Responses of native and invasive fishes to carbon dioxide: potential for a nonphysical barrier to fish dispersal

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Abstract: Upon arrival in a novel environment, invasive species have the potential to cause negative consequences at their new location. Rather than try to eliminate invasive species after introduction, preventing their spread is a more efficient strategy to mitigate impact. The current study used a laboratory setting to quantify the efficacy of elevated carbon dioxide (CO_2) in water to act as a nonphysical barrier to deter fish movement. Our focus was on deterring the movements of silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Hypophthalmichthys nobilis*), but largemouth bass (*Micropterus salmoides*) and bluegill (*Lepomis macrochirus*) were also examined to quantify the impact of elevated CO_2 on native species. Exposure of all species to 30 $mg \cdot L^{-1}$ dissolved CO_2 for 1 h, compared with ambient CO_2 concentrations of 10 $mg \cdot L^{-1}$, resulted in an elevated stress response, along with alterations to ionic–osmotic balance. Exposure of fish to 70 $mg \cdot L^{-1}$ CO_2 caused a reduction in ventilation rates after 1 h, while both silver carp and bighead carp lost equilibrium. Silver carp, largemouth bass, and bluegill also showed avoidance of CO_2 at approximately 100 $mg \cdot L^{-1}$. Together, results suggest that zones of elevated CO_2 have potential to deter the movement of fishes.

Résumé : À leur arrivée dans un nouveau milieu, les espèces envahissantes peuvent potentiellement entraîner des conséquences négatives dans leur nouvel emplacement. Plutôt que d'essayer d'éliminer les espèces envahissantes après leur introduction, la prévention de leur propagation constitue une stratégie plus efficace pour atténuer ces impacts. La présente étude menée en laboratoire avait pour but de quantifier l'efficacité de teneurs élevées en dioxyde de carbone (CO_2) dans l'eau comme barrière non physique au déplacement des poissons. L'exercice visait à empêcher les déplacements de la carpe argentée (*Hypophthalmichthys molitrix*) et de la carpe à grosse tête (*Hypophthalmichthys nobilis*), mais l'achigan à grande bouche (*Micropterus salmoides*) et le crapet arlequin (*Lepomis macrochirus*) ont également été examinés afin de quantifier l'incidence de teneurs élevées en CO_2 sur des espèces indigènes. L'exposition de toutes ces espèces à 30 $mg \cdot L^{-1}$ de CO_2 dissout pendant 1 h, comparativement à une concentration ambiante de CO_2 de 10 $mg \cdot L^{-1}$, s'est traduite par une réaction de stress accrue ainsi que des modifications de l'équilibre ionique–osmotique. L'exposition des poissons à 70 $mg \cdot L^{-1}$ de CO_2 a entraîné une diminution des taux de ventilation après 1 h, la carpe argentée et la carpe à grosse tête perdant toutes deux l'équilibre. La carpe argentée, l'achigan à grande bouche et le crapet arlequin ont également présenté des signes d'évitement du CO_2 à des concentrations d'environ 100 $mg \cdot L^{-1}$. Ensemble, ces résultats suggèrent que des zones riches en CO_2 pourraient empêcher le déplacement des poissons.

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Introduction

Invasion of non-native species can have tremendous negative impacts for the receiving environment. Invasive species have the potential to expand rapidly, reproduce in large numbers, outcompete local species, and disrupt local food webs and ecosystems (Vander Zanden et al. 1999; Clavero and García-Berthou 2005; Ricciardi and MacIsaac 2011). Currently it is believed that approximately 42% of the federally threatened or endangered species in the United States are considered at risk because of the influence of non-native species (Pimentel et al. 2005). Invasive species are also believed to cost the USA approximately $120 billion annually in economic damages (Pimentel et al. 2005), with this figure likely to increase in the future (Lodge et al. 2006). Because it is difficult, if not impossible, to eradicate an invasive species following introduction into a new location, successful management against invasive species should focus on methods to prevent the introduction of non-native species, as this has been proposed as a more cost-effective and long-term approach (Lodge et al. 2006; Finnoff et al. 2007).

In North America, both the Great Lakes and the Mississippi basins have been recipients of large numbers of introduced species. The Great Lakes basin, for example, has been invaded by at least 182 non-native species in the past, translating to a new invader in this system every 28 weeks (Ricciardi 2006), and some of these species are currently not present in the Mississippi River basin (i.e., the round goby (*Neogobius mela-*

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nostomus) is currently confined to the Great Lakes basin; Hoover et al. 2003). These two regions have been economically and ecologically devastated by the establishment of non-native aquatic species, with little hope of mitigating this impact (Patel et al. 2010). Of particular concern are the presence of silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Hypophthalmichthys nobilis*) (hereafter, silver carp and bighead carp will collectively be referred to as Asian carp), which are currently confined to the Mississippi basin. Asian carp numbers have increased dramatically in the Mississippi basin because of a number of factors (reproductive potential, nonselective feeding, rapid growth rate, etc.), and there is potential for these fishes to move from the Mississippi River basin into the Great Lakes. While the predicted impacts of Asian carp have not been well defined (Cooke and Hill 2010; Rasmussen et al. 2011), their presence in the Great Lakes is not expected to have positive impacts and therefore should be avoided (Conover et al. 2007; Patel et al. 2010; Rasmussen et al. 2011).

Currently, it is believed that there are 19 artificial or naturally occurring connections between the Mississippi and Great Lakes basins, and a portion of these could allow passage of Asian carp and other aquatic nuisance species (Asian Carp Regional Coordinating Committee 2010). Of particular interest is the link between the two basins provided by the Chicago Area Waterway System (CAWS), which is an artificial shipping channel that provides a direct, open link that can permit fish passage (Patel et al. 2010; Rasmussen et al. 2011). At present, the movement of Asian carp between the Mississippi and Great Lakes basins in the CAWS is impeded by a pair of electrified barriers (Conover et al. 2007). While these barriers are believed to have been a successful deterrent to date, there is reason to believe the barriers may not be 100% effective at deterring fish movements under all circumstances. More specifically, the electricity from this barrier may not be effective against small fishes (Reynolds 1996), fish may be able to use "protective cover" offered by steel-hulled barges to pass through electric fields (Dettmers et al. 2005), and the design of the current electric barrier is such that fish may be able to bypass the electric field entirely during flood events (US Army Corps of Engineers 2010*a*, 2010*b*). As such, it is imperative that additional barrier technologies be developed, tested, and, where appropriate, implemented to supplement existing electric barriers within the CAWS and to prevent the interbasin movement of invasive species between the Great Lakes and Mississippi basins across all potential exchange points.

The overall goal of this study is to quantify physiological, behavioral, and reflex responses of fish to elevated carbon dioxide (CO_2), with the hope of using elevated dissolved CO_2 as a nonphysical barrier to deter the movement of fishes. Previous work has shown that carbon dioxide dissolved in water can induce fish avoidance (Ross et al. 2001; Clingerman et al. 2007), and a recent report by the United States Army Corps of Engineers identified CO_2 as a potential control mechanism that could be effective against a wide range of vertebrate and invertebrate invaders and as a compound with potential for field application (Great Lakes Mississippi River Interbasin Study Team 2011). Our focus was on Asian carp and preventing their movement into the Great Lakes basin, but, if successful, a zone of elevated CO_2 could potentially act as a barrier that could prevent movement of all potentially invasive fishes between the Mississippi and Great Lakes basins. Responses of both Asian carp and native North American fishes to elevated CO_2 were quantified with three separate yet complementary series of laboratory experiments: observations of reflex responses, blood stress physiology, and avoidance.

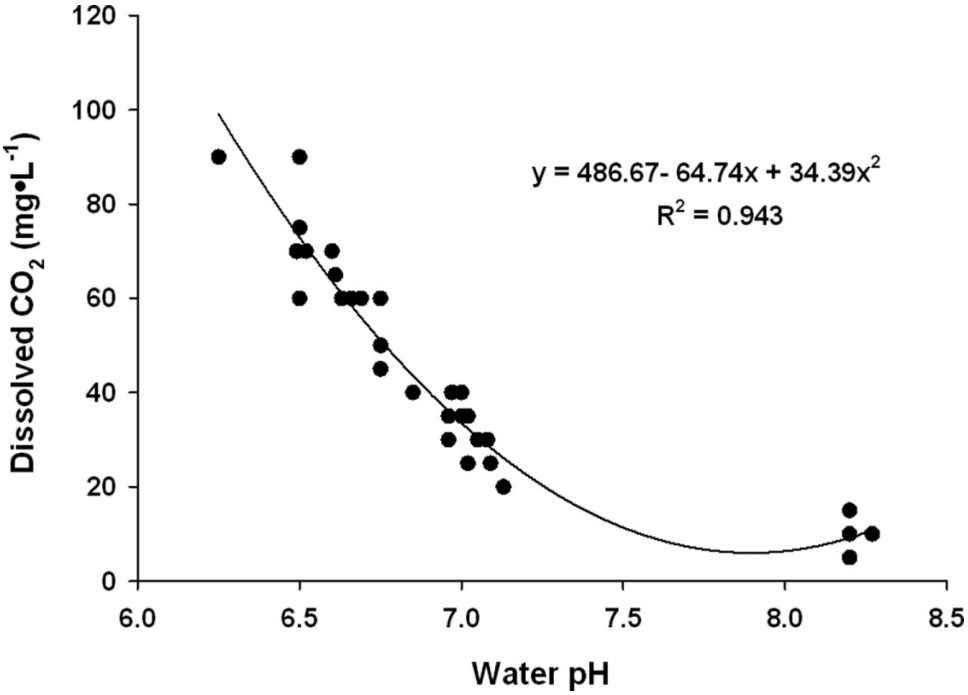
Materials and methods

Four species of fish were used in this study: largemouth bass (*Micropterus salmoides*), bluegill (*Lepomis macrochirus*), silver carp, and bighead carp. Experiments were performed in September–October 2010, March–April 2011, and September–October 2011. Silver carp were collected by using standard pulsed direct current (DC) boat electroshocking in a 5.5 m flat bottom aluminum boat. Hertz and voltage were adjusted daily to perform at approximately 3000 W as per standardized long-term collection protocols (Gutreuter et al. 1995). Bighead carp were collected by trammel netting from a variety of locations in east central Illinois along the Illinois River. Asian carp were then transported to the University of Illinois Aquatic Research Facility, Champaign–Urbana, Illinois, in a 640 L truck bed hauler in ambient water supplied with compressed O_2 gas to near saturation. Largemouth bass and bluegill were purchased from a local fish hatchery (Logan Hollow Fish Farm, Murphysboro, Illinois) and delivered to the Aquatic Research Facility. All fish received a minimum of 48 h acclimation time following transport for recovery prior to being used in experiments; previous work has shown that 48 h is a sufficient duration of time for physiological disturbances associated with capture and hauling to return to control (resting) levels (Milligan 1996; Suski et al. 2006). At the Aquatic Research Facility, all fish were housed in an outdoor, closed recirculating system consisting of six round plastic tanks (1280 L, 1.7 m diameter) connected to a 0.04 ha, earthen-bottom pond with abundant natural vegetation. Water was supplied to the tanks from the pond and allowed to drain back into the pond for oxygenation and removal of waste. Tanks received supplemental aeration from an air blower and were covered with mesh netting to prevent fish from escaping. Largemouth bass and bluegill were fed pelleted food (Dense Culture Food, F2C, Aquatic Ecosystems, Apopka, Florida) until satiation every other day, were held for a maximum of approximately 3 weeks, and were starved for 48 h prior to being used in experiments. Asian carp did not receive food in the lab and were held for a period of 2–4 days prior to being used in experiments. Across all holding periods, water temperatures averaged 18.0 °C (±0.4 °C, standard error, SE) and dissolved oxygen averaged 8.6 $mg \cdot L^{-1}$ (±0.1 $mg \cdot L^{-1}$ SE). Water from this facility drains through the local sanitary sewer system, and the entire site is surrounded by a 3 m chain link fence topped with barbed wire, thus providing biosecurity necessary to prevent accidental introduction of fish into surrounding water courses.

Reflex response observations

Reflex response observations were intended to define concentrations of dissolved CO_2 that begin to cause irregular actions in fishes or a reaction to the noxious stimuli. All trials were conducted between 30 September and 12 November 2010 and followed the general procedures described in Suski et al. (2007). Fish were carefully netted from the outdoor tanks and moved to an indoor experimental facility. The experimen-

Fig. 1. Relationship between water pH and dissolved CO_2 ($mg \cdot L^{-1}$) concentration in the water. Measurements were collected during experimental trials. Equation of the line is given on the figure.



tal apparatus was a flow-through system consisting of individual opaque plastic containers that received water pumped from a central basin. Water was allowed to overflow from each container and drain back into the central basin creating a closed system. Each individual container was outfitted with an airstone, and containers were sized appropriately to hold a single fish of the species being observed (2.25 L for bluegill, 4.75 L for largemouth bass, 24 L for silver carp, and 50 L for bighead carp). After 30 min of acclimation, baseline water quality measurements were taken within the system; water temperature and dissolved oxygen concentrations were taken with a portable meter (YSI, 550A Yellow Springs Instruments, Irvine, California), and pH was quantified using a handheld pH meter (WTW pH 3310 meter with a SenTix 41 probe, Germany). Immediately following acclimation, all fish in the system were monitored by a single researcher for initial reflex responses that included ventilation rates (ventilations per minute) and the occurrence of irregular activities (episodic or intermittent ventilations, twitching, surface ventilations, coughing, or loss of equilibrium; Suski et al. 2007). Quantification of reflex responses for each treatment group occurred every 30 min for a total of 3.5 h, and water quality parameters within the control group did not change during this period. Hypercarbia challenges were conducted at two target CO_2 concentrations, and hypercarbia was achieved by bubbling compressed CO_2 into the water in the central basin following the initial activity and water quality observations (Clingerman et al. 2007). Concentrations of dissolved CO_2 were verified with water samples taken from a separate individual container using a CO_2 digital titration kit (Hach, Titrator model 16900 and kit No. 2272700) (Summerfelt and Sharrer 2004; American Public Health Association, American Water Works Association, and Water Environment Federation 2005). A pH meter was used to develop a relationship between pH and dissolved CO_2 concentrations ($F = 254.7$, $P < 0.0001$, $R^2 = 0.93$; Fig. 1) (Clingerman et al. 2007). For hypercarbia trials, CO_2 was bubbled into the central basin and increased from an ambient level of approximately 10 $mg \cdot L^{-1}$ (10.2 ± 1.0 $mg \cdot L^{-1}$ CO_2; mean $\pm$ SE pH $= 8.2 \pm 0.03$) to either 30 $mg \cdot L^{-1}$ (28.3 ± 1.15 $mg \cdot L^{-1}$ CO_2; mean $\pm$ SE pH $= 7.32 \pm 0.11$) or 70 $mg \cdot L^{-1}$ (67.7 ± 0.9 $mg \cdot L^{-1}$ CO_2; mean $\pm$ SE pH $= 6.6 \pm 0.05$) and verified in an extra test chamber within the system such that monitoring of water quality did not influence test subject activity levels. Ambient (control) values of CO_2 were consistent with previous studies on this topic (Clingerman et al. 2007; Brauner et al. 2000). Target CO_2 levels were maintained manually throughout the trial by monitoring the handheld pH meter and adjusting CO_2 additions as necessary. During hypercarbia trials, aeration was maintained in each individual container to avoid hypoxia (O_2: mean $\pm$ SE $= 8.1 \pm 0.08$ $mg \cdot L^{-1}$). Fish in the hypercarbia treatments were monitored in a manner identical to control treatments. At the conclusion of the observation period, fish were euthanized, measured (total length in mm), and weighed to the nearest gram. Water temperatures across treatments for the different species were as follows: bluegill, 16.4 ± 0.04 °C; largemouth bass, 15.4 ± 0.03 °C; silver carp, 17.4 ± 0.06 °C; bighead carp, 14.7 ± 0.04 °C. Fish size did not vary within species across treatments for largemouth bass (179 ± 3.9 mm) and silver carp (454 ± 6.6 mm) (two-way analysis of variance, F values < 3.0, P values > 0.10). For bluegill, mean size across all treatments was 121 ± 3.4 mm, and bluegill in the control treatment were approximately 25 mm smaller than fish exposed to elevated CO_2 (analysis of variance, ANOVA, $F = 16.6$, $P < 0.05$). For bighead carp, mean size across all treatments was 736 ± 13.9 mm, and fish in the 30 $mg \cdot L^{-1}$

CO_2 trial were 90 mm smaller than fish exposed to 70 mg·L^{-1}. However, there was no significant difference between either of the hypercarbia trials when compared with the control treatment (ANOVA, $F = 5.08$, $P > 0.05$).

Blood stress physiology

Analyses of sublethal blood stress parameters was intended to define the physiological responses of target species to hypercarbia exposure, and methods for the blood stress experiments followed procedures outlined in VanLandeghem et al. (2010). Prior to the start of the blood stress physiology experiment, fish were carefully netted from the outdoor tanks and moved to the same indoor experimental facility as described above. For this series of experiments, however, aerated containers were outfitted with tight-fitting lids to ensure that fish could not escape during trials. Fish were allowed to acclimate to their container for 24 h, and dissolved oxygen concentrations across acclimation periods for all species remained at 8.0 ± 0.2 mg·L^{-1}. Hypercarbia challenges again consisted of bubbling CO_2 gas into the central basin to a free CO_2 concentration of 30 mg·L^{-1} (mean = 33.3 ± 1.3 mg·L^{-1} CO_2 SE; mean pH = 7.03 ± 0.02 SE) and pumping water to the containers. This concentration for physiology experiments was chosen based on preliminary analyses of fish responses from the reflex observation studies described above. After a 1 h exposure to this concentration of CO_2, the flow of water to each individual container was stopped, and fish were euthanized by an overdose of anesthetic (250 mg·L^{-1} tricaine methanesulphonate (MS-222) buffered with 500 mg·L^{-1} sodium bicarbonate) added to each individual chamber. Following cessation of ventilation, fish were weighed, measured, and blood was drawn from the caudle vasculature along the spine of the fish using a 22-gauge needle and a 1 mL syringe rinsed with lithium heparin. To quantify hematocrit, a small volume of whole blood was transferred to a 75 mm microhematocrit tube (Drummond Scientific, Broomall, Pennsylvania), the base of which was sealed with Critoseal putty (McCormick Scientific, St. Louis, Missouri). The sample was spun in a microhematocrit centrifuge (LW Scientific Zippocrit, Atlanta, Georgia) at 4400 × gravity (*g*) for 2 min. The remaining whole blood sample was centrifuged at 2000*g* for 2 min to separate red cells from plasma. Plasma was transferred to 1.5 mL microcentrifuge tubes and immediately placed in liquid nitrogen until later storage at –80 °C. Fish in the control treatment were allowed to remain undisturbed in their containers for 25 h with no change in water parameters and were then sampled in an identical manner. Water temperatures across treatments for different species were as follows: bluegill, 15.9 ± 0.2 °C; largemouth bass, 17.5 ± 0.2 °C; silver carp, 16.7 ± 0.2 °C; bighead carp, 15.3 ± 0.2 °C. Additionally, fish size did not vary across treatment within species; bluegill, 150 ± 2.6 mm; largemouth bass, 216 ± 2.3 mm; silver carp, 461 ± 8.2 mm; bighead carp, 714 ± 11.4 mm; two-way analyses of variance, F values < 4.0, P values > 0.05).

Avoidance

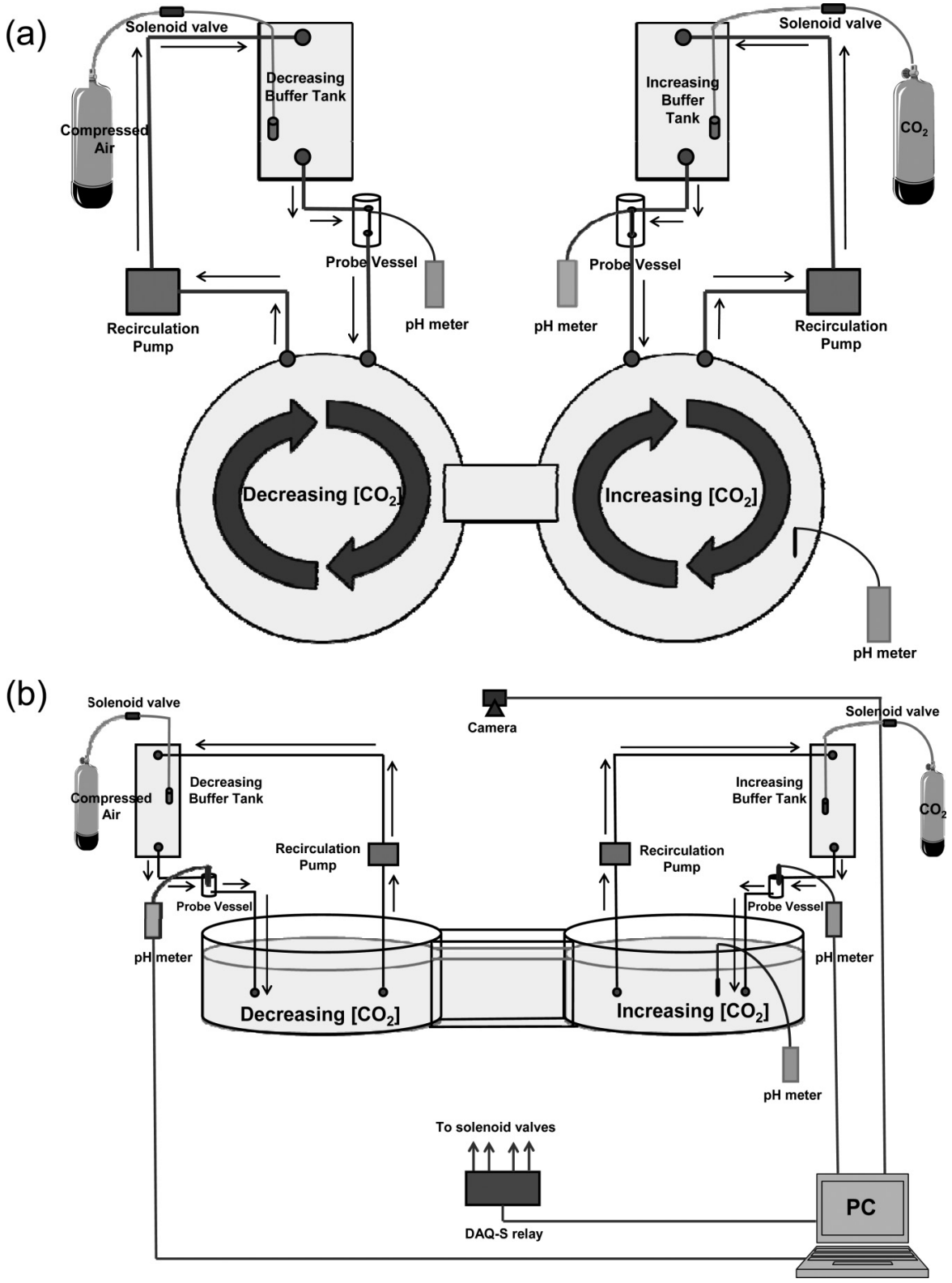
Quantification of hypercarbia avoidance was performed using a "shuttle box" choice arena (Fig. 2; Loligo Inc., Hobro, Denmark) (Serrano et al. 2010). This arena consists of two holding tanks (approximately 1.5 m diameter, 0.5 m deep) connected by a narrow, central tunnel (approximately 20 cm width, 0.5 m deep). Each holding tank has an associated external buffer chamber where water can be treated and returned into the holding tanks without influencing conditions in the opposite tank, and water in each holding tank can be manipulated independently using a computer and software package (ShuttleSoft 2.6.0, Loligo Inc.). A pump continuously drew water from each holding tank into its respective buffer chamber. Gravitational force then drew water out of each buffer chamber and passed it over a pH meter probe contained within an inline probe vessel before being returned to the holding tank. This allowed for continuous, real-time monitoring of water conditions in each tank and also ensured continuous mixing of water within the holding tank. Fish location was monitored remotely using a video camera, and a curtain around the holding tanks ensured that the presence of an observer did not influence fish activity (Serrano et al. 2010).

To quantify avoidance of CO_2, a species of fish was first chosen at random, and an individual of that species was collected from the outdoor holding tank and moved into the shuttle box, located in the indoor experimental facility. A coin flip was used to determine into which of the two holding tanks the fish was placed. Individual fish were allowed to acclimate to the holding tanks for 2 h at ambient temperature, dissolved oxygen, and dissolved CO_2. Water parameters for all species were 17.5 °C (±0.2 °C) and pH 8.0 (±0.13). Following this acclimation period, the external buffer chamber for the holding tank in which the fish had settled received a continuous addition of dissolved CO_2 gas, while the external buffer chamber for the holding tank without the fish received a continuous addition of compressed air. Water from these external buffer chambers was added to the holding tank, thereby achieving a gradual but continuous increase in CO_2 (quantified in real time as a reduction in pH) for the holding tank with the fish, while water quality in the opposite holding tank remained essentially unaltered (i.e., at control pH). During this addition of CO_2 to the holding tank, the time and pH were recorded when the fish became agitated (indicated by surface ventilations, twitching, or elevated swimming activity), shuttled to the opposite holding tank via the central tunnel, or lost equilibrium. If or when the fish moved to the other holding tank through the central tunnel, the external buffer chambers for both holding tanks received compressed air for 10 min to strip CO_2 from the water and increase pH in the tank. After the fish had stopped moving and settled on the bottom of the new holding tank, the tank housing the fish was treated with CO_2 gas. The trial was repeated in this manner for roughly 1 h (or until the fish lost equilibrium), resulting in multiple measurements collected from multiple individuals of each species. At the conclusion of the trials, fish were removed from the system and euthanized as described above to be weighed and measured. Owing to limitations with the size of the behavioral arena, avoidance experiments could not be performed with bighead carp. Fish sizes within species were as follows: bluegill, 132 ± 4.1 mm; largemouth bass, 213 ± 5.8 mm; silver carp, 460 ± 18.3 mm.

Laboratory analyses

Analysis of plasma parameters is described in detail in Suski et al. (2003). Briefly, plasma cortisol was determined using a commercially available kit (cortisol: kit No. 900-071;

Fig. 2. Schematic drawing of the shuttle box system showing major components. An overhead view of the system (*a*) depicting the large choice chamber along with the tanks used to treat the water. Arrows depict the flow of water. A side view (*b*) showing the same tanks, also including the connection to the PC and the manifold controlling the addition of air or CO_2 via solenoid valves. Schematic is not to scale.



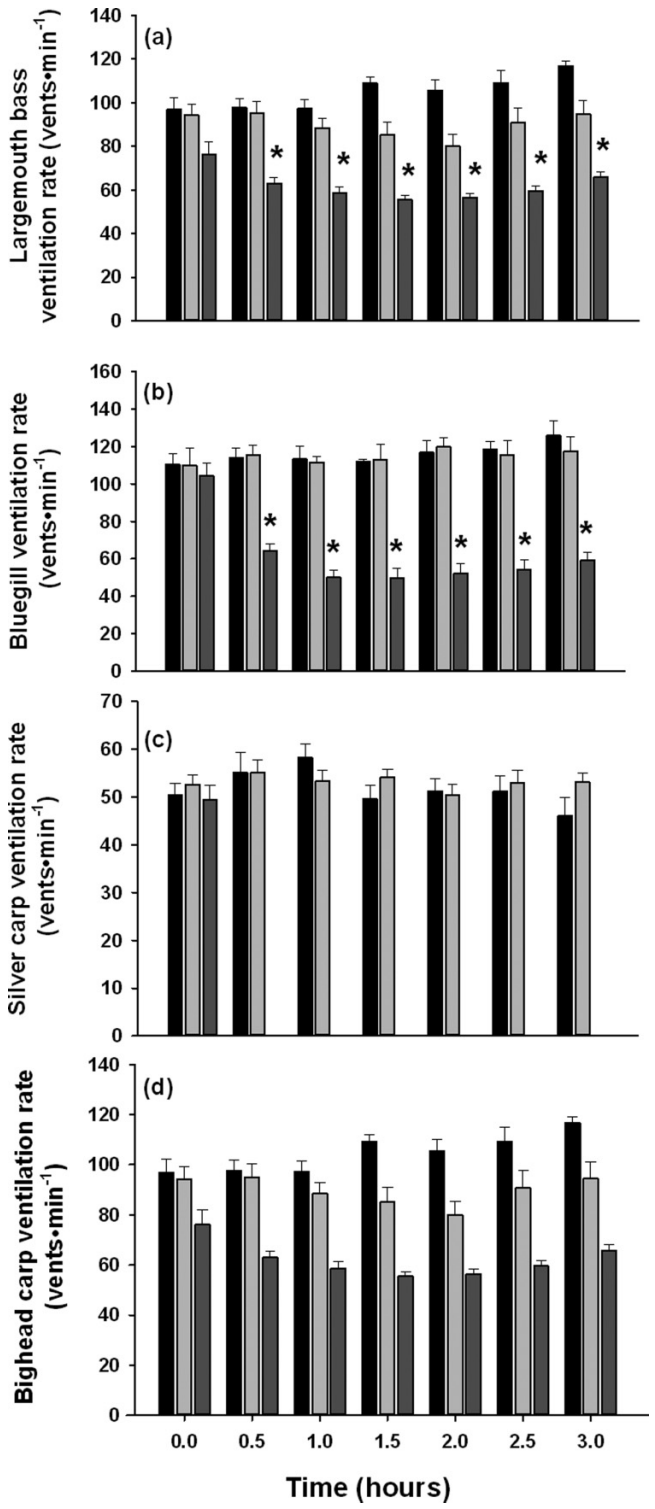


Fig. 3. Ventilation rates for largemouth bass (*a*), bluegill (*b*), silver carp (*c*), and bighead carp (*d*) subjected to one of three different dissolved CO_2 concentrations across a 3 h period. Control CO_2 (5 mg·L^{-1}) is shown with black bars, 30 mg·L^{-1} CO_2 is shown by light gray bars, and 70 mg·L^{-1} CO_2 is shown by dark gray bars. An asterisk denotes a statistically significant difference from control treatment at a time period. Error bars show 1 standard error (SE). Sample size for largemouth bass and bluegill is eight fish per bar. For silver carp, sample sizes are eight fish for time 0.0, but all animals in the 70 mg·L^{-1} CO_2 treatment lost equilibrium after 30 min and were removed from the trial. For bighead carp, sample sizes started at eight at time 0.0, but fell to three fish by 0.5 h for the 70 mg·L^{-1} CO_2 treatment as animals lost equilibrium and were removed from the trial. Results of statistical analyses are given in Table 1.

Enzo Life Sciences, Farmingdale, New York). Plasma sodium and potassium concentrations were determined with a flame photometer (model 2655–00; Cole-Parmer Instrument Company, Chicago, Illinois), and plasma chloride concentrations were determined with a chloridometer (model 4435000; Labconco Corporation, Kansas City, Missouri). Plasma glucose and lactate concentrations were determined enzymatically following the methods of Lowry and Passonneau (1972) in a 96-well microplate read by a commercially available spectrophotometer (Spectra Max Plus 384, model No. 05362; Molecular Devices, Union City, California).

Statistical analyses

Comparisons of ventilation rates across observation times were made using a two-way, repeated measures ANOVA, with time, treatment, and treatment × time entered as fixed effects, and fish identification number entered as a random effect (Sokal and Rohlf 1995). When the interaction was significant, or if the interaction was not significant but at least one of the main effects was significant, a Tukey–Kramer honestly significant difference (HSD) post hoc test was used to separate means (Sokal and Rohlf 1995). Frequency of irregular reflex activities was compared across species using a two-way contingency table analysis, with species, treatment, and treatment × species entered as fixed effects (Sokal and Rohlf 1995). Comparisons of blood physiology parameters were also made using a two-way ANOVA, with species, treatment, and treatment × species entered as fixed effects. When the interaction or any of the individual effects were significant, a Tukey–Kramer HSD post hoc test was used to separate means (Sokal and Rohlf 1995). Comparisons of CO_2 concentration that induced erratic reflex activities (including loss of equilibrium), as well as the CO_2 concentration that induced shuttling, were made across species using a repeated measures ANOVA (with fish identification number entered as a random effect), followed by a Tukey–Kramer HSD post hoc test to separate means (Sokal and Rohlf 1995). All means are reported ±SE where appropriate, and all statistical analyses were performed using JMP version 9.0 (SAS Institute Inc. Cary, North Carolina). The level of significance (α) for all tests was 0.05.

Results

For all four species examined, there was no change in ventilation rates relative to control treatments following 3 h exposure to 30 mg·L^{-1} CO_2 (Figs. 3*a*–3*d*; Table 1). However, after 0.5 h exposure to 70 mg·L^{-1} CO_2, largemouth bass showed a 30% decrease in ventilation rate relative to control treatments, and ventilation rates remained at this level for 3 h (Fig. 3*a*; Table 1). Bluegill showed a similar response to elevated CO_2, but ventilation rates fell by over 50% after 1 h exposure to 70 mg·L^{-1} CO_2 (Fig. 3*b*; Table 1). After 0.5 h exposure to 70 mg·L^{-1} CO_2, all silver carp lost equilibrium in

Table 1. Results for two-way, repeated measures analysis variance (ANOVAs) examining the impact of elevated CO_2 (30 and 70 mg·L^{-1}) concentrations on ventilation rates of largemouth bass, bluegill, and bighead carp.

Species	Main effects	df	*F*	*P*
Largemouth bass	Time	6	4.34	**0.0006**
	Treatment	2	53.2	**<0.0001**
	Treatment × time	12	4.59	**<0.0001**
Bluegill	Time	6	4.29	**0.0006**
	Treatment	2	56.6	**<0.0001**
	Treatment × time	12	6.75	**<0.0001**
Bighead carp	Time	6	1.31	0.2583
	Treatment	2	3.20	0.0580
	Treatment × time	12	1.88	**0.0438**

Note: Ventilation rate data were collected every 30 min over a 3 h exposure period. Silver carp data are not included because all individuals in the 70 mg·L^{-1} CO_2 treatment lost equilibrium within 30 min exposure and were removed from the trial, precluding the collection of data. Bold *p* values highlight significant effects.

their containers. As such, these animals were removed from experiments precluding collection of additional ventilation rate data for this species (Fig. 3*c*; Table 1). Similarly, five of eight bighead carp exposed to 70 mg·L^{-1} CO_2 lost equilibrium after 0.5 h, reducing sample sizes for this treatment. This lack of sample size and power caused ventilation rates for silver carp exposed to 70 mg·L^{-1} to be not significantly lower than controls values despite being 50% lower at some time points (Fig. 3*d*; Table 1).

During 3 h of confinement in individual containers, the proportion of individual fish displaying irregular activities increased with higher concentrations of CO_2 (nominal logistic fit $\chi^2_{11} = 62.7$, $P = < 0.0001$; Fig. 4; Table 2). More specifically, the proportion of fish that showed irregular activities when exposed to 30 mg·L^{-1} varied between 0% and 50% for all four species examined. However, after 3 h of exposure to 70 mg·L^{-1}, 100% of bluegill, silver carp, and bighead carp showed irregular activity, along with 71% of largemouth bass (Fig. 4; Table 2).

One-hour exposure to 30 mg·L^{-1} CO_2 did not cause significant changes to plasma cortisol concentrations for largemouth bass, bluegill, and silver carp (Fig. 5*a*; Table 3). Exposure of bighead carp to 30 mg·L^{-1} CO_2 for 1 h caused a decline in plasma cortisol by almost 50% relative to control values (Fig. 5*a*). Across all four species combined, plasma glucose concentrations increased by approximately 25% after a 1 h exposure to 30 mg·L^{-1} CO_2, but changes within species were not significant (Fig. 5*b*; Table 3). Concentrations of plasma lactate fell by approximately 50% for bighead carp relative to control when exposed to 30 mg·L^{-1} CO_2, but none of the other species displayed any significant change to plasma lactate during trials (Fig. 5*c*; Table 3). One-hour exposure to 30 mg·L^{-1} CO_2 also had a significant effect on hematocrit for all four species examined (Table 3). Largemouth bass and silver carp saw an increase in hematocrit by approximately 40% and approximately 23% in bluegill relative to control values (Table 3). Bighead carp conversely saw a decrease in hematocrit of approximately 14% relative to control fish (Table 3).

Fig. 4. Proportion of irregular activities (episodic or intermittent ventilations, twitching, surface ventilations, coughing, or loss of equilibrium) exhibited by largemouth bass, bluegill, and Asian carp subjected to varying dissolved CO_2 concentrations (control, 30, or 70 mg·L^{-1} CO_2) for 3 h. Sample sizes across all treatments totaled 19 for largemouth bass, 27 for bighead carp, 22 for bluegill, and 22 for silver carp (with 4–10 individuals per individual treatment). Results of statistical analyses are shown in Table 2.

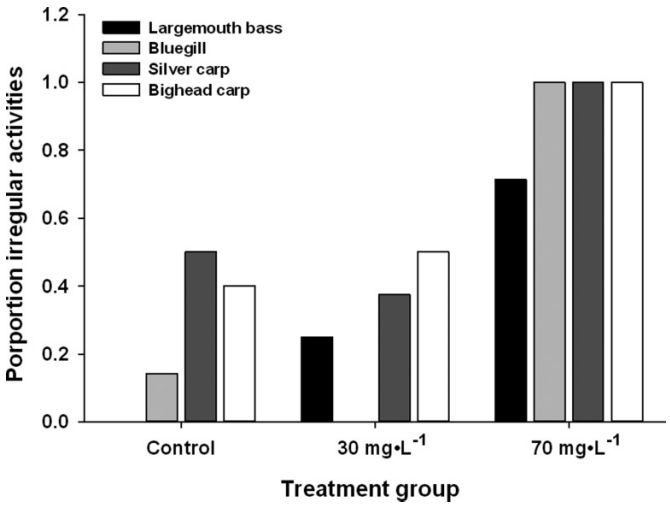


Table 2. Nominal logistic fit model examining the impact of elevated CO_2 concentrations (30 and 70 mg·L^{-1}) on irregular activities (episodic or intermittent ventilations, twitching, surface ventilations, coughing, or loss of equilibrium) of largemouth bass, bluegill, silver carp, and bighead carp.

Main effects	df	χ^2	*P*
Species	3	11.4	**0.0098**
Treatment	2	49.5	**<0.0001**
Treatment × species	6	6.96	0.3243

Note: All animals were held in elevated CO_2 concentrations for 3 h and observations occurred every 30 min. Bold *p* values highlight significant effects.

One-hour exposure to 30 mg·L^{-1} CO_2 did not have a statistically significant impact on plasma potassium, relative to control treatments, for any of the four species examined (Fig. 6*a*; Table 2). Largemouth bass and bighead carp experienced a reduction in plasma sodium of 18% and 14% (respectively), following 1 h exposure to 30 mg·L^{-1} CO_2, but plasma sodium did not change for bluegill and silver carp (Fig. 6*b*; Table 2). Bighead carp also experienced a 20% increase in plasma chloride, but chloride values did not vary significantly for the other three species examined (Fig. 6*c*; Table 2).

Exposure of largemouth bass, bluegill, and silver carp to elevated CO_2 within the shuttle box induced both agitated responses, followed by voluntary shuttling to water with lower CO_2. More specifically, all three species displayed agitated responses beginning at concentrations of approximately 95 mg·L^{-1} CO_2, with no significant difference across species (agitated: $F_{[2]} = 0.03$, $P = 0.97$; shuttled: $F_{[2]} = 0.58$, $P = 0.57$) (Fig. 7*a*). At approximately 110 mg·L^{-1} CO_2, all three

Fig. 5. Concentrations of plasma cortisol (*a*), plasma glucose (*b*), and plasma lactate (*c*) for largemouth bass, bluegill, silver carp, and bighead carp subjected to either control conditions (5 mg·L^{-1} CO_2) or hypercarbia (30 mg·L^{-1} CO_2) for 1 h. An asterisk denotes a statistically significant difference between control and treatment values within a species. Horizontal lines denote a significant treatment effect across all species for a response variable. Error bars show 1 standard error (SE). Sample size is eight fish per treatment group.

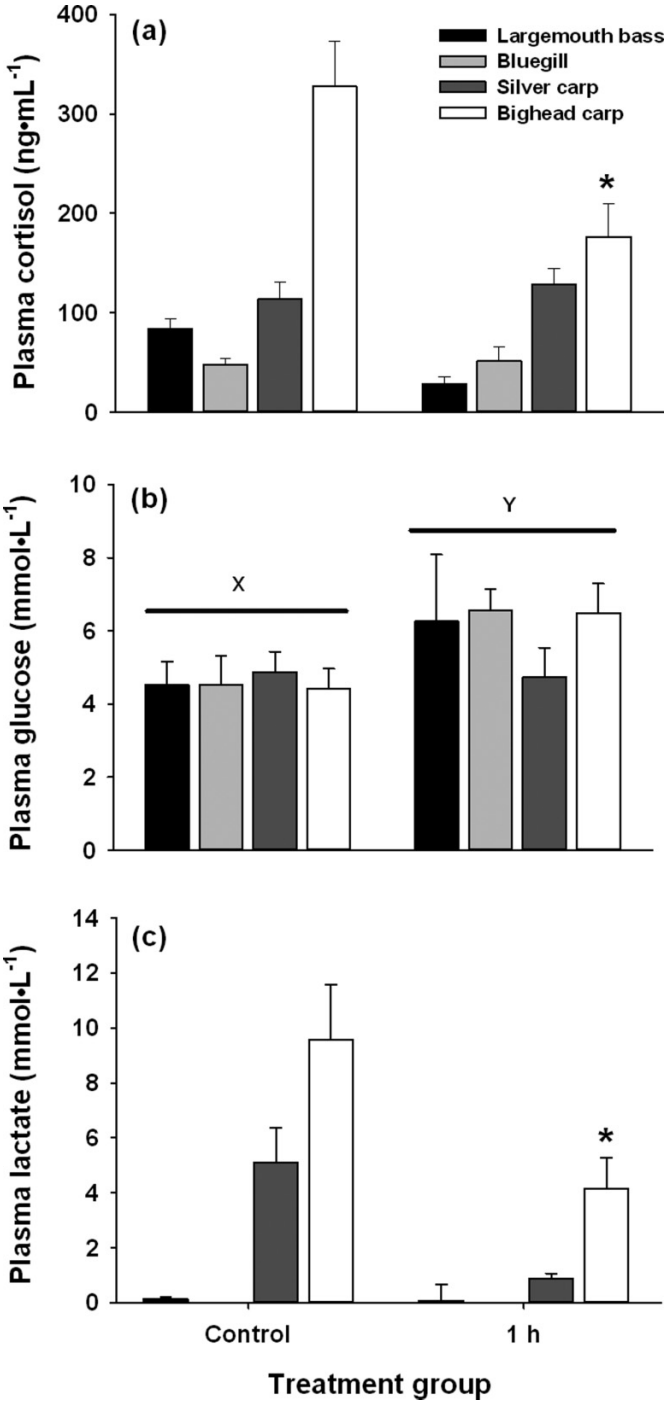


Table 3. Two-way analysis of variance (ANOVA) examining the impact of elevated ambient CO_2 on blood parameters of largemouth bass, bluegill, silver carp, and bighead carp.

Parameter	Main effects	df	*F*	*P*
Plasma cortisol	Species	3	27.8	**<0.0001**
	Treatment	1	6.44	**0.0140**
	Species × treatment	3	4.50	**0.0068**
Plasma glucose	Species	3	0.286	0.8355
	Treatment	1	5.34	**0.0246**
	Species × treatment	3	0.716	0.5467
Plasma lactate	Species	3	19.3	**<0.0001**
	Treatment	1	10.2	**0.0024**
	Species × treatment	3	3.50	**0.213**
Plasma potassium	Species	3	6.64	**0.0007**
	Treatment	1	2.86	0.0967
	Species × treatment	3	2.59	0.0623
Plasma sodium	Species	3	74.0	**<0.0001**
	Treatment	1	10.4	**0.0022**
	Species × treatment	3	16.7	**<0.0001**
Plasma chloride	Species	3	3.81	**0.0152**
	Treatment	1	12.7	**0.0008**
	Species × treatment	3	3.06	**0.0360**
Hematocrit	Species	3	1.54	0.2153
	Treatment	1	30.9	**<0.0001**
	Species × treatment	3	13.5	**<0.0001**

Note: Experiments exposed fish to either control (5 mg·L^{-1} CO_2) conditions or 1 h exposure to 30 mg·L^{-1} CO_2. Bold *p* values highlight significant effects.

species of fish left the choice chamber and moved to water with lower concentrations of CO_2 (Fig. 7*b*).

Discussion

Following acute exposure of largemouth bass, bluegill, and silver carp to concentrations of approximately 100 mg·L^{-1} CO_2, all three species of fish displayed signs of irritation (surface ventilations, elevated swimming activity), followed by voluntary movement to water with lower concentrations of CO_2. Fishes have evolved a number of potential responses to reductions in water quality, and avoidance is one way that animals can avoid costs associated with degraded water quality (Magnuson et al. 1979; Kramer 1987; Kieffer and Cooke 2009). CO_2 is a waste product excreted by fishes into the environment at the gills. Previous studies have shown that several species of fish have chemoreceptors in their gills that are capable of detecting elevated CO_2 in the environment, likely to locate optimal environments that facilitate respiration and allow animals to sense water of poor quality (Perry and Reid 2002; Gilmour et al. 2005). As a result of this ability to sense ambient CO_2, fish have previously been shown to avoid elevated concentrations of CO_2 in their environment. Ross et al. (2001), for example, showed that both brook trout (*Salvelinus fontinalis*) and blacknose dace (*Rhinichthys atratulus*) would actively avoid areas of elevated CO_2 in a laboratory study, while Clingerman et al. (2007) used application of 60–120 mg·L^{-1} CO_2 to induce avoidance in rainbow trout (*Oncorhynchus mykiss*) and facilitate harvest in an aquaculture setting. In addition, the crayfish *Procambarus clarkii* was placed in a choice arena receiving an inflow of water treated

Fig. 6. Concentrations of plasma potassium (*a*), sodium (*b*), and chloride (*c*) for largemouth bass, bluegill, silver carp, and bighead carp subjected to either control conditions (5 mg·L^{-1} CO_2) or hypercarbia (30 mg·L^{-1} CO_2) for 1 h. An asterisk denotes a statistically significant difference between control and treatment values within a species. Error bars show 1 standard error (SE). Sample size is eight fish per treatment group.

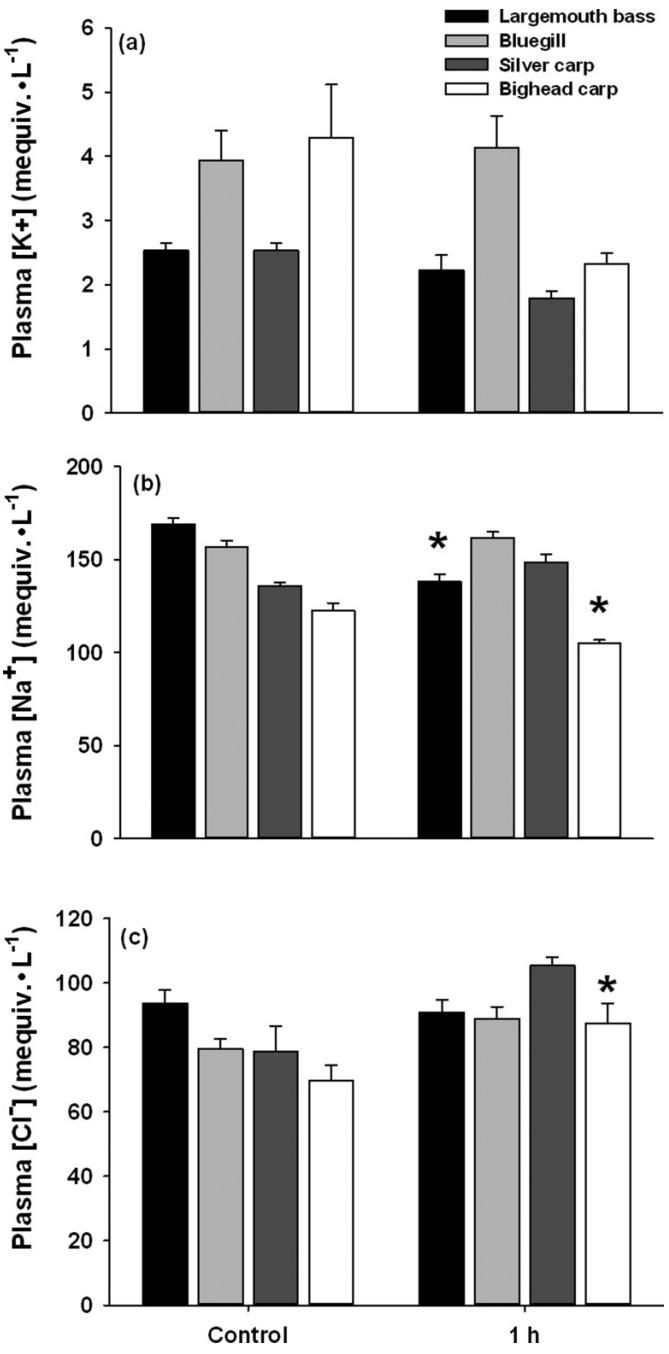
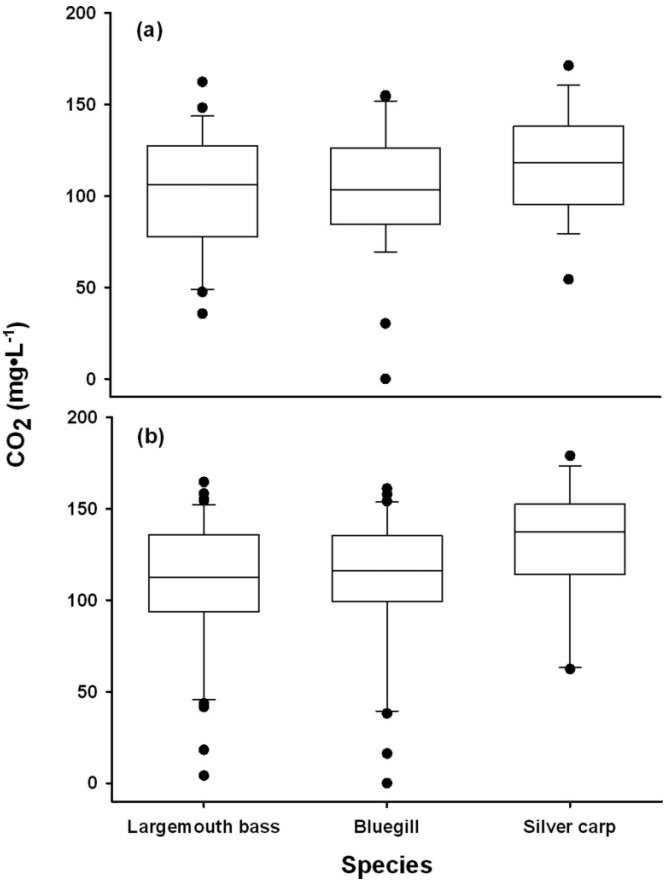


Fig. 7. Concentration of CO_2 at which largemouth bass, bluegill, and silver carp displayed either an agitated activity (surface ventilations, twitching, or elevated swimming activity) (*a*) or movement out of high CO_2 environment to a lower CO_2 environment (*b*) during the course of avoidance trials. Sample size is eight fish for all three species.



with CO_2, and results showed that elevated concentrations of ambient CO_2 acted as a repellant (Bierbower and Cooper 2010). Together, results from the current study showed that largemouth bass, bluegill, and silver carp all demonstrated avoidance of areas of elevated CO_2 once concentrations reached approximately 100 mg·L^{-1}, indicating that this concentration of CO_2 in the environment has potential to repel fish.

In addition to inducing avoidance, exposure of largemouth bass, bluegill, silver carp, and bighead carp to CO_2 concentrations of 70 mg·L^{-1} over extended time periods resulted in increased occurrence of erratic activities, altered ventilation rates, and often equilibrium loss. Previous studies have shown that the ventilatory response of fishes to increased ambient CO_2 is variable and can consist of either an increase or a decrease in ventilation rates (Gilmour 2001; Gilmour and Perry 2007). Often, increases in ventilation frequency occur within minutes of exposure to CO_2 (Bernier and Randall 1998; Gilmour et al. 2005) and is believed to assist with excretion of internal CO_2 that can rise with hypercarbia (Gilmour and Perry 2007). In the current study, monitoring of fish following elevated CO_2 exposure occurred following 30 min intervals over a period of several hours, likely precluding observation of this increase in ventilation previously seen at shorter time intervals. More importantly, ambient concentrations of CO_2 have previously been shown to act as an anesthetic for fish that can lead to a loss of equilibrium and unresponsiveness (Iwama et al. 1989; Yoshikawa et al. 1991; Bernier and Randall 1998). In the current study, all silver carp and over 60% of bighead

carp exposed to 70 $mg \cdot L^{-1}$ CO_2 lost equilibrium (i.e., entered stage I anesthesia) after 30 min (Iwama et al. 1989). It is not likely that reductions in ventilation rates observed in the current study resulted from changes in dissolved oxygen concentration as air stones remained in individual boxes to maintain dissolved oxygen concentrations during CO_2 exposure, and concentrations of lactate in plasma did not increase during trials. Rather, reductions in ventilation rates likely resulted from the anesthetic effects of elevated CO_2. Together, results from the current series of experiments demonstrate that exposure of four species of fish to 70 $mg \cdot L^{-1}$ CO_2 for 30 min results in a reduction in ventilation rates or a loss of equilibrium, likely a result of the anesthetic properties of CO_2.

Exposure of all four species of fish to concentrations of 30 $mg \cdot L^{-1}$ CO_2 for a 1 h period caused an increase in plasma glucose concentration (i.e., statistically significant treatment effect), coupled with alterations to internal physiology. It is likely that a concentration of 30 $mg \cdot L^{-1}$ caused fish to experience the anesthetic effects of CO_2, with bighead carp appearing particularly sensitive. More specifically, all species of fish displayed elevated concentrations of plasma glucose relative to controls after 1 h exposure to 30 $mg \cdot L^{-1}$ CO_2 (i.e., significant treatment effect for all species combined), while bighead carp showed a reduction in plasma cortisol, a reduction in plasma lactate, an increase in plasma Cl^-, and a loss of Na^+ from plasma. Largemouth bass also showed a loss of Cl^- ions from plasma. Previous work has shown that as fish begin to experience the anesthetic effects of CO_2 and ventilation rates decrease, there can be an impairment of oxygen delivery to tissues, which, coupled with elevated levels of CO_2 in blood, can result in disruption to internal blood chemistry and blood acidification (Iwama et al. 1989; Yoshikawa et al. 1991; Bernier and Randall 1998). Iwama et al. (1989), for example, showed that exposure of rainbow trout to elevated concentrations of ambient CO_2 increased concentrations of cortisol in plasma, while Ross et al. (2001) showed an increase in plasma glucose concentrations for brook trout exposed to elevated ambient CO_2. Iwama et al. (1989) also showed increased hematocrit concentrations of rainbow trout exposed to hypercarbia and proposed that this resulted from reduced ventilation rates and impaired oxygen uptake resulting from the anesthetic impacts of hypercarbia. Brauner et al. (2000) noted reductions in blood Cl^- for Atlantic salmon (*Salmo salar*) exposed to hypercarbia, likely due to elevated activity of Cl^-–HCO_3^- exchangers as animals uptake HCO_3^- from the environment in exchange for Cl^- to buffer reductions in blood pH. The mechanism by which Cl^- ions increased during hypercarbia in bighead carp, coupled with a reduction in Na^+ ions for bighead carp and largemouth bass, are not currently clear, but likely result from altered acidification of plasma due to carbonic acid, coupled with alterations to gill structure and function, that influenced ion balance (Bernier and Randall 1998; Brauner et al. 2000). A lack of clear trends in changes in plasma cortisol for largemouth bass, bluegill, or silver carp could be due to a number of factors, including high inter-individual variation within species before and after CO_2 treatments (Pottinger 2008) or sustained elevation of plasma cortisol due to confinement and (or) capture (Barton 2000). Together, results from the current study clearly demonstrate that exposure of largemouth bass, bluegill, silver carp, and bighead carp to 30 $mg \cdot L^{-1}$ CO_2 for a 1 h period caused an increase in plasma glucose concentration, coupled with alterations to internal blood physiology likely due to altered gill structure and function or altered acid–base regulation.

Nonphysical barriers have been used by managers for decades to deter the movements of fishes (e.g., electricity, strobe lights, sound, etc.; Noatch and Suski 2012). Nonphysical barriers to deter fish have a number of strengths relative to physical barriers in that they are often nonpermanent, require less engineering to install, and can be added to a waterway without changing boat traffic or water flow patterns (Noatch and Suski 2012). Results from this series of experiments demonstrate excellent potential for the use of CO_2 as a barrier to prevent the movement of fishes between the Mississippi and Great Lakes basins. All of the species examined in the current study showed avoidance to an acute exposure of 100 $mg \cdot L^{-1}$ CO_2 (i.e., they swam away from it), and they also began to experience the anesthetic effects of elevated CO_2 at lower concentrations (i.e., loss of equilibrium, increased plasma glucose, etc.). In addition, the four species of fish examined in this study have relatively divergent evolutionary histories (Asian carp from the Cyprinid family and native to Asia, largemouth bass and bluegill from the Centrarchid family native to North America), and responses were common and conserved across these two groups. More importantly, CO_2 has been shown to be an effective movement deterrent for small fishes such as the blacknose dace (69 mm, 3 g), suggesting that a CO_2 barrier may provide protection not currently offered by the electrical barrier currently in place in the Chicago Area Waterway (Ross et al. 2001). CO_2 dissolves easily in water, and target concentrations of CO_2 in stationary water remain quite stable for extended periods of time (Wetzel 1983; Clingerman et al. 2007). Additions of CO_2 to links between the Mississippi and Great Lakes basins may function to deter fish of varying sizes and species from moving between the Mississippi and Great Lakes basins.

Despite the potential of CO_2 as a barrier to deter the movement of fishes, there are a number of caveats and unknowns that must be addressed prior to a full-scale implementation into the Chicago Area Waterway or other large field applications. For example, results from the current study were performed only in a controlled laboratory setting, over a narrow range of temperatures. Subsequent tests of CO_2 as a deterrent in a field setting (i.e., a pond using fish telemetry) should be conducted to improve confidence in CO_2 as a barrier and to validate laboratory findings in a more natural setting. In addition, subsequent studies should be performed across a range of water temperatures to define how sensitivity to hypercarbia changes across seasons and (or) temperatures. Our study quantified CO_2 as free dissolved CO_2, similar to methods described in Clingerman et al. (2007) and American Public Health Association, American Water Works Association, and Water Environment Federation (1998). This method of CO_2 quantification was used not only because free dissolved CO_2 has been shown to be the source of physiological disturbances for fishes (Crocker and Cech 1998; Rimoldi et al. 2009), but also because free CO_2 can be easily related to pH, and pH is easily quantified in a field setting using inexpensive, autonomous water quality loggers (Garvey et al. 2007). These loggers are widely used by municipalities, scientists, and resource managers, permitting a reliable method for defining CO_2 concentrations in the field without the need for acquiring additional monitoring tools. The quantity of CO_2 in water in the natural environment can be variable and is influenced by factors

that include pH, alkalinity, temperature, and biological activities (Wetzel 1983), necessitating accurate, precise definitions of control (ambient) conditions in future studies. Similarly, future studies should also be cognizant of monitoring additional water quality parameters as small differences in water chemistry may influence CO_2 concentrations (American Public Health Association, American Water Works Association, and Water Environment Federation 1998; Clingerman et al. 2007). Modeling exercises are also likely needed to provide a sense of the quantity of CO_2 required to achieve target concentrations, delivery methods, expected costs, engineering requirements, and the stability of CO_2 at these concentrations in the field (particularly in turbulent, flowing waters). In addition, matters such as regulatory constraints and the impacts of reduced pH on aquatic ecosystems (nontarget organisms in particular) should be investigated prior to deployment. More importantly, a CO_2 barrier works on the premise of organisms "choosing" to avoid areas with degraded water quality and therefore would likely not be effective against organisms or propagules that float downstream. It is not believed that a zone of CO_2 would be 100% effective at preventing the movement of all fishes, but, following field testing and additional ground truthing, a CO_2 barrier as a part of an integrated system has the potential to impede the spread of invasive fish species between geographically distinct water bodies.

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References

American Public Health Association, American Water Works Association, and Water Environment Federation. 1998. Standard methods for the examination of water and wastewater. 20th ed. American Public Health Association, American Water Works Association, and Water Environment Federation, Washington, D.C.

Asian Carp Regional Coordinating Committee. 2010. Asian carp control strategy framework [online]. Available from http://asiancarp.us/documents/2011Framework.pdf [accessed 16 January 2012].

Barton, B.A. 2000. Salmonid fishes differ in their cortisol and glucose responses to handling and transport stress. Trans. Am. Fish. Soc. **62**: 12–18.

Bernier, N.J., and Randall, D.J. 1998. Carbon dioxide anesthesia in rainbow trout: effects of hypercapnic level and stress on induction and recovery from anesthetic treatment. J. Fish Biol. **52**: 621–637.

Bierbower, S.M., and Cooper, R.L. 2010. The effects of acute carbon dioxide on behavior and physiology in *Procambarus clarkii*. J. Exp. Zool. Part A Ecol. Gen. Physiol. **313A**(8): 484–497. doi:10.1002/jez.620. PMID:20878748.

Brauner, C.J., Seidelin, M., Madsen, S.S., and Jensen, F.B. 2000. Effects of freshwater hyperoxia and hypercapnia and their influences on subsequent seawater transfer in Atlantic salmon (*Salmo salar*) smolts. Can. J. Fish. Aquat. Sci. **57**(10): 2054–2064. doi:10.1139/f00-161.

Clavero, M., and García-Berthou, E. 2005. Invasive species are a leading cause of animal extinctions. Trends Ecol. Evol. **20**(3): 110. doi:10.1016/j.tree.2005.01.003. PMID:16701353.

Clingerman, J., Bebak, J., Mazik, P.M., and Summerfelt, S.T. 2007. Use of avoidance response by rainbow trout to carbon dioxide for fish self-transfer between tanks. Aquacult. Eng. **37**(3): 234–251. doi:10.1016/j.aquaeng.2007.07.001.

Conover, G., Simmonds, R., and Whalen, M. 2007. Management and control plan for bighead, black, grass, and silver carps in the United States. Asian Carp Working Group, Aquatic Nuisance Species Task Force, Washington, D.C.

Cooke, S., and Hill, W.R. 2010. Can filter-feeding Asian carp invade the Laurentian Great Lakes? A bioenergetic modelling exercise. Freshw. Biol. **55**(10): 2138–2152. doi:10.1111/j.1365-2427.2010.02474.x.

Crocker, C.E., and Cech, J.J., Jr. 1998. Effects of hypercapnia on blood-gas and acid–base status in the white sturgeon, *Acipenser transmontanus*. J. Comp. Physiol. **168**: 50–60.

Dettmers, J.M., Boisvert, B.A., Barkley, T., and Sparks, R.E. 2005. Potential impact of steelhulled barges on movement of fish across an electric barrier to prevent the entry of invasive carp into Lake Michigan. Aquatic Ecology Report 2005(14), Illinois State Natural History Survey, Urbana, Ill.

Finnoff, D., Shogren, J.F., Leung, B., and Lodge, D. 2007. Take a risk: preferring prevention over control of biological invaders. Ecol. Econ. **62**(2): 216–222. doi:10.1016/j.ecolecon.2006.03.025.

Garvey, J.E., Whiles, M.R., and Streicher, D. 2007. A hierarchical model for oxygen dynamics in streams. Can. J. Fish. Aquat. Sci. **64**(12): 1816–1827. doi:10.1139/f07-144.

Gilmour, K.M. 2001. The CO_2/pH ventilatory drive in fish. Comp. Biochem. Physiol. A Mol. Integr. Physiol. **130**(2): 219–240. doi:10.1016/S1095-6433(01)00391-9. PMID:11544069.

Gilmour, K.M., and Perry, S.F. 2007. Bronchial chemoreceptor regulation of cardiorespiratory function. *In* Fish Physiology 25: Sensory Systems Neuroscience. *Edited by* T.J. Hara and B. Zielinski. Academic Press, San Diego, Calif. pp. 97–151.

Gilmour, K.M., DiBattista, J., and Thomas, J. 2005. Physiological causes and consequences of social status in salmonid fish. Integr. Comp. Biol. **45**(2): 263–273. doi:10.1093/icb/45.2.263. PMID:21676770.

Great Lakes Mississippi River Interbasin Study Team. 2011. Inventory of available controls for aquatic nuisance species of concern [online]. Chicago Area Waterway System. United States Army Corps of Engineers. Available from http://glmris.anl.gov/documents/docs/ANS_Control_Paper.pdf [accessed 23 January 2012].

Gutreuter, S., Burkhardt, R., and Lubinski, K. 1995. Long term resource monitoring program procedures: fish monitoring [online]. National Biological Service, Onalaska, Wisc. Available from http://www.umesc.usgs.gov/documents/reports/1995/95p00201.pdf [accessed 26 July 2012].

Hoover, J.J., Adams, S.R., and Killgore, K.J. 2003. Can hydraulic barriers stop the spread of the round goby? Aquatic Nuisance Species Program ERDC/TN ANSRP-0301. Vicksburg, Miss.

Iwama, G., McGeer, K., and Pawluk, C.P. 1989. The effects of five fish anesthetics on acid–base balance, hematocrit, blood gases, cortisol, and adrenaline in rainbow trout. Can. J. Zool. **67**(8): 2065–2073. doi:10.1139/z89-294.

Kieffer, J.D., and Cooke, S.J. 2009. Physiology and organismal performance of centrarchids. *In* Centrarchid fishes: diversity, biology, and conservation. *Edited by* S.J. Cooke and D.P. Philipp. Wiley-Blackwell, West Sussex, UK. pp. 207–263.

Kramer, D.L. 1987. Dissolved oxygen and fish behavior. Environ. Biol. Fishes, **18**(2): 81–92. doi:10.1007/BF00002597.

Lodge, D.M., Williams, S., MacIsaac, H.J., Hayes, K.R., Leung, B., Reichard, S., Mack, R.N., Moyle, P.B., Smith, M., Andow, D.A.,

Carlton, J.T., and McMichael, A. 2006. Biological invasions: recommendations for U.S. policy and management. Ecol. Appl. **16**(6): 2035–2054. doi:10.1890/1051-0761(2006)016[2035:BIRFUP]2.0.CO;2. PMID:17205888.

Lowry, O.H., and Passonneau, J.V. 1972. A flexible system of enzymatic analysis. Academic Press, New York.

Magnuson, J.J., Crowder, L.B., and Medvick, P.A. 1979. Temperature as an ecological resource. Am. Zool. **19**: 331–343.

Milligan, C.L. 1996. Metabolic recovery from exhaustive exercise in rainbow trout. Comp. Biochem. Physiol. **113**(1): 51–60. doi:10.1016/0300-9629(95)02060-8.

Noatch, M.R., and Suski, C.D. 2012. Non-physical barriers to deter fish movements. Environ. Rev. **20**(1): 71–82. doi:10.1139/a2012-001.

Patel, A.G., Glassner-Shwayder, K., and Eder, T. 2010. Halting the invasion: maintaining the health of the Great Lakes and Mississippi River basins by preventing further exchange of aquatic invasive species. Environ. Pract. **12**(04): 342–356. doi:10.1017/S1466046610000499.

Perry, S.F., and Reid, S.G. 2002. Cardiorespiratory adjustments during hypercarbia in rainbow trout (*Oncorhynchus mykiss*) are initiated by external CO_2 receptors on the first gill arch. J. Exp. Biol. **205**(Pt. 21): 3357–3365. PMID:12324545.

Pimentel, D., Zuniga, R., and Morrison, D. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol. Econ. **52**(3): 273–288. doi:10.1016/j.ecolecon.2004.10.002.

Pottinger, T.G. 2008. The stress response in fish — mechanisms, effects and measurement. *In* Fish welfare. *Edited by* E.J. Branson. Blackwell Publishing Ltd., Oxford, UK. pp. 32–48.

Rasmussen, J.L., Regier, H.A., Sparks, R.E., and Taylor, W.W. 2011. Dividing the waters: The case for hydrologic separation of the North American Great Lakes and Mississippi River Basins. J. Great Lakes Res. **37**(3): 588–592. doi:10.1016/j.jglr.2011.05.015.

Reynolds, J.B. 1996. Electrofishing. *In* Fisheries techniques, 2nd ed. *Edited by* B.R. Murphy and D.W. Willis. American Fisheries Society, Bethesda, Md. pp 221–253.

Ricciardi, A. 2006. Patterns of invasion in the Laurentian Great Lakes in relation to changes in vector activity. Divers. Distrib. **12**(4): 425–433. doi:10.1111/j.1366-9516.2006.00262.x.

Ricciardi, A., and MacIsaac, H.J. 2011. Impacts of biological invasions on freshwater ecosystems. *In* Fifty years of invasion ecology: the legacy of Charles Elton. *Edited by* D.M. Richardson. Wiley-Blackwell, Oxford, UK. pp. 211–224.

Rimoldi, S., Terova, G., Brambilla, F., Bernardini, G., Gornati, R., and Saroglia, M. 2009. Molecular characterization and expression analysis of Na^+/H^+ exchanger *(NHE)-1* and *c-Fos* genes in sea bass (*Dicentrarchus labrax*, L) exposed to acute and chronic hypercapnia. J. Exp. Mar. Biol. Ecol. **375**(1–2): 32–40. doi:10.1016/j.jembe.2009.05.002.

Ross, R.M., Krise, W.F., Redell, L.A., and Bennett, R.M. 2001. Effects of dissolved carbon dioxide on the physiology and behavior of fish in artificial streams. Environ. Toxicol. **16**(1): 84–95. doi:10.1002/1522-7278(2001)16:1<84::AID-TOX100>3.0.CO;2-1. PMID:11345549.

Serrano, X., Grosell, M., and Serafy, J.E. 2010. Salinity selection and preference of the grey snapper *Lutjanus griseus*: field and laboratory observations. J. Fish Biol. **76**(7): 1592–1608. doi:10.1111/j.1095-8649.2010.02585.x. PMID:20557618.

Sokal, R.R., and Rohlf, F.J. 1995. Biometry, 3rd ed. W.H. Freeman and Company, New York.

Summerfelt, S.T., and Sharrer, M.J. 2004. Design implication of carbon dioxide production within biofilters contained in recirculating salmonid culture systems. Aquacult. Eng. **32**(1): 171–182. doi:10.1016/j.aquaeng.2004.03.006.

Suski, C.D., Killen, S.S., Morrissey, M., Lund, S.G., and Tufts, B.L. 2003. Physiological changes in largemouth bass caused by live-release angling tournaments in southeastern Ontario. N. Am. J. Fish. Manage. **23**(3): 760–769. doi:10.1577/M02-042.

Suski, C.D., Killen, S.S., Kieffer, J.D., and Tufts, B.L. 2006. The influence of environmental temperature and oxygen concentration on the recovery of largemouth bass from exercise: implications for live-release angling tournaments. J. Fish Biol. **68**(1): 120–136. doi:10.1111/j.0022-1112.2006.00882.x.

Suski, C.D., Kieffer, J.D., Killen, S.S., and Tufts, B.L. 2007. Sublethal ammonia toxicity in largemouth bass. Comp. Biochem. Physiol. A Mol. Integr. Physiol. **146**(3): 381–389. doi:10.1016/j.cbpa.2006.11.005. PMID:17258484.

US Army Corps of Engineers. 2010*a*. Dispersal barrier efficacy study, dispersal barrier bypass risk reduction study and integrated environmental assessment [online]. US Army Corps of Engineers, Chicago District, Interim Report I, Chicago, Ill. Available from http://www.lrc.usace.army.mil/Portals/36/docs/projects/ans/docs/ANS_DispersalBarrierEfficacyStudy_Interim_I_FINAL.pdf [accessed 16 January 2012].

US Army Corps of Engineers. 2010*b*. Fish dispersal deterrents, Illinois and Chicago area waterways risk reduction study and integrated environmental assessment [online]. US Army Corps of Engineers, Chicago District, Interim Report IIIA, Chicago, Ill. pp. 28–29. Available from http://www.lrc.usace.army.mil/Portals/36/docs/projects/ans/docs/02June2010_InterimIIIA.pdf [accessed 16 January 2012].

Vander Zanden, M.J., Casselman, J.M., and Rasmussen, J.B. 1999. Stable isotope evidence for the food web consequences of species invasions in lakes. Nature, **401**(6752): 464–467. doi:10.1038/46762.

VanLandeghem, M.M., Wahl, D.H., and Suski, C.D. 2010. Physiological responses of largemouth bass to acute temperature and oxygen stressors. Fish. Manag. Ecol. **17**(5): 414–425. doi:10.1111/j.1365-2400.2010.00740.x.

Wetzel, R.G. 1983. Limnology. Saunders College Publishing, Fort Worth, Tex.

Yoshikawa, H., Yokoyama, Y., Ueno, S., and Mitsuda, H. 1991. Changes of blood gas in carp, *Cyprinus carpio*, anesthetized with carbon dioxide. Comp. Biochem. Physiol. A, **98**(3–4): 431–436. doi:10.1016/0300-9629(91)90427-E.