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The response of two species of unionid mussels to extended exposure to elevated carbon dioxide

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ABSTRACT

Changes in environmental conditions can act as stressors, with potential consequences for the health and fitness of organisms. Rising levels of carbon dioxide (CO_2) is one potential environmental stressor that is occurring more frequently in the environment and can be a stressor for aquatic organisms. In this study, the physiological responses of two species of unionid mussel, *Lampsilis siliquoidea* and *Amblema plicata*, were assessed in response to exposure to two levels of elevated partial pressure of CO_2 (pCO_2) (~20,000 and ~55,000 μatm) over a 28 d period, followed by a subsequent 14 d recovery period. Observations were consistent with responses associated with respiratory acidosis, as demonstrated by changes in hemolymph HCO_3^-, Ca^{2+}, Cl^-, and Na^{2+}. Both species exposed to elevated pCO_2 had elevated hemolymph HCO_3^- during the pCO_2 treatment period compared to control mussels, but recovered once pCO_2 was removed. Similarly, both species had elevated hemolymph Na^+ during exposure to elevated pCO_2, and this returned to control levels for *A. plicata* but remained elevated for *L. siliquoidea* once the pCO_2 stimuli was removed. Changes in hemolymph Ca^{2+} and Cl^- in response to elevated pCO_2 were also observed, but these changes were species-specific. Additional physiological responses to elevated pCO_2 (e.g., changes in hemolymph glucose and Mg^{2+}) were consistent with a stress response in both species. This study demonstrates the importance of considering inter-specific differences in the response of organisms to stress, and also that responses to elevated pCO_2 may be transient and can recover once the stress is removed.

1. Introduction

Changes to the environment can induce physiological responses in organisms, which can have ultimate effects on overall health and fitness (Wikelski and Cooke, 2006). While understanding environmental change and its consequences for overall fitness and survival is critical for predicting future population changes, it is also important to define the sub-lethal impacts of stressors on animals, as sub-lethal physiological changes can provide valuable insight into how organismal physiology predicts population-level effects (Madliger and Love, 2014). With a finite level of resources available to an organism, increasing maintenance costs to respond to an environmental stressor can reduce the potential allocation of these resources to growth and reproduction (Maltby, 1999), thereby providing a link to potential consequences for a population following perturbations (Fefferman and Romero, 2013). Defining physiological endpoints that relate to population-level consequences has become a critical management and conservation tool with increasing human population causing major alterations to the biotic and abiotic environment at an accelerated rate (Bijlsma and Loeschcke, 2005).

One environmental change that has the potential to impact populations, and is occurring more frequently in both the terrestrial and aquatic environments, is an increase in carbon dioxide (CO_2) (Manabe and Wetherald, 1980). Carbon dioxide has increased due to a number of potential sources, both natural (i.e., daily and seasonal fluctuations in CO_2) (Maberly, 1996) and anthropogenic (e.g., climate change, and more recently CO_2 fish barriers) (Hasler et al., 2016; Noatch and Suski, 2012). Due to its effectiveness at deterring fish movement, the use of CO_2 has been considered as a non-physical barrier to prevent the movement of fishes (Donaldson et al., 2016; Noatch and Suski, 2012). In the aquatic environment, these natural and anthropogenic increases in CO_2 raise the partial pressure of CO_2 (pCO_2) in the water, which can cause a number of negative consequences for aquatic organisms (Heuer and Grosell, 2014). Studies that quantify the consequences of elevated aquatic pCO_2 have mainly focused on marine systems, which tend to parallel atmospheric levels of CO_2. However, freshwater systems, unlike marine systems, are highly variable in terms of the levels of pCO_2 that occur (Hasler et al., 2016). These levels can vary from below 100 μatm to over 4000 μatm depending on the substrate, productivity of the surrounding terrestrial environment, precipitation, aquatic respiration, and other factors (reviewed in Hasler et al., 2016). Furthermore, in a review of ~7000 global streams and rivers, the average median values for pCO_2 was ~3100 μatm (Raymond et al., 2013). In another review of 47 large rivers found globally, mean pCO_2 varied

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from 679 ± 543 μatm to 35,617 ± 46,757 μatm, and specifically, the means of rivers in the USA ranged from 679 ± 543 μatm to 9475 ± 993 μatm (Cole and Caraco, 2001). In addition, unlike marine systems, there is currently no clear consensus on how pCO_2 may change in freshwater systems as a result of environmental change, thus increasing the uncertainty of how freshwater biota will be affected by future changes to climate (Hasler et al., 2016).

Due to links between climate change and elevated pCO_2 in the ocean (Kurihara, 2008), the response of marine invertebrates to elevated pCO_2 have been well studied, and are highly variable (Kroeker et al., 2014; Pörtner et al., 2005). The varied responses in marine invertebrates, coupled with the fluctuations/lack of predictions for future changes in freshwater pCO_2, make the impacts of elevated pCO_2 on freshwater mussels difficult to predict. Elevated pCO_2 causes acidosis in the tissues and body fluids of aquatic animals (Pörtner et al., 2004), and marine invertebrates display a suite of physiological disturbances in response to acidosis, including increases and decreases in metabolic rate, reduced protein synthesis, altered ion exchange rates, reduced calcification, and reduced growth (Bibby et al., 2008; Dissanayake et al., 2010; Michaelidis et al., 2005). However, there are mechanisms to counteract the acidosis experienced by aquatic invertebrates, such as the accumulation of bicarbonate (HCO_3^-), excretion of hydrogen ions, and other regulatory processes (Pörtner et al., 2004). One mechanism by which mussels may increase hemolymph HCO_3^- when exposed to elevated pCO_2 is by utilizing $CaCO_3$ released from the shell into their hemolymph. This release of $CaCO_3$ also results in an increase in Ca^{2+} in the hemolymph (Michaelidis et al., 2005; Bibby et al., 2008). Another strategy to increase HCO_3^- in the hemolymph is through regulation of the Cl^-/HCO_3^- exchanger, which may result in changes in hemolymph Cl^- levels (Byrne and Dietz, 1997). An additional mechanism to reduce acidosis of internal fluids is through the active transport and removal of H^+ across cell and epithelial membranes (Gazeau et al., 2013). An increase in the activity of the Na^+/H^+ exchanger to excrete H^+ is a common strategy used by aquatic animals experiencing acidosis, which can result in an increase in hemolymph Na^+ (Byrne and Dietz, 1997; Lannig et al., 2010). Even though regulating acid-base parameters may not be detrimental in the short-term, chronically altering these mechanisms is expected to affect long-term growth (Michaelidis et al., 2005; Kurihara, 2008), calcification (Gazeau et al., 2013), and immune function (Bibby et al., 2008; Beesley et al., 2008), which may translate to negative consequences at the population- and species-levels (Fefferman and Romero, 2013; Pörtner et al., 2004).

Although the negative consequences of elevated pCO_2 have been repeatedly observed in marine mussels (Michaelidis et al., 2005; Kurihara, 2008; Bibby et al., Beesley et al., 2008), a paucity of research has been done on the effects of elevated pCO_2 on freshwater mussels. Studies evaluating the consequences of emersion (air exposure) in freshwater mussels have observed physiological consequences similar to those experienced by marine mussels exposed to elevated pCO_2 (Byrne and McMahon, 1994; Heming et al., 1988); however, the responses of freshwater mussels to elevated pCO_2 in water may differ to those of emersion, as mussels have the capacity to open their valves and exchange ions with the water more freely during exposure to elevated pCO_2, unlike during emersion. Understanding the impacts of elevated pCO_2 on freshwater mussels becomes critical with the ever-increasing CO_2 in aquatic systems (either naturally or due to anthropogenic alterations), coupled with the lack of research on its consequences for already threatened freshwater mussels.

Freshwater mussels have their highest abundance in North American and are one of the most threatened taxa worldwide (Williams et al., 1993). Mussels are generally thought of as characterless animals, however, they have a variety of differences in morphologies (e.g., shell thickness, size, reproductive strategies, etc.) and behaviors (e.g., feeding, righting, movement, burrowing, gaping), especially in response to disturbances (Waller et al., 1999). This high degree of variability in mussel characteristics supports the use of multiple species when defining responses to stressors as this variability can describe how different species respond stressors. Additionally, with increasing threats to their survival, and more than half of freshwater mussel species listed as threatened or endangered (71%), it is critical that precautions be taken to define how environmental changes will affect growth and survival (Williams et al., 1993). The potential exposure of freshwater mussels to elevated pCO_2 may occur due to a number of natural and anthropogenic scenarios, and thus, it is important to understand the potential impacts of CO_2 exposure on this threatened taxon.

Based on this background, the goals of this study were to (1) quantify the physiological impacts of chronic exposure to elevated pCO_2 on two species of freshwater mussels from two different mussel tribes that have different life history strategies, shell thicknesses, and sensitivity to toxicity, and (2) define whether or not physiological disturbances associated with chronic exposure to elevated pCO_2 would recover once the exposure ended. To address these goals, fatmucket (*Lampsilis siliquoidea*) and threeridge (*Amblema plicata*) mussels were exposed to two different CO_2 levels for up to 28 d and then given up to 14 d to recover. It was predicted that changes in hemolymph ion levels will occur as a consequence of the acid-base disturbance experienced during exposure to elevations in pCO_2 similar to those of marine mussels described above.

2. Methods

2.1. Mussel collection and husbandry

Adult fatmucket (*L. siliquoidea*) mussels (65.3 ± 0.04 mm length (standard error), 34.6 ± 0.03 mm width, and 24.2 ± 0.02 mm depth) were delivered overnight from Missouri State University, Springfield, MO, in June 2015 to the Aquatic Research Facility at the University of Illinois, Urbana-Champaign, IL, USA. Adult threeridge mussels (*A. plicata*) (76.4 ± 0.06 mm length, 63.0 ± 0.06 mm width, and 39.8 ± 0.03 mm depth) were collected by benthic grab from the Mississippi River, near Cordova, IL, in July 2015. Following collection, mussels were transported to the Aquatic Research Facility at the University of Illinois, Champaign-Urbana, IL in coolers (travel time < 3 h). Upon arrival at the Aquatic Research Facility, both species of mussels were cleaned of epibionts, tagged for individual identification with Queen Marking Kit tags (The Bee Works, Orillia, ON, CA) (Neves and Moyer, 1988) or marked with a permanent marker. They were then placed in one of three holding tubs (1135.6 L) supplied with water from a 0.04 ha natural, earthen-bottom pond with ample vegetation, where they remained for at least one wk to recover from transport and hauling stressors and acclimate to lab conditions (Dietz et al., 1994; Dietz, 1974; Horohov et al., 1992). All tubs were equipped with a Teco 500 aquarium heater/chiller (TECO-US, Aquarium Specialty, Columbia, SC, USA) to prevent temperature fluctuations, and a low-pressure air blower (Sweetwater, SL24H Pentair, Apopka, FL, USA) for aeration. Fifty percent water changes using pond water were performed weekly to maintain water quality. Mussels were fed a commercial shellfish diet with multiple particle sizes consisting of *Nannochloropsis* sp. 1–2 μm and a mixed diet of *Isochrysis, Pavlova, Thalassiosira,* and *Tertraselmis* spp. 5–12 μm (Instant Algae, Reed Mariculture Inc., Campbell, CA, USA) every other day (Wang et al., 2007); mussels did not receive supplemental food 24 h prior to sampling. Dissolved oxygen (DO) and temperature were recorded daily in all three tubs with a portable meter (YSI 550 A, Yellow Springs Instruments, Irvine, CA, USA) and averaged 8.2 ± 0.02 mg L^{-1} (mean ± standard error, SE) and 21.6 ± 0.1 °C (respectively) throughout both the acclimation and experimental periods. Water pH and total alkalinity (TA) were also measured daily using a handheld meter (WTW pH 3310 meter, Germany) calibrated regularly (Moran, 2014) and a digital titration kit (Titrator model 16,900, cat. no. 2271900, Hach Company, Loveland, CO, USA), respectively, and averaged 8.425 ± 0.031 for pH (<100 μatm) and 1178.9 ± 29.9 μmol/kg for TA during the acclimation period.