Internal carbonic anhydrase activity in the tissue of scleractinian corals is sufficient to support proposed roles in photosynthesis and calcification

Brian M. Hopkinson¹, Anna L. Tansik¹, William K. Fitt²
¹Department of Marine Sciences, University of Georgia, Athens, GA
²School of Ecology, University of Georgia, Athens, GA

Corresponding Author:
Brian M. Hopkinson
Department of Marine Sciences
University of Georgia
Athens, GA, 30602
bmhopkin@uga.edu
706-542-7880

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ABSTRACT

Reef-building corals import inorganic carbon (C\textsubscript{i}) to build their calcium carbonate skeletons and to support photosynthesis by the symbiotic algae that reside in their tissue. The internal pathways that deliver C\textsubscript{i} for both photosynthesis and calcification are known to involve the enzyme carbonic anhydrase (CA), which interconverts CO\textsubscript{2} and HCO\textsubscript{3}-\textsuperscript{−}. We have developed a method for absolute quantification of internal CA (iCA) activity in coral tissue based on the rate of \textsuperscript{18}O-removal from labeled C\textsubscript{i}. The method was applied to three Caribbean corals (\textit{Orbicella faveolata}, \textit{Porites astreoides}, and \textit{Siderastrea radians}) and showed that these species have similar iCA activities per unit surface area, but that \textit{S. radians} had ~10-fold higher iCA activity per unit tissue volume. A model of coral C\textsubscript{i} processing shows that the measured iCA activity is sufficient to support the proposed roles for iCA in C\textsubscript{i} transport for photosynthesis and calcification. This is the case even when iCA activity is homogeneously distributed throughout the coral, but the model indicates that it would be advantageous to concentrate iCA in the spaces where calcification (the calcifying fluid) and photosynthesis (the oral endoderm) take place. We argue that because the rates of photosynthesis and calcification per unit surface area are similar among the corals studied here, the areal iCA activity used to deliver C\textsubscript{i} for these reactions should also be similar. The elevated iCA activity per unit volume of \textit{S. radians} compared to the other species is probably due to the thinner effective tissue thickness in this species.
INTRODUCTION

Active inorganic carbon (C\textsubscript{i}) supply pathways are required to support the high rates of photosynthesis and calcification that have allowed reef-building corals to thrive and create the extensive reefs structures that support coral reef ecosystems. These pathways must deliver C\textsubscript{i} to the symbiotic dinoflagellates (of the genus \textit{Symbiodinium}) that reside primarily within the upper endodermal layer of the coral (Furla et al., 2000b; Bertucci et al., 2013). The algae further concentrate C\textsubscript{i} obtained from the coral using a CO\textsubscript{2} concentrating mechanism and ultimately convert the C\textsubscript{i} to CO\textsubscript{2} for fixation in the Calvin cycle (Leggat et al., 1999). At the same time, C\textsubscript{i} is routed to the calcifying space at the interface between the existing CaCO\textsubscript{3} skeleton and the ectodermal cells of the coral animal where the C\textsubscript{i} is converted to CO\textsubscript{3}\textsuperscript{2-} and combined with Ca\textsuperscript{2+} to form new skeleton (Tambutte et al., 2011). Much of the carbon fixed by the symbiotic algae is transported to and then respired by the coral. The coral recycles most of the respired CO\textsubscript{2}, using it for both photosynthesis and calcification (Furla et al., 2000b). The full details of these C\textsubscript{i} supply and recovery pathways are not fully understood, but it is clear that they are complicated and interconnected (Fig 1; Tambutte et al., 2011; Bertucci et al., 2013).

A critical component of nearly all C\textsubscript{i} supply pathways is the enzyme carbonic anhydrase (CA). This enzyme catalyzes the hydration of CO\textsubscript{2} and dehydration of HCO\textsubscript{3}\textsuperscript{-} moving the CO\textsubscript{2}/HCO\textsubscript{3}\textsuperscript{-} system towards chemical equilibrium. CAs play diverse roles in organisms and are involved in CO\textsubscript{2} uptake, gas exchange, pH homeostasis, and internal C\textsubscript{i} transport pathways (Henry, 1996; Badger, 2003; Boron, 2004). In corals and related cnidarians, CAs are involved in supplying C\textsubscript{i} for both photosynthesis and calcification. A surface-associated extracellular CA is involved in CO\textsubscript{2} uptake (Furla et al., 2000a; Tansik et al. in press), and internal CAs are likely used to convert newly imported CO\textsubscript{2} to HCO\textsubscript{3}\textsuperscript{-}, and for recovery of respired CO\textsubscript{2} as HCO\textsubscript{3}\textsuperscript{-} (Fig. 1; Bertucci et al., 2013). These transformations serve to concentrate C\textsubscript{i} as HCO\textsubscript{3}\textsuperscript{-}, which is the form of C\textsubscript{i} typically concentrated by organisms since as a charged molecule it does not pass easily through membranes. In contrast, biological membranes are highly permeability to CO\textsubscript{2}, a small, uncharged molecule (Gutknecht et al., 1977). Accumulated HCO\textsubscript{3}\textsuperscript{-} can then be transported to the symbiotic algae, possibly via SLC4 type bicarbonate transporters, which have recently been identified in the genomes and transcriptomes of several coral species (Moya et al., 2012; Kenkel et al., 2013), or converted to CO\textsubscript{2} in the oral endoderm for uptake by \textit{Symbiodinium} (Al Moghrabi et al., 1996; Bertucci et al., 2010). Inhibition of CAs reduces the photosynthetic rates of corals, showing that this...
enzyme is essential for maintaining high rates of photosynthesis (Weis et al., 1989; Al-Moghrabi et al., 1996).

CAs are also involved in calcification (Goreau, 1959), probably as part of the C₄ transport system in coral tissue and within the calcifying fluid to enhance calcification. C₄ used for calcification is a mix of respired CO₂, HCO₃⁻ transported through coral tissue, and C₄ obtained from direct seawater import to the calcifying fluid (Furla et al., 2000b; Cohen and McConnaughey, 2003; Gagnon et al., 2012). The C₄ transport pathways for calcification are thus broadly similar to those used to transport C₄ for photosynthesis, and so the likely roles of CA in these transport steps are analogous. In several corals (*Stylophora pistillata*, *Acropora hebes*, and *Tubastrea aurea*), CA has been localized directly to the calicoblastic ectoderm, the cell layer that mediates calcification and is in direct contact with the calcifying fluid, or the skeletal matrix and it has been suggested that CA may be secreted into the calcifying fluid (Isa and Yamazato, 1984; Tambutte et al., 2007; Moya et al., 2008). CA in the calcifying fluid would convert respired CO₂ to HCO₃⁻ for subsequent conversion to CO₃²⁻ (Fig. 1; Cohen and McConnaughey, 2003).

CAs are clearly important in corals and there has been substantial progress in identifying, localizing, and in some case characterizing individual CAs. Weis et al. (1989) found that corals contain substantial amounts of CA activity and that zooxanthellate species had significantly more CA than species lacking symbiotic algae. While this work showed that corals have high levels of CA activity, the approach used was not quantitative in an absolute sense, that would, for example, allow the measured rates to be incorporated into models of photosynthesis and calcification (e.g. Hohn and Merico, 2012; Nakamura et al., 2013). Here, we adapt a method that relies on the rate of $^{18}$O-removal from labeled C₄ to obtain quantitative measurements of internal CA (iCA) activity in corals. Since the $^{18}$O-removal measurement is made on coral homogenate the method provides a measure of the average iCA activity throughout the corals tissue layers including the calcifying fluid. It is highly unlikely that CA is in fact distributed homogenously throughout the tissue, and the need for CA localization is explored using a spatially-resolved model of C₄ processing.

**RESULTS**

**Coral iCA activities**

Coral iCA activity was determined by quantitative analysis of $^{18}$O-removal catalyzed by purified coral tissue homogenate using a model that includes coral iCA and eCA activity, and *Symbiodinium* iCA activity (see Materials and Methods). The method relies on the fact
that all CA isoforms accelerate the hydration of CO₂ and dehydration of HCO₃⁻, which in turn accelerates the exchange of the $^{18}$O label in C₄ for $^{16}$O from water (Silverman, 1982). In this analysis, CA activity in the purified tissue homogenate is partitioned into various experimentally separable fractions. eCA activity is first measured on intact coral fragments (Tansik et al. in press), and then the coral tissue is removed and homogenized (Fig. 2A). Symbiodinium cells are removed from the homogenate by centrifugation and their CA activity is measured. Finally, the purified tissue homogenate is assayed for CA activity, and the iCA activity of the coral is determined by subtracting the contributions of coral eCA and residual Symbiodinium iCA from the total CA activity in the purified homogenate.

Determination of coral iCA activity using this approach first requires measurement of coral eCA activity and the algal iCA activity and mass transfer coefficients. The coral eCA activity was measured by an $^{18}$O-removal assay on intact coral fragments. These data were presented in a companion paper (Tansik et al. in press) and the eCA activities are listed in Table 1. The eCA activity varied substantially between taxa with *O. faveolata* having the highest eCA activities, approximately one order of magnitude greater than that of *S. radians* and *P. astreoides*. Algal iCA activity and mass transfer coefficients were measured using an $^{18}$O-removal assay on cells freshly isolated from coral tissue. Algal iCA activity also differed among taxa, although activities in all taxa were very high compared with other microalgae (Tu et al., 1986; Hopkinson et al., 2013). *Symbiodinium* from *S. radians* had the highest iCA activity, followed by those from *P. astreoides*, and then *Symbiodinium* from *O. faveolata*. CO₂ and HCO₃⁻ mass transfer coefficients were similar among the taxa, and showed that the cell membranes are highly permeable to CO₂ but impermeable to HCO₃⁻ like other microalgae (Table 1).

After obtaining coral eCA and algal parameters, coral iCA activity was determined from analysis of the effect of purified coral tissue homogenate on $^{18}$O-removal rates. The coral homogenate substantially accelerates the rate of $^{18}$O-removal (Fig. 2, Table 2). Addition of the CA inhibitor acetazolamide (AZ) slowed $^{18}$O-removal rates to background rates (or even slightly below background rates) verifying that CA activity was responsible for the acceleration (Table 2). Although the potency of AZ differs with CA isoform, the 100 μM AZ added should be sufficient to fully inhibit most AZ isoforms since typical inhibitory constants are <50 nM (Moya et al. 2008). The fact that $^{18}$O-removal rates returned to background levels after addition of AZ confirms that CA activity was effectively inhibited for these corals. Analysis of this data using the $^{18}$O-removal model presented in equations 1-4 (see Materials and Methods) shows that the model fits the data well for all taxa allowing accurate
determination of coral iCA activity. The coral iCA activity can either be normalized by the coral tissue volume to obtain a first order rate constant, or normalized by coral surface area. The advantage of the first order rate constant is that it is directly relevant to internal C\textsubscript{i} fluxes, can be applied to models of C\textsubscript{i} processing in corals, and is intuitive. However, normalization by volume treats the iCA as being homogeneously distributed throughout the internal volume of the coral, which is not likely to be the case. When normalized to coral tissue volume, S. radians has approximately 10-fold higher iCA activity than both O. faveolata and P. astreoides (t-test, p<0.05), but the iCA activities of O. faveolata and P. astreoides are not significantly different from one another (Fig. 3).

Alternatively, the coral iCA activity normalized to surface area rather than volume. When normalized to coral surface area, O. faveolata and S. radians have similar iCA activity, while P. astreoides has approximately half the iCA activity of the other two species. However, only the difference between S. radians and P. astreoides is statistically significant (t-test, p <0.05). The overall range in coral iCA activity between taxa is lower when normalized to surface area (2.8x) than when normalized by volume (9x), and it is notable that photosynthetic rates per unit area are very similar among the coral species (Fig. 3).

**Contribution of coral eCA and algal iCA to \textsuperscript{18}O-removal**

Obtaining the coral iCA activity requires accounting for the effects of coral eCA and algal iCA on \textsuperscript{18}O-removal catalyzed by the purified tissue homogenate (see Materials and Methods; Supplemental Figure 1). The greater the contribution of these additional components to \textsuperscript{18}O-removal, the more difficult it is to accurately determine coral iCA activity. We assessed the effects of these additional components in two ways: first, graphically, showing how the \textsuperscript{18}O-removal model predicts that \textsuperscript{18}O-CO\textsubscript{2} isotopologues would evolve as components are added, and second by determining how neglecting the additional components in the model would affect inferred coral iCA activity.

We ran the \textsuperscript{18}O-removal model in a forward (predictive) sense first considering how the \textsuperscript{18}O-CO\textsubscript{2} isotopologues would evolve in the absence of any CA activity, and then sequentially adding the effects of algal iCA, coral eCA, and coral iCA. Figure 4 shows representative results for each taxon, but similar results were obtained for each sample within a taxon. The effect of residual *Symbiodinium* on \textsuperscript{18}O-removal is quite small in all taxa. Centrifugation removes more than 98% of the *Symbiodinium* cells from the crude homogenate, which is sufficient to effectively eliminate any interference for determination of coral iCA activity. In general the effects of coral eCA are also small, though in O. faveolata,
which has the highest eCA activity, coral eCA can have a substantial effect on $^{18}$O-removal. This analysis indicates that accounting for the effect of coral eCA activity is important, but that residual *Symbiodinium* contribute little to $^{18}$O-removal as long as the centrifugation is effective.

To make a more quantitative assessment, we examined the effects of neglecting coral eCA and algal iCA on the inferred coral iCA activity. When these components are neglected in the model, their contribution to $^{18}$O-removal will be attributed to coral iCA activity, the unknown model parameter, increasing the inferred coral iCA activity. As shown in Figure 5, neglecting algal iCA results in only minor increases in the inferred coral iCA activity (<7%), consistent with the graphical analysis showing that residual algal cells have negligible effects on $^{18}$O-removal. For *P. astreoides* and *S. radians*, neglecting coral eCA activity led to at most moderate increases in inferred coral iCA activity (<15%), but for *O. faveolata* the effects were much more significant especially for one specimen that had low iCA activity.

**C$_i$ processing model analysis**

A one-dimensional model of coral C$_i$ processing that includes active HCO$_3^-$ transport, passive CO$_2$ transport, CA-catalyzed interconversion of CO$_2$ and HCO$_3^-$, photosynthesis, and calcification was used to help interpret the measured iCA activities. C$_i$ processing pathways in corals are still under study and are in some cases controversial. For example, electron microscopy of the tissue-skeleton interface suggests that there is little to no calcifying fluid (Clode and Marshall, 2002), but more sizable reservoirs of calcifying fluid have been detected as high pH regions in confocal microscopic imaging (Venn et al. 2011). The C$_i$ pathways in the model were chosen from among proposed routes in a way that maximizes iCA requirements (using iCA in all the reactions shown in Fig. 1). The model is schematic and is used here only to assess the suitability of measured iCA activity to perform proposed functions and to explore iCA requirements. It is not a general mechanistic model of coral C$_i$ processing. Most notably the metabolic fluxes (photosynthesis, respiration, calcification, HCO$_3^-$ transport) are specified based on measured rates or to achieve mass balance, rather than being determined mechanistically using enzyme kinetics.

First, the model was run with an iCA activity of 50 s$^{-1}$, approximately what was measured in *O. faveolata* and *P. astreoides*, distributed homogenously throughout the coral tissue layers and calcifying fluid (Fig. 6). This iCA activity was able to support rates of photosynthesis, calcification, and respiration measured in *O. faveolata*, but in the case of calcification the iCA activity was only just sufficient. In the oral ectoderm, iCA catalyzes the
conversion of newly imported and respired CO$_2$ to HCO$_3^-$.

Although CO$_2$ concentrations in this layer are lower than in seawater, driving the CO$_2$ influx, they are in fact above equilibrium concentrations with HCO$_3^-$ because HCO$_3^-$ concentrations are low as a result of active export to the underlying oral endoderm. The active transport of HCO$_3^-$ across membranes would be mediated by transporters such as the SLC4 type HCO$_3^-$ transporters recently identified in corals (Moya et al., 2012; Kenkel et al., 2013). In the oral endoderm, HCO$_3^-$ is imported and converted to CO$_2$ by iCA. The CO$_2$ is then consumed by the *Symbiodinium* that reside in the oral endoderm layer, lowering CO$_2$ concentrations below equilibrium values. CO$_2$ and HCO$_3^-$ are in equilibrium in the coelenteron where no metabolic processes occur. In the aboral tissue layer, respiration produces CO$_2$, part of which is converted to HCO$_3^-$ by CA and the remainder of which diffuses into the calcifying fluid where it then converted to HCO$_3^-$ by CA for use in calcification.

The iCA activity in the calcifying fluid was only just sufficient to support the measured rates of calcification for *O. faveolata*. This can be most clearly seen from the low HCO$_3^-$ concentration in the calcifying fluid, indicating high rates of removal relative to input, and in the nearly 100% deviation of CO$_2$ concentrations from equilibrium with HCO$_3^-$ (Fig. 6C). Greater deviations from equilibrium (either positive or negative) indicate that maximal CO$_2$ hydration or HCO$_3^-$ dehydration fluxes are being reached since the maximal net fluxes occur when the product concentration (HCO$_3^-$ for hydration, CO$_2$ for dehydration) is zero. Consequently, the deviation of CO$_2$ from equilibrium with HCO$_3^-$ is used as an index of the effectiveness of the C$_i$ processing system, with moderate deviation (~25%) being acceptable, but further deviation indicating the system is near its maximal capacity. We note that in our simplistic model, meant only to explore the role of iCA, there is no dependence of the calcification rate on HCO$_3^-$ or CO$_3^{2-}$ concentrations. The low HCO$_3^{2-}$ and CO$_3^{2-}$ concentrations in this model would probably not provide a high enough aragonite saturation state to obtain the observed calcification rates (e.g. Burton and Walter, 1987).

Running the model with higher (500 s$^{-1}$) and lower (5 s$^{-1}$) iCA activities shows that the observed iCA activity is nearly optimal for supporting the required C$_i$ fluxes and keeping CO$_2$ and HCO$_3^-$ concentrations close to equilibrium (Fig. 6). Decreasing iCA 10-fold leads to significant deviations of CO$_2$ from equilibrium with HCO$_3^-$ and the model is only able to support calcification at 40% of the observed rate. This results in lower HCO$_3^-$ concentration in the calcifying fluid compared to that in models with higher iCA activity. Increasing iCA 10-fold does lead to nearly complete equilibrium between CO$_2$ and HCO$_3^-$ throughout the coral, but there are no performance gains directly associated with this increase.
The C\textsubscript{i} processing model was used to explore the effect of changes in photosynthetic rate and tissue thickness on required iCA activity. The model with a homogeneous iCA activity similar to that measured in the reef corals (50 s\textsuperscript{-1}) and metabolic rates of *O. faveolata* was used as a reference point. First the net photosynthetic rate was varied (with the respiration rate being set equal to the net photosynthetic rate) and the iCA activity was optimized to obtain the same average CO\textsubscript{2} deviations in each tissue layer as in the base model. The average CO\textsubscript{2} deviations are used here as a measure of effectiveness of CA at supplying C\textsubscript{i} for transport or metabolism. The required iCA activity scaled approximately linearly with the photosynthetic rate (Fig. 7). Next, the tissue thickness was varied from the reference point (2 mm), keeping the metabolic rates per unit surface area constant, and the iCA activity was optimized to match CO\textsubscript{2} deviations to the base model. The required iCA activity per unit volume increased rapidly as the tissue thickness decreases and conversely decreased as the tissue thickness increased (Fig. 7). However, the iCA activity per unit surface area is nearly constant, increasing slightly with increasing tissue thickness.

**DISCUSSION**

Carbonic anhydrase is a widely distributed enzyme having many isoforms and diverse roles in organisms. It has long been recognized to be important in marine organisms, some of the earliest work being in mollusks (Freeman and Wilbur, 1948; Wilbur and Jodrey, 1955), and more recently its roles in corals and other cnidarians have been investigated (Bertuccci et al. 2013). We have developed a method to determine the iCA activity of corals by measuring the acceleration of 18O-removal from labeled C\textsubscript{i} catalyzed by purified coral tissue homogenate (Fig. 2; Table 2). iCA activity was measured in three species of Caribbean corals. We found that the two species that inhabit offshore reefs (*O. faveolata, P. astreoides*) had similar iCA activity when normalized to tissue volume, whereas a species from Florida Bay (*S. radians*) had ~10-fold higher iCA activity (Fig. 3). In contrast when normalized to coral surface area, *O. faveolata* and *S. radians* had similar iCA activities, while the iCA activity of *P. astreoides* was lower. Determination of coral iCA activity required removing contributions from residual *Symbiodinum* cells and coral eCA from the total CA activity of the purified coral tissue homogenate. Residual *Symbiodinum* cell numbers were always low and did not significantly affect determination of coral iCA activity (Figs 4, 5). In *P. astreoides* and *S. radians*, eCA activity was also not a major issue, but in *O. faveolata*, which had very high eCA activities, accounting for eCA activity was essential for accurate determination of coral iCA activity.
The coral iCA activity measurement includes all CAs in the cytoplasm, on internal membranes, in the coelenteron, and in the calcifying fluid. Membrane-bound CAs have been localized near the symbiosome membrane presumably to facilitate C\textsubscript{i} delivery to *Symbiodinium* (Weis, 1993; Al-Moghrabi et al., 1996), and could also be facing the coelenteron, or attached to the calicoblastic cells to aid calcification (Moya et al., 2008). iCAs have also been identified within the cytoplasm of the oral and aboral tissue (Grasso et al., 2008; Bertucci et al., 2011), and within calicoblastic cells (Moya et al., 2008), but there are many more CAs in coral genomes that have yet to be localized.

Since the iCA measurements required homogenizing the tissue, the measured iCA activity may not precisely match in-vivo activities. This is always a concern with enzyme assays, but is somewhat less of an issue for CA since it does not require an energy source, though its activity can be regulated cellular conditions such as redox status (Kikutani et al. 2012). The in-vivo iCA activity provides complementary rate information to molecular work on coral CAs that has provided more detailed information about CA localization and hence the potential role of individual CA isoforms. While molecular approaches can provide quantitative information about CA activity, this is quite time-consuming and difficult, requiring over-expression of active CA proteins followed by assessment of CA activity, and is only rarely done (Moya et al. 2008; Bertucci et al. 2011).

**Measured iCA activity is sufficient to fulfill hypothesized roles**

A key feature of these measurements is that they are quantitative allowing calculation of CO\textsubscript{2} hydration or HCO\textsubscript{3}\textsuperscript{-} dehydration rates within coral tissue. Consequently, these iCA activities are suitable for incorporation into models of C\textsubscript{i} processing, or for simpler estimates of CO\textsubscript{2} supply for photosynthesis or HCO\textsubscript{3}\textsuperscript{-} production for calcification. Coral CAs have been proposed to play multiple roles in C\textsubscript{i} processing (Fig. 1) and an important question is whether the measured iCA activities are sufficient to support these proposed roles. The gross HCO\textsubscript{3}\textsuperscript{-} generation rate (F\textsubscript{B}) in the coral can be estimated from the measured iCA activity and the CO\textsubscript{2} concentration in coral tissue as:

\[ F_B = k_{if-a}[CO_2] \]

and the gross CO\textsubscript{2} generation rate (F\textsubscript{C}) can be calculated from the iCA activity, the CO\textsubscript{2}/HCO\textsubscript{3}\textsuperscript{-} equilibrium constant (K\textsubscript{1}), the internal pH, and the HCO\textsubscript{3}\textsuperscript{-} concentration in corals tissue as:

\[ F_C = k_{if-a} \frac{[H^+]}{K_1} [HCO_3^-] \]
The net CO$_2$ or HCO$_3^-$ generation rate will depend on the CO$_2$ and HCO$_3^-$ concentrations and pH in the coral tissue, and the rates and net direction of the reaction will likely vary in different regions of the coral. None-the-less, the gross generation rates put bounds on the net generation rates.

Assuming the CO$_2$ concentration in the coral is 10 μM, similar to seawater concentrations, the gross iCA-catalyzed HCO$_3^-$ generation rate for *O. faveolata* would be 3.4 x10$^{-4}$ mol cm$^{-2}$ hr$^{-1}$. This rate is many orders of magnitude higher than the net photosynthetic rate we measured for *O. faveolata* (5 x10$^{-7}$ mol cm$^{-2}$ hr$^{-1}$), and since calcification rates are generally comparable to or less than the net photosynthetic rate, the gross HCO$_3^-$ generation rate also greatly exceeds the calcification rate. Similarly, the gross HCO$_3^-$ generation rates for *P. astreoides* and *S. radians* greatly exceed net photosynthetic rates. Thus there is in principle sufficient iCA activity to convert CO$_2$ imported for photosynthesis to HCO$_3^-$ (Fig. 1 arrow 2), and to convert respired CO$_2$ to HCO$_3^-$ for calcification (Fig. 1 arrows 4,5). The gross CO$_2$ generation rate calculated assuming the average internal pH is 7.4 (Venn et al., 2009; Venn et al., 2011) and that the internal HCO$_3^-$ concentration is 1.8 mM, similar to seawater, is 1.6 x10$^{-3}$ mol cm$^{-2}$ hr$^{-1}$. Again, the gross CO$_2$ production rate is orders of magnitude higher than the net photosynthetic rate, showing there is sufficient iCA activity to produce CO$_2$ for uptake by symbiotic algae (Fig. 1, arrow 3). These gross production rates are of course maximal net production rates, and use estimates for internal CO$_2$, HCO$_3^-$, and H$^+$ concentrations. But the fact that they are many orders of magnitude higher than the required net production rates suggests that there should be enough iCA capacity to fulfill any of these roles.

For a more detailed assessment, a spatially-resolved model of C$_i$ processing in the coral tissue was developed. Because C$_i$ processing pathways have not been fully defined in corals the model is necessarily schematic, and iCAs are used in as many roles as possible to maximize the need for iCA activity. The model was first run with an iCA activity similar to that measured in *O. faveolata* and *P. astreoides* (50 s$^{-1}$) distributed homogeneously throughout the coral tissue. This iCA activity and distribution is sufficient to support realistic rates of photosynthesis and calcification in the model, though it is only just sufficient to support a realistic calcification rate (Fig. 6). The ability of the measured iCA activity to catalyze the multiple hydrations and dehydrations needed to move C$_i$ from seawater to *Symbiodinium* and to the calcifying fluid is consistent with the simpler calculations showing that the potential CO$_2$ and HCO$_3^-$ generation rates from iCA greatly exceed those required for individual steps in the C$_i$ processing pathways. The measured iCA activity is neither
deficient nor greatly excessive as shown by model runs with 10-fold higher or lower iCA activity. Higher iCA activity (500 s\(^{-1}\)) does not offer any direct benefit in the model, and the lower iCA activity (5 s\(^{-1}\)) is only barely sufficient to meet the C\(_i\) requirements of photosynthesis and does not supply enough C\(_i\) to support measured rates of calcification.

The fact that a homogeneous distribution of iCA is capable of meeting C\(_i\) needs does not imply that iCA is or should be homogeneously distributed. The calcifying fluid and oral endoderm would benefit from greater CA activity as shown by the significant deviation of CO\(_2\) from equilibrium with HCO\(_3^-\) in these model layers (Fig. 6). The observed localization of CA in the calicoblastic cells and potentially in the calcifying fluid (Isa and Yamazato, 1984; Tambutte et al., 2007; Moya et al., 2008) may be particularly helpful since CaCO\(_3\) is being rapidly precipitated from a very thin layer of calcifying fluid (~3 \(\mu\)m thick; Venn et al., 2011), necessitating high rates of CO\(_2\) conversion to HCO\(_3^-\) within a very small volume of fluid. It may also be detrimental to have CA activity in all parts of the tissue. For example, cyanobacteria lack CA in the cytoplasm allowing them to accumulate HCO\(_3^-\), and if CA is artificially expressed in the cytoplasm HCO\(_3^-\) is rapidly converted to CO\(_2\), which leaks out of the cell (Price and Badger, 1989). Storage of HCO\(_3^-\) in a tissue layer or vacuole within the coral may require that CA be absent from that space.

Differences among species

Coral iCA activities were determined in three species of coral, two that inhabit offshore reefs (\(O. faveolata\), \(P. astreoides\)) and one that lives in Florida Bay (\(S. radians\)), a shallow, soft-bottomed environment. When normalized by tissue volume, the two reef species show similar iCA activity, while \(S. radians\) has about 10-fold higher iCA activity (Fig. 3). The slow circulation in the bay may limit the transfer of C\(_i\) between bulk seawater and the surface of \(S. radians\), requiring increased iCA activity to help facilitate C\(_i\) acquisition. Lesser et al. (1994) showed that total CA activity of the coral \(Pocillopora damicornis\) increased when exposed to low flow in a controlled flume experiment. However, \(S. radians\) also has the thinnest effective tissue thickness (tissue volume per unit surface area; Table 1) of the corals surveyed and when coral iCA activity is instead normalized by surface area we find that \(S. radians\) and \(O. faveolata\) have similar iCA activities with \(P. astreoides\) having ~50% lower iCA activity (Fig. 3).

Since coral iCA is involved in C\(_i\) supply for photosynthesis and calcification, the similar rates of photosynthesis (and so presumably calcification) per unit surface area for these corals would suggest that they need similar amounts of iCA on an areal basis, an
intuition that we explored with the spatially-resolved model of C\textsubscript{i} processing. When photosynthetic rates were varied, the iCA activity required to maintain constant effectiveness of the C\textsubscript{i} processing system in the model, as indicated by CO\textsubscript{2} deviations from equilibrium, increased or decreased approximately linearly with the photosynthetic rate (Fig. 7). Next photosynthetic rates were kept constant, while coral tissue thickness was varied. The iCA activity per unit surface area required to maintain constant CO\textsubscript{2} deviations from equilibrium remained essentially constant, though there was a slight increase in iCA activity per unit surface area as tissue thickness increased, a result of its role in facilitated diffusion of C\textsubscript{i} through the tissue layers (Fig. 7; Enns, 1967). In contrast, the iCA activity per unit volume must increase dramatically as the metabolic rates are squeezed into a thinner tissue layer and can decrease as the tissue expands. This analysis suggests that the primary reason iCA activity per unit volume is higher in S. radians is because of the thinner effective tissue thickness in this organism and not because of environmental differences, whereas the iCA activity per unit surface area is more similar among species because the metabolic processes iCAs are involved in are relatively constant on an areal basis.

Here we developed a method to measure the absolute iCA activities of corals and applied this method to three species of scleractinian corals found in the Florida Keys. A key advantage of these absolute measurements is that they allowed us to assess the ability of measured iCA activity to fulfill proposed roles in photosynthesis and calcification. Using simple back of the envelope calculations and a more detailed spatially resolved model we showed that the iCA activities are capable of sustaining the rapid rates of CO\textsubscript{2}/HCO\textsubscript{3}⁻ interconversion required to deliver C\textsubscript{i} for photosynthesis and calcification.

MATERIALS AND METHODS

Coral collection and maintenance

Fragments of Orbicella (previously Montastraea) faveolata, Porites astreoides, and Siderastrea radians were collected from Little Grecian reef and Florida Bay in Key Largo, Florida, USA in August of 2013 as permitted by the Florida Keys National Marine Sanctuary (FKNMS-2011-093, FKNMS-2014-015). Exposed skeleton was covered with modeling clay, and the colonies were maintained in closed circulation tanks filled with reef seawater, allowing at least two days of recovery after collection prior to experimentation. The tank was exposed to a natural light regime, with shading added at midday to keep solar irradiance below 600 μmol photons m\textsuperscript{-2} s\textsuperscript{-1}. Coral surface area was measured using an aluminum foil method (Marsh, 1970). Coral tissue thickness was measured on freshly fragmented corals.
with an ocular micrometer on a stereomicroscope (Leica WILD M3Z, Wetzlar, Germany). The thickness varied between polyps and the coenosarc and multiple measurements across the colony were averaged to obtain an average tissue thickness (Lc) for each species (Table 1). The tissue of *P. astreoides* and *S. radians* is intermingled with the skeleton and the fractional occupancy of tissue (ϕ, porosity of the skeleton) was estimated from cross-sectional images of the fragmented coral colonies. The images were analyzed with ImageJ using color and brightness characteristics to segment the image between tissue and skeletal fractions. The effective tissue thickness, the thickness of the tissue if it was not intermingled with skeleton, was calculated as ϕLc. Algal symbiont clades as determined by DGGE analysis of the ITS2 sequence were: *O. faveolata* - B1; *P. astreoides* - A4a; *S. radians* - B5 (LaJeunesse et al., 2003).

**Coral eCA activity**

ECA activity was measured on intact coral fragments using an 18O-removal technique described in Tansik et al. (in press). Briefly, a water-jacketed chamber is interfaced to a membrane inlet mass spectrometer (MIMS; QMS 220 M2, Pfeiffer Vacuum, Asslar, Germany) that allows continuous monitoring of CO2 isotopes. The chamber is filled with C1-free artificial seawater (ASW), buffered with 20 mM Tris at pH 8.0, and 13C-18O labeled C1 is added to the chamber. After monitoring 18O-removal for approximately 15 min to determine the background rate of removal, a coral fragment is added to the chamber and 18O-removal catalyzed by the coral is monitored for a further 10 min. Finally 100 μM of the eCA inhibitor dextran-bound acetazolamide is added to the chamber and 18O-removal is monitored for 10 minutes. The data is fit to a model of 18O-removal that accounts for background removal rates, internal CA activity, and eCA activity, with the measurement of interest being eCA activity.

**iCA activity and mass transfer coefficients of symbiotic algae**

To measure the iCA activity and mass transfer coefficients of the symbiotic algae, the algae were first isolated from the coral. Coral tissue was removed from the skeleton with an airbrush (Paasche, Chicago, IL, USA) using ASW with 20 mM Tris at pH 8.0 and containing a protease inhibitor cocktail (cOmplete Protease Inhibitor Cocktail mini tablets, 1 tablet per 20 mL). The algae were then separated from the coral homogenate by centrifugation at 2350xg for 5 min. iCA activity and the CO2 and HCO3− mass transfer coefficients were then measured on the algae using an 18O-removal method described previously (Tu et al., 1978;
Hopkinson et al., 2011). Absolute iCA activity was inferred by fitting a model that treats the interior of the cell as a single homogenous compartment to the $^{18}$O-removal data. The mass transfer coefficients describe controls on the flux of CO$_2$ and HCO$_3^-$ into the cells, which is primarily controlled by the diffusive boundary layer and cell membrane permeability.

**CA activity of the coral tissue homogenate**

The CA activity of the coral tissue homogenate from which symbiotic algae had been removed (‘purified coral tissue homogenate’) was measured using an $^{18}$O-removal technique. $^{13}$C-$^{18}$O $C_i$ (2 mM) was added to assay buffer (C$_i$-free ASW, 20 mM Tris at pH 8.0) in a small MIMS chamber that holds ~1 mL of solution. Temperature in the chamber was maintained at 28 °C with a water jacket. $^{18}$O-CO$_2$ species were monitored with the MIMS system for approximately 10 min to determine the background rate of removal due to CO$_2$ hydration/HCO$_3^-$ dehydration. Then a sample of the purified homogenate was added to the chamber and the accelerated $^{18}$O-removal rate was monitored for approximately 10 min. In some cases a CA-inhibitor, 10 $\mu$M AZ, was then added to confirm that the accelerated $^{18}$O-removal rate was due to CA activity. A sample of the purified homogenate was preserved in formalin to determine the residual concentration of symbiotic algae in the sample via microscopy.

**$^{18}$O-removal model for coral iCA determination**

The $^{18}$O-removal data obtained from the coral homogenate was analyzed using a model that accounts for the effects of background CO$_2$ hydration/HCO$_3^-$ dehydration, coral eCA activity, and residual algal iCA activity on $^{18}$O-removal, attributing the remaining $^{18}$O-removal to coral iCA. The model treats coral eCA and coral iCA as being homogeneously distributed throughout the assay solution, while the algae are treated as a separate compartment with fluxes between the compartments parameterized using mass transfer coefficients. The model is described by the following system of differential equations:

\[
\frac{dc_e}{dt} = -(k_{uf} + k_{sf,d} + k_{if,d})c_e + (k_{ur} + k_{sr,d} + k_{ir,d})Hb_e + f_c N(c_i - c_e) \quad [1]
\]

\[
\frac{db_e}{dt} = +(k_{uf} + k_{sf,d} + k_{if,d})Gc_e - (k_{ur} + k_{sr,d} + k_{ir,d})b_e + f_b N(b_i - b_e) \quad [2]
\]

\[
\frac{dc_i}{dt} = -k_{cr}c_i + k_{cr}Hb_i + \frac{f_c}{V_{cell}}(c_e - c_i) \quad [3]
\]

\[
\frac{db_i}{dt} = +k_{cr}Gc_i - k_{cr}b_i + \frac{f_b}{V_{cell}}(b_e - b_i) \quad [4]
\]
The notation is detailed in Table 1. For simplicity rate constants for coral eCA and iCA catalyzed hydration and dehydration are given as the first-order rates constants when diluted in the assay. These are related to the intrinsic rates constants in the intact coral by the following equations that correct for dilution in the assay:

\[
k_{sf} = k_{sf-d} \frac{V_{\text{hom}} V_{\text{e}}}{A_{\text{e}} V_{\text{add}}} \tag{5}
\]

\[
k_{if} = k_{if-d} \frac{V_{\text{hom}} V_{\text{e}}}{A_{\text{e}} L_{\text{c}}} \tag{6}
\]

The coral iCA can alternatively be normalized to coral surface area rather than volume:

\[
k_{if-a} = k_{if-d} \frac{V_{\text{hom}} V_{\text{e}}}{A_{\text{e}} V_{\text{add}}} \tag{7}
\]

The only unknown parameters in these equations are the coral iCA-catalyzed CO\(_2\) hydration and HCO\(_3^-\) dehydration rate constants, which are related to each other by the CO\(_2\)/HCO\(_3^-\) equilibrium constant, assuming microscopic reversibility. Consequently, there is effectively only one unknown, \(k_{if}\), the first-order rate constant for coral iCA catalyzed CO\(_2\) hydration, which was determined by optimizing the model fit to the \(^{18}\)O-CO\(_2\) data. The background rate constants for CO\(_2\) hydration/dehydration (\(k_{uf}, k_{ur}\)) were determined from the \(^{18}\)O-removal rate prior to the addition of homogenate. The coral eCA activity (\(k_{sf}, k_{sr}\)) was measured as described above, as was the algal iCA activity (\(k_{cf}, k_{cr}\)) and the CO\(_2\) and HCO\(_3^-\) mass transfer coefficients (\(f_{c}, f_{b}\)). The volume of the algal cells was determined from measurements of cell radius, approximating the cells as spheres.

**Spatially-resolved model of coral C\(_1\) processing**

A one-dimensional model of C\(_1\) processing in coral tissues was developed to assess the potential functions of iCAs and the iCA activity required to perform these roles. The model treats CO\(_2\) and HCO\(_3^-\) in the diffusive boundary layer adjacent to the coral surface, the oral ectoderm, the oral endoderm, the coelenteron, a combined aboral ectoderm and endoderm, and the calcifying fluid (Supplemental Fig S1). CO\(_3^{2-}\) is treated implicitly as part of the HCO\(_3^-\) pool since equilibrium between these two species is rapidly established (Zeebe and Wolf-Gladrow, 2001). CO\(_2\) and HCO\(_3^-\) are transported by diffusion within the model compartments, and CO\(_2\) diffuses passively through membrane barriers separating compartments while HCO\(_3^-\) only traverses membranes via active membrane-embedded transporters. Photosynthesis occurs in the oral endoderm; respiration occurs in all the tissue layers and is distributed between the compartments based on the thickness of each tissue
layer; calcification occurs in the calcifying fluid. In each layer of the model the following
generic equations are solved:

$$\frac{d[CO_2]}{dt} = -k_f[CO_2] + k_r[HCO_3^-] + \frac{D_c}{z} \left(\frac{d[CO_2]}{dz}\right) + T + M$$  \[8\]

$$\frac{d[HCO_3^-]}{dt} = +k_f[CO_2] - k_r[HCO_3^-] + \frac{D_b}{z} \left(\frac{d[HCO_3^-]}{dz}\right) + T + M$$  \[9\]

The first two terms describe the chemical reactions: hydration of CO\(_2\) and dehydration of
HCO\(_3^-\), while the third term describes diffusion, and the final two terms represent transport
(T) and the metabolic reactions (M): photosynthesis, respiration, and calcification. \(k_f\) and \(k_r\)
are the first order rate constants for CO\(_2\) hydration and HCO\(_3^-\) dehydration. \(D_c\) and \(D_b\) are the
diffusion coefficients for CO\(_2\) and HCO\(_3^-\) respectively, and \(z\) is the thickness of the model
layer. pH in each tissue layer was set as shown in Supplemental Fig. S1, based on literature
values (Venn et al., 2009; Ries, 2011; Venn et al., 2011) except that the pH in the oral tissue
layers had to be elevated somewhat to achieve a net CO\(_2\) influx. HCO\(_3^-\) transport rates
between the tissue layers were set to obtain steady-state concentrations in each layer,
delivering \(C_i\) as needed for photosynthesis and calcification, and removing respired CO\(_2\)
(Supplemental Fig S1).

Rates of gross photosynthesis (1 x10\(^{-6}\) mol cm\(^{-2}\) hr\(^{-1}\)), respiration (5 x10\(^{-7}\) mol cm\(^{-2}\) hr\(^{-1}\)), and calcification (1.7 x10\(^{-7}\) mol cm\(^{-2}\) hr\(^{-1}\)) measured for \(O. faveolata\) were used as base
rates in the model. The model was designed to maximize the need for iCA, employing iCA
in as many consistent roles as possible as diagramed in Supplemental Fig. S1: conversion of
imported and respired CO\(_2\) to HCO\(_3^-\) in the oral ectoderm, conversion of HCO\(_3^-\) to CO\(_2\) for
uptake by \(Symbiodinium\) in the oral endoderm, and conversion of respired CO\(_2\) to HCO\(_3^-\) in
the aboral tissue and calcifying fluid. CO\(_2\) and HCO\(_3^-\) concentrations were set at typical
seawater values at the outer edge of the diffusive boundary layer and the model was then run
until a steady-state was reached.
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We thank Dustin Kemp and Clinton Oakley for assistance with coral collection and maintenance, and Virginia Weis for advice on measurement of carbonic anhydrase activity in corals. Permission to collect corals was granted by the Florida Keys National Marine Sanctuary.

COMPETING INTERESTS
The authors declare that there are no competing financial interests.

AUTHOR CONTRIBUTIONS

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REFERENCES


## Tables

Table 1. Description of parameters used in the model and values for each coral species.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>O. faveolata</th>
<th>P. astreoides</th>
<th>S. radians</th>
<th>units</th>
</tr>
</thead>
<tbody>
<tr>
<td>$c_x$</td>
<td>CO$_2$ concentrations</td>
<td></td>
<td></td>
<td></td>
<td>mol cm$^{-3}$</td>
</tr>
<tr>
<td>$b_x$</td>
<td>HCO$_3^-$ concentrations</td>
<td></td>
<td></td>
<td></td>
<td>mol cm$^{-3}$</td>
</tr>
<tr>
<td>G, H$^2$</td>
<td>stoichiometric matrices for interconversion of $^{18}$O-CO$_2$ and $^{18}$O-HCO$_3^-$ species</td>
<td></td>
<td></td>
<td></td>
<td>--</td>
</tr>
<tr>
<td>$k_{uf}$, $k_{ur}$</td>
<td>background CO$_2$ hydration, HCO$_3^-$ dehydration rate constants</td>
<td>0.14</td>
<td>0.16</td>
<td>0.14</td>
<td>s$^{-1}$</td>
</tr>
<tr>
<td>$k_{sf}$, $k_{sr}$</td>
<td>coral eCA catalyzed CO$_2$ hydration, HCO$_3^-$ dehydration rate constants</td>
<td>6.3 ± 0.3</td>
<td>0.45 ± 0.29</td>
<td>0.26 ± 0.44</td>
<td>cm s$^{-1}$</td>
</tr>
<tr>
<td>$k_{if}$, $k_{ir}$</td>
<td>coral iCA catalyzed CO$_2$ hydration, HCO$_3^-$ dehydration rate constants</td>
<td></td>
<td></td>
<td></td>
<td>s$^{-1}$</td>
</tr>
<tr>
<td>$k_{cf}$, $k_{cr}$</td>
<td>Symbiodinium iCA catalyzed CO$_2$ hydration, HCO$_3^-$ dehydration rate constants</td>
<td>970 ± 350</td>
<td>1450 ± 370</td>
<td>2120 ± 230</td>
<td>s$^{-1}$</td>
</tr>
<tr>
<td>$f_c$</td>
<td>Symbiodinium CO$_2$ mass transfer coefficient</td>
<td>1.6 ± 0.1 x10$^{-7}$</td>
<td>1.7 ± 0.3 x10$^{-7}$</td>
<td>1.5 ± 0.1 x10$^{-7}$</td>
<td>cm$^3$ s$^{-1}$</td>
</tr>
<tr>
<td>$f_b$</td>
<td>Symbiodinium HCO$_3^-$ mass transfer coefficient</td>
<td>1.1 ± 0.7 x10$^{-11}$</td>
<td>1.4 ± 0.4 x10$^{-12}$</td>
<td>6.7 ± 9.0 x10$^{-12}$</td>
<td>cm$^3$ s$^{-1}$</td>
</tr>
<tr>
<td>N</td>
<td>concentration of Symbiodinium</td>
<td></td>
<td></td>
<td></td>
<td>cells cm$^{-3}$</td>
</tr>
<tr>
<td>$V_{cell}$</td>
<td>volume of Symbiodinium cell</td>
<td>2.7 x10$^{-10}$</td>
<td>2.7 x10$^{-10}$</td>
<td>2.7 x10$^{-10}$</td>
<td>cm$^3$</td>
</tr>
<tr>
<td>$V_{add}$</td>
<td>volume of homogenate added to assay</td>
<td>0.1 − 0.2</td>
<td>0.1 − 0.2</td>
<td>0.1 − 0.2</td>
<td>cm$^3$</td>
</tr>
<tr>
<td>$V_{hom}$</td>
<td>total volume of homogenate</td>
<td>24 − 38</td>
<td>40</td>
<td>27 − 37</td>
<td>cm$^3$</td>
</tr>
<tr>
<td>$V_e$</td>
<td>assay volume</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>cm$^3$</td>
</tr>
<tr>
<td>$A_c$</td>
<td>coral surface area removed</td>
<td>7.7 − 11.2</td>
<td>8.1 − 11.3</td>
<td>6.0 − 8.4</td>
<td>cm$^2$</td>
</tr>
<tr>
<td>$L_c$</td>
<td>average coral tissue thickness</td>
<td>1.9</td>
<td>2.3</td>
<td>1.3</td>
<td>mm</td>
</tr>
<tr>
<td>$\phi$</td>
<td>porosity</td>
<td>1</td>
<td>0.36</td>
<td>0.20</td>
<td>mm</td>
</tr>
<tr>
<td>$\phi L_c$</td>
<td>effective tissue thickness (tissue volume per unit surface area)</td>
<td>1.9</td>
<td>0.83</td>
<td>0.26</td>
<td>mm</td>
</tr>
</tbody>
</table>

1 e = bulk solution, i = inside Symbiodinium cells
2 see (Hopkinson et al., 2011)
Table 2. $^{18}$O-removal rates calculated as the slope of a linear fit through natural log-transformed $^{18}$O atom fraction data. Background rates are averages ± standard deviations obtained prior to the addition of coral homogenate. Rates after the addition of coral homogenate are reported as the range among samples, since these rates are not expected to be constant due to variation in CA activity and volume of homogenate added. AZ was then added to selected samples and the average ± standard deviations are reported for this phase.

<table>
<thead>
<tr>
<th>coral</th>
<th>background (s$^{-1}$)</th>
<th>homogenate (s$^{-1}$)</th>
<th>homogenate +AZ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. faveolata</em></td>
<td>$3.5 \pm 0.3 \times 10^{-4}$</td>
<td>$3.9 - 14.2 \times 10^{-4}$</td>
<td>$2.5 \pm 0.2 \times 10^{-4}$</td>
</tr>
<tr>
<td><em>P. astreoides</em></td>
<td>$2.6 \pm 0.3 \times 10^{-4}$</td>
<td>$3.5 - 6.5 \times 10^{-4}$</td>
<td>$2.1 \pm 0.1 \times 10^{-4}$</td>
</tr>
<tr>
<td><em>S. radians</em></td>
<td>$2.8 \pm 0.4 \times 10^{-4}$</td>
<td>$5.0 - 12.5 \times 10^{-4}$</td>
<td>$1.7 \pm 0.1 \times 10^{-4}$</td>
</tr>
</tbody>
</table>
Figure 1. C<sub>i</sub> fluxes and potential roles of CA in coral C<sub>i</sub> processing based primarily on Bertucci et al. (2013). (1) eCA on the oral surface converts HCO<sub>3</sub><sup>-</sup> to CO<sub>2</sub>, which then passively diffuses in the oral ectoderm due to a concentration gradient. (2) CA in the oral ectoderm may convert imported and respired CO<sub>2</sub> to HCO<sub>3</sub><sup>-</sup>, which is then transported into the oral endoderm. Respiration occurs in the mitochondria (mito, orange ovals). (3) In the oral endoderm HCO<sub>3</sub><sup>-</sup> can be converted to CO<sub>2</sub> by CA for uptake by Symbiodinium (Sym, green circle). (4) Respired CO<sub>2</sub> can be converted to HCO<sub>3</sub><sup>-</sup> using CA for use in calcification or photosynthesis. (5) CA in the calcifying fluid converts respired CO<sub>2</sub> to HCO<sub>3</sub><sup>-</sup> for subsequent use in calcification.
Figure 2. (A) Diagram illustrating iCA measurement procedure. The intact coral fragment contains eCA (e) on the outer surface, iCA (i) within the tissue, and CA within Symbiodinium (s, Sym). The tissue is homogenized and then centrifuged to remove Symbiodinium. The purified tissue homogenate is then assayed for CA activity using an $^{18}$O-removal assay. Key model terms affecting $^{18}$O-removal are indicated: $k_{if-d}$, the diluted coral iCA CO$_2$ hydration rate constant; $k_{sf-d}$, the diluted coral eCA CO$_2$ hydration rate constant; $f_c$, the Symbiodinium CO$_2$ mass transfer coefficient; $k_c$, the Symbiodinium CO$_2$ hydration rate constant (see also Table 1). (B-D) Examples of $^{18}$O-CO$_2$ data for each coral taxa (B: O. faveolata, C: P. astreoides, D: S. radians) showing background rates of $^{18}$O-removal prior to time zero, acceleration $^{18}$O-removal upon addition of coral tissue homogenate at time zero (first dotted vertical line), and a slowdown in the $^{18}$O-removal rate after addition of a CA inhibitor (AZ) at 7-10 min (second dotted vertical line). The solid lines are fits of the $^{18}$O-removal model used to determine coral iCA activity to the data.
Figure 3. Coral iCA activity normalized to coral tissue volume ($k_{if}$) and to coral surface area ($k_{if-a}$) and net photosynthetic rates for each coral taxon. Error bars represent standard deviations.
Figure 4. Contribution of CA components in the coral homogenate to total $^{18}$O-removal. The $^{18}$O-removal model was run in a predictive sense sequentially adding components to the model (sym = *Symbiodinium* iCA; eCA = coral eCA; iCA = coral iCA). A: *O. faveolata*, B: *P. astreoides*, C: *S. radians*
Figure 5. Effect of neglecting contributions of *Symbiodinium* iCA and coral eCA on the determination of coral iCA activity for samples of *O. faveolata* (Of), *P. astreoides* (Pa) and *S. radians* (Sr).
Figure 6. Results of the spatially resolved C1 processing model. iCA activity was homogeneously distributed throughout the tissue at the rates indicated. Photosynthetic and calcification rates were the same in the models with measured (50 s⁻¹) and elevated (500 s⁻¹) iCA activity but the calcification rate was reduced to 40% of the original rate in the model with lowered (5 s⁻¹) iCA activity. Plots show the (A) CO₂ concentration, (B) HCO₃⁻ concentration, and (C) CO₂ deviations from equilibrium with HCO₃⁻ as a function of depth in the coral tissue. Background colors and abbreviations in panel A indicate the model layers: diffusive boundary layer (DBL), oral ectoderm (OEc), oral endoderm (OEn), coelenteron (Coel), aboral tissue (Ab), calcifying fluid (CF). The thickness of the calcifying fluid has been exaggerated (60x) in the plot for clarity.
Figure 7. Effect of (A) photosynthetic rate (P) and (B) tissue thickness (L_c) on iCA activity required to maintain constant CO₂ deviations from equilibrium in the spatially-resolved model of coral C₃ processing. On the x-axes, the parameters are plotted relative to base values for the photosynthetic rate (P_base = 1 x 10⁻⁶ mol cm⁻² hr⁻¹) and tissue thickness (L_c-base = 2 mm).