Stimulated Respiration and Net Photosynthesis in *Cassiopeia* sp. during Glucose Enrichment Suggests *in hospite* CO$_2$ Limitation of Algal Endosymbionts

Nils Rädecker[1†], Claudia Pogoreutz[1†], Christian Wild[2] and Christian R. Voolstra[1]*

[1] *Biological and Environmental Sciences and Engineering Division, Red Sea Research Center, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia,* [2] *Marine Ecology Working Group, Faculty of Biology and Chemistry (FB 2), University of Bremen, Bremen, Germany*

The endosymbiosis between cnidarians and dinoflagellates of the genus *Symbiodinium* is key to the high productivity of tropical coral reefs. In this endosymbiosis, *Symbiodinium* translocate most of their photosynthates to their animal host in exchange for inorganic nutrients. Among these, carbon dioxide (CO$_2$) derived from host respiration helps to meet the carbon requirements to sustain photosynthesis of the dinoflagellates. Nonetheless, recent studies suggest that productivity in symbiotic cnidarians such as corals is CO$_2$-limited. Here we show that glucose enrichment stimulates respiration and gross photosynthesis rates by 80 and 140%, respectively, in the symbiotic upside-down jellyfish *Cassiopeia* sp. from the Central Red Sea. Our findings show that glucose was rapidly consumed and respired within the *Cassiopeia* sp. holobiont. The resulting increase of CO$_2$ availability *in hospite* in turn likely stimulated photosynthesis in *Symbiodinium*. Hence, the increase of photosynthesis under these conditions suggests that CO$_2$ limitation of *Symbiodinium* is a common feature of stable cnidarian holobionts and that the stimulation of holobiont metabolism may attenuate this CO$_2$ limitation.

Keywords: symbiosis, *Symbiodinium*, carbon limitation, heterotrophy, upside-down jellyfish

INTRODUCTION

Despite being surrounded by highly nutrient-poor (oligotrophic) waters, tropical coral reefs are among the most productive marine ecosystems (Hatcher, 1988). Reef ecosystems are sustained by an efficient uptake, retention, and reuse of nutrients on all levels of biological organization (Hatcher, 1990; Wild et al., 2004). In particular, the symbiosis between cnidarian hosts and endosymbiotic algae of the genus *Symbiodinium* facilitates the recycling of nutrients as it sustains primary productivity in the absence of major nutrient sources (Muscatine and Porter, 1977; Rädecker et al., 2015). In this symbiosis, *Symbiodinium* translocate most of their photosynthates to the cnidarian host that in turn provides inorganic nutrients derived from its metabolism (Muscatine et al., 1989). Thereby, this tight nutrient-exchange relationship, particularly in stony corals, is the functional basis for the ecological success of tropical coral reefs over millions of years.

Being surrounded by host membranes, *Symbiodinium* rely on their host to fulfill their photosynthetic carbon dioxide (CO$_2$) requirements. The supply of CO$_2$ to the symbiont

is controlled by two major processes: (1) CO_2 is produced during holobiont respiration (Muscatine et al., 1989). (2) Active carbon concentrating mechanisms (CCMs) by the host facilitate the uptake of dissolved inorganic carbon from surrounding seawater (Furla et al., 2000).

Despite these processes, several studies suggest that productivity in *Symbiodinium* may be carbon-limited even in stable symbiotic systems (Muscatine et al., 1989; Herfort et al., 2008; Klein et al., 2017). Hence, understanding the processes and environmental controls of *in hospite* CO_2 availability is crucial for our understanding of the cnidarian—alga symbiosis.

To address this issue, we experimentally tested whether photosynthesis of *Symbiodinium in hospite* is carbon-limited. Specifically, we investigated photosynthetic activity during glucose-stimulated holobiont respiration in the upside-down jellyfish *Cassiopeia* sp.. Unlike most other Scyphozoa, *Cassiopeia* spp. are mixotrophic, i.e. draw energy and nutrients from both heterotrophic and autotrophic sources (Rahav et al., 1989; Muscatine, 1990), as they form a close endosymbiotic relationship with *Symbiodinium*. Thereby, *Cassiopeia* spp. offer distinct advantages for the study of the cnidarian—alga symbiosis, similar to the Aiptasia model system (Baumgarten et al., 2015). For instance, they are easy to rear in aquaria cultures, are non-calcifying, have motile medusa stages and can be infected with various algal symbionts (Klein et al., 2017). Using this emerging cnidarian model system allowed us to tackle the issue of CO_2 limitation in the cnidarian—*Symbiodinium* symbiosis in a straightforward experiment.

METHODS

Collection and Maintenance

A total of 14 individuals of *Cassiopeia* sp. (mean bell diameter of 6.9 ± 0.3 cm) were collected with a dip net in the KAUST Harbor Lagoon, Saudi Arabia (N22°18′18.63″, E39°6′10.45″) in the Central Red Sea in September 2014. After collection, animals were immediately transferred to 2 recirculation aquaria (each filled with 20 L of ambient seawater) and acclimated to aquaria conditions for 7 days (salinity of 40, 28°C, 12:12 h light/dark cycle with ~ 100 µmol m^{-2} s^{-1}). Stability of water parameters was ensured by exchanging 50% of aquaria seawater daily.
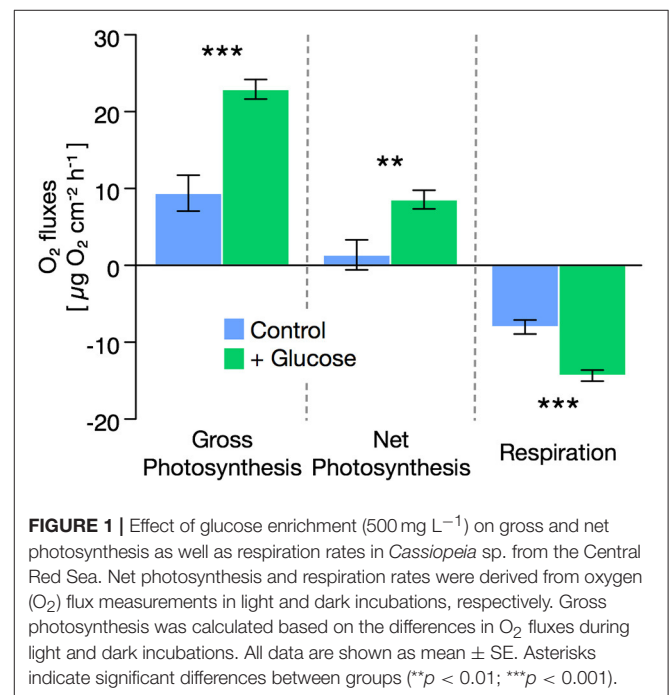
Incubations and Glucose Enrichment

Following acclimation, net photosynthesis and respiration rates of animals were directly assessed from oxygen (O_2) evolution/depletion measurements in 2 h light and dark incubations in 1 L gas-tight glass chambers, respectively. During these incubations, half of the animals were incubated in ambient seawater freshly enriched with glucose (500 mg L^{-1}). The other half of the animals served as a control and were incubated in ambient seawater. To correct jellyfish O_2 fluxes for planktonic background metabolism, two seawater controls (i.e., ambient seawater without jellyfish) were included for each treatment. Importantly, the dissolved organic carbon concentrations used here do not reflect naturally occurring ambient reef water conditions (Vaccaro et al., 1968; Kline et al., 2006). Rather, the level of enrichment was chosen to avoid glucose depletion

over the course of the incubation and to ensure that effects of increased carbon availability were not buffered within the holobiont framework, in order to gain mechanistic insights into the cnidarian—alga symbiosis.

O_2 fluxes were assessed based on differences in O_2 concentrations before and after the incubation using an optical oxygen multiprobe (WTW, Germany). O_2 production/consumption rates were corrected for seawater controls and normalized to bell surface area of animals and incubation time. Gross photosynthesis rates were calculated based on differences in O_2 fluxes during light and dark incubations (gross photosynthesis = net photosynthesis + |respiration|). Differences between treatments for the individual response parameters were tested for significance using an unpaired Student's *t*-test with a significance level (α) of 0.05.

RESULTS AND DISCUSSION

Glucose enrichment stimulated respiration rates in seawater during both light and dark incubations (Supplementary Table S1). Still, seawater respiration rates were ~ 5-fold below *Cassiopeia* sp. respiration rates at all times. Holobiont respiration rates of *Cassiopeia* sp. increased by $\sim 80\%$ under glucose-enriched conditions compared to untreated controls $[t_{(13)} = 5.27, \ P < 0.001, $ **Figure 1**]. Despite this increase in respiratory O_2 consumption, net photosynthesis rates during glucose-enriched conditions showed a significant increase of nearly 400% compared to controls $[t_{(13)} = 3.08, P = 0.008]$. Consequently, gross photosynthesis rates increased by $\sim 140\%$ under glucose-enriched conditions compared to untreated controls $[t_{(13)} = 4.94, P < 0.001]$.

FIGURE 1 | Effect of glucose enrichment (500 mg L^{-1}) on gross and net photosynthesis as well as respiration rates in *Cassiopeia* sp. from the Central Red Sea. Net photosynthesis and respiration rates were derived from oxygen (O_2) flux measurements in light and dark incubations, respectively. Gross photosynthesis was calculated based on the differences in O_2 fluxes during light and dark incubations. All data are shown as mean ± SE. Asterisks indicate significant differences between groups (**$p < 0.01$; ***$p < 0.001$).

Glucose enrichment, hence, not only stimulated respiration rates but also caused a stark increase in photosynthetic activity in the mixotrophic cnidarian holobiont *Cassiopeia* sp.. The increase in respiration rates indicates that glucose was rapidly taken up and consumed (i.e., respired) within the holobiont (Pogoreutz et al., 2017). Given our current understanding of cnidarian holobionts, there is no reason to assume that glucose enrichment directly affected photosynthetic activity in *Symbiodinium*. Rather, the observed increase in net and gross photosynthesis can be attributed to an increase in CO_2 availability *in hospite*, stemming from increased respiration in the *Cassiopeia* holobiont and seawater planktonic communities within the incubation chamber. In the case of *Cassiopeia* sp. this CO_2 limitation may be potentially attenuated by their continuous pumping motion facilitating increased gas exchange with the surrounding seawater (Wild and Naumann, 2013).

On a broader scale, these results could have implications for our understanding of the mechanisms underlying the cnidarian—alga symbiosis. The observation of glucose-stimulated photosynthesis implies that productivity of *Symbiodinium in hospite* may be tightly limited by CO_2 derived from holobiont metabolism.

Wooldridge (2009) proposed that a failure of coral CCMs during heat stress may ultimately result in a CO_2 limitation of photosynthetic dark reactions in *Symbiodinium*, ultimately leading to coral bleaching. Direct empirical evidence for this theory is missing to date. Our results, therefore, add to a growing emerging body of work suggesting that *Symbiodinium* may be CO_2-limited even in stable symbiotic systems (Muscatine et al., 1989; Herfort et al., 2008; Buxton et al., 2009; Klein et al., 2017). Hence, environmental stressors which alter metabolic processes in the holobiont may indeed lead to severe CO_2 limitation as predicted by Wooldridge (2009). Furthermore, we could show that the stimulation of host heterotrophy may attenuate CO_2 limitation in *Symbiodinium*. In this context, several studies reported that increased heterotrophic feeding may mitigate the effects of thermal stress in reef-building corals, resulting in increased bleaching resilience (Grottoli et al., 2006; Baird et al., 2009; Houlbrèque and Ferrier-Pagès, 2009; Ezzat et al., 2016). While this effect was mostly attributed to a compensation of autotrophic with heterotrophic energy sources by the host, here we show that heterotrophy may also increase bleaching resilience by increasing CO_2 availability *in hospite*.

Taken together, our study highlights that the role of CO_2 availability within the cnidarian—algae symbiosis deserves further in-depth assessment. Further work will be necessary to understand the effects of environmental conditions on CO_2 availability *in hospite*, along with their implications for the cnidarian—alga symbiosis.

AUTHOR CONTRIBUTIONS

NR and CP designed and conducted the experiment. All authors analyzed the data and wrote and revised the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmars.2017.00267/full#supplementary-material

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