Bicarbonate enrichment may mitigate oxidative stress in two octocorals and their photosynthetic symbionts

Jeffrey Golladay

Follow this and additional works at: https://huskiecommons.lib.niu.edu/allgraduate-thesesdissertations

Recommended Citation
Golladay, Jeffrey, "Bicarbonate enrichment may mitigate oxidative stress in two octocorals and their photosynthetic symbionts" (2018). Graduate Research Theses & Dissertations. 1638.
https://huskiecommons.lib.niu.edu/allgraduate-thesesdissertations/1638

This Dissertation/Thesis is brought to you for free and open access by the Graduate Research & Artistry at Huskie Commons. It has been accepted for inclusion in Graduate Research Theses & Dissertations by an authorized administrator of Huskie Commons. For more information, please contact jschumacher@niu.edu.
ABSTRACT

BICARBONATE ENRICHMENT MAY MITIGATE OXIDATIVE STRESS IN TWO OCTOCORALS AND THEIR PHOTOSYNTHETIC SYMBIONTS

Jeffrey Golladay, M.S.
Department of Biological Sciences
Northern Illinois University, 2018
Neil W. Blackstone, Director

The ecological communities built by corals are some of the most biodiverse in the world, and the current rate of climate change threatens their long-term persistence, with mass coral bleaching events becoming more frequent. Ocean acidification (OA) is one consequence of increased atmospheric CO₂ that results in changes in seawater carbon chemistry with the potential to impact organisms that rely on seawater dissolved inorganic carbon (DIC) for calcification, photosynthesis, or both. Corals are unique animals that participate in a symbiotic relationship with dinoflagellate algae of the genus Symbiodinium spp., in which the algal symbionts translocate most of the products of photosynthesis to their host coral. To fuel photosynthesis, the algal symbionts must obtain inorganic carbon from seawater via their coral host. As such, symbionts may be particularly sensitive to the changes in the seawater DIC pool that will result from ongoing OA. Projected increases in seawater bicarbonate (HCO₃⁻) ion concentration associated with OA may act to supplement symbiont photosynthesis, and could potentially mitigate that effects of other stressors that lead to photoinhibition and subsequent production of reactive oxygen species (ROS). ROS may ultimately trigger coral bleaching.

The research presented here focuses on the effects of ocean acidification, specifically bicarbonate enrichment, on the photo-physiological stress response of two
representative octocoral species, *Sympodium* sp. and *Sarcothelia* sp., and their symbionts. Bicarbonate enrichment experiments were carried out and fluorescence microscopy was used to visualize the production of ROS *in hospite* (within the host) in light stressed colonies of both octocoral species. Additional bicarbonate enrichment experiments were conducted in which oxygen metabolism was measured in colonies of *Sympodium* sp. The results presented here suggest that bicarbonate enrichment may act to alleviate oxidative stress resulting from photoinhibition, possibly by supplementing symbiont photosynthesis under saturating irradiance.
NORTHERN ILLINOIS UNIVERSITY
DE KALB, ILLINOIS

AUGUST 2018

BICARBONATE ENRICHMENT MAY MITIGATE OXIDATIVE STRESS IN
TWO OCTOCORALS AND THEIR PHOTOSYNTHETIC SYMBIONTS

BY

JEFFREY GOLLADAY
© 2018 Jeffrey Golladay

A THESIS SUBMITTED TO THE GRADUATE SCHOOL
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE
MASTER OF SCIENCE

DEPARTMENT OF BIOLOGICAL SCIENCES

Thesis Director:
Dr. Neil W. Blackstone
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>LIST OF FIGURES</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>iii</td>
</tr>
</tbody>
</table>

**BICARBONATE ENRICHMENT MAY MITIGATE OXIDATIVE STRESS IN TWO OCTOCORALS AND THEIR PHOTOSYNTHETIC SYMBIONTS** .... 1  

- Introduction ........................................................................... 1  
- Methods .................................................................................. 8  
- Results ..................................................................................... 14  
- Discussion ............................................................................... 23  

**LITERATURE CITED** ..................................................................... 31
LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Photosynthesis</td>
<td>4</td>
</tr>
<tr>
<td>2.</td>
<td>ROS in colonies of <em>Sympodium</em> sp.</td>
<td>17</td>
</tr>
<tr>
<td>3.</td>
<td>ROS in colonies of <em>Sarcothelia</em> sp.</td>
<td>18</td>
</tr>
<tr>
<td>4.</td>
<td>Oxygen metabolism of <em>Sympodium</em> sp. colonies before and after incubation</td>
<td>22</td>
</tr>
</tbody>
</table>
BICARBONATE ENRICHMENT MAY MITIGATE OXIDATIVE STRESS IN TWO OCTOCORALS AND THEIR PHOTOSYNTHETIC SYMBIONTS

Introduction

The current rate at which the global climate is shifting threatens the stability of many ecosystems, but perhaps none more than coral reefs. The corals that underpin reef ecosystems are highly specialized members of the phylum Cnidaria, many of which have developed finely-tuned symbiotic relationships with dinoflagellate algae of the genus *Symbiodinium* (Muscatine and Porter, 1977). Countless studies have examined the sensitivity of this relationship to the predicted effects of climate change, typically focusing on elevated seawater temperature (Jokiel and Coles, 1990; Jones et al., 1998; Nielsen et al., 2018) or UV radiation (Lesser and Farrell, 2004; Drohan et al., 2005; Ferrier-Pages et al., 2007). Another facet of climate change is ocean acidification, in which seawater pH and carbonate chemistry shift as the ocean absorbs CO$_2$ from the atmosphere. The ocean sequesters between one-quarter to one-third of the CO$_2$ produced from human activities (Sabine et al., 2004; Gabay et al., 2012), and as the partial pressure of CO$_2$ ($p$CO$_2$) in the atmosphere continues to increase, recently surpassing 400 ppm (IPCC, 2014), there is greater dissolution of CO$_2$ into the ocean. In brief, as CO$_2$ dissolves into the ocean it reacts with H$_2$O to form carbonic acid (H$_2$CO$_3$), which rapidly dissociates into a proton (H$^+$) and bicarbonate (HCO$_3^-$). The result is a decline in seawater pH, a decrease in [CO$_3^{2-}$], and an
increase in $[\text{HCO}_3^-]$ (Caldeira and Wickett, 2005). So far, ocean pH has dropped 0.1 units since the pre-industrial era (Raven et al., 2005; Gabay et al., 2012), and projections estimate that by 2100 it will drop an additional 0.2-0.3 units, a change that corresponds to a $[\text{H}^+]$ increase of nearly 150% and a $[\text{CO}_3^{2-}]$ decrease of roughly 50% (Doney et al., 2009; Mackey et al., 2015). Many questions remain about how these changes in the chemistry of the ocean will affect the coral/$\text{Symbiodinium}$ symbiosis.

Concerns over the effects of ocean acidification on coral reefs has primarily centered around calcifying organisms due to projected declines in seawater $[\text{CO}_3^{2-}]$ and its effects on the calcium carbonate ($\text{CaCO}_3$) saturation state, a critical factor affecting shell and skeleton formation (Putron et al., 2011). As a result, a majority of the research examining the effects of ocean acidification on corals has focused on calcification in scleractinians - the major reef-builders (Schneider and Erez, 2006; Marubini et al., 2008; Edmunds et al., 2012, 2013; Comeau et al., 2013a, b; Dufault et al., 2013; Jokiel, 2013). Octocorals represent another important group in coral reefs, often occupying a majority of the reef space (McFadden et al., 2010). Nonetheless, studies examining how they will respond to ocean acidification have not kept pace with those examining the scleractinians. In addition to calcification, ocean acidification has the potential to impact the photo-physiology of coral/$\text{Symbiodinium}$ symbioses by altering the speciation of dissolved inorganic carbon (DIC) in seawater, the ultimate source of the substrate, CO$_2$, necessary for symbiont photosynthesis. Nevertheless, details on the photo-physiological response of the coral/$\text{Symbiodinium}$ symbiosis to ocean acidification also remain scarce.

The coral/$\text{Symbiodinium}$ relationship has long been considered mutualistic (for review, see Blackstone and Golladay, 2018). Symbionts carry out oxygenic photosynthesis and export up to 95% of the associated products, hereafter referred to as “photosynthate,” to their coral host
(Yellowlees et al., 2008; Tremblay et al., 2012), while the symbionts receive metabolic waste products from their host, shelter, and consistent access to sunlight. However, the fragility of the coral/Symbiodinium symbiosis to environmental stressors has led to a recent reevaluation of the symbiosis as one that lies along a mutualistic to parasitic continuum (Lesser et al., 2013; Baker et al., 2018; Blackstone and Golladay, 2018). Some Symbiodinium strains have been shown to display a greater tendency towards parasitism (Stat et al., 2008), while others may shift towards parasitism during periods of stress (Baker et al., 2018). For example, symbionts in the coral Orbicella faveolata produced more photosynthate when exposed to elevated seawater temperatures, but transport of photosynthate to the host coral did not increase proportionally (Baker et al., 2018). Symbionts that do not participate in the symbiosis by retaining photosynthate for their own selfish replication may be considered “defectors”, while those that share photosynthate are “cooperators”. To prevent the proliferation of defectors, it has been postulated that features of photosynthesis have been co-opted into mechanisms of conflict mediation between corals and their symbionts (Blackstone and Golladay, 2018). These mechanisms function to reconcile evolutionary conflict inherent in coral/Symbiodinium symbioses, with selection at the level of the symbiont favoring retention of photosynthate for selfish growth and replication, while selection at the level of the higher unit (host/symbiont community) favors the sharing of photosynthate with the host and greater symbionts community.

When conditions are favorable photosynthesis proceeds normally, converting light energy into electron flow which is ultimately stored as chemical energy (figure 1). In short, light is captured by photosystem II in the thylakoid membrane of symbiont chloroplasts, and this energy is used to split water, which releases electrons that go on to power an electron transport chain that terminates in the formation of ATP. Meanwhile, photosystem I captures additional light
which re-energizes electrons that go on to reduce NADP\(^+\) to NADPH. Ultimately, ATP and NADPH provide the energy and reducing power necessary for the fixation of CO\(_2\) in the Calvin cycle. Symbionts transport a majority of the photosynthate they produced to the host coral.

Figure 1. Photosynthesis.
Schematic of photosynthesis showing electrons originating from the splitting of water at photosystem II (PSII), flowing through the electron transport chain (ETC), generating a proton gradient that ultimately powers the formation of ATP by ATP synthase. The electrons then continue along the ETC to photosystem I (PSI) where they are reenergized and reduce NADP\(^+\) to NADPH. The ATP and NADPH generated by electron transport provide the power reduce and fix carbon in the Calvin Cycle (Modified by Parrin, 2016 from Thylakoid Membrane, In Wikipedia, n.d., https://commons.wikimedia.org/wiki/File%3AThylakoid_membrane.png).
During stressful environmental conditions, typically too much light or heat, symbiont photosynthesis may be disrupted (Downs et al., 2002; Lesser, 2006; Buxton et al., 2009), leading to a backup of electrons onto photosystems I and II. Overreduction of the photosystems results in alternative electron flow, favoring the formation of reactive oxygen species (ROS), and is an integral part of the initiating stages of the cnidarian stress response (Parrin et al., 2017). The input of light is critical in this pathway, as light provides the necessary energy to split water, and water is the ultimate source of the electrons that lead to the formation of ROS. However, singlet oxygen deserves special consideration here because it is a ROS that is generated by an input of energy other than that derived from electrons (Foyer and Noctor, 2009). Energy in photosynthetic systems, however, is ultimately from light, and it is this energy that can lead to the formation of singlet oxygen. During conditions in which photosystems I and II are reduced, the input of this energy into the formation of singlet oxygen occurs more readily. In these cases, singlet oxygen is also more likely to gain additional electrons, forming other ROS (Foyer and Noctor, 2009). The proliferation of ROS may trigger programmed cell death and the collapse of the mechanisms of conflict mediation (Blackstone and Golladay, 2018), ultimately resulting in coral bleaching (i.e., the expulsion or death of symbionts; Weis, 2008).

The formation of ROS is an integral step in the “canonical” pathway of bleaching. Symbiont migration, however, represents a “non-canonical” bleaching pathway. Parrin et al. (2012, 2016) showed that during periods of stress, although polyps appear to be bleached, most symbionts were not expelled from their host. Rather, in the octocorals Sarcothelia sp. and Phenganax parrini, symbionts were observed migrating from the polyps into the stolons following perturbation by light or temperature (Parrin et al., 2012, 2016; Netherton et al., 2014). The relationship between ROS formation and migration has yet to be determined, but these
results indicate that symbiont migration may be one of the first indications of stress in perturbed colonies, and is possibly triggered by ROS. This suggests that stressed colonies may have a greater capacity to recover after a period of stress if symbionts remain in host stoloniferous tissue. However, accumulation of symbionts in the stolon could prove detrimental to the colony if they are emitting high levels of ROS (Parrin et al., 2012).

The reliance of symbiotic octocorals on their photosynthetic symbionts makes it difficult to predict how they will respond to ocean acidification. Symbionts reside in host endodermal cells and rely on DIC from an external source (seawater) for photosynthetic substrate (Davy et al., 2012), forcing symbionts to depend on their host to supply them with CO$_2$ (Goiran et al., 1996; Wooldridge, 2013). Indeed, several mechanisms for the uptake of DIC from seawater by corals have been described, including active HCO$_3^-$ transport or the conversion of external HCO$_3^-$ to CO$_2$ via a H$^+$-ATPase in coral ectoderm (Allemand et al., 1998). Commonly referred to as carbon concentrating mechanisms (CCMs), these mechanisms ultimately function to shuttle DIC from the surrounding seawater into symbiont chloroplasts near the active site of ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco), the main photosynthetic enzyme responsible for carbon fixation (Badger, 1985). It is widely accepted that HCO$_3^-$ is the dominant species of DIC taken up by coral CCMs (Goiran et al., 1996; Allemand et al., 1998). For instance, in the scleractinian coral *Galaxea fascicularis*, Al-Moghrabi et al. (1996) demonstrated that carrier proteins in the animal membrane selectively take up HCO$_3^-$ from seawater. Once inside the coral, carbonic anhydrases in the cytoplasm convert it to CO$_2$, which may then be used in symbiont photosynthesis (Allemand et al., 1998; Reinfelder, 2011). Changes in the speciation of seawater DIC resulting from ocean acidification ultimately alters photosynthetic substrate availability and may affect symbiont productivity.
Projected increases in seawater [HCO$_3^-$] under ocean acidification could potentially supplement symbiont photosynthesis (Mackey et al., 2015). For instance, bicarbonate enrichment was shown to increase photosynthetic oxygen evolution in the scleractinian corals *Stylophora pistillata* (Marubini et al., 2008), *Porites porites* and *Acropora* sp. (Herfort et al., 2008). These reports suggest that current seawater DIC levels may be carbon-limiting for symbiont photosynthesis under saturating irradiance. In this context, increases in the DIC supply could potentially mitigate the effects of photoinhibition in stressed colonies, especially during exposure to excess light when photosynthesis is proceeding rapidly. Increased availability of HCO$_3^-$, and in turn CO$_2$, may result in greater carbon fixation, providing a “sink” for electrons while minimizing alternative electron flow, therefore decreasing the likelihood of ROS formation. Thus, bicarbonate enrichment, through the addition of sodium bicarbonate (NaHCO$_3$), may mitigate impaired symbiont photosynthesis and alleviate the collapse of mechanisms of conflict mediation during stressful conditions. Based on the rationale presented above, it is predicted that bicarbonate enrichment will (I) decrease ROS formation and (II) lead to an increase in oxygen formation in colonies exposed to excessive light.

Progress in elucidating the role of ROS in the cnidarian stress response has been limited, partly due to a lack of appropriate laboratory models that allow microscopic visualization of ROS formation in stressed colonies using fluorescent probes. Such methods can be used to shed light on the physiology of the cnidarian stress response that other methods cannot adequately account for. The use of microscopic visualization to examine stress response in octocorals has largely been pioneered through the work of Parrin et al. (2012, 2016, 2017), Netherton et al. (2014), and more recently in scleractinians by Nielsen et al. (2018). The research presented here focuses on the effects of ocean acidification, specifically bicarbonate enrichment, on the photo-
physiological stress response of two representative octocoral species, *Sympodium* sp. and *Sarcothelia* sp., and their symbionts using fluorescence microscopy and imaging techniques, and oxygen metabolism experiments.

### Methods

#### Study species

All experiments were performed on colonies of *Sympodium* sp. and *Sarcothelia* sp. Both species are closely related members of the family Xeniidae belonging to the Holaxonia-Alcyoniina clade of octocorals, each containing dinoflagellate symbionts in the genus *Symbiodinium*. *Sympodium* sp. harbor a Clade C1 variant, while *Sarcothelia* sp. contain Clade D4-5-9 symbionts (Parrin et al., 2016). Small colonies of each species were explanted from a single parental clone and grown on 12 mm (oxygen metabolism experiments) or 15 mm (ROS experiments) diameter cover glass. Explants were taken from one parental clone for each species to limit genotypic variability. Stock colonies and explants were maintained in aquaria in standard culture conditions (Netherton et al., 2014) at 27°C under a natural light cycle provided by metal halide and fluorescent lights (12 h dark, 8 h illumination at 30 μmol photons m$^{-2}$ s$^{-1}$, 4 h illumination at 110 μmol photons m$^{-2}$ s$^{-1}$). Water chemistry was tested weekly to ensure stable culture conditions.
Bicarbonate enrichment

Colonies of each species were incubated in bicarbonate enriched (BE) seawater and exposure to excessive light was used to induce stress as described below. BE seawater was produced fresh for each trial by mixing 300 mL of artificial seawater with sufficient NaHCO$_3$ to bring [HCO$_3^-$] up to the appropriate treatment concentration. The two BE treatments used were +1mM HCO$_3^-$ ([HCO$_3^-$] $\approx$ 3mM) and +3mM HCO$_3^-$ ([HCO$_3^-$] $\approx$ 5mM). In each experiment, an equal number of colonies were incubated in bicarbonate ambient (BA) seawater ([HCO$_3^-$] $\approx$ 2.1mM). BA seawater was taken directly from aquaria and had no additional bicarbonate added to it.

Bicarbonate enrichment through the addition of NaHCO$_3$ to artificial seawater is frequently used to increase DIC (Marubini and Thake, 1999; Herfort et al., 2008; Ramsby et al., 2014), however it does produce an initial drop in pH, potentially exposing colonies incubated in BE seawater to a pH shock (Gattuso and Lavigne, 2009). Nonetheless, decreases in pH this small have been shown to have little effect on octocoral growth (Gabay et al., 2014; Gomez et al., 2015) and symbiont photosynthesis (Marubini et al., 2008). Small samples of seawater to which NaHCO$_3$ is added typically equilibrate to original pH levels while maintaining the increased concentration of HCO$_3^-$ ions (Gattuso and Lavigne, 2009). To ensure any changes in pH were temporary and not substantial, pH was measured in freshly prepared samples of BE seawater at both treatment levels (i.e., +1mM and +3 mM HCO$_3^-$). These samples were then left in the incubator for the same amount of time as experimental incubations, and then pH was measured again. Initial measurements confirmed that the addition of NaHCO$_3$ did produce a drop in pH (pH 8.1 to pH 8.0 in +1mM HCO$_3^-$ samples; pH 8.1 to pH 7.9 in +3mM HCO$_3^-$ samples). After
the incubation period, +1mM HCO$_3^-$ samples remained at pH 8.0, while the pH of +3mM HCO$_3^-$ samples increased to 8.0.

**Incubation procedures**

In each experiment, colonies were incubated overnight (18:00 h to 06:00 h) in the dark in glass finger bowls in their respective treatments. At 06:00 h, colonies were illuminated (140 μmol photons m$^{-2}$ s$^{-1}$) and incubated for another 4 hours. At 10:00 h the fluorescent probe 2′,7′-dichlorodihydro-fluorescein diacetate (H$_2$DCFDA) dissolved in dimethyl sulfoxide (DMSO) was added to the fingerbowls and colonies were incubated in the dark for an additional hour. H$_2$DCFDA is commonly used to assay ROS (Cherry-Vogt et al., 2011; Wu and Yotnda, 2011; Netherton et al., 2014; Durand et al., 2017; Parrin et al., 2017; Schlosser et al., 2017). Colonies were then visualized using fluorescence microscopy and imaged following the methods described below. Temperature during incubations was maintained at 27° C for all experiments. Each species was exposed to two BE seawater treatments, with either +1 mM NaHCO$_3$ added ([HCO$_3^-$] ≈ 3 mM; *Sympodium* sp. n = 15; *Sarcothelia* sp. n = 14) or +3 mM NaHCO$_3$ added ([HCO$_3^-$] ≈ 5 mM; *Sympodium* sp. n = 15; *Sarcothelia* sp. n = 15). Trials involved 3 colonies incubated in BA seawater (negative control) and 3 colonies incubated in BE seawater. Small sample sizes were used in each experiment so that colonies could be paired closely in time and to minimize the difference in time between the first and last colony imaged.

To better assess the effects of bicarbonate enrichment alone on ROS formation, two additional experiments were carried out in which colonies were incubated entirely in the dark (18:00 h to 11:00 h, with the fluorescent probe added at 10:00 h). Each species was exposed to
BE seawater with +3 mM NaHCO$_3$ ([HCO$_3^-$] $\approx$ 5 mM; *Sympodium* sp. n = 15; *Sarcothelia* sp. n = 15). Incubations for these experiments were conducted in the same manner as described above, with 3 treated colonies paired with 3 negative control colonies. Colonies were then visualized and imaged following the methods described below.

**ROS visualization**

ROS was visualized *in vivo* using the fluorescent probe H$_2$DCFDA (Cherry-Vogt et al., 2011; Wu and Yotnda, 2011; Netherton et al., 2014; Durand et al., 2017; Parrin et al., 2017; Schlosser et al., 2017). After the final hour of incubation with the fluorescent probe, colonies were confined to 5 ml microscopic chambers, observed using fluorescence microscopy (blue excitation at 900 μmol photons m$^{-2}$ s$^{-1}$ and green emission) and imaged following the methods of Parrin et al. (2017) using a Zeiss Axiovert 135 inverted microscope (Carl Zeiss, Jena, Germany) with an attached Hamamatsu Orca-100 camera (Hamamatsu Photonics, Hamamatsu City, Japan). Three similar-sized areas of coenenchyme were imaged for each colony. Relative luminance of coenenchyme area, an indirect assessment of ROS formation, was measured by subtracting background luminance from coenenchyme luminance using Image Pro Plus v. 6.3 (Media Cybernetics, Silver Spring, MD, USA). H$_2$DCFDA is a non-selective ROS probe, but was most likely detecting H$_2$O$_2$ (Setsukinai et al., 2003; Foyer and Noctor, 2009).

Retrieving a colony from the incubator and obtaining the three images took approximately three minutes. To minimize variation in ROS formation due to length of incubation, colonies from each treatment were paired as closely as possible in time. For example, if the first colony imaged was BA colony number one, the second colony imaged was BE colony
number one followed by BA colony number two, and so on. For the same reason, the first colony imaged at the start of each new experiment was alternated (i.e., BA first in one trial, then BE first in the next trial).

**Oxygen Metabolism**

To examine the effects of bicarbonate enrichment on symbiont photosynthesis, experiments were carried out in which oxygen metabolism was measured before and after colonies were incubated in BE seawater (+1mM HCO$_3^-$). A repeated-measures design was employed for these experiments in which each colony served as its own control. Two colonies were first taken from the aquarium and measured for oxygen metabolism, as described below, and then returned to the aquarium. At 18:00 h, the two colonies that were used were then confined to separate finger bowls and incubated following the bicarbonate enrichment incubation procedures described above. One colony was incubated in BA seawater and the other was incubated in BE seawater. At 10:00 h the following morning, the colonies were taken from the incubator and measured for oxygen metabolism again. Due to the amount of time it took to measure oxygen metabolism in the dark and in the light for a single colony, the colony that was measured second endured an additional hour in the incubator. To minimize the effects of an additional hour of incubation, the first colony measured was alternated each trial (i.e., if a BA colony was measured first, then a BE colony would be measured first in the next trial).

Measurements were made using a Strathkelvin 1302 electrode and 781 oxygen meter (North Lanarkshire, Scotland, UK). Colonies of *Sympodium* sp. were grown on 12 mm cover glass for this experiment. Cover glass containing colonies were attached with silicone grease to a
second coverslip that had a magnetic stir bar cemented to it, so that water could be stirred in the chamber. The chamber was maintained at 27°C using a Neslab (Oak Park, IL, USA) RTE-100D recirculating water bath. Oxygen metabolism (mg l\(^{-1}\) min\(^{-1}\)) was measured for 30 minutes in the dark and then for 30 minutes in the light (50 \(\mu\)mol m\(^{-2}\) s\(^{-1}\)). Measurements taken in the dark were attributed to respiration while measurements taken in the light were attributed to a combination of both respiration and photosynthesis (Blankenship, 2002; Netherton et al., 2014).

**Statistical analyses**

Measures of ROS were analyzed using a nested analysis of variance (ANOVA), with colonies nested within treatments. In a nested ANOVA, the F-ratio for the treatment effect is formed using the colonies-within-treatment effect as the error variance. In all of the experiments, this latter effect was highly significant, usually but not always obscuring the between-treatment effect. Inspection of the data suggested that despite the short duration of the experiments, ROS tended to increase during the experiments. This within-treatment effect can be alleviated by pairing measures in time and using a paired sample t-test for statistical analysis. During imaging, colonies were paired in time as closely as possible by alternating colony treatment during imaging (i.e., if the first colony imaged was a BA colony, the second colony imaged would be a BE colony, and so on). To further account for the effects of time, image sequence was also paired within colony pairs (e.g., ROS image 1 from BA colony 1 is compared to ROS image 1 from BE colony 1, etc.). The paired sample t-test compares the mean of the differences to zero, rather than the difference of the means.
For oxygen metabolism experiments, change in the slopes of oxygen concentration versus time in the dark (respiration) and in the light (respiration and photosynthesis) was calculated by subtracting slopes of oxygen concentration versus time before incubation from slopes of oxygen concentration versus time after incubation (Netherton et al., 2014). Colonies incubated in BA seawater served as negative controls. For within-treatment analyses, a paired sample t-test was employed to compare differences in oxygen metabolism in the dark and in the light for each colony before and after incubation. Between-treatment comparisons were performed using student’s t-test.

Results

**ROS Measures**

In experiments with +1mM HCO₃⁻, nested ANOVA revealed that there was a significant within-treatment effect for BE incubated colonies of both species (Sympodium sp., F₂₈,₆₀=4.27, P<0.05; Sarcothelia sp., F₂₆,₅₆=5.25, P<0.05). In other words, ROS production between colonies within the same treatment varied significantly. For Sympodium sp., BA incubated colonies produced significantly more ROS then BE incubated colonies (F₁,₆₀=5.74, P<0.05), however, when the mean square of the within-treatment effect was used as an error term, the significant effect disappeared (F₁,₆₀=1.34, P>0.05). No significant difference in ROS formation was found between colonies of BA or BE incubated colonies of Sarcothelia sp., both with (F₁,₅₆=0.14, P>0.05) and without (F₁,₅₆=0.75, P>0.05) consideration of the significant within-treatment effect.
Similar trends were found for BE colonies in experiments with +3mM HCO$_3^-$ . Again, a highly significant within-treatment effect was revealed for both species (Sympodium sp., $F_{28,60}=5.60$, $P<0.05$; Sarcothelia sp., $F_{28,60}=5.77$, $P<0.05$). Between-treatment analyses for Sympodium sp. revealed no significant difference in ROS formation for BA and BE incubated colonies ($F_{1,60}=2.43$, $P>0.05$). In contrast, BA incubated colonies of Sarcothelia sp. produced significantly more ROS than BE incubated colonies ($F_{1,60}=30.7$, $P<0.05$) and this effect remained significant when the mean square of the significant within-treatment effect was used as an error term ($F_{1,28}=5.32$, $P<0.05$).

The next set of experiments involved incubating entirely in the dark with seawater enriched with +3mM HCO$_3^-$ for BE incubated colonies. A significant within-treatment effect was also found for both species (Sympodium sp., $F_{28,60}=3.60$, $P<0.05$; Sarcothelia sp., $F_{28,60}=1.79$, $P<0.05$). For Sympodium sp., colonies incubated in the dark in BE seawater produced significantly more ROS than BA incubated colonies ($F_{1,60}=56.0$, $P<0.05$), and this effect remained highly significant even when the mean square of the significant within-treatment effect was used as an error term ($F_{1,60}=15.5$, $P<0.05$). This was not the case for Sarcothelia sp., where BA and BE colonies incubated in the dark produced similar amounts of ROS ($F_{1,60}=1.20$, $P>0.05$).

Analyses of ROS data using a nested ANOVA, with colonies nested within treatments, revealed a significant colonies-within-treatment effect in all experiments. Pairing ROS measures in time and comparing the mean of their differences to zero can alleviate the significant colonies-within-treatment effects, and provided the rationale for further analyzing ROS data with a paired sample t-test. For experiments with +1mM HCO$_3^-$, BE colonies of Sympodium sp. produced significantly less ROS than BA colonies (paired sample t-test, $t=-2.48$, d.f.=44, $p<0.05$; figure 2),
whereas there was no difference in ROS production between BA and BE colonies of *Sarcothelia* sp. (*t*=-0.601, d.f.=41, *P*<0.05; figure 3). In experiments with +3mM HCO$_3^-$, ROS production did not differ significantly between BA and BE colonies of *Sympodium* sp. (*t*=1.33, d.f.=44, *P*<0.05). However, the difference was highly significant between BA and BE colonies of *Sarcothelia* sp. (*t*=-4.65, d.f.=44, *P*<0.05), with BE colonies producing less ROS. When colonies were incubated entirely in the dark and BE colonies were exposed to +3mM HCO$_3^-$, a significant increase in ROS formation was found in BE colonies of *Sympodium* sp. relative to BA colonies (*t*=4.17, d.f.=44, *P*<0.001), but there was no significant difference between BA and BE colonies of *Sarcothelia* sp. (*t*=1.03, d.f.=44, *P*>0.05).
Figure 2. ROS in colonies of *Sympodium* sp.
Relative luminance of fluorescence due to ROS was measured by subtracting background luminance from foreground (coenenchyme) luminance, thus eliminating the effect of background luminance. All three bicarbonate enrichment treatments are represented with negative control and treated results from each experiment placed together for comparison. Bold horizontal lines represent the mean, upper and lower limits of boxes represent 95% confidence intervals, and vertical lines indicate minimum and maximum values. “+ x mM” represents the experiment. BA = bicarbonate ambient (negative control, no added bicarbonate) and BE = bicarbonate enriched (treated).
Figure 3. ROS in colonies of Sarcothelia sp. Relative luminance of fluorescence due to ROS was measured by subtracting background luminance from foreground (stolon) luminance, thus eliminating the effect of background luminance. All three bicarbonate enrichment treatments are represented with negative control and treated results from each experiment placed together for comparison. Bold horizontal lines represent the mean, upper and lower limits of boxes represent 95% confidence intervals, and vertical lines indicate minimum and maximum values. “+ x mM” represents the experiment. BA = bicarbonate ambient (negative control, no added bicarbonate) and BE = bicarbonate enriched (treated).

Due to the importance of timing in capturing ROS formation, comparisons between treatments that were carried out at different times were not explored explicitly using statistical tests. Comparisons of mean relative luminance ± 95% confidence intervals (CI) are best suited for these analyses. ROS production was similar for BA incubated colonies (negative controls) in both +1mM HCO$_3^-$ and +3mM HCO$_3^-$ experiments for Sympodium sp. (means ± 95% CI; +1mM...
HCO$_3^-$, 941 ± 161; +3mM HCO$_3^-$, 925 ± 174; figure 2) and *Sarcothelia* sp. (+1mM HCO$_3^-$, 1290 ± 241; +3mM HCO$_3^-$, 1350 ± 209; figure 3). Since BA colonies served as negative controls, the similarity of ROS formation between experiments confirms that the incubation procedures were consistent and provides support for these comparisons. In BE colonies of *Sympodium* sp., ROS formation was higher in colonies treated with +3mM HCO$_3^-$ (1060 ± 217; figure 3) relative to those treated with +1mM HCO$_3^-$ (763 ± 141). *Sarcothelia* sp. demonstrated the opposite, with colonies incubated with +1mM HCO$_3^-$ (1200 ± 213; figure 3) producing more ROS than colonies incubated with +3mM HCO$_3^-$ (919 ± 130).

Within species comparisons of ROS formation between the two experiments in which 3mM HCO$_3^-$ was added provides insight into the role of light when DIC levels are elevated. Differences in relative luminance for *Sympodium* sp. (figure 2) show that colonies exposed to excessive light with +3mM HCO$_3^-$ (means ± 95% CI; 1060 ± 217; figure 2) produced more ROS than colonies incubated entirely in the dark with +3mM HCO$_3^-$ (427 ± 88.9). The same difference in ROS formation was also found for colonies of *Sarcothelia* sp. exposed to excessive light with +3mM HCO$_3^-$ (919 ± 130; figure 3) versus the same treatment in the dark (344 ± 57.9). Differences in ROS formation between colonies exposed to excessive light and colonies incubated the dark at the same [HCO$_3^-$] suggests that the input of light plays a critical role in the response of symbionts to excess HCO$_3^-$.

Of course, all colonies were exposed to significant light levels (900 μmol photons m$^{-2}$ s$^{-1}$) during the imaging process, which has been shown to trigger ROS formation even in dark adapted colonies (Parrin et al. 2017).

Comparisons of the means ± 95% CI are also informative in considering differences in ROS formation between species. In the +1mM HCO$_3^-$ experiments with excessive light exposure, BA colonies of *Sympodium* sp. produced less ROS than BA incubated colonies of
Sarcothelia sp. (means ± 95% CI; Sympodium sp., 941 ± 161, figure 2; Sarcothelia sp., 1290 ± 241; figure 3). The same trend was seen in BA colonies of both species in the +3mM HCO$_3^-$ experiments, with Sympodium sp. producing less ROS than Sarcothelia sp. (Sympodium sp., 925 ± 174; Sarcothelia sp., 1350 ± 209). Between species comparisons for treated colonies reveal that with +1mM HCO$_3^-$, BE colonies of Sympodium sp. produced less ROS than BE colonies of Sarcothelia sp. (Sympodium sp., 763 ± 141; Sarcothelia sp., 1200 ± 213). Interestingly though, this difference disappeared in experiments with +3mM HCO$_3^-$ and BE colonies of both species produced similar amounts of ROS (Sympodium sp., 1060 ± 217; Sarcothelia sp., 919 ± 130). When incubated in the dark with +3mM HCO$_3^-$, ROS formation was also similar between colonies of Sympodium sp. and Sarcothelia sp. (Sympodium sp., 427 ± 88.9; Sarcothelia sp., 344 ± 57.9).

**Oxygen Metabolism Measures**

For colonies incubated in BA seawater, there was no significant difference in oxygen metabolism in the dark before and after the incubation period (mean ± s.e.m.: before, -0.021 ± 0.0023 mg l$^{-1}$ min$^{-1}$; after, -0.023 ± 0.0022; paired sample t-test, $t$=2.0, d.f.=14, $P$>0.05; figure 4). This was not true for colonies incubated in BE seawater, which showed a significant increase in respiration after the incubation period (before, -0.020 ± 0.0019; after, -0.024 ± 0.0016; $t$=3.5, d.f.=14, $P$<0.05; figure 4). This indicates that incubation in bicarbonate enriched seawater may impose a physiological stress leading to an increase in respiration. Increase in respiration has been a consistent response in stressed colonies of Sarcothelia sp. and the octocoral Phenganyx parrinii (Netherton et al., 2014). Oxygen metabolism in the light was significantly depressed
after incubation in both BA (before, 0.055 ± 0.0068; after, 0.026 ± 0.0039; \( t=6.2, \) d.f.=13, \( P<0.05; \) figure 4) and BE (before, 0.055 ± 0.0071; after, 0.028 ± 0.0047; \( t=5.7, \) d.f.=14, \( P<0.05; \) figure 4) incubated colonies. These data show that BA and BE incubated colonies both experienced a highly significant decline in oxygen evolution after the incubation period, a clear indication that symbiont photosynthesis was impaired. However, incubation in the BE seawater did not appear to exacerbate or mitigate photoinhibition due to excessive light exposure; both treatments resulted in a roughly 50% decrease in oxygen evolution.

Changes in the slopes of oxygen concentration versus time measured in the dark for colonies of *Sympodium* sp. incubated in BA seawater (negative controls) were not significantly different compared to colonies incubated in BE seawater (BA, -0.0024 ± 0.0012, N=15; BE, -0.0039 ± 0.0011, N=15; t-test with equal variances, \( t=0.91, \) d.f.=28, \( P>0.05 \)), however a trend of increased respiration following the incubation was noted. Changes in oxygen evolution in the light were also not significantly different between colonies incubated in BA seawater and those incubated in BE seawater (BA, -0.030 ± 0.0047, N=14; BE, -0.027 ± 0.019, N=14; \( t=-0.38, \) d.f.=26, \( P>0.05 \)). This suggests that the confinement in finger bowls and the incubation period had the same effect on oxygen metabolism both in the dark and in the light for BA and BE incubated colonies.
Figure 4. Oxygen metabolism of *Sympodium* sp. colonies before and after incubation. Changes in oxygen concentration (mean ± s.e.m) following 30 minutes in the dark and 30 minutes in the light are shown. For “before” measures, two colonies were taken directly from the aquarium in which they were grown and measured for oxygen metabolism. For “after” measures, one colony was incubated in bicarbonate ambient seawater (BA), while the other was incubated in bicarbonate enriched seawater (BE; \[\text{HCO}_3^-\] ≈ 3mM). Incubations occurred for 12 h in the dark followed by 4 h under excessive light (140 µmol photons m\(^{-2}\) s\(^{-1}\)) at 27ºC. Colonies were then remeasured for oxygen metabolism. Both BA and BE incubated colonies showed small increases in oxygen metabolism in the dark (i.e., respiration) and a significant decline in oxygen metabolism in the light (i.e., photosynthesis plus respiration) following the incubation.
Due to the length of time it took to measure oxygen metabolism, the second colony measured remained in the incubator for an additional hour. To determine whether this additional hour of incubation influenced oxygen metabolism, within treatment comparisons were made between colonies measured first and those measured second. The order of measurement had no significant effect on the change in oxygen metabolism in the dark for BA colonies (first, -0.0013 ± -0.0018, N=8; second, -0.0030 ± 0.0017, N=6; t-test with equal variances, \( t = 0.69 \), d.f.=12, \( P > 0.05 \)) or BE colonies (first, -0.0042 ± 0.0019, N=6; second, -0.0041 ± 0.0016, N=8; \( t = 0.04 \), d.f.=12, \( P > 0.05 \)). Similar results were found for oxygen metabolism in the light, with the additional hour of incubation having no significant effect on the change in oxygen evolution for BA colonies (first, -0.027 ± 0.0049, N=8; second, -0.033 ± 0.0094, N=6; \( t = 0.60 \), d.f.=12, \( P > 0.05 \)) and BE colonies (first, -0.021 ± 0.010, N=6; second, -0.031 ± 0.0048, N=8; \( t = -0.98 \), d.f.=12, \( P > 0.05 \)). Thus, the extra hour of incubation endured by colonies measured second did not significantly impact changes in oxygen metabolism before and after the incubation period, both in the dark or in the light, in BA or BE incubated colonies.

Discussion

Ecological communities built by corals are some of the most biodiverse in the world, and the current rate of climate change threatens their long-term persistence. The future of coral reefs is still uncertain (Pandolfi et al., 2011); however, it is generally accepted that coral bleaching increases the risk of death in affected corals and may have ecosystem-scale effects (Weis, 2008, Hoegh-Guldberg, 2007). Elevated sea surface temperatures is the most well-studied stressor of the coral/Symbiodinium symbiosis, but another major aspect of climate change is ocean
acidification, which occurs as the ocean absorbs CO₂ from the atmosphere. The result of ocean acidification is a decline in seawater pH and a shift in the speciation of seawater DIC, most notably reducing the concentration of CO₃²⁻ ions and increasing the concentration of HCO₃⁻ ions (Caldeira and Wickett, 2005). The seawater DIC pool is the ultimate source of photosynthetic substrate for *Symbiodinium* symbionts in corals, thus the change in seawater DIC speciation that results from ocean acidification stimulates many questions about how it will impact the coral/*Symbiodinium* symbiosis and symbiont photosynthesis.

Corals remain one of the few animals that can harness the energetic output of photosynthesis, achieved through maintaining their partnership with dinoflagellate algae of the genus *Symbiodinium*. These symbioses are the foundation on which coral reef communities rely (Ramsby et al., 2014), and the success of the host coral depends on symbiont productivity. Nevertheless, the coral/*Symbiodinium* partnership is surprisingly sensitive to adverse environmental conditions, such as too much heat or light (Weis 2008; Parrin et al. 2017), given that it has persisted through 10’s of millions of years of natural selection (Pochon et al., 2006). The collapse of mechanisms of conflict mediation and bleaching may represent the failure of an attempt at evolutionary “individuality” in corals (Blackstone and Golladay 2018). Bleaching is rarely conceptualized in this manner, yet experiments relevant to this notion have been carried out (Fitt and Trench, 1983; Wilkerson et al., 1988; Baghdasarian and Muscatine, 2000; Stat et al., 2008; Baker et al., 2018). Nonetheless, if the failure of the symbiosis initiates with the breakdown of symbiont photosynthesis and subsequent ROS formation, increasing the availability of CO₂ could potentially provide a “sink” for electrons, thereby reducing the likelihood that ROS form and mitigating oxidative stress.
Octocorals are an important constituent of coral reef communities and are often the dominant group represented (McFadden et al., 2010), yet research exploring the effects of ocean acidification on these critical reef animals is still lacking. The study presented here is the first to examine how bicarbonate enrichment influences oxidative stress in octocorals utilizing fluorescence microscopy. Results suggest that bicarbonate enrichment may mitigate oxidative stress resulting from impaired symbiont photosynthesis in perturbed octocorals. Nevertheless, there are many aspects to climate change, presenting a myriad of potential interactions, and bicarbonate enrichment may elicit different responses in conjunction with other environmental stressors.

Predicting how ocean acidification will affect symbiotic octocorals is complicated by their dependence on photosynthetic symbionts. Symbionts rely on DIC in seawater for photosynthetic substrate (Davy et al., 2012), and changes in the speciation of DIC may alter substrate availability, ultimately affecting symbiont productivity and the coral/Symbiodinium symbiosis. The availability of substrate for symbiont photosynthesis is further complicated by their location inside endodermal cells within their hosts, where they rely on host CCMs to supply substrate for photosynthesis (Goiran et al., 1996; Wooldridge, 2013). Since the CCMs actively transport HCO$_3^-$ into the coral where it is converted into CO$_2$ by carbonic anhydrases, it is thought that increases in the HCO$_3^-$ ion concentration in seawater associated with ocean acidification may supplement symbiont photosynthesis (Mackey et al., 2015; Cole et al., 2018), so long as host coral CCMs do not fail (Wooldridge, 2013). If host CCMs fail, then no amount of additional substrate will alleviate impaired symbiont photosynthesis. However, the results presented here do not suggest the failure of host CCMs during exposure to excessive light stress, as both species showed either similar or reduced production of ROS when incubated in BE.
seawater with +1 mM HCO$_3^-$ relative to colonies incubated in BA seawater. Colonies of *Sarcothelia* sp. also produced significantly less ROS when incubated in BE seawater with +3 mM HCO$_3^-$ compared to colonies incubated in BA seawater. This suggests that host CCMs were functioning normally and increased concentration of HCO$_3^-$ may have supplemented impaired symbiont photosynthesis, preventing alternative electron flow and subsequent ROS production. Importantly, though, if water splitting fails, the formation of ROS other than singlet oxygen ceases. Nevertheless, increasing seawater temperatures are a significant aspect of climate change and the results reported here should not be interpreted to imply that coral CCMs will not fail under prolonged exposure to elevated seawater temperature.

Oxidative stress is a well-known instigator of programmed cell death (Weis, 2008) and has been implicated as one of the first signs of stress in corals, potentially acting as a trigger for symbiont expulsion (Downs et al., 2002). The sequence of events for colonies stressed by excessive light in the current study may be briefly summarized as follows: (1) prolonged exposure to excessive light led to photoinhibition and alternative electron flow, resulting in the formation of ROS; (2) ROS triggered symbiont migration, programmed cell death, or both; and (3) dead or damaged symbionts are either digested by the host or migrate through the gastrovascular cavity and out of the mouth. Visual observations in this study during the ROS experiments support this sequence, with symbiont migration seen in nearly all colonies of *Sympodium* sp. and *Sarcothelia* sp., however the expulsion of symbionts was not common. The lack of expelled symbionts, though, supports the theory that ROS formation and migration precede expulsion. Recently, though, the role of ROS as one of the first signals of the cnidarian stress response and as a driver of symbiont migration and expulsion has been called into question. For instance, Nielsen et al. (2018) incubated colonies of *Pocillopora damicornis* for
two weeks at elevated temperatures and found significant amounts of ROS in symbiont cells but not in host cells, and concluded that damage associated with ROS-leakage was not an initiator of symbiont expulsion. However, in these experiments cells containing symbionts were isolated from the host coral prior to ROS visualization, potentially limiting the applicability of their results to the entire host/symbiont assembly. The results presented here, as well as the work of Netherton et al. (2014) and Parrin et al. (2017), suggest that both timing and *in vivo* measurements are critical when attempting to visualize ROS in stressed corals and their symbionts.

Bursts of ROS in stressed colonies of both species occurred shortly after the onset of illumination. In some instances, treated colonies would show little ROS formation but still looked visually perturbed, with contracted polyps and coenenchyme edges, suggesting the burst of ROS possibly occurred before imaging. In addition, a trend was noticed during each imaging session in which ROS formation typically increased throughout the imaging process for each colony and as the incubation period grew longer (i.e. the first image captured for a colony typically showed less ROS than the third image captured, and the first colony imaged usually had less ROS than the last colony imaged), further supporting the importance of timing in visualizing ROS via fluorescence microscopy. Nevertheless, this does not detract from the significance of ROS formation as one of the early signs of stress in symbiotic octocorals.

In *Sympodium* sp. ROS formation was lower in colonies exposed to the low bicarbonate treatment but higher in those exposed to the moderate bicarbonate treatment. Potentially, symbiont photosynthesis is DIC limited at the current levels of seawater [HCO$_3^-$], and small increases in HCO$_3^-$ may serve as a supplement, while moderate increases (e.g. up to [HCO$_3^-$] $\approx$ 5 mM) surpass the saturation point of symbiont photosynthesis, and the associated decline in pH
potentially counteracts the benefits of an increased DIC supply (Mackey et al., 2015). In fact, Weis (1993) demonstrated that rates of photosynthesis in the sea anemone *Aiptasia pulchella*, a symbiotic cnidarian also harboring *Symbiodinium* spp., increased with DIC concentration up to \([\text{HCO}_3^-] \approx 5 \text{ mM}\), at which point photosynthesis appeared to be saturated. As well, Herfort et al. (2008) found that bicarbonate enrichment up to 6 mM stimulated photosynthesis in the scleractinian corals *Porites porites* and *Acropora* sp. Interestingly though, comparison of the +3mM HCO\(_3^-\) experiments with excessive light exposure to those conducted in the dark highlight the role of light when DIC concentrations are increased. For instance, BA and BE colonies of *Sympodium* sp. produced similar amounts of ROS when exposed to excessive light, but when incubated in the dark BE colonies produced more ROS than BA colonies. This suggests that in the dark or during conditions when photosynthesis is not proceeding rapidly, an increased DIC supply may not provide any benefit and could be detrimental. However, for colonies of *Sarcothelia* sp., ROS formation was the same in BE and BA incubated in the dark, but when exposed to excessive light BA colonies produced significantly more ROS than BE colonies. For *Sarcothelia* sp. it appears that increased DIC had no effect in the dark but may have alleviated oxidative stress in the light. Additional experiments in which light levels are varied (e.g., low, moderate, high) at different levels of bicarbonate enrichment (e.g., +1mM, +2mM, +3mM) should be considered to further elucidate the synergistic effects of light and DIC enrichment on oxidative stress in corals.

Differences in response to bicarbonate enrichment between *Sympodium* sp. and *Sarcothelia* sp. may also be dependent upon the strains of symbionts present in each species. *Sarcothelia* sp. contain Clade D4-5-9 symbionts and *Sympodium* sp. contain a Clade C1 variant (Parrin et al., 2016). Here, in experiments conducted with +1mM HCO\(_3^-\), *Sarcothelia* sp. (clade
D symbionts) produced more ROS than *Sympodium* sp. (clade C symbionts). However, at higher levels of HCO$_3^-$ (+3mM), *Sarcothelia* sp. produce less ROS than *Sympodium* sp. Some studies have found differences in photosynthetic productivity and bleaching in coral containing clade D symbionts versus clade C symbionts (McGinty et al., 2012; Lesser et al., 2013; Parrin et al. 2016). For example, McGinty et al. 2012 exposed various *Symbiodinium* spp. types (A1, B1, B2, C1, D, E1, and F2) to elevated temperatures and found that clade C symbionts produced the most ROS, while clade D symbionts were the only type to remain unaffected. However, these results should be interpreted with caution because they were obtained using cultured *Symbiodinium* spp. Isolated symbionts may eventually acclimate to culture conditions and not accurately represent the response of symbionts *in hospite* (within the host).

Oxygen metabolism experiments involving *Sympodium* sp. demonstrated that the addition of HCO$_3^-$ did not exacerbate or mitigate impaired photosynthesis. For both BA and BE incubated colonies, oxygen metabolism in the light decreased by approximately 50% after the incubation period, suggesting that symbiont photosynthesis was impaired. Exposure to excessive light and UV radiation is well known to lead to photoinhibition of symbiont photosynthesis and the production of ROS (Dykens et al., 1992; Lesser, 2004; Ferrier-Pages et al., 2007). These results demonstrate that photoinhibition due to excessive light, however, was not exacerbated by the additional HCO$_3^-$ or any associated initial changes in pH. Oxygen metabolism in the dark did not follow the same trend and remained the same before and after incubation for BA colonies, but significantly increased in BE incubated colonies relative to measures before incubation. Increased respiration suggests that the added HCO$_3^-$ imposed some additional physiological stress to BE incubated colonies, a finding that has been consistent in stressed octocorals (Netherton et al., 2014).
The results presented here suggest that bicarbonate enrichment may act to alleviate oxidative stress resulting from photoinhibition, possibly by supplementing symbiont photosynthesis under saturating irradiance. Nevertheless, changes in the speciation of DIC resulting from ocean acidification is only one small aspect of climate change. Increasing sea surface temperatures, changes in sea level, and ocean acidification all present unique challenges to corals and their symbionts, and the interactive effects of these stressors must be further explored to better assess future of reefs. The laboratory models implemented here allowed for detailed visualization of symbiont migration and ROS formation *in vivo* at the microscopic scale. They should continue to be used in future experiments examining the interactive effects of environmental stressors because they can provide new insights into the physiology of the cnidarian stress response.
LITERATURE CITED


