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Physiological responses of corals to ocean acidification and copper exposure

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ABSTRACT

Acidification and land-based sources of pollution have been linked to widespread declines of coral cover in coastal reef ecosystems. In this study, two coral species, *Acropora cervicornis* and *Pocillopora damicornis* were exposed to increased copper at two CO_2 levels for 96 h. Copper accumulation and anti-oxidant enzyme activities were measured. Copper accumulation only increased in *A. cervicornis* zooxanthellae and corresponded with photosynthetic toxicity. Enzyme activities in both coral species were affected; however, *A. cervicornis* was more sensitive than *P. damicornis*, and zooxanthellae were more affected than animal fractions of holobionts. Generally, activities of all anti-oxidant enzymes increased, with copper exposure in corals; whereas, activities of glutathione reductase and to some degree glutathione peroxidase were observed due to increasing CO_2 exposure alone. Exposure to copper in combination with higher CO_2 resulted in a synergistic response in some cases. These results provide insight into mechanisms of copper and CO_2 impacts in corals.

1. Introduction

Rising atmospheric carbon dioxide (CO_2), mainly due to anthropogenic activities, is rapidly changing the oceanic carbonate system (partial pressure of CO_2, pH, and alkalinity), resulting in ocean acidification (Hoegh-Guldberg, 1999; Anthony et al., 2011; Takahashi et al., 2012). Globally, oceanic CO_2 has increased by 30% over pre-industrial levels resulting in a 0.1 pH decrease (Caldeira and Wickett, 2003; Siegenthaler et al., 2005). At the current rate of change, atmospheric CO_2 levels are predicted to increase up to 1000 μAtm (~0.4 pH decrease) by the end of this century and up to 1900 μAtm (~0.8 pH decrease) by 2300 (Caldeira and Wickett, 2003; Orr et al., 2005). The carbonate system is such that CO_2 and water react to form carbonic acid, which then can dissociate to bicarbonate, and further dissociate to carbonate. Shifting the balance between these different carbon species may have dramatic effects on aquatic life, depending on which carbon species dominates. Corals and other marine calcifying organisms are particularly threatened due to the decreased calcium carbonate ($CaCO_3$) saturation state caused by ocean acidification (Orr et al., 2005; Fabry, 2008; Guinotte and Fabry, 2008; Anthony et al., 2011; Ateweberhan et al., 2013). Calcification involves precipitation of dissolved ions into solid $CaCO_3$ structures, which can be subsequently dissolved if seawater does not contain saturating carbonate ion concentrations. This problem may be exacerbated by increased pollution.

Coral reefs provide essential habitats for a wide array of marine life and are also among the world's most fragile and endangered ecosystems (Howard and Brown, 1984). Scleractinian (stony) corals have a mutualistic relationship with endosymbiotic dinoflagellates in the genus *Symbiodinium* (often referred to as "zooxanthellae"). The decline of coral reef ecosystems has been linked with global climate change and disease, ocean acidification, habitat destruction, pollution, and poor water quality (Hughes et al., 2003; Ateweberhan et al., 2013). Reefs in near shore environments close to heavily populated areas with substantial anthropogenic inputs are particularly threatened from combined exposure of multiple interacting stressors. Global climate change and other stressors have been found to disrupt the mutualistic relationship of corals and their endosymbiotic dinoflagellate (zooxanthellae), resulting in coral "bleaching" (loss of algal symbionts, or a reduction in their per-cell pigment concentrations) which causes visible paling of coral colonies (Brown and Howard, 1985; Guzman and Jimenez, 1992; Gardner et al., 2003; Baker et al., 2008). Corals may recover from bleaching, depending on the intensity and duration of the stress, but if the algal symbiont communities are not restored relatively quickly, corals may die.

The effects of ocean acidification on coral reefs has been extensively researched in the past two decades (Langdon et al., 2000; Leclercq et al., 2000; Schneider and Erez, 2006; Hoegh-Guldberg et al., 2007; Kuffner et al., 2008; Doney et al., 2009; Anthony et al., 2008, 2011; Albright and Langdon, 2011; Krief et al., 2010; Gómez et al., 2015). Reported effects include decreased.

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productivity and bleaching in corals and crustose coralline algae (Anthony et al., 2008; Albright et al., 2010); decreased coral recruitment and settlement (Anthony et al., 2008; Albright et al., 2010; Ateweberhan et al., 2013); reduced primary polyp growth in corals (Anlauf et al., 2010); and effects on early life history processes of coral (Albright et al., 2008; Albright and Langdon, 2011). In contrast, very little attention has been given to effects caused by both global changes in ocean acidification and local changes in water quality, such as metal pollution to coral reef organisms (Houlbrèque et al., 2011; Horwitz et al., 2014; Siddiqui and Bielmyer-Fraser, 2015; Biscéré et al., 2015; Marangoni et al., 2017).

Metals enter aquatic systems via industrial effluent, agricultural and stormwater runoff, sewage treatment discharge, fossil fuel combustion, mining processes, marine disposal of municipal solid waste, sacrificial anodes on boats, and shipwrecks (Bryan, 1974; Guzman and Jimenez, 1992; Howard and Brown, 1984; Brown and Howard, 1985; Peters et al., 1997; Ross and DeLorenzo, 1997; Knap et al., 1991; Richmond, 1993; Davis et al., 2001; Prego and Cobelo-Garcia, 2004; Flint and Davis, 2007). Copper is a commonly used metal in corrosion prevention and antifouling, therefore leachate from metal-based paints can be substantial in local areas such as marinas and ports (Reichelt and Jones, 1994; Evans et al., 2000; Voulvoulis et al., 2000). Waste disposal is also particularly problematic, especially in densely populated small islands, such as Bermuda (Jones, 2007, 2010). Much of the metallic bulk waste and municipal solid waste has been dumped directly into the ocean. As a result of this uncontained marine landfill, continuous contaminant leaching occurs onto nearby reefs, at levels exceeding water quality guidelines for copper in particular (Jones, 2010). Copper concentrations in seawater generally range from 0.13 to 9.5 μg/L (Kozelka and Bruland, 1998) but have been documented at nearly 30 μg/L in more polluted areas (Sadiq, 1992; Jones, 2010). The presence of heavy metals in coral tissue, water, and sediment samples collected from the coast of Florida and Hawaii (Glynn et al., 1984, 1989; Hanna and Muir, 1990), Puerto Rico (Pait et al., 2008), as well as Australia (Esslemont, 2000; Denton and Burdon-Jones, 1986a, 1986b; Haynes and Johnson, 2000) has been reported.

Copper is an essential element for all living organisms; however, at elevated concentrations copper may accumulate and cause toxicity in marine organisms (Bielmyer et al., 2005, 2010, 2012; Bielmyer and Grosell, 2011; Main et al., 2010; Patel and Bielmyer-Fraser, 2015; Siddiqui and Bielmyer-Fraser, 2015; Siddiqui et al., 2015). Copper generally exerts toxicity by altering enzyme function, causing oxidative stress, disrupting ionoregulation, and/or disrupting acid/base balance in aquatic organisms (Crespo and Karnaky Jr., 1983; McGeer et al., 2000; Bielmyer et al., 2005; Grosell, 2011; Patel and Bielmyer-Fraser, 2015; Siddiqui and Bielmyer-Fraser, 2015; Siddiqui et al., 2015). In the laboratory, copper has been shown to accumulate in corals and zooxanthellae and cause deleterious effects (Mitchelmore et al., 2007; Bielmyer et al., 2010; Marangoni et al., 2017). Biological effects in adult coral exposed to metals include reduced coral and zooxanthellae growth and coral bleaching (Howard and Brown, 1984; Goh and Chou, 1997; Jones, 1997; Peters et al., 1997; Brown, 2000; Bielmyer et al., 2010). Both metal pollution and ocean acidification have been shown to cause deleterious effects in aquatic organisms individually; however, the problem of dual exposure may be exacerbated because lower pH (increased acidification) causes changes in metal speciation, resulting in a shift to more toxic ionic metal species.

Physiological responses, such as reduced photosynthesis in zooxanthellae (Jones, 1997; Bielmyer et al., 2010; Siddiqui and Bielmyer-Fraser, 2015; Patel and Bielmyer-Fraser, 2015) and oxidative stress, in both host and symbiont have been reported consequences of metal exposure in cnidarians (Gilbert and Guzman, 2001; Mitchelmore et al., 2003; Main et al., 2010; Bielmyer et al., 2010; Brock and Bielmyer, 2013; Patel and Bielmyer-Fraser, 2015; Siddiqui and Bielmyer-Fraser, 2015). To neutralize the potentially harmful effects of reactive oxygen species (ROS) from metals and other stressors, cnidarians and other organisms produce antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) (Main et al., 2010; Brock and Bielmyer, 2013; Patel and Bielmyer-Fraser, 2015; Siddiqui et al., 2015). Carbonic anhydrase (CA) is another important enzyme that has been measured in recent studies to assess coral stress due to metal exposure (Bielmyer et al., 2010). CA catalyzes the interconversion of CO_2 to HCO_3 and is therefore important in respiration and metabolism processes (Weis et al., 1989; Bundy, 1977; Henry, 1996). Additionally, CA facilitates the formation of CO_3^{2-} which acts as a substrate for $CaCO_3$ formation.

Acropora cervicornis (Staghorn coral) and *Pocillopora damicornis* (cauliflower coral) are important reef building species and have been shown to be sensitive to copper in previous studies (Bielmyer et al., 2010). The goals of this project were to quantify copper accumulation and assess physiological responses in the corals, *A. cervicornis* and *P. damicornis*, and their symbionts after acute exposure to copper and increased CO_2 in the laboratory. We hypothesized that exposure to increasing atmospheric CO_2 would cause impairment to these organisms, which would further be affected by combined copper exposure.

2. Methods

2.1. Test organisms

In 2005, *A. cervicornis* was collected from Biscayne National Park, and in 2003, *P. damicornis* was purchased from The Coral Nursery. Since that time, all coral species have been maintained and cultured at the Coral Resource Facility at the University of Miami's Rosenstiel School of Marine and Atmospheric Sciences (RSMAS). The reef building clones are genetically identified, routinely fragmented, and housed in an isolated re-circulating seawater system. For each species, coral fragments were cut from a single colony and then mounted on tiles to facilitate handling. The fragments were allowed to recover from handling and observed for normal growth and performance such that only healthy coral fragments were used for experimentation. Coral fragments are uniquely suited for toxicological testing because they have > 90% survival using approved protocols (Shafir et al., 2002) and they have been shown to be sensitive to copper in previous studies (Bielmyer et al., 2010).

2.2. Experimental design

Coral fragments were exposed to a control or 20 μg/L copper (nominal concentration), as $CuCl_2$ at ambient or 1000 μAtm CO_2 levels in a flow-through system for 96 h. Measured copper values were 25 ± 11.2 μg/L and 17 ± 4.48 μg/L for the copper treatments in ambient and 1000 μAtm CO_2 respectively. Copper concentrations in the controls were below detection (< 2 μg/L).

There were three replicate tanks per treatment, each with two coral fragment of each species. Concentrated copper solutions and gravity fed natural filtered seawater (untreated or treated with CO_2) were continually mixed and then distributed into testing chambers (10 gal aquaria). A pH/pCO_2 stat system (Loligo system) was used to maintain the required amount of pCO_2 in the water of different tanks, where pH was continuously monitored and each tank was also aerated. The 1000 μAtm exposure CO_2 concentration was controlled using a commercially available PCO_2/pH feedback controller (DAQ-S; Loligo Systems Inc.) connected to a wtw pH 3310 meter and SenTix 41 pH electrode (Loligo Systems Inc.) and controlled using CapCTRL software (Loligo Systems Inc.). The automated system used measured pH data and an input PCO_2 – pH standard curve to determine water PCO_2 and has been employed previously for climate change relevant CO_2 exposures to marine organisms (Esbaugh et al., 2012; Heuer et al., 2012; Heuer et al., 2016; Heuer and Grosell, 2016). When PCO_2 in the aquaria drops below the set point, as determined by pH measurements, the system adds pure CO_2 gas via an airstone until the PCO_2 returns to the