boundary layers which control local pH gradients and inorganic carbon speciation in addition to proton flux. Results of the present investigation support this model.
ADDITIONAL INFORMATION AND DECLARATIONS

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Author Contributions
• Paul L. Jokiel conceived and designed the experiments, performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, prepared figures and/or tables, reviewed drafts of the paper.
• Christopher P. Jury analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, reviewed drafts of the paper.
• Ku’ulei S. Rodgers performed the experiments, analyzed the data, contributed reagents/materials/analysis tools, wrote the paper, reviewed drafts of the paper.

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