ALKALINITY TO CALCIUM FLUX RATIOS FOR CORALS AND CORAL REEF COMMUNITIES: VARIANCES BETWEEN ISOLATED AND COMMUNITY CONDITIONS

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Liana Jean Auliʻi Murillo

Thesis Committee:

Marlin Atkinson, Chairperson-deceased
Paul Jokiel, Chairperson-acting
Fred Mackenzie
Matthew Church

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Abstract:

A series of outdoor flume experiments was conducted using a range of experimental "reef communities" in near natural conditions to test whether the alkalinity anomaly technique accurately predicts calcification. Such calcification measurements have been used to describe coral reef metabolism and to monitor reef health. For the past 40 years it has been assumed that half the total alkalinity flux ($\Delta TA/2$) equals calcification and that there is no significant effect of organic metabolism on the overall alkalinity. The hypothesis that $\Delta TA/2$ equals calcification was tested by determining if the relationship between alkalinity and calcium uptake is constant for various communities and community components. Assumptions of the alkalinity anomaly technique were tested by measuring the alkalinity and calcium fluxes of isolated components (corals, phytoplankton, algae and the sediment) in reference to that of the combined community. Natural sunlight, realistic hydrodynamic regime and natural levels of nutrients, plankton and organic matter were available to the organisms. Groups of corals were run separately and in conjunction with other reef components (live rock, filamentous algae and sandy sediment). The alkalinity to calcium flux ratios were consistently higher during coral-only runs (2.01 ± 0.19) than in the mixed community (1.61 ± 0.14, *p*-value =0 .011) where additional sources of alkalinity from the sediment and algae caused a depressed ratio. Additionally, pH was higher and more stable when sediment was included with the corals (7.52 ± 0.07 vs. 7.94 ± 0.03, *p*-value = 3×10^{-5}). Aragonite saturation state (Ω_{arag}) showed the same pattern (1.12 ± 0.14 vs. 2.51 ± 0.2, *p*-value = 2×10^{-6}), indicating corals may receive benefits from living in the same environment with carbonate sediments and photosynthetic organisms. Additional experiments where macro-algae and a sediment community were tested separately revealed that these components of alkalinity can be a significant source of error for calcification measurements when they are dominant on a reef. Alterations of nutrients and organic matter due to photosynthesis, oxidation, reduction and remineralization in the non-coral components can cause deviations from $\Delta TA/\Delta Ca^{2+} = 2$.

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1. Introduction:

Accurate net calcification measurements of coral reef organisms and communities are necessary if we are to understand the carbon cycle on reefs and to predict and monitor the effects of ocean acidification (OA) in these crucial coastal ecosystems. Rising atmospheric CO_2, and the consequent changes in seawater chemistry makes it more difficult for corals to form their skeletons (Scheider & Erez 2006, Atkinson & Cuet 2008). Changes in carbonate ion (CO_3^{2-}) concentration (Yates & Halley 2006, Silverman et al. 2009), bicarbonate ion (HCO_3^-) concentration (Edmunds et al. 2012, Comeau et al. 2013) as well as proton (H^+) concentration (Jury et al. 2010, Jokiel 2011) influence coral calcification and are expected to shift in response to climatic forcing. Thus, understanding coral and coral reef metabolism is presently a top priority for marine science. During the last few decades, experimental and monitoring efforts on coral reefs have primarily deduced calcification rate from measured changes in dissolved inorganic carbon (DIC) and total alkalinity (TA) (Smith & Key 1975, Kinsey 1978). Total alkalinity can be defined as the total buffering capacity of the water, or the excess of proton acceptors over proton donors (Dickson 1981):

$$TA = [HCO_3^-] + 2[CO_3^{2-}] + [B(OH)_4^-] + [OH^-] + [HPO_4^{2-}] + 2[PO_4^{3-}] + [SiO(OH)_3^-] \quad (1)$$
$$+ [NH_3] + [HS^-] - [HSO_4^-] - [H^+]_F - [HF] - [H_3PO_4] + [\text{minor bases} - \text{minor acids}]$$

TA is composed predominantly by the carbonate ions along with a myriad of other compounds that are usually in lesser concentration in seawater. A simple relationship exists between TA and calcification when treated as an isolated system (coral in a beaker with no sediment, nutrient or algal input). Under these conditions the release or consumption of carbonate ions creates a shift in alkalinity (Smith & Key 1975). The basic reaction can be described as follows:

$$Ca^{2+} + 2HCO_3^- \Leftrightarrow CaCO_3 + CO_2 + H_2O \Leftrightarrow CaCO_3 + HCO_3^- + H^+ \quad (2)$$

From the above equation, one can assume that for every two moles of bicarbonate ions or alkalinity consumed, one molar equivalent of calcium carbonate is produced and the reverse reaction occurs during dissolution. This is the basis of the alkalinity anomaly technique. The metabolic reaction is only involved in the construction of calcium carbonate skeletons, and is not influenced by the animal's respiration or by photosynthesis from the zooxanthellae and other symbionts. The processes of photosynthesis and respiration alone do not change the total

alkalinity (Smith & Key 1975, Brewer & Goldman 1976, Boucher et al. 1998, Kim & Lee 2009, Wolf-Gladrow et al. 2007), but photosynthetic organisms can alter alkalinity through processes such as nutrient uptake and metabolism.

Direct calcium measurements have seldom been utilized as an alternative for measuring calcification because changes in the large pool of calcium in open ocean water are usually very small and difficult to quantify precisely. Most investigators have determined calcification from changes in alkalinity (Kinsey 1978, Smith & Kinsey 1978, Barnes 1983, Gattuso et al. 1998, Leclercq et al. 2002). Direct calcium measurements such as calcium fluorescence and ^{45}Ca tagging are now available (Howe & Marshall 2002) along with methods such as titration with EGTA and calcium selective electrodes. A widely used and practical direct measurement of skeletal growth in corals is buoyant weighing (Jokiel et al. 2008). While $\Delta TA/2$ will give a reasonable estimate of calcification in the incubation of a coral in filtered sea water, it may not be valid on a biogeochemically complex reef where many chemical processes contribute to alkalinity. Alterations of dissolved organic matter arising from photosynthesis, grazing, viral lysis, transformations of nutrient speciation and organic diagenesis in sediments alter the ionic balance in seawater. These processes are potentially important pathways for changes in alkalinity production. When alkalinity is produced and calcium is unaltered (by non-calcification sources), this offsets the ratio of $\Delta TA/\Delta Ca^{2+}$, which are usually both negative numbers. This creates a lower $\Delta TA/\Delta Ca^{2+}$ ratio (< 2) and would result in an underestimation of reef net calcification.

1.1 Limitations of the alkalinity anomaly technique

The alkalinity anomaly technique assumes that the observed alkalinity shift is entirely due to the net change in calcification and dissolution of $CaCO_3$. The limitations of this technique were recognized when it was first developed (Kinsey 1978, Smith and Kinsey 1978). It was acknowledged that "specialized reefs may show poor correlations between alkalinity and calcium flux" and that denitrification as well as ammonia and sulfide release during organic matter diagenesis in sediments also contributes to the alkalinity signal (Davies & Kinsey 1973). Current researchers have shown directly that the $\Delta TA/\Delta Ca^{2+}$ relationship is not 2/1 in some systems (Andersson et al. 2007). Few studies have been directed at validation of the alkalinity anomaly technique and none to date with mixed natural communities. Chisholm & Gattuso (1991) measured ΔTA versus skeletal weight gain with a colony of the coral *Pocillopora*

damicornis in a beaker with filtered seawater to validate the technique. Heterotrophic feeding is a substantial part of a coral's metabolic requirements, which filtered water does not provide. Isolating an organism from its environment, natural food sources, and biogeochemical partners assumes that its' metabolism is the same as in nature. Although careful scientific procedures are taken to replicate natural conditions, ecosystems are complex and interconnected. Reef communities that encompass coral rubble, sand, and coralline or filamentous algae have not yet been investigated in detail and the metabolic response of whole communities may act differently than isolated coral colonies.

The alkalinity signal in typical coral reef ecosystems is likely to be predominantly from calcium carbonate formation and dissolution. However, other processes such as organic matter production from photosynthesis (Kim & Lee 2009), anaerobic diagenesis in sediments (Thomas et al. 2009, Mackenzie & Andersson 2011), and nutrient transformation in macro-algae, sediments and plankton (Brewer & Goldman 1976, Wolf-Gladrow et al. 2007) can further alter ion concentrations that compose TA. The level of impact of these processes depends on environmental parameters such as surface area, organic input, temperature and hydrodynamic regime. The net effect these outside-coral biogeochemical processes create is usually an increase in TA over time. This would create a $\Delta TA/\Delta Ca^{2+}$ ratio that is lower than 2:1. Failure to evaluate the alkalinity technique under natural ecosystem conditions may lead to an underestimate of reef net calcification in the field as discussed by Smith and Kinsey (1978).

1.2 Interpretation and Re-evaluation of the Technique

The most accessible technique to date has been the use of observed changes in alkalinity to estimate calcium carbonate calcification and dissolution. Until recently, our understanding of the reef ecosystem has largely excluded metabolic contributions by microbes and organic matter, or treated them as a black box. Due to recent advancements in technology, a more complete understanding of the importance of the microscopic biological components in the ocean has been obtained and should be accounted for in our older constructs. This is especially important when models are used to extrapolate conditions in the field. A more detailed understanding of carbon cycling is crucial to modern environmental challenges.

Changes in alkalinity can result from the alteration of other chemical components other than those normally included in the carbonate system. While biologically mediated changes in

alkalinity are complex, they can be derived from sedimentary diagenesis (Emerson & Hedges 2003, Mackenzie & Andersson 2011) transformations in the nitrogen system (Brewer & Goldman 1976) or through production of organic matter complexes (Kim & Lee 2009). In sediments with an appreciable amount of Fe and organic matter, sulfate reduction is likely to be a major source of alkalinity. Many experiments have demonstrated the crucial role played by the microscopic community in primary production, nutrient cycling and carbon transport (Brewer & Goldman 1976, Boucher et al. 1998, Kim & Lee 2006, Werner et al. 2008). Microbes living within the plankton and sediment can make a significant contribution to alkalinity in shallow water systems because of their vast abundance in the environment. On the scale of an entire reef system, their contributions may be on the same order of magnitude as the production estimated for corals (Werner et al. 2008). Negatively charged ions arising from plankton metabolism and sedimentary processes can absorb H^+ in seawater and increase alkalinity.

Using the concept of electro neutrality, Wolf-Gladrow et al. (2007) describe an explicitly conservative definition of alkalinity, using nutrient concentrations and other non-carbonate compounds. The reasoning follows a nutrient - proton compensation principal from balanced metabolic processes occurring in both plant and animal cells, and can be set equal to Dickson's equation for alkalinity. Canceling out like terms (such as the carbonate species) yields the explicitly conservative form of alkalinity (TA_{ec}) where the species involved mix conservatively with their respective volumes. TA_{ec} is written as follows:

$$TA_{ec} = [Na^+] + 2[Mg^{2+}] + 2[Ca^{2+}] + [K^+] + 2[Sr^{2+}] + ... - [Cl^-] - [Br^-] - [NO_3^-] - ... \quad (3)$$
$$TPO_4 + TNH_3 - TSO_4 - THF - THNO_2$$

The lesser species: total phosphate, ammonium, sulphate, fluoride and nitrate, are defined as:

$$TPO_4 = [H_3PO_4] + [H_2PO_4^-] + [HPO_4^{2-}] + [PO_4^{3-}] \quad (4a)$$
$$TNH_3 = [NH_3] + [NH_4^+] \quad (4b)$$
$$TSO_4 = [SO_4^{2-}] + [HSO_4^-] \quad (4c)$$
$$THF = [F^-] + [HF] \quad (4d)$$
$$THNO_2 = [NO_2^-] + [HNO_2] \quad (4e)$$

This new equation can be used to calculate changes in alkalinity due to biogeochemical processes that require transformations of the nutrient systems or from organic matter diagenesis. It is important to note that the concentrations of these ions and changes in their abundance in

typical reef environments are usually low. To simplify the equation for the most dominant components in the reef environment, changes in calcium concentrations can be predicted from changes in alkalinity and nitrate combined as described by Kanamori & Ikegami (1982) and Wolf-Gladrow et al. (2007).

$$\Delta Ca^{2+} = 0.5\ \Delta TA + 0.68\ \Delta NO_3^- \quad (5)$$

A recent debate has focused on whether the source of nitrogen used in photosynthesis has a significant effect on alkalinity and therefore calcification data. In general, the concentrations and changes in nitrate are small compared to the magnitude of alkalinity uptake driven by corals. NO_3^- in coral reef systems is typically very low (~1 μmole), and changes are typically very small in comparison to changes in TA. Average coral nutrient uptake for phosphate (0.3 ± 0.2 mmol $m^{-2}\ d^{-1}$), ammonium (1.4 ± 0.6 mmol $m^{-2}\ d^{-1}$), and nitrate is (2.7 ± 0.8 mmol $m^{-2}\ d^{-1}$) (Langdon & Atkinson, 2005). These rates were, however, deduced in a coral flume separated from other benthic sources of nitrogen and it has been shown that sedimentary nutrient flux can stimulate higher rates of production (Stimson & Larned 2000). While this only represents 1% of the strength of the total alkalinity changes seen in coral reef ecosystems (200-400 mmol $m^{-2}\ d^{-1}$), many other organisms in coastal benthic habitats are also transforming nutrients. Nitrate is produced and consumed during both oxic and anoxic diagenesis, making it an important tracer in biogeochemical reactions that affect the ionic balance (Emerson & Hedges 2003). Changes in alkalinity generated by a variety of biogeochemical processes can be calculated easily through the use of TA_{ec}. Use of the historical alkalinity anomaly technique could be continued with these corrections in environmental situations that warrant their use.

2. Transformations leading to alterations of alkalinity:

2.1 Planktonic and algal photosynthetic metabolism:

Photosynthetic metabolism affects alkalinity in several ways. Firstly, plankton and bacterial cells and their macromolecules such as carboxyl, phosphate and amino groups have negatively charged surfaces (Gonzalez-Davila & Millero 1990, Kim et al. 2006). If alkalinity titrations are performed on unfiltered water the functional groups will absorb the protons in the acid used for titration. The net charge that these macromolecules exhibit is dependent on pH. The higher the pH the more negative the surfaces and at low pH's (below 4-5) the cells can have a net

positive surface charge (Kleijn & van Leeuwen 2000). The contribution of planktonic surfaces to alkalinity was shown to be larger than that of $CaCO_3$ particles suspended in seawater, which were previously recognized as the only significant input of alkalinity in unfiltered samples. The increase in alkalinity created by the surface charge of these organisms is on the order of 5-15 µmol L^{-1} depending on the concentration of particulate organics (Fig 1). In general, the higher the nutrient and particulate organic pool, the larger the effect that plankton have on alkalinity (Kim & Lee 2006). Levels of POC in these experiments are larger than those normally found on reefs, but depict changes that can occur during blooms.

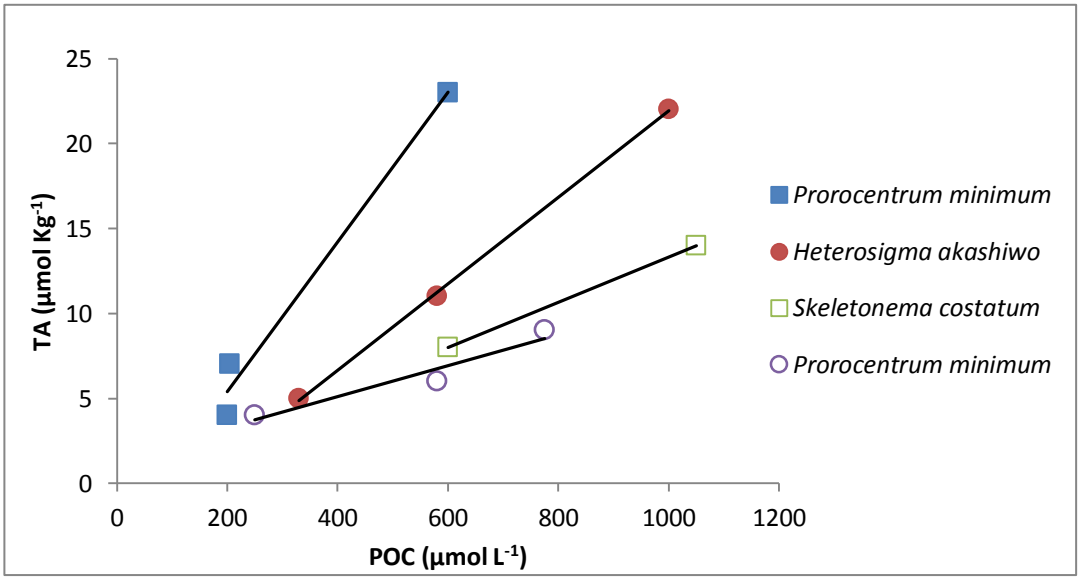

Fig 1. Alkalinity differences between unfiltered seawater with respective species of cultured phytoplankton and the same seawater filtered through a 0.7µm filter. Effect on measured alkalinity is strongly dependent on amount of particulate organic carbon available. Figure after Kim & Lee 2006.

Secondly, photosynthesis by algae and microbes both in the plankton and in the upper sedimentary layers contributes to seawater alkalinity through the production of dissolved organic matter matrices and through nutrient transformations. The addition or removal of CO_2 during photosynthesis and respiration does not affect alkalinity, but this does not mean that the photosynthetic, respirational and decompositional processes do not alter alkalinity through the use of other compounds, such as nutrients (Brewer & Goldman 1976). The photosynthetic community in the benthos is dominated by diatoms, dinoflagellates and cyanobacteria. While the per unit surface area of microphytobenthic photosynthesis is at least six times lower than

photosynthetic rates reported for corals (Werner et al. 2008), sediments often occupy extensive map areas on reefs. The ionic balance can be shifted by these organisms through the production of organic matter matrices that are often negatively charged and through the assimilation and remineralization of ionically charged nutrients (Wolf-Gladrow et al. 2007). The extent of this process is species and interaction dependent and is influenced by grazing, lysing and the solubilization of particles. Kim & Lee (2009) demonstrated that with increasing nitrate uptake the effect on the measured alkalinity increased; validating the relationship between nutrient transformation and alkalinity generation. For example, during photosynthesis, the uptake of nitrate and phosphate from the water column raises TA through the transfer of protons from the reagents to create organic matter. On the other hand, the oxidation of organic matter during respiration releases the protons and decreases TA (Brewer & Goldman 1976).

$$106\ CO_2 + 122\ H_2O + 16\ HNO_3 \Leftrightarrow (CH_2O)_{106}(NH_3)_{16} + 138\ O_2 \quad (6)$$

The net microbial or algal photosynthetic effect on alkalinity is dependent on the dominant species, water advection and source of nitrogen being taken up by photosynthetic organisms. Uptake of NO_3^- generates a strong base, OH^- whereas NH_4^+ assimilation leads to acid production, H^+ (Brewer & Goldman 1976) (Eq. 7). This means that phytoplankton and algae using nitrate increase TA and those utilizing ammonia generally decrease TA. If CO_2 decreased and TA increased due to nitrate assimilation, this trend would generally be attributed to $CaCO_3$ dissolution, but the same combination can occur from nutrient uptake. A previously mentioned adaptation of the alkalinity anomaly technique can be utilized to account for nitrate assimilation (Eq. 5).

$$106\ CO_2 + 138\ H_2O + 16\ NO_3^- \leftrightarrow (CH_2O)_{106}\ (NH_3)_{16} + 16\ OH^- + 138\ O_2 \quad (7)$$

Macro-algae are often very abundant in reef environments and have a large impact on nutrient concentrations and pH. *Sargassum* macro-algae have been used as a nutrient sink for inorganic nitrogen in aquaculture tanks, because they uptake significant amounts of ammonium, nitrite and nitrate which can alter the charge balance and alkalinity (Mai et al. 2010). Algal nutrient uptake rates are widely variable depending on species and nutrient concentration. Bulk water processes such as planktonic photosynthesis can produce an increase in alkalinity equal to a calcification rate of -1.0 ± 0.5 mol $CaCO_3$ m^{-2} yr^{-1} (Small & Adey 2001). On the other hand,

photosynthesis in the sediments can be a strong signal, ranging from 19-85 mmol m^{-2} d^{-1} (Werner et al, 2008). Benthic photosynthesis often uses sedimentary nutrient sources to fuel production at higher rates than the oligotrophic waters alone would allow (Stimson & Larned 2000). Changes in seawater nutrient concentrations may not be entirely indicative of production rates because high productivity ecosystems in oligotrophic waters such as coral reefs rely on rapid nutrient recycling.

2.2 Anaerobic degradation in the sediments

Dissolved and particulate organic matter produced by reef organisms and from terrestrial runoff is remineralized and oxidized within the interstitial waters of the sediments. The alkalinity rapidly increases deeper in sediments where the oxidation of organics occurs (Kuivila & Murray 1984, Alongi et al. 1996, Thomas et al. 2009, Mackenzie & Andersson 2011). Thus sediments can act as a source of alkalinity to the overlying water column. Sediments can cover up to 70% of the area on a coral reef and play a crucial role on the reef, facilitating nutrient cycling, diagenesis and system buffering. Sediments are significant sinks of carbon, sites of organic matter storage and support bacterial activity with consequent diagenesis (Morse & Mackenzie 1990, Boucher et al. 1998, Mackenzie & Andersson 2011). It has been estimated that on a global scale, anaerobic alkalinity generation such as sulphate reduction and denitrification could be accountable for as much as 60% of the uptake of CO_2 in shelf and marginal seas (Thomas et al. 2009). Thus shallow systems are important drivers of the global carbon cycle which regulates pH and the abundance of $CaCO_3$ in the ocean (Emerson & Hedges 2003). Reef sediments are usually sandy and highly permeable. Reefs are often exposed to strong hydrodynamic forces that facilitate the exchange of chemical compounds through the sediment water interface (Werner et al. 2008). These shallow environments are an important biogeochemical link between the land, open-ocean and atmosphere. The processes of $CaCO_3$ dissolution, sulfate reduction and denitrification tend to be the most dominant reactions that incur alkalinity changes in sediments, leading to a net increase in alkalinity. Thomas et al. (2009) showed that the sediments act as a buffer that keeps TA and pH more stable. They estimate that benthic alkalinity generation has the potential to facilitate as much as 20-25% of the CO_2 uptake of the North Sea.

The effect that organic matter diagenesis has on ecosystem alkalinity depends on many factors including: grain size and composition of the sediment, nutrient and organic input,

temperature and hydrodynamics. Alongi et al. (1996) experimentally demonstrated significant differences in sedimentary metabolism based on environment. They found faster rates of diagenic activity and alkalinity generation observed in Mangrove Bay (compared to the fore-reef) due mainly to restricted water circulation and organic input. Additionally, sediments with smaller grain sizes and more restricted water flow tended to have higher rates of anaerobic diagenesis. For example, observed increases in dissolved nitrogen content within algal mats and heightened sedimentary nutrient concentrations in protected reef flats were not found to be prevalent in exposed barrier reefs (Larned 1998). Therefore, metabolic measurements from within lagoonal reefs or barrier reefs with high organic input would need to consider the sedimentary and algal components of alkalinity more so than experiments being done on isolated coral atolls or open flow fore-reefs.

In another experimental example, a large increase in alkalinity was observed by Kuivila & Murray (1984) in the top 4 cm of sediment, and continued to increase with depth through the sediment. Many other studies have confirmed the same trend (Alongi et al. 1996, Boucher et al. 1998, Thomas et al. 2009) demonstrating that sediments can act as a source of alkalinity to the overlying water column. However, even with the Redfield stoichiometric equations for sulfate reduction, ammonia assimilation, iron reduction, denitrification and calcium carbonate dissolution, Kuivila & Murray (1984) could not account for 40-50% of the increased alkalinity in the top 4cm (the aerobic surface portion) of the sediment. It is possible that photosynthetic byproducts were not considered as a plausible source of alkalinity. The Kim & Lee (2009) and Werner (2008) experiments suggest that microbial photosynthesis could make up part of the remaining portion of alkalinity signal.

There are a myriad of metabolic and geochemical reactions affecting alkalinity that occur in sediments both anaerobically and aerobically in the surface sediment. Bioturbation increases the depth that the aerobic layer penetrates and can stimulate degradation up to a moderate level of disturbance with a slowing of diagenesis at intense disturbance levels (Emerson & Hedges 2003). These processes affect alkalinity in different ways and will be discussed separately. The effect these transformations have on alkalinity can be calculated by using TA_{ec} as described by Wolf-Gladrow et al. 2007 (Eq. 3).

2.2.1 Calcium carbonate precipitation and dissolution: Calcium carbonate is deposited, precipitated and dissolved within sediments as it does in living coral. H_2CO_3 produced by organic matter oxidation is utilized in $CaCO_3$ dissolution in sedimentary pore waters (Mackenzie & Andersson 2011). The fraction of $CaCO_3$ that is buried in sediments dramatically affects the TA and DIC of seawater (Emerson & Hedges 2003). This can become an important local buffer for coral reefs where the sediment can dissolve to balance pH or Ω_{arag} state in lieu of dissolving of live coral skeletons. In a closed system the precipitation of 1 mole of $CaCO_3$ always leads to a decrease of 1 mole in DIC and 2 moles in TA (Wolf-Gladrow et al. 2007).

$$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O \quad (8)$$

2.2.2 Denitrification, is often fueled by riverine or other terrestrial nitrate inputs due to low concentrations in the reef environment. The process consumes H^+ ions and releases the quasi-inert gas of N_2 which is then released to the atmosphere through the air-sea interface. For every mole of N denitrified, 0.99 moles of alkalinity are released.

$$CH_2O + NO_3^- + 2H^+ \rightarrow CO_2 + 0.5\ N_2 + 2H_2O \text{ (Thomas 2009)} \quad (9)$$

2.2.3 Nitrification occurs in aerobic environments where ammonia is ultimately oxidized to nitrate. This leads to a decrease of alkalinity by 2 moles per mole of NO_3^- formed (Schlesinger 1997).

$$NH_4^+ + 1.5\ O_2 \rightarrow NO_2^- + H_2O + 2H^+ \ : \ NO_2^- + 0.5\ O_2 \rightarrow NO_3^- \quad (10)$$

2.2.4 Sulfate Reduction predominates nitrate and manganese reduction in near shore environments due to an increase in organic matter flux (Emerson & Hedges 2003). It occurs in the anaerobic layer of sediments using sulfate as an electron acceptor for bacteria obtaining energy from oxidizing organic material. This process converts the sulfate to hydrogen sulfide gas, absorbs H^+ and releases alkalinity. For every mole of SO_4^{2-} reduced 1.98 moles of alkalinity are released.

$$CH_3COO^- + SO_4^{2-} + H^+ \rightarrow 2HCO_3^- + H_2S \quad (11)$$

2.2.5 Phosphorus assimilation and remineralization. Uptake of 1 mole of phosphate (H_3PO_4, $H_2PO_4^-$, HPO_4^{2-} or PO_4^{3-}) by algae will increase alkalinity by 1 mole per mole P (Wolf-Gladrow

et al. 2007). The effect on alkalinity of phosphate uptake is small compared to the changes seen by the uptake of nitrate.

2.2.6 Sulfur assimilation and remineralization. Uptake and assimilation of sulfate into particulate organic matter leads to an increase of alkalinity by 2 moles per mole of S. On the other hand, when sulfate is remineralized the reverse occurs and alkalinity is consumed in the same proportions (Brewer et al. 1975).

2.2.7 Methane oxidation by sulphate reduction can occur in anoxic sediments. During this process the concentration of total sulphate decreases by 1 mole per mole of methane oxidized and therefore alkalinity is increased by 2 moles per mole of methane oxidized.

$$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O \quad (12)$$

This process affects alkalinity because it consumes sulfate. Methane concentrations do not directly affect alkalinity, but do increase DIC by 1 mole per mole methane oxidized. (Wolf-Gladrow et al. 2007)

The processes of detnitrification and sulfate reduction are mostly irreversible, and when their products are buried or escape to the atmosphere, the anaerobic degradation of organic matter leads to a net alkalinity gain on annual time scales. Thus benthic anaerobic alkalinity generation may be an important biogeochemical pump that leads to the uptake of atmospheric CO_2 in shallow coastal areas. (Thomas et al. 2009, Mackenzie & Andersson 2011). Increases in TA but not Ca^{2+}would lead to a lower $\Delta TA/\Delta Ca^{2+}$ratio (less than 2:1) and an underestimation of net calcification using the alkalinity anomaly technique.

3. Other benefits of living in a natural, mixed community:

Sediments cover a large surface area in reef systems and therefore may be an important compartment for system buffering, microphytobenthic primary production and nutrient cycling (Werner et al. 2008). Benthic photosynthesis also absorbs CO_2 and raises the pH, which increases Ω_{arag} and stimulates coral calcification, as described by the proton flux hypothesis presented by Jokiel (2011). This is how corals internally regulate CO_2 and pH within the cells in order to precipitate calcium carbonate, but the same effects can be seen on an ecosystem scale. It has been long known that photosynthesis and calcification are synergistic, forming basis for the

symbiosis between zooxanthellae and their coral hosts. However, it seems that zooxanthellae are not capable of completely removing all the excess CO_2 produced by the coral. Photosynthesis from other organisms in the reef environment help to speed up calcification by removing further CO_2 and elevating pH (Small & Adey 2001, Scheider & Erez 2006, Anthony et al. 2011). Photosynthesis and calcification support each other through this internal and external pH regulation, which provides CO_3^{-2} ions for calcification and CO_2 (aq) for photosynthesis (Schneider & Erez 2006). Anthony et al (2011) demonstrated this role that macro-algae on reefs have and their effect on driving higher and less variable Ω_{arag} that can stimulate coral calcification. During Small & Adey (2001)'s experiment, coral calcification increased 60% for *Acropora* and 120% for *Montipora* when the free living algae *Chondria* was added to the tanks. During the same experiment, the *Chondria* algae maintained a steady TA but converted bicarbonate to carbonate ions. The McConnaughey and Whelen (1997), or the Jokiel (2011) model of proton flux could be a likely source of protons for bicarbonate assimilation from carbonate ions generated by algae (Small & Adey 2001). Photosynthetic organisms and carbonate sediments both offer system stabilizing effects that coral-only experiments lack, and provide community buffering and resilience to increasing pCO_2 levels.

Nutrient recycling is another contribution of microbes on coral reefs in addition to their influence on alkalinity and buffering. Corals grow in highly productive yet oligotrophic waters and energy and nutrients need to cycle rapidly in order to maintain their high biomass and primary production. Sedimentary sources of nutrients include microbial nitrogen fixation, excretion by macrofauna and remineralization (Capone et al. 1992, Stimson & Larned 2000). Microbes in the sediments break down and turn over refractory compounds and fix nitrogen. Coral reefs are generally depleted in nitrogen relative to phosphorus so this source of fixed nitrogen is essential. It is often assumed that water column nutrient concentrations fuel benthic primary production, but it has been shown that normal rates of production from algae require access to benthic nutrient sources (Larned 1998). Dissolved inorganic nitrogen in sedimentary pore-water is 30-60 times more concentrated than in the overlying water column (50-100 μM vs. 1.5 μM). In tropical, oligotrophic systems, it is very probable that the low nutrient concentrations in the water and the change in those concentrations over time are not indicative of the actual uptake by organisms. Nitrogen efflux from the sediments in Kaneohe Bay ranges from 250-1500 $\mu mol\ m^{-2}\ d^{-1}\ NH_4^+$ and 0-1000 $\mu mol\ m^{-2}\ d^{-1}\ NO_3^-$ (Stimson & Larned 2000) and can be generated

by nitrogen fixation, macrofauna excretion and remineralization. A coral reef without sediment is like a forest without soil. Sediment is the compartment where organic and inorganic material are recycled and transformed into reusable compounds by a complex community of microbes and detritivores.

Sediments also act as a buffer that stabilizes coral reef environmental conditions. The vast pool of calcium carbonate and Mg-calcite sands serve to buffer the Ω_{arag} of the ecosystem and on a local scale can help counteract OA on local scales (Andersson & Mackenzie 2012). For example, many diurnal coral experiments have shown that coral reef net carbonate dissolution can occur at night (Clavier et al. 2008, Shamberger et al. 2011, Atkinson & Cuet 2008, Silverman et al. 2007, Andersson et al.2009). However, with a carbonate sand reservoir in the sediment, this can be a source of calcium and bicarbonate ions that do not have to come from live coral skeleton. $CaCO_3$ dissolves in sediments during organic matter oxidation which can be very active in coral reef sediments (Werner et al. 2008). Anthony et al. (2011) included 3 cm deep sand in their coral flumes along with macro-algae and demonstrated a high stable pH with net calcification at night.

Sediments can also act as a microbial buffer, or as a repository of microbes that can be used as a source for acquiring symbionts in corals. After bleaching events, corals may need to reacquire zooxanthellae from their environment and some species do not produce eggs with the symbionts inside. Being separated from their natural environment may hinder this acquisition process. During and after stress events, corals secrete large quantities of mucus. Microbes living within coral tissue are continuously lost to the water column and the surrounding sediments through this mucus excretion (Wild et al. 2004). This adaptation is useful when microbial symbionts need to be exchanged during times of environmental changes, when they can readily attain new microbes from the water or sediment. The fast generational times of microbes make the coral biome, as a whole, more adaptive (Gates & Ainsworth 2011). However, this could be an important confounding factor in coral tank experiments - where corals are put under stress during collection, excrete mucus and release their microbial partners but are then isolated from the sediments where new microbes could be attained. The sediment compartment is a crucial component to reef systems which is often overlooked in favor of water column processes that are assumed to interact more directly with the organisms in the environment.

4. Experimental Design:

An outdoor flume was used with a volume of 2.28 m^3, total air to water surface area of 8 m^2 and a flow rate of 10 cm s^{-1}, which is comparable to the hydrodynamic regime of Kaneohe Bay reefs. Five flume incubations were performed: 1.) the "coral-only" run consisted of 2 m^2 of mixed corals, 2.) the "mixed community" run consisted of the exact same corals from the coral-only run combined with 2 m^2 of fine carbonate sediment and 2 m^2 of "filamentous algae" that had been allowed to develop on the flume walls, 3.) the "sediment-only" run with the same sediment as in the mixed community, 4.) the "macro-algae algae only run" with 2 m^2 of the marco-alga *Gracillaria salicornis* and 5.) the "plankton run" with the unfiltered water in a clean flume without any other organisms. Temperature, pH and changes in calcium and total alkalinity were measured concurrently. All of these incubations intrinsically include the microbial plankton community in the unfiltered seawater. The separate controls of the seawater, algae and sediment were run on their own to test the background alkalinity and calcium metabolism from phytoplankton, algal primary production and sedimentary diagenesis. The relationship between the calcium and alkalinity fluxes were then examined to see if the relationship holds to or deviates from $\Delta TA/\Delta Ca^{2+} = 2$ and if a significant alkalinity signal is received from planktonic, algal and sedimentary biochemical reactions.

The corals collected were a representative community from Moku-o-loe, Kaneohe Bay (Coconut Island, or HIMB) (Fig. 2). The spatial map area of the coral colonies was estimated using pixel counts performed in Photoshop C4 (49% *Porities compressa*, 41% *Montipora capitata*, 9% *Pocillopora damicornis* and 1% *Fungia scutaria*). Two square meters of corals were collected, in a 2.28 cubic meter flume tank, leading to a 1.14 volume to surface area ratio. In the mixed community incubation, the live rock was representative with filamentous algae, tubeworms, and small crustaceans that inhabit live rock. Two square meters of sediment was collected from a near shore lagoonal reef community off of Moku-o-loe. This sediment was 12 cm deep in the flume and consisted of fine, carbonate sands with a relatively high amount of organic matter and anoxic layering, allowing a significant signal from organic matter diagenesis. The sediment was allowed to adjust to conditions in the flume for a week under conditions of flowing water at a rate of 10 cm s^{-1} before experimentation to allow natural anoxic diagenesis layers to reform after being disturbed in the collection process.

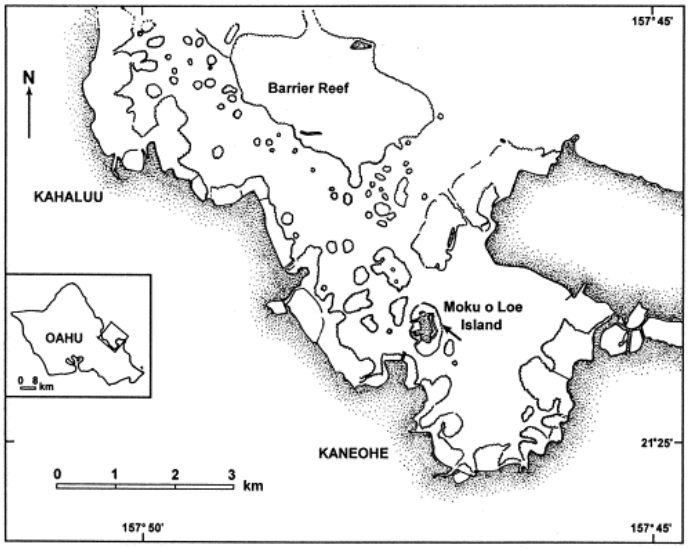

Fig. 2: Map of the collection site inside the marine sanctuary of the Hawaii Institute of Marine Biology within Kaneohe Bay on Moku-o-loe Island.

The macro-algae-only run contained calcifying epiphytes on the surfaces of the algae while the mixed community had filamentous algae devoid of these epibionts. Corals released mucus when first collected, but rapidly returned to normal during the acclimation period before the start of the experiment. Calcification rates were within the normal range (170-230 mmol m^{-2} d^{-1}) reported in the literature (Gattuso et al. 1998, Langdon & Atkinson 2005, Atkinson & Cuet 2008, Andersson et al. 2009), and did not change significantly between the coral only and mixed community runs. The calcification rate for the first two days of each week stayed consistently high for all six trials, indicating that the corals remained in good health. There was no evidence of disease or bleaching. Corals did not show stress response of excess mucous production and appeared to be healthy with extended polyps for feeding, especially at night.

Irradiance in the shallow outdoor flumes was reduced by 50% with shade cloth in order to mimic reef conditions at greater depths. Sunlight, temperature and hydrodynamic flow were within the typical range found on Kaneohe Bay reefs. The flume was filled with unfiltered water from the HIMB sea water system that is pumped from a depth of 2 m off the Coconut Island windward reef. This water provided the organisms with natural levels of plankton and nutrients from their natural environment. The water was aerated during all experiments in order to stabilize dissolved oxygen levels and keep the communities from going anoxic at night. Water in the flume was re-circulated in a closed loop with a current of 10 cm s^{-1}, which is within the average flow for reefs in the bay (Langdon & Atkinson 2005). During the period of closed flow –

"incubation"- the residence time of 5 days permitted the accurate measurement of calcium flux. Salinity was measured throughout the experiment. Integrated samples were taken every other day over the 5 day static incubation through use of a 20 L subsampling bucket. Water from the recirculating flume was slowly siphoned into the bucket using 1 cm diameter tubing for a period of 10 minutes. Bottles were rinsed three times with sample water before being filled from the integrating sampling bucket, and the remaining integrated sample returned to the flume. Samples were placed in glass bottles, sealed with rubber stoppers, unfiltered and stored at 25°C. After the 5 day incubation period the flume was opened and flushed for 2 days with unfiltered seawater from the HIMB seawater system to refresh the supply of nutrients and plankton. This process was then repeated three times for each community in the flume (3-4 wks. each, 12-16 wks. total for the experiment component). A 24 hour study during which samples were taken every two hours was conducted to obtain diel data in order to evaluate net calcification which is high during daylight due to light-enhanced calcification and low or negative at night due to dissolution.

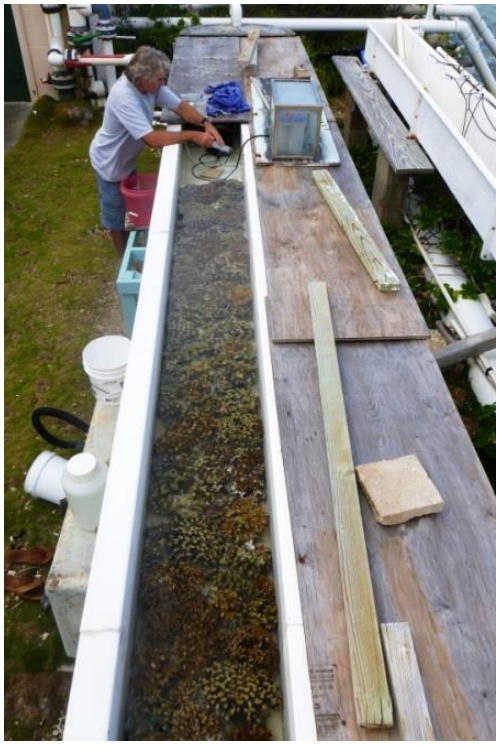

Fig. 3: The flume system with the shade cloths taken off showing the subsample bucket in use.

4.1 Measurements:

Water mixing time was approximated using mean flow rates and volume. Total alkalinity was measured in duplicate by auto titration with 0.1N HCl solution on a Metrohm titrator, using Dickson standards for alkalinity (Dickson et al. 2007). The samples were kept in a water bath of 25^{o}C beforehand and placed in a water-jacketed container held at that temperature during analysis. Standard error in measurement was 5 μM alkalinity. Calcium and alkalinity values were normalized to the starting salinity at the beginning of each incubation experiment. Salinity and pH were measured using a Dickson standardized conductivity probe. Calcium was measured in duplicate with the standard titration of Ca^{2+} with EGTA and calcium selective electrodes (Edmond 1970). Standard error in measurement was 0.02 mM Ca^{2+}. All fluxes in the experiments were at least four to five times the standard error, and in most cases the magnitude of change was more than twenty fold the standard error. HCO_3^-, CO_3^{2-}, pCO_2 and Ω_{arag} were calculated from salinity, temperature, TA and pH data using the CO2SYS program developed by Lewis and Wallace (1998). Average Kaneohe Bay phosphate and silicate concentrations along with constants from Mehrbach et al. (1973) refit by Dickson and Millero (1987) were used, as described in (Shamberger et al 2011).

Statistical testing was carried out by using Origin Pro 8 Data Analysis and Graphing Software. Data are reported as mean ± standard error of the mean. Significant differences between parameters were tested by using two-tailed student t-tests where H_o= no difference between mesocosms, significance at $P<0.05$. If the P value was less than 0.05 for a 95% confidence level, the null hypothesis was rejected.

5. Results:

5.1 Alkalinity flux to calcium flux ratios:

The concentrations of alkalinity and calcium were determined for the first, third and fifth days of each week's experiment. Concentrations were normalized to the starting salinity for each incubation experiment to correct for changes in concentration due to evaporation and precipitation. The ratio between alkalinity flux and calcium flux ($\Delta TA:\Delta Ca^{2+}$) was consistent regardless of the method used to generate them. The ratios for the coral only and mixed communities (coral + sediment + algae) were significantly different from each other ($p<0.05$). The ratio of change between successive individual samples was determined ($\Delta TA:\Delta Ca^{2+}$ratio)

with a mean of 2.02 ± 0.05 for corals and 1.55 ± 0.09 for the mixed community, *p*-value for the difference in the ratios = 0.01. Additionally, the paired concentrations of alkalinity and calcium for each sample was plotted and the corresponding slope ($\Delta TA:\Delta Ca^{2+}$ ratio) shows very similar values: 2.01 ± 0.19 for corals and 1.61 ± 0.14 for the mixed community (Fig. 4). Since the rate of calcium uptake was the same for both runs, this offset (18%) indicates additional sources of alkalinity to the water column (i.e. buffering).

Table 1: Mean community uptake of alkalinity and calcium ions during incubation and the resulting $\Delta TA:\Delta Ca^{2+}$ ratio. Fluxes are reported in units of mmol m^{-2} d^{-1}. Ratios are mean ± SE.

	Ca^{2+} uptake	TA uptake	Ratio
Coral	-228 ± 64	-470 ± 126	2.06 ± 0.19
Mix	-171 ± 30	-274 ± 57	1.60 ± 0.14

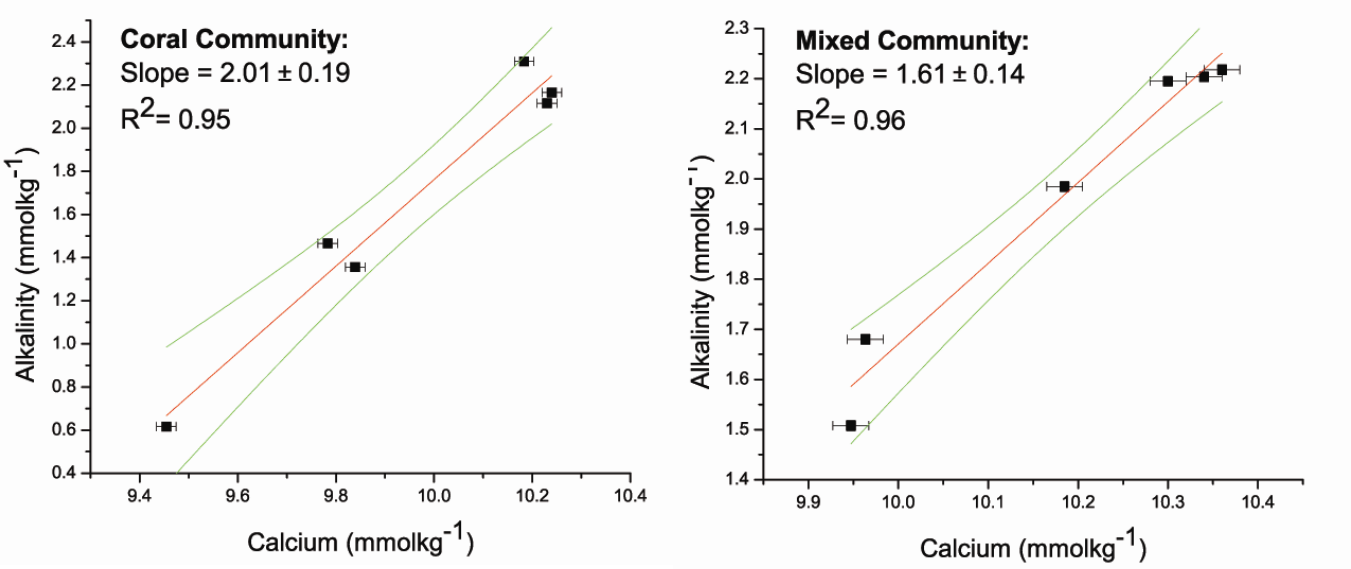

Fig. 4. ΔTA and ΔCa^{2+} fluxes for a coral-only (left) and a mixed community (right). The slope of the line corresponds to the $\Delta TA/\Delta Ca^{2+}$ ratio for each mesocosm. For corals, this is 2:1, $r^2 = 0.95$ for the mixed community the ratio is 1.61:1, $r^2 = 0.96$

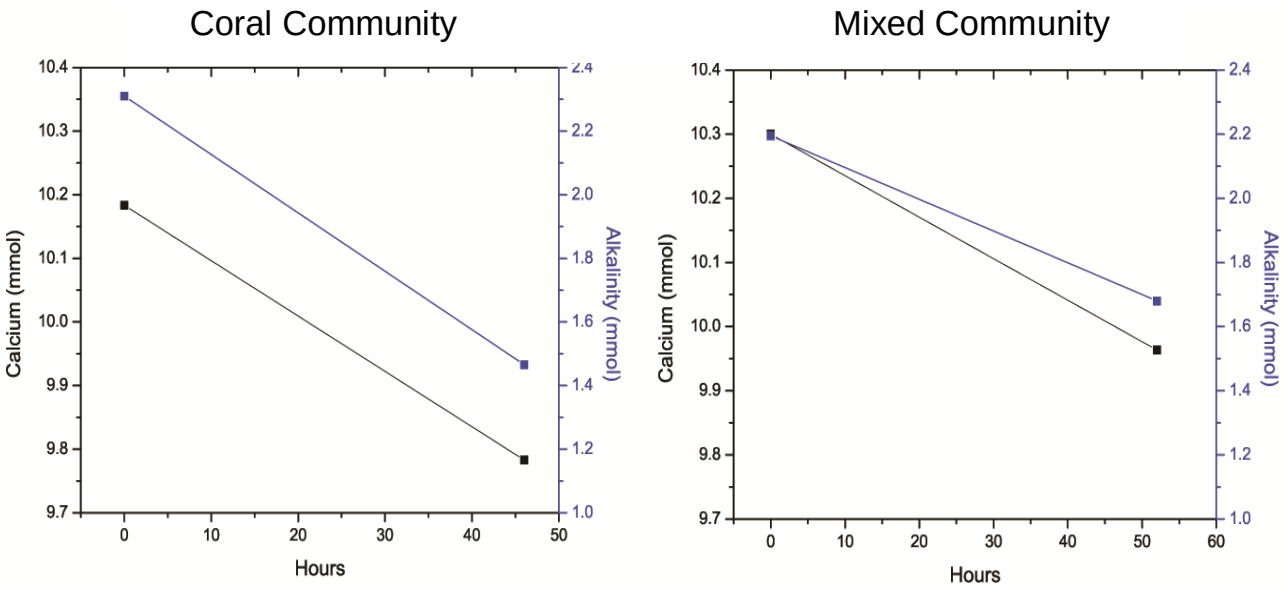

Fig. 5. Show ΔTA and ΔCa^{2+} uptake over the course of five days for a coral community (left) and a mixed community (right). The axes are scaled to 2:1, so the linear relationship at the beginning in the left figure demonstrates a 2:1 ΔTA:ΔCa^{2+} ratio whereas the mixed incubation has a 1.6:1 ratio.

5.2 Water chemistry conditions

pH in the flume was higher and more stable when sediment and algae were included with the corals. Mean pH was 7.52 ± 0.07 for isolated corals and 7.94 ± 0.03 when corals were combined with their community partners, *p*-value: 3 x 10^{-5} (Figure 5). Ω_{arag} was also consistently higher with sediment and algal addition; for isolated corals the mean Ω_{arag} was 1.12 ± 0.14 verses 2.51 ± 0.2 in the mixed community incubation, *p*-value = 2 x 10^{-6} (Fig. 5). Ω_{arag} never fell below 1 when the corals were in the mixed community with the sediment and filamentous algae components. However, Ω_{arag} dropped below 1 for 30% of the samples when the corals were incubated alone. Both incubations had the same corals, so these changes are not due to alterations in coral biomass or community composition. Calcium levels were higher with sediment addition: 9.75 ± 0.068 for corals alone and 10.05 ± 0.070 in the mixed community, *p*-value = 0.0054. Average CO_2 concentration was twice as high in the coral-only experiments (42 ± 6.15 µmol kg^{-1} compared to 20.5 ± 7.3 µmol kg^{-1} in the mixed community, *p*-value = 0.03) despite the fact that both flume runs were aerated at the same rate. Results are summarized in Table 5.2. Most of these correlations are linked to the fact that pH was higher and more consistent in the coral + sediment + algae flume experiment. As pH shifts, the carbonate speciation changes, which in turn affects Ω_{arag}.

Table 2: Summary of pH, Ω and carbonate ion speciation for the different communities. P-values indicate significance of difference between the coral and mixed incubations. Units reported in µmol m^{-2} d^{-1} with mean ± SE

Mesocosm	pH	Ω_{Ar}	HCO_3	CO_3	CO_2	Temp °C	N
Coral	7.5 ± 0.07	1.12 ± 0.14	1651 ± 59	72 ± 9	42 ± 6.2	23.8 ± 0.28	20
Mix	7.9 ± 0.035	2.51 ± 0.20	1622 ± 60	159 ± 12	20.5 ± 7.3	23.8 ± 0.34	14
***p*-value**	3 x 10^{-5} **	2 x 10^{-6} **	0.74	6 x 10^{-6}**	0.03 *	0.97	
Sediment	8.0 ± 0.02	2.73 ± 0.144	1609 ± 31	172 ± 9.8	11.4 ± 0.78	24 ± 0.56	7
Algae	8.56 ± 0.13	5.89 ± 0.68	1028 ± 181	370 ± 41	3.39 ± 1.8	23.5 ± 0.39	6
Control	8.05 ± 0.025	3.4 ± 0.43	1707 ± 75	215 ± 28	10.45 ± 0.46	24.5 ± 0.54	4

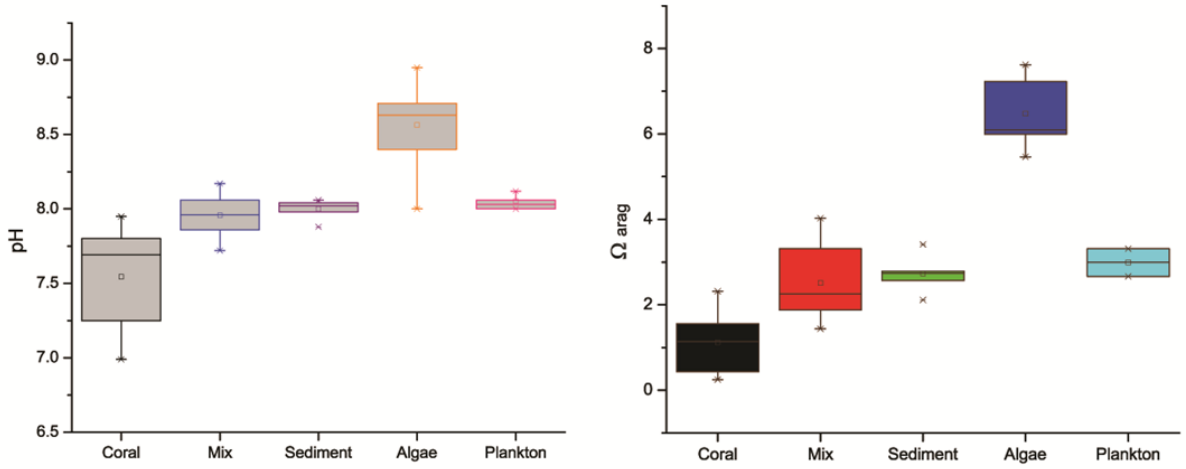

Fig. 6. Range of daytime pH values for all five incubations (left) and the aragonite saturation states, Ω_{arag} (right). Note that the coral-only incubations had lower and more variable pH which led to depressed Ω_{arag}. Water chemistry during the sediment run was more stable indicating sediment in the mixed community elevated and buffered pH for the corals. The macro algae *Gracillaria salicornis* created a high pH environment that favors coral calcification according to the proton flux hypothesis (Jokiel 2011).

5.3 Diel Cycle

Daytime calcium uptake over the week showed a similar pattern in both coral and mixed incubations. However, during a diel cycle, corals dissolved more when isolated than when in a community setting (Fig. 7). Observed diel cycle changes in carbonate ion speciation and Ω_{arag} are shown in Fig. 8 with notable differences during night dissolution. The diel calcification rates reported in this section were calculated from calcium data alone. In the coral-only flume run, average night dissolution was +142 ± 39 μmol Ca^{2+} m^{-2} hr^{-1}, and when sediment was included night dissolution was ten-fold lower at +9.57 ± 1.5 μmol Ca^{2+} m^{-2} hr^{-1} (a measured 50 μmol increase was averaged over 4 hours) *p*-value = 0.053. During night dissolution, in both experiments, Ω_{arag} increased from the time of sunset to around 11 pm. A spike in calcification occurred between 11 pm and 1 am as both systems underwent midnight calcification, as seen in Andersson et al. 2009. Midnight calcification was higher for the coral-only tank (-78.8 μmol Ca^{2+} m^{-2} hr^{-1}) than the mixed community (-44.3 μmol Ca^{2+} m^{-2} hr^{-1}). From 7 pm to 7 am the net change in calcium concentrations were +164 μmol Ca^{2+} for corals (net dissolution) and -39 μmol Ca^{2+} (small net calcification) for the mixed community. The diel study results are summarized in Table 3. Both diel studies were conducted during the summer months, one month apart from each other at the same time in the lunar cycle (1-2 days after first quarter moon). Only one night time period was observed for each community.

Table 3: Summary of main differences in calcium ion uptake and release (calcification and dissolution) between coral and mixed night-time incubations.

Mesocosm community	Mean Dissolution μmol m^{-2} hr^{-1}	Calcification at midnight μmol m^{-2} hr^{-1}	1900 h to 0700 hours Net Δ Ca^{2+} μmol
Coral	142 ± 39	78.75	+164
Mixed	9.57 ± 1.5	44.3	-39
p-value	0.053		

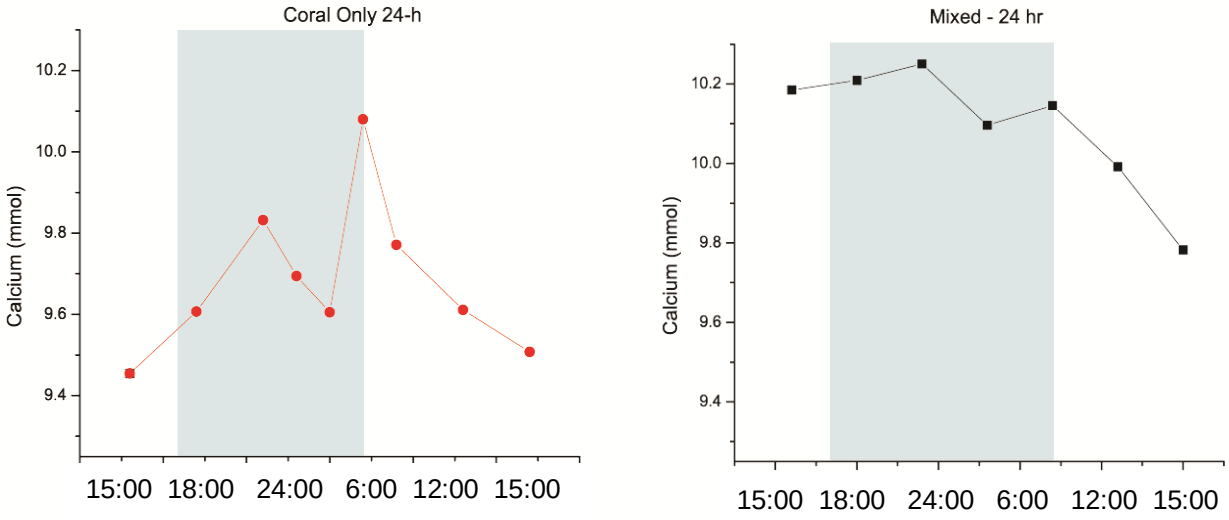

Fig. 7. Calcium uptake & release over 24h for coral (left) and mixed communities (right). Shaded region corresponds to night time, 24 hour time on x-axis; increases in calcium correspond to net dissolution.

5.4 Component comparison:

The sediment, unfiltered water from the HIMB water system and macro-algae were all run separately to measure the "background" alkalinity of the mixed community which was compared to the coral-only community. Two square meters of sediment and algae were collected in a 2.28 m^3 flume for separate runs. This was done in order to compare signal magnitude generated by the of the 2 m^2 of sediment, collected from the same reef flat, and 2 m^2 of filamentous algae growing on the sides of the tank, that were in the flume during the mixed community. Water velocity (10 cm s^{-1}) and average sample temperature (24 ^oC) were consistent with the coral and mixed incubations. Sediment was a source of alkalinity to the flume during the course of the experiment. The separate sediment-only run depicted an increase in alkalinity of 12.8 mmol TA m^{-2} d^{-1}, which matches rates found by Boucher et al. (1998). This magnitude of signal is only 5% the average total alkalinity signal from the mixed experiment, but there were equal map surface areas of coral and sediment. However, when taking into account that corals exhibit a 3-dimensional structure, the actual "surface area" of uptake was much higher on the corals than in the flat 2 m^2 of sediment. All together the sediment + algae + plankton signal strength was about 10-15% of the total community alkalinity signal.

The separate algae-only run used the macro-algae, *Gracillaria salicornis*, which is common in Kaneohe Bay. This species is a thick-branched macro-algae that has calcareous

epibionts and some crustose coralline algae on the branch bases. The algae showed both a release and uptake in alkalinity on the order of -34.8 to +41 mmol TA m^{-2} d^{-1} depending on weather conditions and source of nitrogen. The macro-algae in the control was a different species than the mixed filamentous algal community that developed on the tank walls during the mixed community incubation. A summary of alkalinity and calcium flux strength is presented in Fig. 8.

A seawater-control was performed to measure the background alkalinity signal of the plankton. This signal was small and averaged + 5.6 mmol TA $m^{-3}d^{-1}$, which is comparable to the magnitude of controls run by Small & Adey (2001). After excess alkalinity was added to the flume, the unfiltered seawater absorbed ten times more alkalinity at -40 mmol TA $m^{-3}d^{-1}$. This background absorption or possible outgassing of TA could be a major source of the increased ratios observed after periods of alkalinity addition in the other experiments. Average temperature was consistent for all experiments and was between 23.5 ^{o}C and 24.0 ^{o}C.

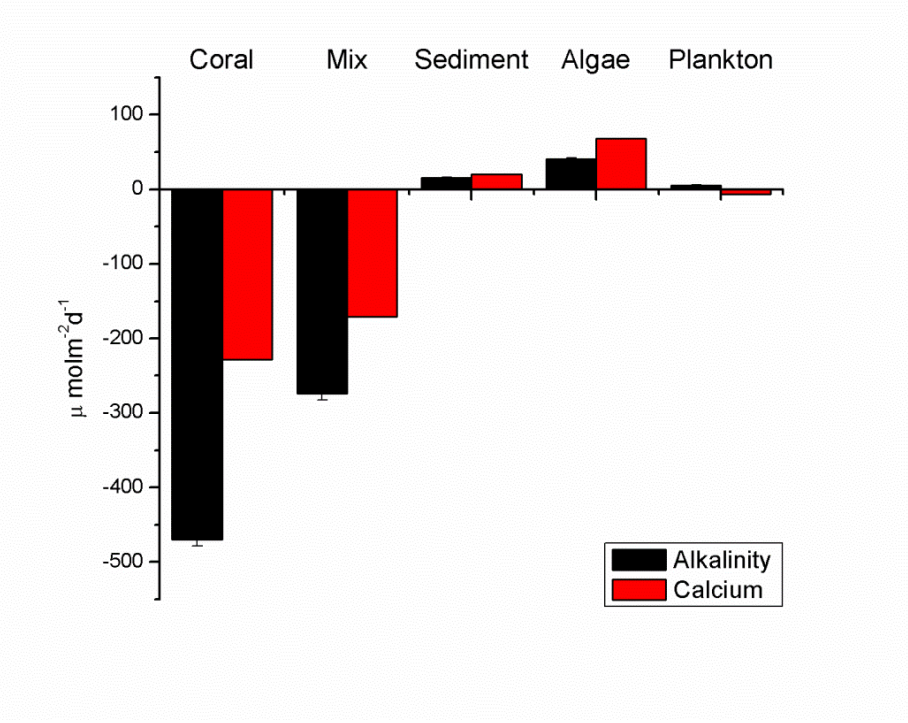

Fig. 8. Alkalinity and calcium fluxes for each incubation in μmol m^{-2} d^{-1}. Sediment and plankton increased alkalinity while algae and corals decrease alkalinity.

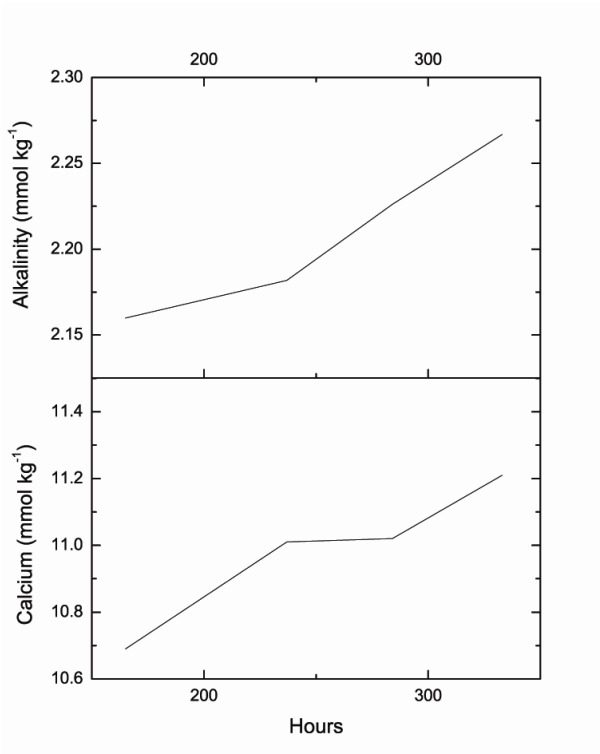

Fig. 9. Calcium and alkalinity increases from the sediment community, which increase average concentrations for these ions in the mixed community as shown in Table 4.

Table 4: Calcium and alkalinity mean concentrations ± standard error for each incubation. Sediment and algae incubations show the highest concentrations, indicating they may be a source of these ions for the mixed community – which is elevated over the isolated corals.

Mesocosm	**Ca^{2+} mmol kg^{-1}**	**TA µmol kg^{-1}**
Coral	9.75 ± 0.068	1765 ± 68
Mixed Community	10.05 ± 0.070	2015 ± 76
p-value	0.0054 *	0.023 *
Sediment	11.5 ± 0.21	2185 ± 17
Algae	10.67 ± 0.08	2126 ± 56
Control	10.4 ± 0.03	2153 ± 69

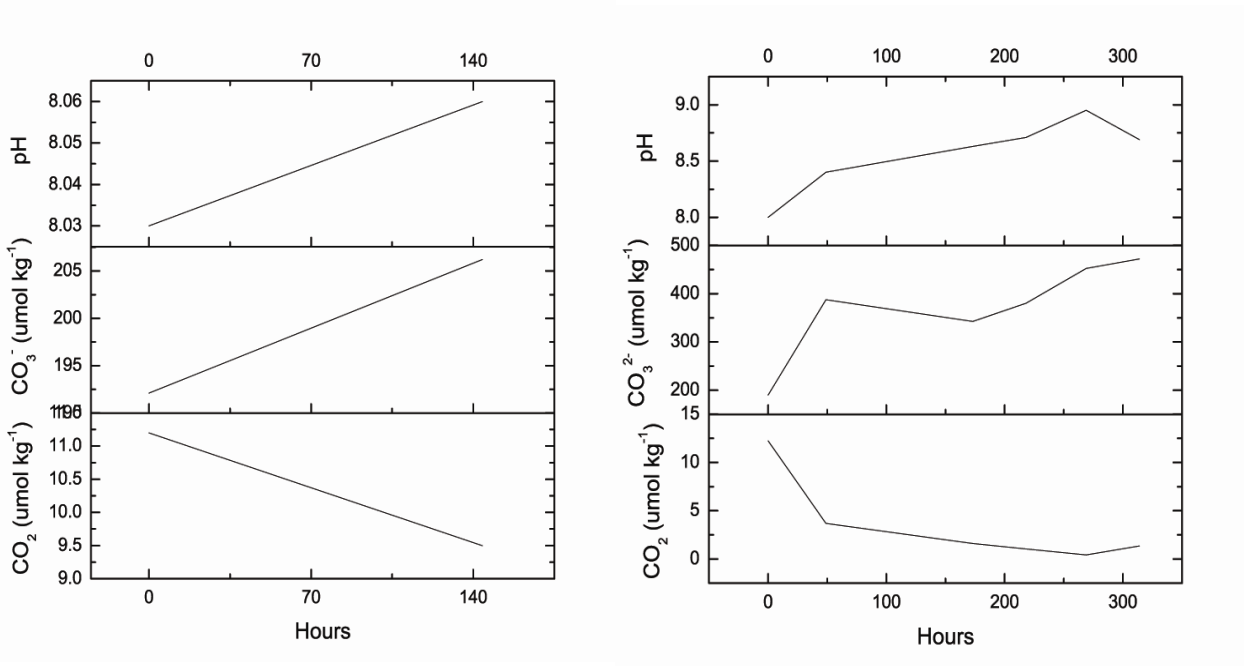

Fig. 10. Carbonate ion speciation and pH for the planktonic community (left) and the macro-algae community (right). Both convert CO_2 to CO_3^{2-} and raise pH.

6. Discussion:

6.1 $\Delta TA:\Delta Ca^{2+}$ flux ratios:

Since the resulting $\Delta TA:\Delta Ca^{2+}$ ratio for the coral community was very close to 2.0 (1.98-2.01), the alkalinity anomaly technique appears to be valid for the coral only experiments and environments dominated by coral cover. This is fortuitous because calcium fluxes can often be very difficult to measure due to the small changes in concentration unless there is sufficient residence times and coral biomass as in these experiments. However, since the $\Delta TA:\Delta Ca^{2+}$ ratio seems to be lower than 2:1 (1.55-1.7) when sediments and algae are combined with corals, extra precautions should be used when estimating reef calcification in the field or in mixed community laboratory experiments. In systems where corals make up the vast majority of the surface area with a strong hydrodynamic regime (e.g. exposed barrier reef) using the 2:1 $\Delta TA:\Delta Ca^{2+}$ ratio may still be adequate. However, if estimating reef calcification in lagoons, reef flats, or shallow coastal areas with high sedimentary or algal cover, it is advisable to use a lower ratio, track $[Ca^{2+}]$ directly (with high water residence time), employ Ca^{45} tagging, use buoyant weight changes for individual colonies, or use TA_{ec} (Wolf-Gladrow et al. 2007). Measuring changes in

nitrate in addition to alkalinity would result in more accurate estimations, except the magnitude of nitrate changes in most shallow water settings (unless they are polluted, strongly affected by upwelling, etc.) is typically low.

The lowered $\Delta TA:\Delta Ca^{2+}$ ratios observed in the mixed community (1.6 vs. 2.0 in treatment with corals alone) indicate that corals are calcifying 2:1 to create $CaCO_3$ but additional sources of alkalinity from the sediment, plankton, or algae. This additional source of TA dampens the decrease of TA resulting from calcification and creates a lower $\Delta TA:\Delta Ca^{2+}$. Estimated calcification rates in field studies where sedimentary or algal surface areas are dominant may be underestimating reef calcification when using a 2:1 ratio. This underestimation of coral growth was also seen when the technique was first discovered. When measuring coral calcification by $\Delta TA/2$, the result was a chronic underestimation of 50-75% of the growth measured by increases in dry skeletal weight (Smith & Kinsey 1978). In retrospect they reported that a probable explanation for this consistent underestimate by the alkalinity approach was that the corals were given only an hour or two of acclimatization time in the incubation chambers prior to alkalinity depletion measurements. The corals also only spent one day per month in those chambers and the rest of the time the corals lived free in the lagoonal environment. Results of the present experiments provide a possible alternate explanation for the chronic underestimation. The buffering services provided by sediments and algae are not present in the incubation jars and there would be diffusional restrictions due to boundary layer thickness in the incubation jars. Regardless of the mechanism, this offset in measurement represents an artifact from removing the organism from its natural environment.

There was a 15-20% difference between the isolated coral and the coral community $\Delta TA:\Delta Ca^{2+}$ ratios and the combined magnitudes of the plankton, sediment and algal alkalinity signals were between 10-15% of the average total alkalinity signal from the coral community. This further suggests that the shift in ratio is due to sources of alkalinity outside the corals. This 15% sediment/algae signal error increases from 15% when the map surface areas are equal to each other to 48% error when the sediment or algae occupies 75% of the map area on a reef. Other factors such as temperature, nutrient loading, plankton blooms, hydrodynamics and organic matter input to the sediments could further increase the non-coral alkalinity error. Additionally, this error could be higher at nighttime when corals are no longer calcifying as

strongly but anaerobic diagenesis in the sediments increases. During periods of nighttime net dissolution the average $\Delta TA:\Delta Ca^{2+}$ ratio was higher (>2) and more variable. The heightened ratio during dissolution may be partially due to the fact that some calcifying organisms consist of magnesium calcites which dissolve more readily (Kuffner et al. 2007, Andersson & Mackenzie 2012).

6.2 Water chemistry conditions:

The significantly higher, more stable pH and lower pCO_2 that was observed in the flume when algae and sediment was included with corals support the findings by Thomas et al. (2009) who found that sediments uptake DIC and release TA, synergistically increasing pH and decreasing pCO_2. Higher pH levels promote calcium carbonate precipitation (Alongi et al. 1996, Jokiel 2011). The stable, elevated levels of pH and low levels of CO_2 seen in the mixed community were also highly affected by the presence of the photosynthetic algae. The algae only run had the highest pH's and aragonite saturation states, demonstrating the importance of their input in the mixed community (Fig. 6). Generally, photosynthesis and calcification balance each other through internal and external pH regulation, which provides CO_3^{-2} ions for calcification and CO_2 (aq) for photosynthesis (Schneider & Erez 2006). This can explain why night dissolution was so much lower in the mixed community where pH remained higher due to the algal and sedimentary buffering processes. It has been found that some systems undergo net community dissolution while corals themselves are increasing in biomass (Andersson et al. 2009). This indicates that net dissolution of the system is not necessarily indicative to coral decline and that carbonate sediments and coral rubble may play an important role in buffering the ionic concentrations of the community. The average calcium and carbonate ion concentrations were higher when corals were combined with carbonate sediments, most likely from the dissolution of calcium carbonate at the sediment water interface. When sediments were incubated alone the sea water exhibited the highest average calcium and alkalinity concentrations. This source of calcium and carbonate ions to the water column could be important for maintaining a consistent high Ω_{arag} and sediments could dissolve in lieu of coral skeletons. Further investigations of how the sedimentary and algal components affect night dissolution would shed light not only on conditions in the field but on how coral reefs may respond to climate change.

6.3 Diel Cycle

The well observed diel cycle on coral reefs (Boucher et al. 1988, Gattuso et al. 1998, Atkinson & Cuet 2008, Clavier et al 2008, Shamberger et al. 2011) demonstrate the connectivity between photosynthesis and respiration with calcification and dissolution. During the day, community primary production consumes CO_2 faster than the respiring organisms produce it, resulting in an increased Ω_{arag} that promotes $CaCO_3$ precipitation (Gattuso et al. 1998, Langdon & Atkinson 2005). Thus, coral-external photosynthetic and respiratory processes affect the [pCO_2], pH, Ω_{arag}, and DIC of the ecosystem to either enhance calcification or the dissolution processes as seen by Anthony et al. (2011) and Small & Adey (2001) where addition of macro-algae enhanced coral calcification. Algal addition also increased pH and thus changed the speciation of carbonate ions, converting CO_2 to CO_3^{2-}. This is analogous to the increase in pH within coral cells caused by photosynthesis of zooxanthellae. Outside sources of photosynthesis can provide the same ecosystem service (Small & Adey 2001, Anthony et al. 2011).

It has long been assumed that reefs undergo net dissolution at night. In the field, night net "coral dissolution" could reflect higher rates of anoxic degradation in the sediments overpowering a decreased or nonexistent coral calcification. Sand dissolution can also occur at night during anoxic, low pH conditions, and the dissolution of the sands may protect the living corals from dissolution. In these experiments and others (Schnieder & Erez 2006) nighttime net calcification can occur at sufficiently high pH's fostered by additional outside-coral photosynthetic organisms or sedimentary input. This suggests that combining carbonate sediments with corals reduces night dissolution rates for the corals and can even result in overnight net calcification. This is perhaps due to the source of calcium and carbon ions from the sediments or through the buffering of pH. However, only one night study was conducted for each mesocosm and samples were only taken every 2 hours. Further diel cycle studies that compare isolated corals to corals with carbonate sediments and algae would be very beneficial

Furthermore, organic matter that is produced by bacteria, phytoplankton and algae during photosynthesis not only contributes to seawater alkalinity but represents a newly identified buffering component as well (Kim & Lee, 2009).Therefore the results of many laboratory ocean acidification experiments could be confounded by the fact that the corals are being observed in

filtered seawater with no sediment or algae present, essentially removing the buffering system that keeps the ecosystem stable and assists in the recycling of nutrients.

The affect algal photosynthesis has on alkalinity and pH in a reef system can be highly variable, depending on the species and environmental conditions. Different species of algae utilize different sources of nitrogen for photosynthesis (Larned et at. 1998, Small & Adey 2001, Mai et al. 2010) and may shift nutrient source depending on what is more readily available in the environment. During the mixed community incubation, the algae in the flume were filamentous algae that grew naturally along the sides of the tank. During the coral-only experiment, the algae were cleaned off the sides every other day. The algae-only run, using *Gracillaria salicornia*, depicted both a decrease in alkalinity of 34.8 mmol TA m^{-2} d^{-1} after heavy rains and an increase on the order of 41mmol TA m^{-2} d^{-1} during other days. When using nitrate for photosynthesis the result is to increase alkalinity while uptake of ammonia decreases alkalinity, and reef macro-algae have been shown to employ both systems (Mai et al. 2010). The large change in the magnitude of alkalinity uptake (-34.8 to +41 mmol TA m^{-2} d^{-1}) suggests that the algal alkalinity signal can vary greatly according to Ω_{arag}, irradiance strength and source of nitrogen used for photosynthesis. Additionally, *G. salicornia* can house calcareous epibionts that could have been absorbing alkalinity to form calcareous crusts.

6.4 Importance of micro-organism symbionts:

In addition to the benefits that sediments provide in maintaining consistent ion concentrations, they are also important habitats for micro-organisms. The mucus that corals shed after stressful events (such as being collected) release their microbial symbionts to the water column and nearby sediments (Gates & Ainsworth 2011). This loss of internal symbionts could cause negative health affects to the corals overall from decreased nutrient cycling or resistance to disease (Amend et al. 2011). When corals are isolated from the sediment component, where many microbes live, it could hamper their ability to re-acquire microbial symbionts from the environment. With the loss of these symbionts coupled with the loss of geochemical buffering from carbonate sands, additional sources of photosynthesis, external nutrient cycling and lessened heterotrophic feeding, it is no wonder that CO_2 addition experiments show such drastic results. Experiments on corals isolated from the reef community are crucial for understanding specific animal physiology, but Ω_{arag} may not fall as low with a certain input of pCO_2 if there

was a sedimentary buffering component or algal photosynthesis. While lower pH's due to increased CO_2 and increased temperature will be detrimental to some coral systems, the breadth of the effects will probably not be as dire as buffer-less, short term, drastic rate increase CO_2 experiments make them seem. Projections of ocean acidification are often based on air-sea fluxes in the open ocean and are not indicative for shallow systems such as coral reefs (Anthony et al. 2011). These assumptions are dangerous, because if the common belief is that coral reefs will die out no matter what we do, they could be considered a "lost cause" and conservation efforts and land use development will not cater to improving reef health.

6.5 Limitations of extrapolating models:

Saturation state may be descriptive of calcification rates on a single reef or in a single system, but they cannot always be used to extrapolate results to a different system. Shamberger et al. (2011) found that predictions of calcification rates by using a predictive Ω_{arag} model developed by Silverman et al. (2009) calculated a calcification rate for Kaneohe bay that was only 10% of the measured in-situ rates. This demonstrates the limitations of using laboratory experimental models to extrapolate to conditions in the field, especially for processes that will unfold over decades to hundreds of years. It is also interesting to note that despite Ω_{arag} dropping below 1 quite often for the coral-only community in the experiments described in this paper, the system continued to uptake Ca^{2+} and calcify. The calculation of Ω_{arag} can be done according to different methods. The software CO2SYS is commonly used for calculating the carbonate speciation of reef and lab experiments where Ω_{arag} is calculated by $[Ca^{2+}][CO_3^{2-}]/K_{sp.}$ However, there is no input in this program for $[Ca^{2+}]$ and changes are assumed from salinity. While this assumption may be true for open ocean calcium concentrations, which are nearly conservative, shallow systems with high coral biomass and high residence time or static lab incubations can have variable $[Ca^{2+}]$ due to strong calcification signals. This combined with the fact that corals can uptake CO_3^{2-} or HCO_3^- have led researchers to find other expressions for Ω_{arag} that hold more consistently. The proton flux hypothesis presented by Jokiel (2011) describes $\Omega_{arag} = k[DIC]/[H^+]$ which accounts for the concentrations of multiple carbonate species depending on the $[H^+]$, which is directly related to pH.

The Ω_{arag} relationship to calcification rate does not always hold between ecosystems and ocean acidification will not affect reefs uniformly (Shamberger et al. 2011). This nonconformity

in calcification response was also demonstrated by Edmunds et al. (2012) where the response and resistance to changing pH, [HCO_3^-] and [CO_3^{2-}] varied among species. This is not surprising because corals are complex, multi-specie symbiomes. Inside these organisms many complex metabolisms and microbial interactions compose the community characteristics and response to environmental conditions. Due to this complexity both within the corals and in the coral community, it is difficult to isolate the effects from CO_2 chemistry from other parameters affecting calcification such as light, temperature, nutrients and heterotrophic coral feeding (Atkinson & Cuet, 2008). Furthermore, other factors such as expanding island population sizes result in direct contact damage, foreign chemical introduction, overfishing herbivorous fish, and high nutrient and sedimentary runoff affect reefs. The runoff problem is exacerbated from decreased watershed retention from installation of concrete lined canals in lieu of natural, slowly flowing streams. Many of these confounding influences make corals more susceptible to diseases as well. During times of increased stress it is possible that coral symbionts phase shift from being mutalists to competing for resources within the coral (Gates & Ainsworth 2011).

In summary, sediments buffer the water and provide nutrient and ionic sources of alkalinity and calcium, along with being a habitat for microbial life that may move into the coral symbiome. Macro-algae raise Ω_{arag} by removing CO_2 and increasing pH, fostering coral growth. Further studies would benefit from experimenting on whole coral reef communities to examine the community level effects of increased pCO^2 and temperature as well as other parameters. The balance and interrelations between organisms on a coral reef is the reason it can support such a diverse proliferation of life. Symbiosis has continuously led to the evolution of organisms and communities and has been the backbone for increasing complexity on our planet. If symbiosis had never occurred we would still all be single cells. In the spirit of these theories, I think it is wise to continue the recent attention in coral endosymbionts other than zooxanthellae. The understanding of the additional microbial metabolisms occurring within coral tissue could shed light on coral stress management and adaptation to change as these microbes have very fast generation times and could provide the corals with enhanced adaptability. Coral reefs have survived on the planet for 250 million years and while they went through species composition shifts during the mass extinction event between the Cretaceous and Palaeocene they reradiated back out to proliferate again. Corals evolved and live in conjunction with ecosystem partners who complete parts of the biogeochemical cycles they operate within. While increased CO_2 and

temperature will negatively affect corals, the natural mixed community offers negative feedback biogeochemical loops that can help mitigate some of these stressors on a local reef to reef scale.

7. Conclusions:

- Corals calcify at a 2:1 ratio of alkalinity in respect to calcium ($\Delta TA:\Delta Ca^{2+} = 2.0$).
- Coral communities (consisting of corals, sediment, algae, and plankton) calcify at a lower ratio ($\Delta TA:\Delta Ca^{2+} = 1.6$) which demonstrates the presence of additional sources of alkalinity (i.e. buffering) from non-coral components of the coral community.
- Carbonate sediments buffer the water column, maintaining higher and more stable levels of pH and Ω_{arag}. Furthermore, sediments act as a source of calcium and carbonate ions, so presence of carbonate sands and sediments can be viewed as factors beneficial to coral growth.
- Daytime photosynthesis by macro-algae, turf algae and corals lowers the seawater pCO_2 and raises the pH – providing an environment more favorable to coral calcification.
- The combined additional alkalinity produced by sediments and algae is 10-15% of that consumed by the corals and on some reefs the immense map area occupied by sediments and algae could contribute a significant source of alkalinity to the water column.
- Ignoring the various sources of alkalinity to the system can lead to an underestimation of reef growth in the field if the traditional alkalinity anomaly technique is used alone.
- Experiments extrapolated to predictive models of future CO_2 conditions would benefit from including carbonate sediments and or algae in the experimental design.
- Experiments involving isolated coral incubations are also crucial for understanding the chemical physiology of the organisms (temperature and Ω_{arag} thresholds), but precautions should be taken in extrapolating organism-based response to whole-ecosystem response or directly comparing one ecosystem to another based on a single number (i.e. Ω_{arag}).

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