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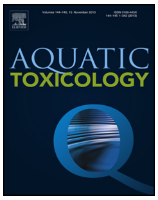

Responses of the sea anemone, *Exaiptasia pallida*, to ocean acidification conditions and zinc or nickel exposure

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ABSTRACT

Ocean acidification, caused by increasing atmospheric carbon dioxide (CO_2), is a growing concern in marine environments. Land-based sources of pollution, such as metals, have also been a noted problem; however, little research has addressed the combined exposure of both pollutants to coral reef organisms. In this study we examined tissue metal accumulation and physiological effects (activity of anti-oxidant enzymes, catalase and glutathione reductase) in the sea anemone, *Exaiptasia pallida* after exposure to increased CO_2, as well as zinc (Zn) or nickel (Ni). After exposure to four concentrations (nominal values = control, 10, 50, 100 µg/L) of Zn or Ni over 7 days, both metals accumulated in the tissues of *E. pallida* in a concentration-dependent manner. Anemones exposed to elevated CO_2 (1000 ppm) accumulated significant tissue burdens of Zn or Ni faster (by 48 h) than those exposed to the same metal concentrations at ambient CO_2. No differences were observed in catalase activity due to Zn exposure; however, 50 µg/L Ni caused a significant increase in catalase activity at ambient CO_2. No significant effect on catalase activity from CO_2 exposure alone was observed. Glutathione reductase activity was affected by increased Zn or Ni exposure and those effects were influenced by increased CO_2. Results of this study provide insight into the toxic mechanisms and environmental implications of CO_2 and Zn or Ni exposure to the cnidarian *E. pallida*.

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1. Introduction

Coral reefs are among the most diverse and productive ecosystems on Earth, containing a wealth of species complexity and substantial economic importance (Brown and Howard, 1985). Despite their vital role in the global economy and ecology, these valuable marine environments have experienced vast degradation in recent years (Brown and Howard, 1985; Albright and Langdon, 2011). Ocean acidification has become a prevalent environmental stressor to many coral reef species. Current oceanic values of CO_2 approximate 380 µatm, and by the years 2100 and 2300 these values are expected to rise to 1000 and 1900 µatm, respectively, which would cause a decrease of up to 0.77 pH units with the higher CO_2 level (Caldeira and Wickett 2003; Meehl et al., 2007; Fabry et al., 2008; Esbaugh et al., 2012; Albright and Langdon, 2011). The uptake of atmospheric CO_2 by the ocean alters the ocean carbonate chemistry, lowering the concentration and saturation states of calcium carbonate ($CaCO_3$) minerals (Gomez et al., 2014). Several studies have demonstrated a negative correlation with higher concentrations of CO_2 in coral reef organisms that rely on a saturated state of CO_3^{2-} for calcification and growth (Gomez et al., 2014; Ateweberhan et al., 2013). Towle et al. (2015) reported a decline of as much as 98% in the dominant reef-building coral *A. cervicornis* in the Florida Reef Tract since the 1970s. Environmental stressors affecting calcification and the coral-algal symbiosis may induce a phase shift from communities of hard, reef-building corals to more resilient species of soft corals and fleshy macroalgae (Peters et al., 1997; Gomez et al., 2014; Anthony et al., 2011).

In addition to the global stressors of ocean acidification, coral reef ecosystems are also faced with more localized stressors, such as heavy metals, from land-based anthropogenic sources (Howard and Brown, 1984; Anthony et al., 2011). Many reefs are located near densely populated areas with substantial industrialization and coastal development. Sewage discharge, dredging, coastal petroleum refineries, fossil fuel combustion, sacrificial anodes on boats, leachate from metal-based antifouling paints, marine disposal of municipal solid waste and metallic bulk waste, mining,

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smelting, refining, and alloy processing, and many other agricultural and industrial activities contribute to increased heavy metal pollution in nearshore marine environments (Howard and Brown, 1984; Guzmán and Jiménez, 1992; Reichelt and Jones, 1994; Gonzalez et al., 1999; Evans et al., 2000; Voulvoulis et al., 2000; Naoum et al., 2001; Stylianous et al., 2007; Jones, 2010). Zinc and nickel are both essential metals to marine organisms and function in multiple roles as a cofactorsfor enzymes and in DNA. As essential metals, they are needed in low concentrations, but may become toxic at concentrations above certain thresholds (Poonkothai and Vijayavathi, 2012; Ferrier-Pages et al., 2005). High concentrations of zinc can affect main physiological functions, such as calcification rates, and can affect photosynthetic efficiency and proper enzyme function in the coral symbionts, zooxanthellae (Houlbrèque et al., 2012; Ferrier-Pages et al., 2005).

Heavy metals have been shown to accumulate in coral reef organisms, particularly those containing algal symbionts such as zooxanthellae (Peters et al., 1997; Main et al., 2010; Horwitz et al., 2014). In many cases, corals and anemones exposed to elevated concentrations of metals will release their symbiotic algae as a possible method of detoxification, since algae have been shown to accumulate some metals to a larger extent than their animal symbiont (Bielmyer et al., 2010; Horwitz et al., 2014). This process of "bleaching" is a potentially detrimental condition and can occur in conjunction with physiological disruptions in the holobiont (Bielmyer et al., 2010; Main et al., 2010; Brock and Bielmyer, 2013; Patel and Bielmyer-Fraser, 2015). Physiological effects of metal exposure include changes in the activity of antioxidant enzymes (Main et al., 2010; Brock and Bielmyer, 2013). When exposed to metals, corals and anemones containing a photosynthetic symbiont are susceptible to increased production of reactive oxygen species (ROS) that can denature proteins, mutate DNA, and cause lipid peroxidation (Yakovleva et al., 2004). Antioxidant defenses utilizing the enzymes glutathione peroxidase (GPx), glutathione reductase (GR), and catalase (CAT), have been shown to reduce these harmful effects (Yakovleva et al., 2004; Brock and Bielmyer, 2013; Patel and Bielmyer-Fraser, 2015; Siddiqui et al., 2015; Siddiqui and Bielmyer-Fraser, 2015). GPx and CAT are produced to catalyze the conversion of hydrogen peroxide into water and oxygen, thus combating the effects of ROS (Higuchi et al., 2010; Forman et al., 1990; Sies, 1999; Sunagawa et al., 2008; Brosnan and Brosnan, 2009). GR is then produced to reduce glutathione so that it may be recycled for the reaction above (Forman et al., 1990; Sies, 1999; Sunagawa et al., 2008; Brosnan and Brosnan, 2009).

Though several studies have examined the effects of ocean acidification or metal exposure, few studies have analyzed the effects of both stressors combined (Houlbrèque et al., 2012; Gomez et al., 2014; Peters et al., 1997; Horwitz et al., 2014). The lower pH in marine ecosystems can increase the solubility of some metals thus increasing their bioavailability and/or toxicity to the organism, while also directly inducing physiological stress, such as decreased photosynthesis, respiration, calcification rates, and the rate of nitrogen-fixation (Ateweberhan et al., 2013; Horwitz et al., 2014; Houlbrèque et al., 2012).

Exaiptasia pallida (formerly *Aiptasia pallida* and now synonymous with *Aiptasia pulchella*) contain photosynethetic dinoflagellate zooxanthellae, and occupy a range of nearshore environments in southeastern United States where metal pollution is more prevalent (Leal et al., 2012). This species has also been shown to be affected by both metals and ocean acidification (Siddiqui and Bielmyer-Fraser, 2015). For all of these reasons, *E. pallida* serve as useful surrogates for corals in toxicity testing. The objectives of this research were to assess tissue metal accumulation and physiological effects of elevated CO_2 with Zn or Ni exposure in the sea anemone *Exaiptasia pallida*.

2. Materials and methods

2.1. Test organisms

E. pallida were shipped from University of Miami (Miami, FL, USA) and maintained in a 30 L holding tank at Valdosta State University filled with synthetic saltwater with continuous filtration and aeration. Synthetic saltwater at a salinity of 30 ppt was prepared by mixing Instant Ocean salt and 18.2 mΩ Milli-Q water 24 h prior to use. Anemones were fed brine shrimp (*Artemia* sp.) ad libitum daily and acclimated to testing conditions for at least two weeks prior to testing. Salinity was measured using a portable refractometer (Aquatic Ecosystems, Inc.) and temperature and dissolved oxygen (DO) were measured using a YSI 85 Meter (YSI, OH, USA) daily. Measured values (mean ± standard deviations) for salinity, temperature and DO were as follows: 29.9 ± 0.45 ppt, 24.2 ± 0.48 °C, and 8.28 ± 0.19 mg/L, respectively. Anemone wet and dry weight averaged 0.09 ± 0.05 g and 0.01 ± 0.007 g, respectively.

2.2. Experimental design

Sixteen 10-gallon tanks were used in the experiments. Eight of these tanks contained a nominal CO_2 value of 1000 ppm and the remaining eight tanks had no added CO_2 and were at ambient conditions. The pH/pCO_2 stat system used for this experiment is from Loligo systems with CAPCTRL software and set up by manual instruction. This system has been used in our laboratory previously (Siddiqui and Bielmyer-Fraser, 2015) and by several other investigators (Heuer et al., 2012). A standard curve for the calibration was prepared using a known CO_2/O_2 gas mixture of 2250 ppm. The pH of each tank was continuously monitored and regulated using an automated negative feedback system which dispensed pure CO_2 gas into the water when needed. Each tank was also continuously aerated.

Metal exposure concentrations were prepared from mixing 10 g/L stock solutions of $NiCl_2$ and $ZnCl_2$ (Ricca Chemical Company) with 30 g/L synthetic saltwater 24 h prior to the beginning of the experiment and 24 h prior to each water change. At ambient CO_2 or 1000 ppm CO_2, anemones were exposed to nominal concentrations of 0 (control), 10, 50, and 100 μg/L Ni or Zn in two sequential experiments and there were two replicate tanks for each metal concentration.

At the start of each experiment, eight anemones were transferred from the holding tank to each experimental tank in the Ni experiment; and in the Zn experiment, six anemones were transferred to each control tank and four anemones to each Zn treatment. Additionally, five *E. pallida* were obtained from the holding tank, anesthetized with Tricaine methanesulfonate (MS-222), and cut in half using a scalpel. Half of the anemone was collected for enzyme analysis and immediately frozen at −80 °C. After dissection, the other halves were dried in an oven at 65 °C for 24 h, weighed, and acidified using trace metal grade nitric acid for later metal analysis. This procedure was followed on 2, 4, and 7 d with two anemones collected per tank in the Ni experiment. For the Zn experiment, two anemones were collected from the control tank on 2 d, while on 4 and 7 d one anemone was collected. For all other tanks, one anemone was sampled on 2 and 4 d and two anemones were collected on 7 d. This pattern was due to the number of anemones available for the experiment. Behavior was monitored and recorded daily by taking pictures of every tank. Water samples were collected from each tank on each sampling day and before each 50% water change. Temperature and DO were measured daily using a YSI 85 Meter (YSI, OH, USA). Salinity was measured daily using a portable refractometer (Aquatic Eco-Systems, Inc.). Ammonia,

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