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Molecular responses of fishes to elevated carbon dioxide

Clark E. Dennis III *, Daniel F. Kates, Matthew R. Noatch, Cory D. Suski

Department of Natural Resources and Environmental Sciences, University of Illinois, 1102 S. Goodwin Ave., Urbana, IL 61801, USA

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ABSTRACT

Hypercarbia, or elevated carbon dioxide, is an environmental challenge that can have detrimental effects on the physiology and performance of aquatic organisms. With aquatic hypercarbia predicted to become more prevalent in the future due to global climate change, it is important to quantify how hypercarbia impacts aquatic organisms, especially fish. The impact of hypercarbia on the behavior and physiology of fishes has been well studied, but relatively few studies have examined the molecular processes that underlie resulting behavioral and physiological changes. In an effort to define the molecular response of fishes to acute hypercarbia exposure, bluegill (*Lepomis macrochirus*) and silver carp (*Hypophthalmichthys molitrix*) were exposed to either 30 mg L^{-1} CO_2 ($pCO_2 \approx 15{,}700$ μatm) or ambient (10 mg L^{-1} CO_2; $pCO_2 \approx 920$ μatm) conditions for 1 h and the expression of a variety of genes, across three tissues, were compared. Exposure to 30 mg L^{-1} CO_2 in bluegill and silver carp resulted in an increase in *c-fos*, *hif1-α*, and *gr-2* transcripts, while silver carp alone showed increases in *hsp70* and *hsc70-2* mRNA. This study demonstrates that acute hypercarbia exposure impacts gene expression in a species and tissue specific manner, which can be useful in identifying potential mechanisms for hypercarbia tolerance between species, and pinpoint specific tissues that are sensitive to hypercarbia exposure.

1. Introduction

Environmental stress is a ubiquitous challenge for aquatic organisms, and exposure to stress can translate into a suite of molecular, biochemical, and behavioral changes. The perception of an external stressor initially involves the activation of receptors and release of corticosteroids, which can lead to changes in the molecular expression of processes, involved in oxygen transport, metabolism, osmoregulation, and eventually changes in the whole animal performance and behavior (Barton, 2002). Following the perception of a stressor, organisms have the ability to alter genetic and physiological systems to maintain homeostasis in the face of environmental challenges (Barton, 2002; McEwen and Wingfield, 2003). In addition, animals can display behavioral avoidance to escape poor quality environments and avoid potential energetic costs associated with inhabiting sub-optimal areas (Kieffer and Cooke, 2009). Due to anthropogenic-driven environmental challenges, such as global climate change and degraded water quality, determining the capacity of aquatic organisms to respond to environmental stressors will become increasingly important in the future.

Aquatic hypercarbia, both naturally-occurring and anthropogenically induced, can be an environmental challenge for aquatic organisms. Elevated dissolved CO_2 concentrations can occur in both freshwater and marine environments, especially in estuarine waters and coastal upwelling zones (Feely et al., 2008; Thomsen et al., 2010), due to a wide range of factors including thick surface vegetation, insufficient water mixing, and the respiratory processes of microbes (Heisler et al., 1982; Ultsch, 1996). In addition to natural occurrences, aquatic hypercarbia can be created through anthropogenic means, such as through intensive aquaculture practices (Colt and Orwicz, 1991; Kristensen et al., 2009) or novel chemical fish deterrent systems (Clingerman et al., 2007; Kates et al., 2012). Due to the wealth of sources for aquatic hypercarbic environments, many studies have investigated the impact of elevated CO_2 on marine and freshwater fishes and demonstrated a variety of physiological impacts on fishes including respiratory acidosis (Iwama et al., 1989; Bernier and Randall, 1998), metabolic acidosis (Bernier and Randall, 1998), ion imbalance (Brauner et al., 2000), and activation of stress hormones (Iwama et al., 1989). Exposure to hypercarbia has also been shown to influence ventilation amplitude and frequency in an effort to offset elevated internal CO_2 concentrations (Gilmour, 2001; Gilmour and Perry, 2007). Elevated dissolved CO_2 concentrations can also impair the ability of larval and juvenile marine fishes to detect auditory cues, locate refuge and settlement areas, and avoid predation, resulting in potential reductions in fish populations and recruitment (Munday et al., 2009, 2010; Dixson et al., 2010; Simpson et al., 2011). More importantly, hypercarbic environments are expected to become more prevalent in the future due to global climate change (Feely et al., 2008), making elevated environmental CO_2 a growing concern that has potential to impart negative impacts on both individual fish (Baumann et al., 2012; Esbaugh et al., 2012) and fish populations (Munday et al., 2010).

* Corresponding author at: Department of Natural Resources and Environmental Sciences, University of Illinois, 1102 S. Goodwin Ave, W401-C Turner Hall, Urbana, IL 61801, USA. Tel.: +1 217 721 8420.
E-mail address: clarkdennis3@gmail.com (C.E. Dennis).

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While the physiological and behavioral impacts of hypercarbia exposure for fishes are well understood, currently relatively little work has been performed to define the molecular underpinnings that drive many of the physiological and behavioral adjustments that have been observed following hypercarbia exposure (Rimoldi et al., 2009). Therefore, determining the molecular response of fishes to elevated carbon dioxide will provide critical information on 1) how hypercarbia exposure affects different fish species at the molecular level; 2) what specific genetic pathways are being activated following hypercarbia exposure; and 3) mechanisms behind species-specific tolerance to elevated CO_2 environments.

Based on this background, the objective of the current study was to characterize stress-related gene expression patterns (i.e., upregulation/downregulation, tissue-specific expression, species-specific expression) in fishes exposed to an elevated carbon dioxide environment. In an effort to better understand the transcriptional changes induced upon an acute hypercarbia stressor, a suite of functionally distinct gene transcripts were examined to provide a broad perspective of the molecular stress responses in adult fishes, compared to many previous studies that often focus on candidate genes within a target gene family (Ali et al., 2003; Lund et al., 2003; Rimoldi et al., 2012). One of the few gene transcripts that have been linked to hypercarbia exposure is *c-fos*, an immediate early gene transcript that is rapidly induced once animals are exposed to hypercarbia (Sato et al., 1992; Tankersley et al., 2002; Rimoldi et al., 2009). The product of this gene, the c-Fos protein, is a nuclear factor that enhances the transcription of multiple genes (Curran and Franza, 1988) and may potentially modify ventilation behavior in response to hypercarbia (Rimoldi et al., 2009). Hypoxia-inducible factor 1 alpha (*hif1-α*) is another transcription factor that enhances the expression of several genes, however this gene transcript is typically induced following hypoxia stress (Nikinmaa and Rees, 2005). Previous research has demonstrated that acute hypercarbia in fishes will decrease blood pH (Iwama et al., 1989), which theoretically results in a loss of efficiency in hemoglobin oxygen uptake and delivery to the tissues (Root and Bohr effects). For adult bluegill and silver carp, Kates et al. (2012) found that exposure to 30 mg L^{-1} CO_2 resulted in an increased hematocrit concentration with the authors suggesting that this resulted from impaired oxygen uptake coupled with reductions in ventilation rates. Therefore, *hif1-α* mRNA expression was quantified to determine whether oxygen transport or uptake was impacted due to blood acidosis caused by an acute exposure to hypercarbia. Along with potential impairments to oxygen delivery mechanisms, previous work has also shown that acute exposure to elevated CO_2 environments can result in disruptions in blood chemistry, such as increased cortisol and glucose concentrations (Iwama et al., 1989; Ross et al., 2001). To determine whether cortisol may be directly influencing transcriptional regulation, glucocorticoid receptor isoform 2 (*gr-2*) mRNA expression was examined. The product of this gene, the GR-2 protein, is localized on the cell membrane of all tissues and binds free cortisol in the blood stream resulting in the activation of several effector genes that regulate responses to a general stressor (i.e., ion maintenance, increased metabolism, decreased growth, and changes in behavior) (Mommsen et al., 1999). Heat-shock protein 70, which mediates the repair and degradation of altered proteins (Iwama et al., 2004), can also be induced following a stressor of sufficient intensity and duration to cause proteins to denature or become altered and lose functionality. As such, heat-shock protein 70 (*hsp70*) and heat-shock cognate 70 isoform 2 (*hsc70-2*) transcripts were examined to determine whether exposure to an acute hypercarbia stressor impacted cellular protein functioning resulting in the upregulation of heat shock proteins. While the synthesis of these transcripts (mRNA) does not confirm any functional impact and does not always correlate with changes in protein concentrations, they do represent the initial cellular response to an acute stressor. We hypothesized that an acute hypercarbia exposure would increase transcript abundance of *c-fos*, *hif1-α*, and *gr-2* due to molecular responses targeting hypercarbia-specific, hypoxia-specific, and general-stress related transcription factors. Expression of *hsp70* and *hsc70-2* transcripts, however, were expected to remain at control levels following hypercarbia exposure due to previous research on acute thermal stress showing that *hsp70* can take several hours to reach peak expression levels (Lund et al., 2003; Lewis et al., 2010). We also hypothesized that gene expression within the gill tissue would be altered to a greater extent than the heart or erythrocyte tissue due to the gills' direct contact with the hypercarbic stressor.

To test these hypotheses, two species of fish, bluegill (*Lepomis macrochirus*) and silver carp (*Hypophthalmichthys molitrix*), were given an acute sub-lethal hypercarbia challenge, and the expression of several genes, across three tissues, were quantified. Silver carp and bluegill were ideal species to use in this study for the following reasons: 1) these are evolutionarily divergent species (Betancur et al., 2013), making general trends in gene expression data applicable across a broad range of species; 2) previous research by Kates et al. (2012) has shown that during a common hypercarbia exposure, bluegill reduced ventilation rates while all silver carp lost consciousness during the trial suggesting that silver carp have greater sensitivity to elevated CO_2 relative to bluegill; and 3) assessing the impact of realistic acute elevated CO_2 concentrations on bluegill may be useful for aquaculture managers rearing bluegill. Results will also have important implications for understanding how fish respond at the molecular level to acute hypercarbia exposure, and how these changes in gene expression might confer hypercarbia tolerance.

2. Materials and methods

2.1. Experimental animals

Bluegill were purchased from a commercial supplier (Logan Hallow Fish Farm, Murphysboro, IL, USA) and delivered to the Aquatic Research Facility at the University of Illinois, Champaign-Urbana, Illinois, in October–November, 2010. Silver carp were collected from a variety of locations in east central Illinois along the Illinois River by using standard pulsed direct current (DC) boat electroshocking in a 5.5 m flat bottom aluminum boat. As per standardized long-term collection protocols, electroshocking parameters (i.e. voltage, amperage, and wavelength) were adjusted daily to maintain a performance of approximately 3000 Watts (W) based on water chemistry parameters on site (Gutreuter et al., 1995). Once captured, silver carp were transported to the Aquatic Research Facility in a 640 L truck bed hauler in ambient water supplied with compressed oxygen gas to near saturation to minimize transport stress. At the Aquatic Research Facility, all fish were housed outdoors in round plastic holding tanks (1280 L, 1.7 m diameter) connected to a 0.04 ha natural, earthen-bottom pond with abundant vegetation. Water was supplied to the tanks from the pond, and allowed to drain back into the pond providing sufficient water replacement and nitrogenous waste removal. Tanks also received supplemental aeration from a low-pressure air blower. Bluegill were acclimated to the outdoor holding system for 4 weeks and fed pelleted food (Dense Culture Food, F2C, Aquatic Ecosystems, Apopka, FL, USA) until satiation every other day, while silver carp were only held for a period of 2–4 days prior to being used in this experiment and did not receive supplemental food during laboratory confinement. All fish received a minimum of 48 h acclimation time, without food, prior to experiments to ensure sufficient time for recovery from disturbances associated with capture, hauling, acute stress, and food digestion (Milligan, 1996; Lund et al., 2003; Suski et al., 2006). During holding, water temperatures averaged 14.1 °C (±1.1 °C, standard error, SE) and dissolved oxygen averaged 9.5 mg L^{-1} (±0.4 mg L^{-1} SE).

2.2. Hypercarbia challenge

Prior to the start of the hypercarbia challenge, fish were carefully netted from the holding tank and placed into individual opaque, sensory