Management of Biological Invasions (2017) Volume 8 Article in press

Research Article CORRECTED PROOF

Using dissolved carbon dioxide to alter the behavior of invasive round goby

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Received: 18 April 2017 / Accepted: 4 August 2017 / Published online: 17 August 2017

Handling editor: Matthew A. Barnes

Abstract

Fisheries managers need effective methods to limit the spread of invasive round goby *Neogobius melanostomus* in North America. Elevating carbon dioxide (CO_2) in water at pinch points of rivers (e.g., inside locks) is one approach showing potential to deter the passage of invasive fishes, such as bigheaded carps *Hypophthalmichthys* spp., but the effectiveness of this method to alter round goby behavior has not been determined. The goal for this study was to determine CO_2 concentrations that alter round goby behavior across a range of water temperatures. Free-swimming avoidance (voluntary response) and loss of equilibrium (involuntary response) were quantified by exposing round goby to increasing CO_2 concentrations at 5, 15, and 25 °C using a shuttle box choice arena and static tank. Water chemistry was measured concurrent with behavioral endpoints and showed that round goby avoided a threshold of 99–169 mg/L CO_2 (79,000–178,000 µatm) and lost equilibrium at 197–280 mg/L CO_2 (163,000–303,000 µatm). Approximately 50% lower CO_2 concentrations were found to modify behavior at 5 °C relative to 25 °C, suggesting greater effectiveness at lower water temperatures. We conclude that CO_2 modified round goby behavior and concentrations determined in this study are intended to guide field testing of CO_2 as an invasive fish deterrent.

Key words: Great Lakes, fish passage, migration, deterrent, barrier

Introduction

Round goby (*Neogobius melanostomus* Pallas, 1814) are prolific invasive fish in North America. Native to the Ponto-Caspian region, round goby were first detected in the St. Clair River in 1990 and subsequently became established in all five Laurentian Great Lakes and many adjacent tributaries (Corkum et al. 2004). These small-bodied (2–20 cm total length) brown to gray fish opportunistically consume a broad diet of benthic invertebrates, fish eggs, small fish, and are one of few species known to forage on invasive zebra mussels (*Dreissena polymorpha* Pallas, 1771) (Ray and Corkum 1997; Coulter et al. 2011). Successful adaptation to a broad range of freshwater habitats and food sources has facilitated the colonization of round goby in Great Lakes ecosystems.

Fisheries managers have realized the importance of preventing further inland spread of round goby outside of the Great Lakes (Leung et al. 2002; Lodge et al. 2006; Bronnenhuber et al. 2011; Kornis et al. 2012). For example, forecast models in Wisconsin predict that round goby will inhabit 1,369 km of Lake Michigan tributaries up to the first impassible structure, but an additional 8,878 km upstream would also provide suitable habitat if round goby pass current physical barriers (Kornis and Vander Zanden 2010). Upstream risks identified using these models suggest that interconnected waterbodies have a higher likelihood for round goby invasion because they solely rely on physical structures (e.g., locks and dams) to block access (Beyer et al. 2011). Managers of these systems need additional barrier strategies to reduce upstream movement, particularly where aging dams become scheduled for maintenance or removal (Noatch and Suski 2012).

Physical separation of rivers would be effective to prevent round goby passage (Rahel 2013), but this approach conflicts with economic and social interests (Schwieterman 2015). Hydrologic fragmentation of the Chicago Area Water System (CAWS), a direct connection from the Great Lakes basin to the Mississippi River basin, was estimated to cost the Chicago metropolitan area more than $1.3 billion USD annually in lost revenue related to the commercial shipping industry (Schwieterman 2010). In areas where separation of rivers (e.g., permanent lock closure) is not an option, non-physical barriers are being developed to limit invasive species movement without obstructing navigation (Noatch and Suski 2012). Perhaps the most well-known non-physical barrier near the Great Lakes is the electric fish dispersal barrier operated by the U.S. Army Corps of Engineers in the CAWS near Romeoville, IL (Moy et al. 2011). Originally implemented to prevent the inter-basin transfer of round goby between the Great Lakes and Mississippi River, the electric fish dispersal barrier is now critical to reducing the bi-directional movements of several invasive fish species (Parker et al. 2015). Unfortunately, concerns for human safety with hazardous electrical fields in water has limited the utility of this barrier method in other key management locations (Slater et al. 2011).

Dissolved carbon dioxide (CO_2) could be a viable alternative to electrical barriers in areas with public access. Fish detect CO_2 through branchial chemoreceptors (Ishimatsu et al. 2005; Perry and Abdallah 2012) and respond by avoiding those areas or becoming incapacitated through narcosis (Noatch and Suski 2012). A few laboratory studies have found that CO_2 was effective to alter the behavior of invasive sea lamprey (*Petromyzon marinus* Linnaeus, 1758), silver carp (*Hypophthalmichthys molitrix* Valenciennes, 1844), bighead carp (*Hypophthalmichthys nobilis* Richardson, 1845) and several native fishes (Kates et al. 2012; Dennis et al. 2015, 2016). Tracking of telemetered fish in outdoor ponds also showed that CO_2 could be used to exclude bigheaded carps (*Hypophthalmichthys* spp. Bleeker, 1860) from specific areas (Donaldson et al. 2016) and reduce upstream movement by approximately 50% (Cupp et al. 2017a). Although most research has focused on using CO_2 to stop bigheaded carps from entering the Great Lakes, the utility of this method to deter movements of other invasive fishes, like round goby, has not been investigated. Furthermore, the influence of water temperature on fish behavior in response to elevated CO_2 has not been previously reported for any fish species. Addressing this knowledge gap is critical to the success of CO_2 as an invasive fish deterrent as water temperatures differ spatially and temporally across management sites.

The objective of this study was to quantify CO_2 concentrations that modify round goby behavior across multiple water temperatures. Based on behavioral responses described in the literature for other species, we hypothesized that round goby would voluntarily avoid elevated CO_2 when given access to untreated (ambient) water and involuntarily lose equilibrium during prolonged CO_2 exposure. We further hypothesized that because round goby occupy broad thermal habitats, behavioral responses to CO_2 would not differ across water temperatures. To test our hypotheses, we quantified free-swimming avoidance responses of round goby to CO_2 at 5, 15, and 25 °C water temperatures using a shuttle box choice arena. We conducted separate trials to quantify CO_2 concentrations that cause round goby to lose equilibrium using static exposures across the same three water temperatures. Results from this study define CO_2 concentrations that can be used to modify round goby behavior and are intended to guide field testing of a CO_2 fish passage barrier.

Materials and methods

Study animals

Round goby were harvested using hook-and-line angling by the Wisconsin Department of Natural Resources (WDNR) from Lake Michigan near Sturgeon Bay, WI, USA. Fish were held indoors in a large aquarium for two weeks, then truck transported to the U.S. Geological Survey (USGS) Upper Midwest Environmental Sciences Center (UMESC) in La Crosse, WI, USA. A sub-sample of fish were euthanized and taken to the U.S. Fish and Wildlife Service La Crosse Fish Health Center for pathogen screening. The remaining population was held in quarantine at UMESC for 30 d until certified specific-pathogen free. Fish were then held in flow-through culture tanks at 12 °C and maintained on a forage diet (rainbow trout *Onchorhynchus mykiss* Walbaum, 1792) until testing. Water temperatures were modified at < 3 °C per day and fish were held for a minimum of 3 days at testing temperature before testing. Transport and possession of round goby for testing was conducted under an approved WDNR NR 40 permit. Experimental methods for vertebrate testing were approved by the UMESC Animal Care and Use Committee (protocol

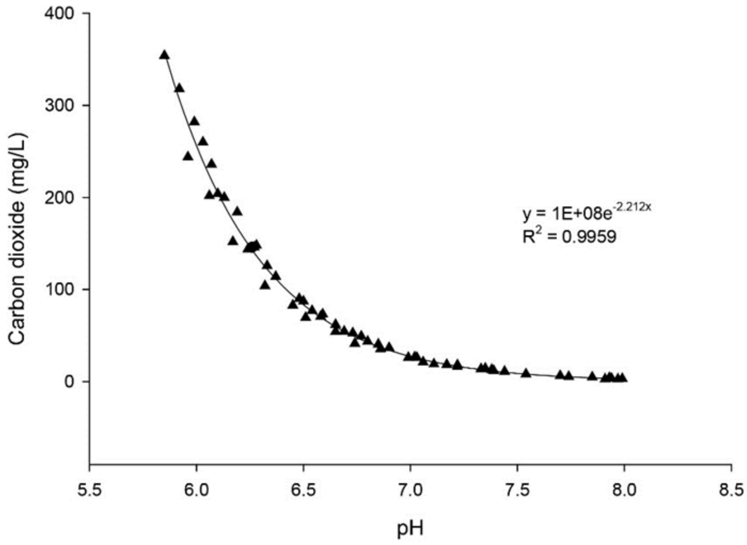

Figure 1. Relationship between pH and dissolved CO_2 (mg/L) concentrations in experimental water. Curve was used to determine CO_2 concentrations during behavioral testing using pH sensors placed inside tanks. Equation of the line is included on the figure.

number: AEH-16-GOBY-01). At the conclusion of each trial, fish were euthanized in a 200 mg/L solution of MS-222 and morphometric measurements recorded. Ten fish were individually tested at 5, 15, and 25 °C for a total of 30 observations per behavioral endpoint (60 fish total for this study).

Avoidance trials

Round goby avoidance was determined using a shuttle box choice arena (Loligo Systems Inc., Viborg, Denmark) from modified methods described in Kates et al. (2012). Briefly, the experimental setup consisted of two circular tanks (dimensions: 1.5 m wide × 0.5 m deep) connected by a narrow, rectangular passageway (dimensions: 0.2 m wide × 0.5 m deep). Water was drawn from each circular tank using small electric pumps and delivered into external buffer columns. Buffer columns were used to manipulate CO_2 in one of the circular tanks without influencing water conditions in the adjacent tank. Water then passed through the buffer column and was returned to the original tank via gravitational flow (3.5–3.6 L/min). Fish movement and position within the choice arena was observed remotely using a single overhead camera (Loligo® uEye USB video camera, Viborg, Denmark). Tanks were surrounded by a black wall to prevent external stimuli from influencing fish behavior.

For each trial, the shuttle box arena was filled with 270 L of water at a predetermined temperature (5, 15, or 25 °C). A single fish was netted from the source tank and placed directly into one of the circular tanks. Location of stocking for the first fish was determined using a coin-flip and subsequent trials alternated the location of fish stocking. Small (approx. 2.5 cm diameter) rock substrate was added to one tank while the other remained empty. Preliminary testing without CO_2 found that round goby spent > 82% of time in the tank containing rock substrate over the opposite empty tank, regardless of which side the rock was added. Tank preference was necessary to minimize the potential for random swimming movements between tanks for reasons not due to CO_2 avoidance and to give incentive for fish to persist in a high CO_2 environment. The tank containing substrate was randomized for each trial.

Each fish remained in the shuttle box arena for up to 90 min. Fish were acclimated for 60 min before CO_2 was added. Carbon dioxide was then continuously administered for up to 30 min, or until avoidance behavior was observed, into the buffer column of the occupied tank. Observers remotely monitored fish movement and recorded pH values during the first movement into the opposite (no CO_2 added) tank. Carbon dioxide was injected from a compressed cylinder at a flow rate of 1 L/min through a single ceramic diffuser located at the bottom of the buffer column. The buffer column for the adjacent non-treated tank received a continuous supply of blown air (Sweetwater® Model: SL94A pump, Pentair Aquatic Ecosystems Inc., Apopka, FL, USA) to facilitate off-gassing of any CO_2 that mixed into the untreated tank.

Carbon dioxide concentrations were monitored using pH sensors located mid-depth in each circular tank. Relationships between pH and CO_2 (mg/L) were determined using a standard curve developed before testing (Figure 1). Carbon dioxide (mg/L) was measured with a HACH digital titrator using a pH 8.3 endpoint with 0.3636 N or 3.636 N NaOH titrant (HACH Method 8205), and pH was measured using a handheld meter with a pH sensor (HACH Model: HQ40d meter, Model: PHC20101 probe; HACH Inc., Loveland, CO, USA). Other parameters measured within the shuttlebox before and after testing were dissolved oxygen, water temperature, alkalinity, hardness, light intensity, and total ammonia nitrogen (TAN). Alkalinity and TAN were quantified using a bench-top spectrophotometer (HACH® Model: DR3900, HACH Inc., Loveland, CO, USA). Hardness was measured using a colorimetric endpoint titration with 0.01 M Na_2EDTA titrant (APHA 1998). Light intensity was measured directly above tank using a handheld meter (Milwaukee Light Meter Model: MW700, Rocky Mount, NC, USA). Partial pressures of CO_2 (μatm) were calculated from alkalinity, pH, and temperature using CO2Calc software (Robbins et al. 2010). Round goby mean ± standard deviation total length during avoidance trials was 123 ± 19 mm and wet weight was 28.5 ± 16.0 g.

Loss of equilibrium trials

Carbon dioxide concentrations that narcotized round goby were quantified using loss of equilibrium as the behavioral endpoint. For these trials, a single fish was stocked into a 100-L stainless steel tank (dimensions: 61 cm long × 61 cm wide × 36 cm deep) containing 30 L of temperature acclimated well water. Fish were given 15 min to acclimate to tanks before initiating trials. Carbon dioxide gas was then continuously administered into the tank at a flow rate of 1 L/min through a single ceramic diffuser. Loss of equilibrium was assessed by applying light pressure to one side of the fish using a small net every 30–40 s. The loss of equilibrium endpoint was defined as the failure to maintain an upright dorsoventral orientation for > 10 s (Cupp et al. 2017b). Dissolved oxygen, temperature, and pH were recorded before fish were stocked and immediately after fish lost equilibrium. Carbon dioxide concentration and partial pressure was calculated from pH at the time fish lost equilibrium using the standard curve equation and CO2Calc as previously described (Robbins et al. 2010). Alkalinity and light intensity were measured once for each temperature. Round goby mean ± standard deviation total length during these trials was 130 ± 17 mm and wet weight was 33.1 ± 12.9 g.

Statistical analyses

Carbon dioxide concentrations that round goby avoided and lost equilibrium were compared using two-way analysis of variance (ANOVA) in R (Zar 2010; R Core Team 2016). Fixed effects in the statistical model were behavioral endpoint (avoidance and loss of equilibrium) and temperature (5, 15, and 25 °C). The assumption of normality was assessed through visual inspection of fitted residuals using a normal probability plot, and the assumption of homogeneity of variances across groups was assessed using a visual inspection of fitted residuals plots (Zar 2010). Pairwise comparisons of significant effects were evaluated using Tukey's honestly significant difference test (Zar 2010). Data figures were developed using the ggplot2 package (Wickham 2009). Fish lengths and weights were compared using one-way ANOVA. Relationships between pH and CO_2 were calculated using SigmaPlot Version 13.0 and fit to an exponential line (Systat Software, San Jose, CA). Water chemistry and morphometric measurements are reported using descriptive measures in text and tables. Statistical significance for comparisons was declared at $\alpha < 0.05$.

Results

Carbon dioxide concentrations (mean [range]) that round goby avoided (63 mg/L CO_2 [14–168 mg/L] or 59,500 μatm [10,300–178,300 μatm]) were significantly lower than CO_2 concentrations that caused round goby to lose equilibrium (216 mg/L CO_2 [144–280 mg/L] or 207,000 μatm [118,200–303,900 μatm]) (ANOVA, $F_{1,53} = 501.6$, $P < 0.001$; Figure 2). Carbon dioxide concentrations modifying behavior for both response endpoints differed with water temperature (ANOVA, $F_{2,53} = 22.9$, $P < 0.001$). Specifically, avoidance and loss of equilibrium were attained using lower CO_2 concentrations at 5 °C than at higher temperatures. Round goby avoided approximately 50% lower CO_2 concentrations at 5 °C relative to 25 °C (Tukey's HSD, $P = 0.004$), but concentrations that causing avoidance at 5°C did not differ from 15 °C (Tukey's HSD, $P = 0.95$). Round goby also avoided lower CO_2 concentrations at 15 °C relative to 25 °C (Tukey's HSD, $P = 0.04$). Loss of equilibrium was observed at approximately 30% lower CO_2 concentrations at 5 °C relative to 15 °C (Tukey's HSD, $P < 0.001$) and 25 °C (Tukey's HSD, $P < 0.001$), but CO_2 concentrations did not differ between 15 °C and 25 °C (Tukey's HSD, $P = 0.99$, Figure 2).

Water chemistry before CO_2 injection was stable across all trials (Table 1). However, CO_2 injection decreased the pH of water by 1.5–2 units (Figure 1). Fish did not differ in length (ANOVA, $F_{5,54} = 0.713$, $P = 0.62$) or weight (ANOVA, $F_{5,54} = 0.38$, $P = 0.86$) across treatment groups.

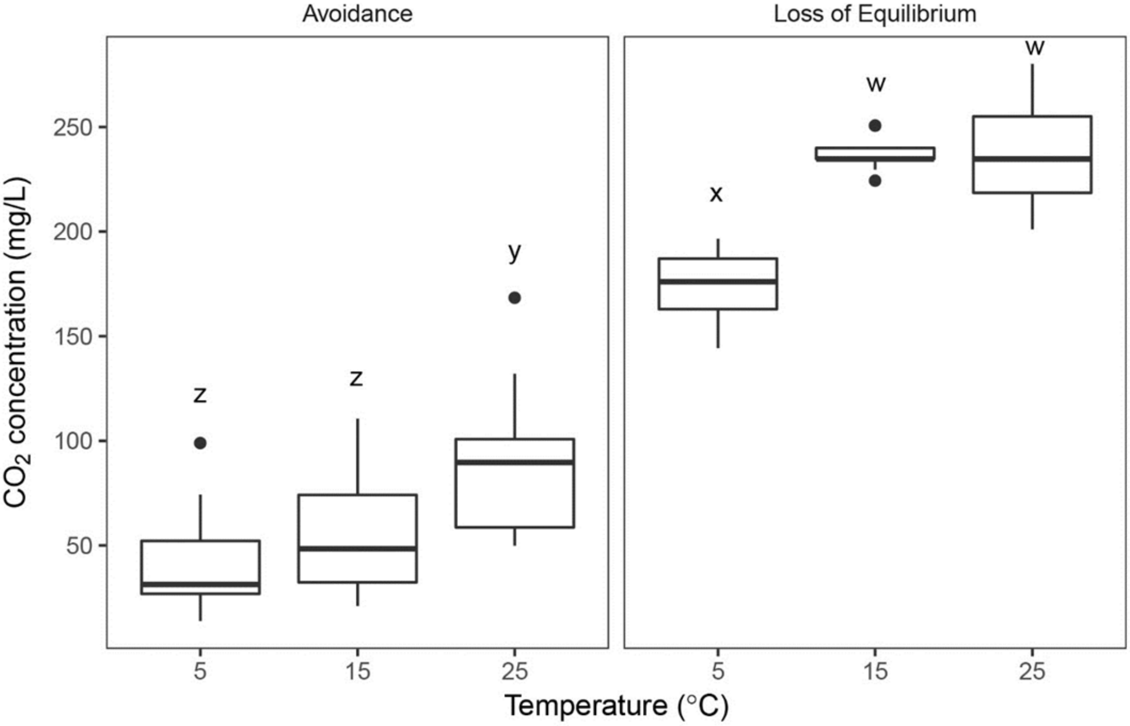

Figure 2. Carbon dioxide concentrations that modified round goby behavior during laboratory testing. Avoidance (left) was defined as a voluntary movement away from the area where carbon dioxide was added. Loss of equilibrium (right) was defined as the involuntary loss of normal swimming behavior and an inability to maintain an upright orientation during continuous carbon dioxide exposure. Response endpoints were observed at three water temperatures (5, 15, and 25 °C) with ten fish observed per temperature. Distribution of box-and-whiskers indicate median, interquartile range, maximum/minimum and outlier values for carbon dioxide concentrations within each treatment group. Dissimilar letters denote statistical differences across treatment groups ($P < 0.05$).

Table 1. Water chemistry and lighting conditions (mean ± standard deviation) inside tanks before evaluating round goby behavioral responses to carbon dioxide (CO_2) at three testing temperatures.

	5 °C Trials	15 °C Trials	25 °C Trials
Temperature (°C)	5.9 ± 0.4	14.8 ± 0.7	24.9 ± 0.2
Dissolved oxygen (mg/L)	12.8 ± 0.5	9.2 ± 0.2	7.6 ± 0.2
pH	7.9 ± 0.1	7.8 ± 0.0	7.7 ± 0.3
Alkalinity (mg $CaCO_3$/L)	166 ± 1	154 ± 12	135 ± 1
Hardness (mg $CaCO_3$/L)	186 ± 4	185 ± 1	180 ± 2
Ammonia-N (mg/L)	0.063 ± 0.003	0.207 ± 0.000	Not recorded
Overhead lighting (lux)	2.0 ± 0.0	2.5 ± 0.7	1.5 ± 0.7
Carbon dioxide (mg/L)	3.7 ± 0.4	4.2 ± 0.0	4.6 ± 1.4

Discussion

Carbon dioxide has received considerable recent attention as a non-physical deterrent to invasive bigheaded carp movement (Kates et al. 2012; Donaldson et al. 2016; Cupp et al. 2017a), and this study found similar behavioral responses with round goby. Across all temperatures, round goby swam away from CO_2 once a threshold of 99–169 mg/L (79,000–178,000 µatm) was reached and lost equilibrium at 197–280 mg/L CO_2 (163,000–303,000 µatm). Aside from one fish at 5 °C, fish during avoidance trials voluntarily moved into the untreated tank before losing equilibrium suggesting round goby leave CO_2-enriched water before the onset of severe physiological impairment. Deterring invasive fish movement, indicated through voluntary CO_2 avoidance, has previously been hypothesized as an approach to reduce movement and abundance in specific locations without causing undue mortality of nontarget species because outcomes are attained at lower concentrations than would otherwise cause adverse effects (Noatch and Suski 2012). However, physical immobilization, characterized using loss of equilibrium, is also a beneficial behavioral endpoint to reduce fish passage due to the loss of controlled swimming, although the CO_2 concentrations were much higher. Altering behavior with CO_2 could be used to reduce round goby movement and further research is needed to determine the feasibility of this method at a larger field-scale.

This was one of the first studies to evaluate the effects of temperature on fish behavior in CO_2-enriched water. Round goby are ectothermic poikilotherms, and the relation between environmental temperature and metabolic rate is directly correlated (Hazel 1993). Our study found that round goby avoided and lost equilibrium from lower CO_2 concentrations at 5 °C relative to 25 °C. One possible explanation is that the concentration gradient between PCO_2 (partial pressure of CO_2 in blood) and P_wCO_2 (partial pressure of CO_2 in water) is greater at lower temperatures as cellular respiration and production of CO_2 is reduced and more CO_2 gas is dissolved in colder water (Perry and Gilmour 2006). The steeper diffusion gradient of CO_2 between water and blood would increase the influx diffusion rate (Fick's Laws of Diffusion) resulting in narcotizing levels of CO_2 in blood over a shorter time period (Willmer et al. 2000). More specifically, elevated environmental CO_2 likely resulted in a net influx of CO_2 (Heuer and Grosell 2014), and elevated internal CO_2 likely lead to narcosis from the movement of CO_2 across the blood/brain barrier, resulting in altered brain pH and an impairment of brain activity (Yoshikawa et al. 1991; Hiromasa et al. 1994). The removal of CO_2 from blood occurs through the actions of the enzyme carbonic anhydrase, which converts CO_2 into HCO_3^- and H^+, and each of these components are excreted separately (Gilmour and Perry 2009). If acclimation to colder water temperatures has slowed the ability of carbonic anhydrase to excrete CO_2, then it is possible that the influx of ambient CO_2 can outpace the ability carbonic anhydrase to excrete CO_2, resulting in a decline of CO_2 level necessary for narcosis relative to warmer water.

Round goby behavior could have also been modified from other water chemistry constituents. When added to water, CO_2 hydrates to form a weak acid (carbonic acid, H_2CO_3) that readily dissociates (H^+), decreasing pH. At CO_2 concentrations causing avoidance and loss of equilibrium, pH decreased by 1.5–2 units from ambient water (see Figure 1). Although increased acidity can alter fish behavior due to its detrimental effects on blood chemistry and acid-base regulation (Perry and Gilmour 2006; Ern and Esbaugh 2016), research suggests that gill chemoreceptors allow fish to sense and differentiate between H^+ and CO_2 (Jones et al. 1985; Ishimatsu et al. 2005; Perry and Abdallah 2012). A few studies have demonstrated that fish specifically avoid areas of elevated CO_2 when given access to ambient fresh water (Clingerman et al. 2007; Dennis et al. 2016; Cupp et al. 2017a). Furthermore, others have hypothesized that a CO_2 barrier could reduce invasive fish passage by creating hypoxic or anoxic conditions through displacement of dissolved oxygen (Noatch and Suski 2012). Dissolved oxygen in our study remained above 5 mg/L and was unchanged (Δ 0.5 mg/L) over the short 30 min exposure, supporting elevated CO_2, and not hypoxia, as a mechanism causing avoidance.

Weakly acidic conditions during CO_2 injection suggests field trials could affect nontarget organisms (Leduc et al. 2004, 2010; Tix et al. 2017). Waller et al. (2017) found that juvenile fat mucket mussels (*Lampsillis siliquoidea* Barnes, 1823) had a 28-d LC50 of 87 mg/L CO_2 during continuous exposure in laboratory tanks. Sensitivity of native mussels to CO_2 levels found to modify round goby behavior has also been shown to decrease shell formation, increase energy demand, increase stress levels and cause substantial ion imbalances (Hannan et al. 2016a, b; Jeffrey et al. 2017). Similarly, toxicity to several freshwater fishes, namely cyprinids, was found during chronic under-ice exposure to approximately 100 mg/L (Cupp et al. 2017c). Although it is important to recognize that these studies were conducted using conditions where animals could not escape or avoid CO_2 exposure, documented toxicity to macroinvertebrates and other fishes suggests that long-term CO_2 use for round goby control could affect nontarget organisms, particularly those unable to quickly relocate away treatment areas. Identifying and mitigating impacts to species of concern will be imperative when selecting suitable locations for employing CO_2 as a non-physical deterrent to round goby. Threatened and endangered species for Federal Agencies in the United States are governed by the U.S. Fish and Wildlife Service Section 7 Consultation under the Endangered Species Act: Section 7(a)(2). State agencies follow similar regulatory requirements but specific guidance will vary with jurisdiction.

Conclusions

Round goby avoided 99–169 mg/L CO_2 (79,000–178,000 μatm) and lost equilibrium at 197–280 mg/L CO_2 (163,000–303,000 μatm) at 5–25 °C. These concentrations may inform field testing.

Acknowledgements

Research funding was provided by the WDNR and the USGS Ecosystem Mission Area Invasive Species Program. We thank Dave Boyarski and Sturgeon Bay WDNR staff for fish collection, Maureen Ferry and Michelle Nault (WDNR) for contributions to study design and Caleb Hasler (University of Illinois) for guidance with shuttle box tank setup. Any use of trade, firm, or product name is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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