Tolerance to Hypercarbia Is Repeatable and Related to a Component of the Metabolic Phenotype in a Freshwater Fish

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ABSTRACT

Freshwater fish may be exposed to high levels of carbon dioxide (CO₂) because of several actions, including anesthesia and high levels of aquatic respiration and potentially as the result of using high-CO₂ plumes as a barrier to the movements of invasive species. Metabolic phenotype can potentially drive how freshwater fish respond to high CO₂. We therefore quantified how tolerance (measured using time to equilibrium loss [ELT]) was driven by metabolic phenotype in a cosmopolitan freshwater fish species, Micropterus salmoides. ELT was repeatable, with 60% of the variance across trials attributable to individual differences. For each fish, standard metabolic rate and maximum metabolic rate were measured using respirometers and time to exhaustion after a chase test was recorded. Fish with high anaerobic performance were less tolerant to elevated CO₂, potentially as a result of preexisting metabolic acidosis. Standard metabolic rate and aerobic scope did not predict ELT. Our findings define which fish may be more vulnerable to high CO₂, a potential mechanism for this tolerance, and show that tolerance to high CO₂ may be acted on by natural selection. Should freshwater ecosystems become elevated in CO₂, by either natural means or anthropogenic means, it is possible that there is potential for heritable selection of CO₂ tolerance, evidenced by the fact that ELT was found to be repeatable.

Keywords: acidification, anaerobic activity, aerobic scope, equilibrium loss.

Introduction

The past century has seen a rise in the amount of carbon dioxide (CO₂) in the atmosphere, along with a concomitant rise in the partial pressure of CO₂ (PₖCO₂) in marine, and potentially freshwater, ecosystems (Hasler et al. 2016; McNeil and Sasse 2016). Research has demonstrated negative outcomes for aquatic taxa to elevated PₖCO₂, particularly in marine fishes (Kroeker et al. 2013; Heuer and Grosell 2014). However, freshwater fishes are much more likely to experience high environmental PₖCO₂ from actions such as anesthesia (Mitsuda et al. 1980; Marking and Meyer 1985), crowding at aquaculture facilities (e.g., Santos et al. 2013), and potential deployment of high-CO₂ plumes to control the movements of invasive fishes (Noatch and Suski 2012; Donaldson et al. 2016; Cupp et al. 2017). Currently, a potential CO₂ plume that would be approximately 50–100 times the CO₂ of surrounding waters has been reviewed for the Illinois River to control the movements of bigheaded carp (Hypophthalmichthys spp.; US Army Corps of Engineers 2014).

Freshwater fishes respond negatively to elevated PₖCO₂ and show high individual variation in the magnitude of their behavioral and physiological responses. For example, shutting away from elevated PₖCO₂ of three freshwater species showed considerable within-species variation (threefold difference between minimum and maximum shuttling PₖCO₂; Kates et al. 2012) despite PₖCO₂ levels being found to cause a range of negative physiological responses (Dennis et al. 2015). To assess within-species variation, determining the repeatability of a response to elevated PₖCO₂ is necessary, which specifically requires quantifying the proportion of the total variation among measurements within individuals (Wolak et al. 2012). Once within-species variation for a response to elevated PₖCO₂ is measured, the mechanisms responsible for within-species variation to high PₖCO₂ can be determined and used to assess vulnerabilities of freshwater fisheries to environmental change (Seebacher and Franklin 2012), including the potential deployment of high-CO₂ plumes (Noatch and Suski 2012).

A potential mechanism for the responses of freshwater fishes to elevated PₖCO₂ may involve metabolic phenotype, which is composed of standard metabolic rate (SMR), maximum metabolic rate (MMR), aerobic scope (AS), and anaerobic activity (Metcalfe et al. 2016). Metabolic phenotype demonstrates intraspecific variation (Burton et al. 2011) and has also been shown to drive both performance and behavioral responses to changes in environmental conditions for fishes, making it relevant to use in studies focused on environmental factors (Claireaux and Lefrancois 2007; Metcalfe et al. 2016). More importantly, aspects of an individual’s metabolic phenotype can be affected by ambient levels of PₖCO₂,

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potentially driving within-species variation in tolerance. For example, high ambient Pco2 can reduce both metabolic rate and aerobic swimming performance in fish (Basu 1959; Dahlberg et al. 1968), and anaerobic activity may predict tolerance to high Pco2, as the ability to withstand metabolic acidosis resulting from exhaustive exercise may lead to a similar capability to endure CO2-induced respiratory acidosis (Wood 1991). Together, several aspects of an individual’s metabolic phenotype may predict tolerance and performance of fish in areas of elevated Pco2.

The objectives of this study were to define whether physiological tolerance to high Pco2 in a freshwater fish (largemouth bass [LMB] Micropterus salmoides Lacépède) is a repeatable trait and whether tolerance was predicted by metabolic phenotype. Trait repeatability is important because it suggests heritability, and thus there is potential for natural selection to act on the trait (Killen et al. 2016). We chose to use time to equilibrium loss (ELT) as a metric of tolerance because it is easily measured and occurs before death, and fish exposed to high Pco2 can still recover if placed in freshwater with low Pco2 following equilibrium loss (Yoshihikawa et al. 1994). Given that within-species variation has been found in several behavioral and physiological responses to elevated Pco2, we predicted that tolerance to elevated Pco2 would be repeatable. Further, because metabolic rates are influenced by exposure to elevated Pco2, we predicted that at least part of the metabolic phenotype, if not the entire metabolic phenotype, would influence tolerance.

### Material and Methods

Twenty-five hatchery-reared subadult LMB (181 ± 12 mm total length, mean ± SD; 81.6 ± 16.6 g mass) were held at 19.8° ± 0.4°C for 2 wk before the experiments in one of two tanks. For each fish, four experiments were conducted over four consecutive days. First, fish underwent intermittent-flow respirometry to measure SMR (Chabot et al. 2016), MMR (Norin and Clark 2016), and AS (MMR-SMR). SMR was measured overnight on the first day and was calculated using the mean lowest normal distribution method (Chabot et al. 2016). On the second day following completion of the SMR measurements, fish were chased to exhaustion using tail pinching and air exposed for 3 min before being transferred to respirometers for at least three metabolic rate measurement cycles to determine MMR. Following MMR measurements, fish were given 24 h to recover from MMR assessment (Gustaveson et al. 1991) and then on the third day exposed to high Pco2 to quantify trait tolerance. Tolerance to high Pco2 was measured by exposing fish, individually, in a 15-L cooler to 174, 000 ± 2, 000 μatm of Pco2 and measuring ELT (for comparative purposes, 174, 000 μatm is equal to 17.63 kPa or 132.24 mmHg). The target partial pressure was chosen as it has previously been shown to induce physiological changes in freshwater fishes, including equilibrium loss (Mitsuda et al. 1980; Yoshihikawa et al. 1991, 1994), and should not compromise the cardiorespiratory system because of hemoglobin denaturing (Manwell et al. 1963). On the fourth day, after 24 h had elapsed from the first tolerance test, fish were subjected to the same conditions to measure a second ELT to define repeatability of this response. ELT was tested twice to limit the potential for prolonged fish holding, habituation, learning, and fasting to potentially influence ELT (Tierney 2016). All fish were fasted for 24 h before the commencement of the metabolic rate determination and were not fed until after the second tolerance test (i.e., each fish was fasted for a total of 5 d).

Mass-specific rates of oxygen consumption (M02; mg O2 kg”–1 h”) were measured in 740-mL resting respirometry chambers (6.2–14.8 times animal volume; 9.7 ± 2.4; four chambers were used throughout the study). Automated measurement cycles consisted of a 4-min open/flush period, a 1-min closed/wait period, and a 5-min closed/measure period (Svensen et al. 2016). Dissolved oxygen levels, on average, declined to 88.1% ± 9.0% of fully saturated freshwater (9.09 mg L”–1 at 20°C) during measure periods. To account for background respiration, a single oxygen consumption measurement was taken in sealed chambers without fish before SMR measurement and after MMR measurement (Rodgers et al. 2016). Background respiration was negligible if coefficients of determination for rates of oxygen consumption were less than 0.95 (Svensen et al. 2016). If background respiration values were considerable, a linear increase in background M02 was assumed, and values for background respiration were subtracted from each M02 measurement using the linear equation generated for background M02 against time (Rodgers et al. 2016).

The repeatability of ELT following two exposures to elevated Pco2 was quantified using the intraclass correlation coefficient (ICC) using the ICC package in R (Lessells and Boag 1987; Cicchetti 1994; Wolak et al. 2012). The influence of aerobic (SMR and AS) and anaerobic (time to exhaustion) metrics on ELT was assessed using a linear mixed-effects (LME) model with a Gaussian error distribution. Fish mass was included as a covariate, and tank of origin and fish ID were included as random effects. The LME was constructed using the “lme4” package in R (Bates 2010). The importance of fixed effects was determined by generating credible intervals from 1,000 posterior simulations of each fixed effect using the “sim” function in the “arm” package in R (Bates et al. 2015; Gelman and Su 2016). Credible intervals that did not over-

![Figure 1. Time to equilibrium loss (s) during exposure to two trials of high carbon dioxide partial pressure. Repeatability = 0.60. Each data point represents one largemouth bass.](image-url)
ELT after being placed in a bath of high Pco2 (time following anaerobic swimming had marginally shorter
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higher values (suggesting that interindividual tolerance to high Pco2 in fresh-
differences among individuals between trials (ICC
60% of the variance in the data set was attributable to consistent
ates. A Pearson-
the effects of body mass and to obtain relative parameter esti-
fi
Results
ELT after hatchery-reared fish were placed in a bath of high Pco2 demonstrated a twofold difference between the lowest and highest values (fig. 1) and tended to show strong repeatability, as 60% of the variance in the data set was attributable to consistent differences among individuals between trials (ICC = 0.60, 95% confidence interval [CI] 0.29–0.80). Fish that had longer exhaustion time following anaerobic swimming had marginally shorter ELT after being placed in a bath of high Pco2 (fig. 2; table 1). SMR, AS, and mass were not found to predict ELT (table 1).

Discussion
ELT is often used by biologists as a diagnostic tool to define physiological tolerances of animals to environmental variables (Gibert et al. 2001). Equilibrium loss in fish exposed to high Pco2 likely occurs because of a lowering of brain pH when environmental CO2 enters the bloodstream of the fish and crosses the blood-brain barrier, which induces loss of motor function and anesthesia (Mitsuda et al. 1980; Yoshikawa et al. 1994). More importantly, situations that result in loss of equilibrium may ultimately lead to mortality (Raby et al. 2015); therefore, within populations, some individuals may be more vulnerable to the physiological and behavioral changes previously found in fishes exposed to high Pco2 (Clements and Hunt 2015). Hatchery-reared LMB exposed to high Pco2 exhibited repeatability for ELT, suggesting that interindividual tolerance to high Pco2 in freshwater fish is a stable response that could then be influenced by
natural selection (Killen et al. 2016). Should responses to high Pco2 be adaptive and linked to fitness, fish populations may become more tolerant to prolonged periods of high Pco2.

Fish that had longer exhaustion time following anaerobic swimming had shorter ELT after being placed in a bath of high Pco2. This relationship, however, was only marginally significant (95% credible interval, −0.75 to −0.01) and therefore potentially coincidental. Exhaustive exercise endpoints are often subjective, and if changes in levels of biomarkers of anaerobic metabolism (e.g., muscle lactate dehydrogenase activity, blood lactate levels, and pH; Kieffer 2000) are not quantified, exhaustion, as defined by this study, may not be indicative of true physiological exhaustion (Norin and Clark 2016). Furthermore, time to exhaustion observed in LMB may have been a behavioral response to anaerobic metabolism or fish lacking motivation to swim (Peake and Farrell 2006). Conversely, the respiratory acidosis induced by external Pco2 from the CO2 bath may have exacerbated a metabolic acidosis caused by chasing (Wood 1991), though it is unlikely that chasing resulted in an acidosis that persisted 24 or even 48 h postexercise when fish were exposed to high Pco2 (Milligan 1996). Additionally, the water source used in the holding tanks is considered to be hard on the basis of geographic location; therefore, compensation of respiratory acidosis should not have been hindered by holding conditions (Larsen and Jensen 1997). Thus, time to exhaustion had a negative relationship with CO2 tolerance, though it is unclear whether this result occurred by happen-

![Figure 2. Relationship between time to equilibrium loss (s) and time to exhaustion for largemouth bass. Regression line is \( y = 385.11 + (-0.39x) \), \( r^2 = 0.16 \). The shaded area represents the 95% confidence interval of the regression line. Each data point represents the mean time to equilibrium loss for one individual fish over two trials.](image)

<table>
<thead>
<tr>
<th>Term</th>
<th>Measured value (mean ± SD)</th>
<th>Mean estimate/change in intercept</th>
<th>Credible interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>NA</td>
<td>528.79</td>
<td>213.29 to 816.42</td>
</tr>
<tr>
<td>SMR</td>
<td>117.40 ± 12.08</td>
<td>−1.21</td>
<td>−3.58 to .93</td>
</tr>
<tr>
<td>AS</td>
<td>150.26 ± 38.26</td>
<td>−.22</td>
<td>−.86 to .51</td>
</tr>
<tr>
<td>Exhaust</td>
<td>219.52 ± 65.76</td>
<td>−.39</td>
<td>−.75 to −.01</td>
</tr>
<tr>
<td>Mass (g)</td>
<td>80.24 ± 20.90</td>
<td>.37</td>
<td>−.85 to 1.62</td>
</tr>
</tbody>
</table>

Note. SMR = standard metabolic rate (mg O2 kg−1·h−1); AS = aerobic scope (mg O2 kg−1·h−1·); exhaust = time to exhaustion (s). Terms in bold denote statistical significance. NA = not applicable.
in brain pH, ultimately resulting in equilibrium loss (Yoshikawa et al. 1994). Though it is uncertain whether aerobic capacity would be altered during acute or chronic exposure to moderate or low PCO₂ (Couturier et al. 2013), the absence of observed relationships between SMR and AS with CO₂ tolerance reflects a lack of understanding of how high PCO₂ influences fish.

In conclusion, tolerance to high PCO₂ in a freshwater fish was found to be repeatable and predicted by a fish’s capacity for anaerobic activity. These findings indicate that fish exposed to high PCO₂, either because of environmental change or as a result of the use of CO₂ plumes to limit the distribution of invasive species, may demonstrate a repeatable variation in reactions to this CO₂ exposure, which ultimately may be heritable and therefore influenced by natural selection. Future studies should aim to understand the potential for responses to CO₂ to be repeatable and the likelihood that they are influenced by natural selection. Other measurements of tolerance, including behavioral avoidance and the potential for interactive effects with other environmental stressors, might also be assessed and related to tolerance to high PCO₂.

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