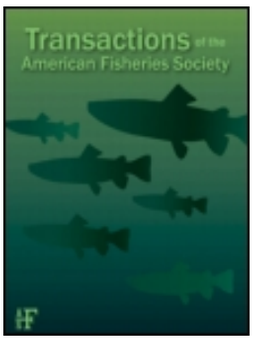

Transactions of the American Fisheries Society

ISSN: 0002-8487 (Print) 1548-8659 (Online) Journal homepage: http://www.tandfonline.com/loi/utaf20

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To cite this article: Michael R. Donaldson, Jon Amberg, Shivani Adhikari, Aaron Cupp, Nathan Jensen, Jason Romine, Adam Wright, Mark Gaikowski & Cory D. Suski (2016) Carbon Dioxide as a Tool to Deter the Movement of Invasive Bigheaded Carps, Transactions of the American Fisheries Society, 145:3, 657-670, DOI: 10.1080/00028487.2016.1143397

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Transactions of the American Fisheries Society 145:657–670, 2016
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ISSN: 0002-8487 print / 1548-8659 online
DOI: 10.1080/00028487.2016.1143397

ARTICLE

Carbon Dioxide as a Tool to Deter the Movement of Invasive Bigheaded Carps

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Abstract

Nonnative bigheaded carps are established in the Mississippi River and there is substantial concern about their potential entry into the interconnected Laurentian Great Lakes. While electrical barriers currently exist as a preventative measure, there is need for additional control mechanisms to promote barrier security through redundancy. We tested the effectiveness of infused carbon dioxide gas (CO_2) as a tool to influence the movement and behavior invasive bigheaded carps, namely Bighead Carp *Hypophthalmichthys nobilis* and Silver Carp *H. molitrix*, as well as native Bigmouth Buffalo *Ictiobus cyprinellus*, Channel Catfish *Ictalurus punctatus*,

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Received July 27, 2015; accepted January 11, 2016

Paddlefish *Polyodon spathula*, and Yellow Perch *Perca flavescens* in an experimental pond. Individuals were monitored with acoustic telemetry before, during, and after CO_2 addition to the pond. We noted distinct changes in fish behavior following CO_2 addition. Each species except Paddlefish maintained farther distances from the CO_2 infusion manifold relative to controls. Both bigheaded carp species had slower persistence velocities (persistence of a movement in a given direction) following CO_2 infusion and Bighead Carp used a smaller area of the pond immediately after CO_2 addition. Pond pH progressively decreased up to 1.5 units following CO_2 infusion. This work provides evidence that could inform future research to enhance existing control measures used to deter high-risk invasive fishes, such as bigheaded carps.

Biological invasions are a component of rapid global environmental changes (Mack et al. 2000), placing increased demands on policy makers, resource managers, and scientists alike (Simberloff et al. 2013). In aquatic systems, biological invasions can result in profound ecological consequences (Ricciardi and MacIsaac 2011) and high economic costs (Pimentel et al. 2005). The growing number of species introductions is linked with a range of dispersal pathways created by global shipping networks and canals (Ruiz et al. 1997; Levine and D'Antonio 2003), sport fish and baitfish introductions, and escapes and releases from aquaculture, aquaria, and the ornamental fish trade (Padilla and Williams 2004). The secondary spread of introduced species poses considerable challenges to managers (Vander Zanden and Olden 2008). There are numerous examples of invasive species becoming established in the North American Laurentian Great Lakes, resulting in a range of ecological outcomes (Rothlisberger and Lodge 2013).

The risk of two nonnative invasive Asian bigheaded carp species, the Silver Carp *Hypophthalmichthys molitrix* and Bighead Carp *H. nobilis*, entering and establishing in the Great Lakes via interconnected waters is high, and the potential impacts therein are uncertain (DFO 2012). This concern has resulted in widespread attention from the media and public (Hinterthuer 2012). These concerns have likewise generated considerable scientific discourse, as well as proposed policy and management interventions (Patel et al. 2010; Rasmussen et al. 2011). Bigheaded carps are well established in the Mississippi River basin (Patel et al. 2010). While the risk of bigheaded carps entering the Great Lakes is high, their potential impacts there are largely unknown (Cooke and Hill 2010) and could be ecologically and economically deleterious because they can compete with native planktivores and are a nuisance for boaters (Irons et al. 2007; Wittmann et al. 2014).

Prevention is the preferred tactic when managing invasive species (Finnoff et al. 2007) as control after establishment can be extremely challenging, particularly for bigheaded carps (Tsehaye et al. 2013). Deterrents have been proposed to reduce the risk of bigheaded carps entering the Great Lakes from the Mississippi River system. Currently, a series of electrical barriers in the Chicago Area Waterway System (CAWS) appears to be an effective deterrent preventing bigheaded carps from entering into Lake Michigan (Patel et al. 2010). However, such barriers may not impede the movement of all fishes (Sparks et al. 2010; Noatch and Suski 2012). For example, electricity loses effectiveness as fish size decreases and movements of very small fish may thus not be adequately prevented (Reynolds 1996). In addition, electrical barriers must be shut down periodically for routine maintenance or other problems (Rasmussen et al. 2011). In such circumstances, additional mechanisms for impeding fish movements would provide critical redundancy to insure overall barrier effectiveness and security.

Multiple fish deterrence methods have been explored and include physical, chemical, and sonic deterrents and barriers, among others (Noatch and Suski 2012). For example, seismic water gun firing in an experimental pond resulted in Bighead and Silver carps maintaining a greater distance from the water guns and using a smaller area of the pond during firing compared with the prefiring periods (Romine et al. 2015). Romine et al. (2015) also noted that persistence velocity (i.e., persistence of a movement in a given direction) decreased during the firing period as carp movement became more tortuous (i.e., a path full of twists and turns rather than a direct path), suggesting the guns elicited a change in swimming behavior during firing periods.

The infusion of carbon dioxide gas (CO_2) into water has recently been explored as a deterrent to bigheaded carps (Kates et al. 2012). The ability of animals to sense environmental CO_2 is highly conserved across taxa (Cummins et al. 2014) and fish have externally oriented, branchial CO_2-sensitive chemoreceptors that enable the detection of CO_2 (Gilmour 2001). Fish will avoid locations that contain CO_2 that falls outside their preferred concentration range (Fromm 1980; Gilmour 2001). In a laboratory study, Kates et al. (2012) found that the addition of 100 mg/L of CO_2 to water resulted in the exclusion of adult Bighead and Silver carps and native fish species from the infusion area, and even lower concentrations resulted in reflex, behavioral (e.g., avoidance responses), and physiological responses. Carbon dioxide exposure has been shown to trigger a physiological response and to limit the movement of several species, including bigheaded carps (Dennis et al. 2015a, 2015b). Despite these promising laboratory results, CO_2 has yet to be tested as a deterrent to movement of free-swimming bigheaded carps in a larger spatial area. If CO_2 deterrents are to be used effectively, it is also critical to understand the impacts of this approach on nontarget, native species. It is therefore essential to understand

the impacts of CO_2 barriers on both Bighead and Silver carps and on representative nontarget native fishes, beyond small-scale laboratory studies, in order to determine the efficacy of using this method for in situ invasive species control.

The goal of this study was to determine whether a zone of elevated CO_2 influenced the movements and behaviors of free-swimming fishes in an earthen pond. This study has provided the first assessment of CO_2 as a potential tool to influence the behavior and movement of free-swimming bigheaded carps in a mesocosm environment, an essential step before conducting field trials. In addition to Bighead Carp and Silver Carp, we investigated how CO_2 influenced the behavior of selected native fish species: Bigmouth Buffalo *Ictiobus cyprinellus*, Channel Catfish *Ictalurus punctatus*, Paddlefish *Polyodon spathula*, and Yellow Perch *Perca flavescens*. We hypothesized that a zone of elevated CO_2 would cause fish to move out of that area (i.e., increased distance from diffusion manifolds) and remain in a smaller, non-CO_2 enriched portion of the pond (i.e., decreased distribution of the usable pond volume, or utilization distribution). We also hypothesized that the zone of elevated CO_2 would decrease the fish's persistence of a movement in a given direction (i.e., persistence velocities). Together, these metrics will provide an indication of whether CO_2 can be used as a tool to deter the movement of bigheaded carps.

METHODS

Study site.—The experiment was conducted in a rectangular earthen pond at the U.S. Geological Survey (USGS), Upper Midwest Environmental Sciences Center (UMESC) in La Crosse, Wisconsin (Figure 1). The rectangular experimental pond measured 64.6 m long × 33.5 m wide × 1.8 m deep (volume ≈ 3,962.1 m^3). The bottom of the pond was sloped so that the depth ranged between 1.8 and 2.4 m (Romine et al. 2015).

CO_2 infusion.—A custom-designed CO_2-infusion system used a centrifugal pump (3 hp; Goulds, model 30SPM20, Xylem, Seneca Falls, New York) to draw pond water into a distribution manifold and send the water through three distribution lines. Each line fed a commercially available Venturi injection chamber (Mazzei Injector Company, Bakersfield, California) where CO_2 was added to the water from lines connected to air regulators on the gas ports of 160-L liquid-CO_2 cylinders. The CO_2-infused water leaving each Venturi chamber flowed into one of three pairs of 208-L contact drums, which allowed extra time for CO_2 to diffuse into the water. From the contact drums, CO_2-rich water flowed into a common discharge line that fed the manifold on the bottom of the pond. The custom-built discharge manifold, constructed from PVC pipes with vertical arms reaching to the surface of the pond, was placed along the bottom of one end of the pond, covering approximately one-quarter of the pond bottom. The manifold was 21.4 m wide and 10.7 m long (from the south end of the pond).The arms were located at 3.0 m depth, with the top-most nozzle height at 2.1 m. The infusion system drew water from one portion of the pond and returned CO_2-rich water to the same area of the pond to create a zone of high CO_2.

Water quality measures.—Water pH was monitored as a surrogate for CO_2 concentration, using Hydrolab MS5 multiparameter sondes (Hach, Loveland, Colorado) and HQ40d portable pH meters (Hach), outfitted with IntelliCAL PHC101 rugged gel-filled pH electrodes (Hach). A CO_2 digital titration kit (Hach, titrator model 16900, kit number 227270) was also used to test water samples drawn from the peristaltic pump. The use of pH as a proxy for CO_2 is common, because pH measurements are rapid and relatively inexpensive to measure with field meters, and there is a strong, near-linear relationship between pH and CO_2 concentrations (Wurts and Durborow 1992; Kates et al. 2012; Esbaugh et al. 2012). Water pH inside the manifold was monitored using a single sonde at the midline and two pH probes placed on either side. Two more sondes were placed outside the manifold on each side of the pond. The other two sondes and pH probes were placed alternately and equidistant from one other to cover the remainder of the northern end of the pond. Together, this provided a total of eight pH probes distributed both within the distribution manifold and throughout the entire pond to monitor pH during the study. All probes were calibrated before each trial according to the manufacturer's instructions, mounted securely on the pond bottom, and set to record pH data at a rate of four recordings per hour. In addition to pH monitoring, two three-channel peristaltic pumps (Cole-Parmer, model 7553-70, Vernon Hills, Illinois) combined with approximately 30 m of vinyl tubing were used to draw water samples from fixed locations within the pond to a common collection point on land. The vinyl tubing was placed on the pond bottom adjacent to six of the pH meters, thereby allowing for near-simultaneous measurements of pH along with collection of water samples from the pond bottom. Total alkalinity in the water samples was measured with a commercially available kit (Hach digital titrator, model AL-DT). In addition, the behavioral responses of fishes to elevated CO_2 environments are believed to result from the presence of CO_2 in the environment, not reduced pH (Clingerman et al. 2007; Kates et al. 2012; Heuer and Grosell 2014). Data were subsequently entered into the program CO_2Calc (Robbins et al. 2010) to generate partial pressures of carbon dioxide (pCO_2) in microatmospheres (μatm; 1 atm = 101,325 Pa) to facilitate comparisons of pH additions across pond locations. The pCO_2 values should be interpreted cautiously, however, because, due to the rapid addition of large quantities of CO_2 coupled with relatively short time frames of the study (i.e., hours), CO_2 pressures may not have fully equilibrated at the time of measurements (Cole et al. 1994; Gattuso et al. 2010).

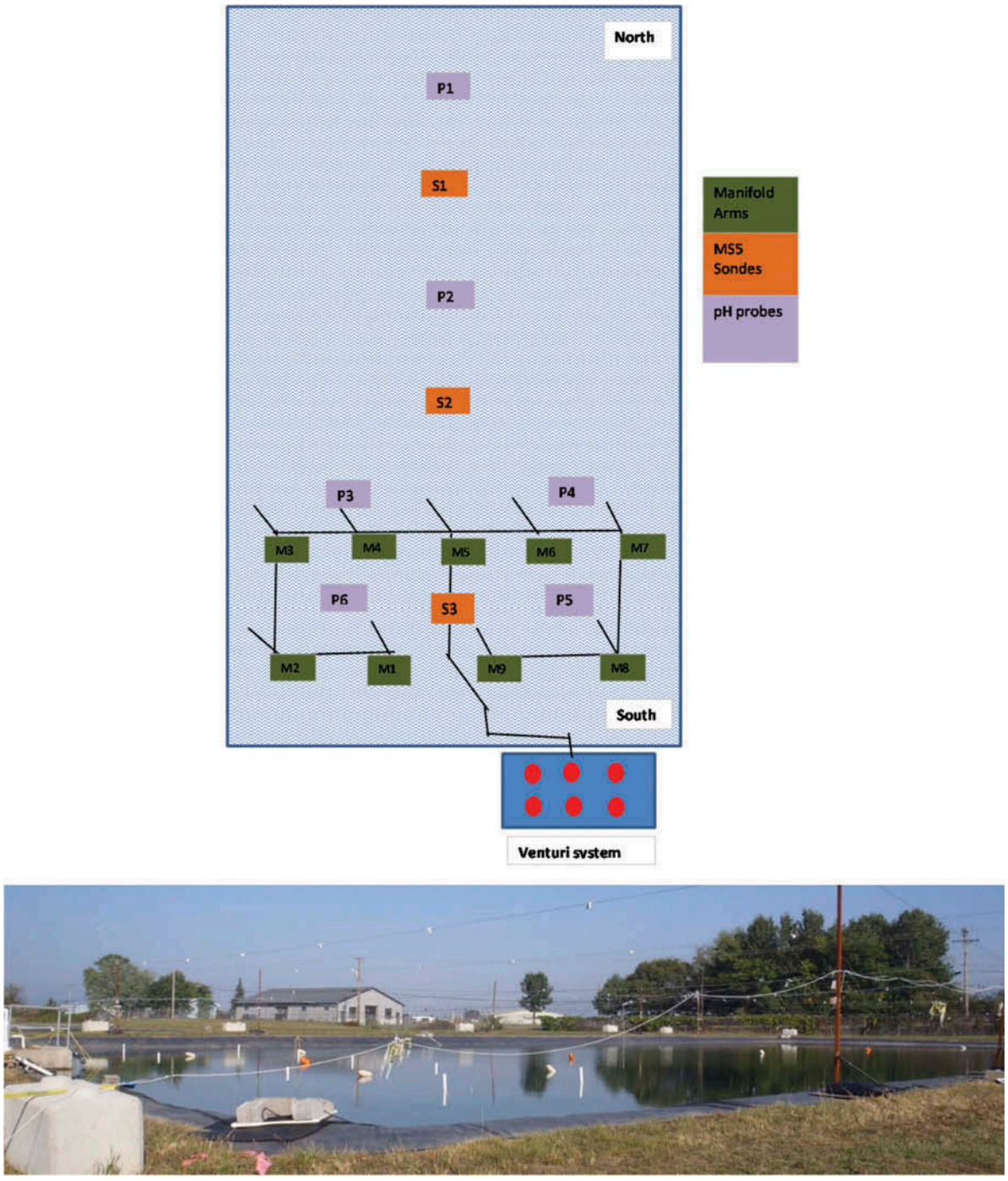

FIGURE 1. Schematic showing the experimental pond indicating positions of the manifold arms, sondes, and pH probes. The pond dimensions were 64.6 m long × 33.5 m wide. Photograph shows a panoramic view of the experimental pond taken from the south side to indicate the position of the manifold arms.

Water temperature was collected using iButton thermal data loggers (Maxim Integrated Products, Sunnyvale, California) and from the sondes. Sunrise and sunset times were determined using the National Oceanic and Atmospheric Administration solar calculator using latitude and longitude for La Crosse, Wisconsin (http://www.esrl.noaa.gov/gmd/grad/solcalc/). Water pH data were first combined with alkalinity and temperature data to generate values for the concentration of CO_2 (mg/L).

Fishes studied.—Silver Carp used in the study were captured using seine nets at Big Muddy National Wildlife Refuge located on the Missouri River near Columbia, Missouri. Bighead Carp were obtained from a commercial

aquaculture facility in Osage Beach, Missouri. Bigmouth Buffalo, Channel Catfish, Paddlefish, and Yellow Perch were cultured on-site at the UMESC facility. Bigheaded carps were transported to UMESC by USGS personnel and then reared separately in closed recirculating aquaculture systems for a minimum of 6 months before use. All other species tested were held in flow-through culture systems prior to use.

Bighead Carp ranged from 195 to 224 mm TL and averaged 205.6 mm TL, and Silver Carp ranged from 221 to 324 mm TL and averaged 254.2 mm TL. Bigmouth Buffalo ranged from 83 to 204 mm TL and averaged 177.4 mm TL, Channel Catfish ranged from 92 to 238 mm TL and averaged 187.5 mm TL, Paddlefish ranged from 394 to 546 mm TL and averaged 467.5 mm TL, and Yellow Perch ranged from 77 to 208 mm TL and averaged 171.2 mm TL. Food was withheld from fish for 24 h prior to transmitter attachment. All fish were released into the experimental pond from a single location following tagging. Total length and weight measurements were collected at the end of each trial.

Tagging methods.—Tagging details are provided in Romine et al. (2015); briefly, acoustic transmitters were model 795LD manufactured by Hydroacoustic Technology Incorporated (HTI, Seattle). The transmitters were 6.8 mm in diameter × 20 mm long and weighed 1.1 g in air. The tags emitted a primary and secondary pulse separated by ~800 ms that is used to identify individual tags, and both pulses can be resolved for position; if a valid position was derived for the first pulse, the second pulse was discarded. All fish were sedated in a 100-mg/L solution of AQUI-S 20E (AQUI-S New Zealand, Lower Hutt, New Zealand). All tags were disinfected prior to attachment by means of a 15-min immersion in a 3% (v/v) solution of Nolvasan (active ingredient: chlorohexidine diacetate [2%]; Fort Dodge Animal Health, Fort Dodge, Iowa) and then rinsed with deionized water. Transmitters were made neutrally buoyant and anchored using a T-bar anchor-tagging gun into the dorsal musculature approximately 1 cm lateral and posterior to the midline of the dorsal fin. Transmitters were programmed with an HTI model 490-LP acoustic tag programmer.

Telemetry methods.—Fish locations were determined from a whole-pond acoustic telemetry array, based on methods validated by Romine et al. (2015). Two model 290 acoustic tag receivers (HTI) were wired to a total of 24 hydrophones. Eighteen hydrophones were mounted on the pond bottom at intervals of 10–15 m. An additional six hydrophones were positioned adjacent to the upper banks of the pond so that they would be 0.5 m below the water surface when the pond was filled. Each receiver was connected to a separate computer that continuously monitored and recorded the data from each hydrophone. Raw data were bundled into hourly files and then further processed to derive the geographic coordinates for the acoustic tags using USGS-developed (public) and HTI (proprietary) codes. Hyperbolic positioning algorithms used time difference of arrival of transmitter signals at a minimum of three hydrophones. The coordinate position for the second pulse was discarded if a valid position was derived for the first pulse.

Experimental protocol.—The experiment was conducted over two independent trial periods, with separate naive individual fish, from September 20 to 23, 2013 (trial 1), and from September 25 to 28, 2013 (trial 2). Trial 1 included 10 Silver Carp, 10 Bighead Carp, 4 Paddlefish, and 5 each of Yellow Perch, Channel Catfish, and Bigmouth Buffalo. Trial 2 included the same number of fish per species as trial 1, but 10 Silver Carp, 10 Bighead Carp, 5 Channel Catfish, 2 Paddlefish, 1 Yellow Perch, and 2 Bigmouth Buffalo were used for analyses (reduced sample sizes for Paddlefish, Yellow Perch, and Bigmouth Buffalo in trial 2 were due to problems associated with predation and transmitter loss). During both trials, naive fish were provided with an acclimation period of at least 14 h to enable recovery following transfer into the pond. Following acclimation, telemetry monitoring occurred for a period of 22 h, during which time the CO_2 infusion pump drew water from the pond but no CO_2 gas was added to the water, representing the control period. Following the control period, CO_2 infusion occurred over a period of 22 h, representing the CO_2 treatment period. After a 6-h posttreatment recovery period following the cessation of CO_2 infusion, the pond was drained and all fish were collected, weighed, and measured.

Statistical analysis.—We applied a maximum-rate-of-movement filter to remove location data that had a high probability of error (Romine et al. 2015). This was done by determining the speeds between each successive relocation. Burst swimming speed of Bighead Carp and Silver Carp have been reported to be approximately 1.28 m/s to 1.66 m/s (Hoover et al. 2012). Relocation errors were identified with a modified version of the algorithm from McConnell et al. (1992) and implemented using the trip package for R (R Development Core Team 2013).

We measured three main variables: (1) distance from infusion manifold (DistM5; m); (2) persistence velocity (PV; m/s); and (3) utilization distribution (UD; m^2). The DistM5 was calculated as the Euclidean distance from each location to the center point of the infusion manifold. Persistence velocity is the measure of the persistence of a movement in a direction, and was estimated as

$$\mathrm{PV} = V(t)\cos[\theta(t)],$$

where $V(t)$ is velocity at time t and $\cos\theta(t)$ is the turning angle at time t. We then calculated the areas of UD for each fish in m^2. Utilization distributions were created for the 50% and 95% kernel isopleths (a line connecting data points of the same value) for each of the periods within each trial. The 50% and 95% kernel isopleths for UDs represent the probability of relocating the tagged fish within the bounds of the isopleth. The UDs were estimated with the adehabitatHR

home range estimation package for R using a bivariate normal kernel with the reference bandwidth.

Time period was binned into 4-h blocks (e.g., starting at 0000 hours to 0400 hours for block 1, and so on). Changes in water pH during each trial were analyzed using a two-way, repeated-measures ANOVA with time period, treatment (CO_2 application versus control period), and the time period × treatment interactions as fixed effects and hour of the day as a random effect, nested within treatment. The use of a random effect (essentially a repeated-measures design) was necessary because multiple measurements were taken from each pH meter during a trial, and each measurement was not independent and potentially autocorrelated within the trial (Laird and Ware 1982; Lindstrom and Bates 1990).

Comparisons of DistM5, PV, and UD were made using a two-way, repeated-measures, mixed-model ANOVA with time period, treatment, and the treatment × time period interaction as fixed effects, and fish identification number, again added as a random effect to account for autocorrelation, was nested within treatment. To account for intertrial differences, data from trials were pooled and trial number was included as a random effect in each ANOVA model. Variables of interest were $\log_{10}$ transformed, if necessary, to meet assumptions of normality and homogeneity of variances following visual inspection of the data, Kolmogorov–Smirnov tests for normality on the residuals of the analysis, and Bartlett's test for equal variances (Zar 1984). If one of the main effects in the ANOVA was significant, or if the interaction term was significant, a Tukey's honestly significant difference post hoc test was used to separate means ($\alpha < 0.05$). Values are reported as means ± SE. Statistical analyses were conducted in R version 3.1.0 and JMP version 11 (SAS Institute, Cary, North Carolina).

RESULTS

During the control period water pH remained stable between 8.43 and 8.13 pH units (Figure 2). During both the control and treatment periods of both trials, dissolved oxygen taken from the center of the pond did not fall below 9.92 mg/L. Carbon dioxide infused slowly into the pond and the apparatus did not cause additional noise or vibrations. As CO_2 was added, there was a continual, significant decline in water pH from control values to the lowest observed value of 6.78 pH units ($F = 301.94$, df = 6, $P < 0.001$; Figure 2). In the hours following infusion, pH was lowest near the infusion manifold, but over time the pH decreased throughout the entirety of the pond, resulting in a near-homogenous mixture of pH throughout the pond approximately 13 h after infusion (Figure 2). Initial CO_2 concentrations averaged approximately 30.3 mg/L (2,165.31 µatm), and each increase in CO_2 of approximately 10 mg/L (5,627.73 µatm) resulted in a reduction in pH of 0.25 pH units. The lowest pH observed during the trial (6.78 pH units) corresponds to a CO_2 concentration of approximately 59 mg/L (29,193.05 µatm). During trial 1,

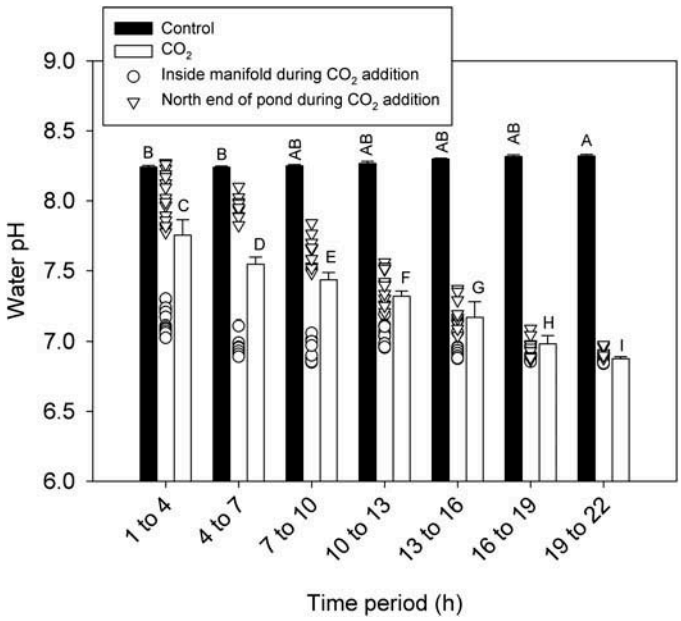

FIGURE 2. Water pH (mean + SE) over time during control (black bars) and CO_2 treatment (white bars) in the experimental pond. The overlaid data points represent pH measured inside the manifold (white circles) and the area at the opposite end of the pond (white triangles) during CO_2 addition. Dissimilar letters above each bar represent significant differences ($P < 0.05$) among groups.

water temperature averaged 15.8°C (range, 15.5°C to 17.0°C) across both the control and CO_2-infusion components of the study, with temperatures varying by less than 1°C across the pond within a 4-h time period. For trial 2, water temperature averaged 15.8°C (range, 14.0°C to 18.5°C) across both the control and CO_2-infusion period, with no more than 3°C differences within the pond during each 4-h time period.

During the control period, both Silver Carp and Bighead Carp were closest in proximity to the distribution manifold (typically <20 m) during daylight hours, with this distance increasing to almost 30 m at 16–22 h into the monitoring period (Figure 3; Table 1). The addition of CO_2 to the pond resulted in both Bighead and Silver carps remaining significantly farther from the center of the distribution manifold, which comprised ~30% of the experimental pond, at each time point relative to control periods, averaging approximately 10% farther from the manifold across all time periods combined (Figure 3; Table 1). In the period 1–4 h after the addition of CO_2 to the pond and the subsequent time periods during the day, both species were almost 50% farther from the manifold than during the control period.

Similar to distance from the manifold, there was a significant influence of the addition of CO_2 on the PV of both Bighead Carp and Silver Carp (Figure 4; Table 1). More specifically, in the first 7 h after CO_2 was added to the pond and before sunrise, the PV of Bighead Carp was approximately half that of the control period, and was approximately 25% lower for

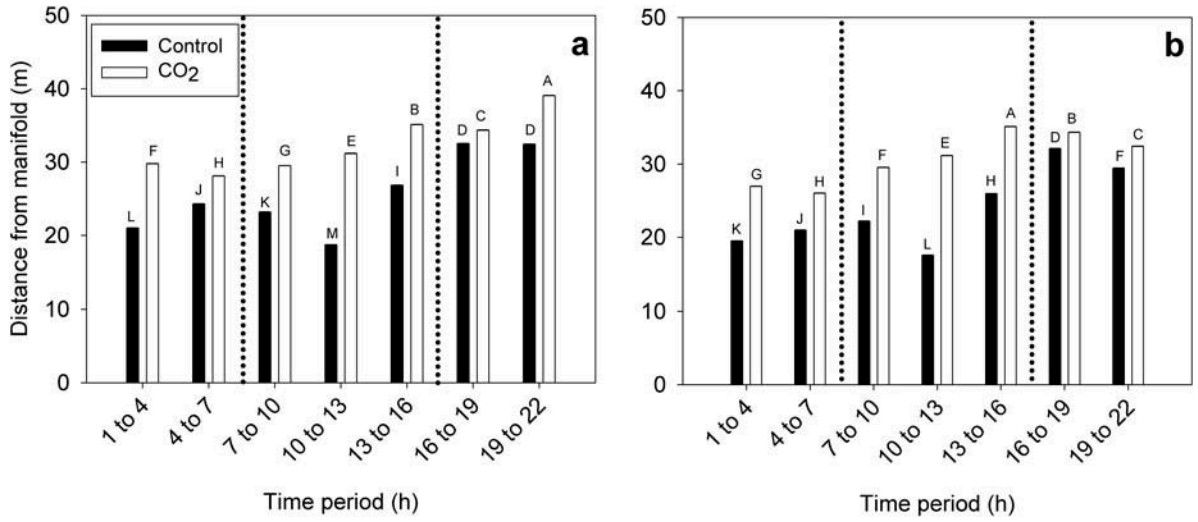

FIGURE 3. Distance (m) from the CO_2-infusion manifold over time for (**a**) Bighead Carp and (**b**) Silver Carp during control (black bars) and CO_2 treatment (white bars). Vertical dotted lines represent sunrise (left line) and sunset (right line), and bins along the *x*-axis represent time since the beginning of either the control or CO_2 treatment (aligned to match time of day). Dissimilar letters represent significant differences ($P < 0.05$) between groups for the treatment × time period interaction. Values are shown as means + SE; error bars may not be apparent due to small SE.

Silver Carp during the same time. After sunrise, the PV of both species doubled, independent of CO_2 addition. During this daylight period, the presence of CO_2 again caused a significant reduction in PV for Silver Carp relative to control periods, while patterns in PV for Bighead Carp during the daylight period did not reveal consistent patterns. After sunset, PV of Bighead Carp were lower in the presence of CO_2 relative to control conditions, while PV for Silver Carp were reduced in the presence of CO_2, but only for the first 3 h after sunset.

For Bighead Carp, additions of CO_2 to the pond resulted in reduced UD relative to controls, particularly during the first 3 h during which UD decreased by approximately threefold (Figure 5; Table 2). During the remainder of the experiment, differences in UD between the control and test periods were minimal, with little pattern emerging. The UD of Silver Carp remained largely unchanged throughout the monitoring period, but a higher UD for the treatment group during the final time period of

TABLE 1. Results of a two-way, repeated-measures ANOVA examining the influence of CO_2 treatment and time for Bighead Carp and Silver Carp. Variables of interest were mean distance from manifold (DistM5; m), mean persistence velocity (PV; m/s), and utilization distribution (UD; m^2). *P*-values in bold italics are significant ($P < 0.05$).

	Bighead Carp			Silver Carp		
Main effects	df	*F*	*P*	df	*F*	*P*
			DistM5			
Treatment	1, 1.0×10^6	59646.85	***<0.0001***	1, 1.0×10^6	54008.54	***<0.0001***
Time period	6, 1.0×10^6	10689.93	***<0.0001***	6, 1.0×10^6	10477.78	***<0.0001***
Treatment × Time period	6, 1.0×10^6	3507.55	***<0.0001***	6, 1.0×10^6	3943.96	***<0.0001***
			PV			
Treatment	1, 1.0×10^6	28763.96	***<0.0001***	1, 1.0×10^6	10582.16	***<0.0001***
Time period	6, 1.0×10^6	49869.44	***<0.0001***	6, 1.0×10^6	43497.85	***<0.0001***
Treatment × Time period	6, 1.0×10^6	6106.28	***<0.0001***	6, 1.0×10^6	1306.26	***<0.0001***
			UD			
Treatment	1, 38	6.97	***0.0121***	1, 38	0.07	0.794
Time Period	6, 228	1.97	0.071	6, 228	9.50	***<0.0001***
Treatment × Time period	6, 228	9.90	***<0.0001***	6, 228	3.27	***0.004***

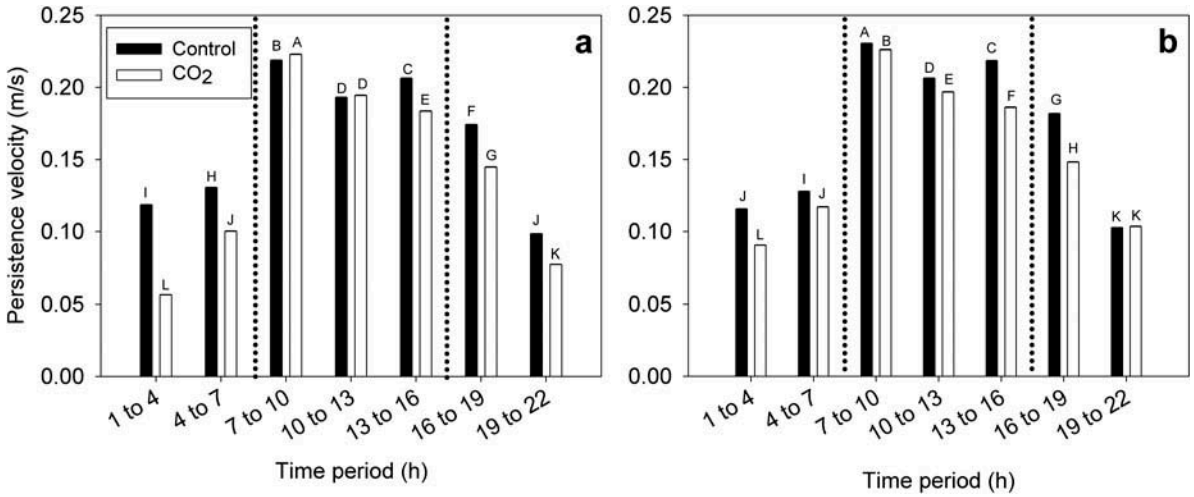

FIGURE 4. Persistence velocity (m/s) for (**a**) Bighead Carp and (**b**) Silver Carp during control (black bars) and CO_2 treatment (white bars). Vertical dotted lines represent sunrise (left line) and sunset (right line), and bins along the *x*-axis represent time since the beginning of either the control or CO_2 treatment (aligned to match time of day). Dissimilar letters represent significant differences ($P < 0.05$) between groups for the treatment × time period interaction. Values are shown as means + SE; error bars may not be apparent due to small SE.

the experiment (from 19 to 22 h) was observed relative to earlier time periods (Figure 5; Table 2).

Additions of CO_2 to the pond caused significant changes to the spatial distribution of all four native fish species. During the entire control period, Bigmouth Buffalo remained approximately 25 m from the distribution manifold across all time periods examined (Figure 6a; Table 2). Additions of CO_2 caused Bigmouth Buffalo to move significantly farther from the distribution manifold relative to control times in six of the seven periods monitored, with distances during 10–19 h after CO_2 injection being almost 15% greater than during control periods (Figure 6a; Table 2). Similarly, additions of CO_2 caused Channel Catfish to move approximately 50% farther from the manifold than during the control monitoring periods (Figure 6b; Table 2). In the first 10 h after CO_2 addition, the distance from the distribution manifold maintained by Yellow Perch was almost double that of control periods, and distances from the manifold remained

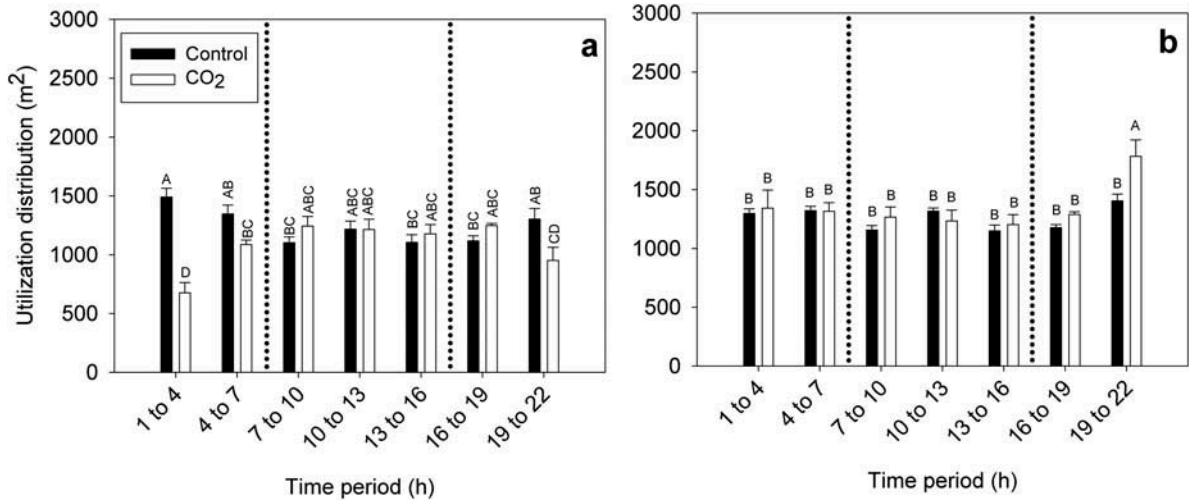

FIGURE 5. Utilization distributions (UD; m^2) for (**a**) Bighead Carp and (**b**) Silver Carp during control (black bars) and CO_2 treatment (white bars). Vertical dotted lines represent sunrise (left line) and sunset (right line), and bins along the *x*-axis represent time since the beginning of either the control or CO_2 treatment (aligned to match time of day). Dissimilar letters represent significant differences ($P < 0.05$) between groups for the treatment × time period interaction. Values are shown as means + SE.

TABLE 2. Results of a two-way, repeated-measures ANOVA examining the influence of CO_2 treatment and time for four native species: Bigmouth Buffalo, Channel Catfish, Paddlefish, and Yellow Perch. Variables of interest were mean distance from manifold (DistM5; m), mean persistence velocity (PV; m/s), and utilization distribution (UD; m^2). *P*-values in bold italics are significant ($P < 0.05$).

	Bigmouth Buffalo			Channel Catfish			Paddlefish			Yellow Perch		
Main effects	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
						DistM5						
Treatment	1, 12	20,634.31	***<0.0001***	1, 16	66,418.46	***<0.0001***	1, 12	0.08	0.7813	1, 10	29,180.51	***<0.0001***
Time period	6, 3.5×10^5	3,454.72	***<0.0001***	6, 4.5×10^5	2,019.10	***<0.0001***	6, 3.5×10^5	1,726.97	***<0.0001***	6, 3.0×10^5	4,449.98	***<0.0001***
Treatment × Time period	6, 3.5×10^5	1,519.10	***<0.0001***	6, 4.5×10^5	370.25	***<0.0001***	6, 3.5×10^5	1,124.25	***<0.0001***	6, 3.0×10^5	1,523.91	***<0.0001***
						PV						
Treatment	1, 12	4,173.10	***<0.0001***	1, 16	7,950.71	***<0.0001***	1, 12	541.30	***<0.0001***	1, 10	9,454.78	***<0.0001***
Time period	6, 3.5×10^5	6,818.22	***<0.0001***	6, 4.5×10^5	22,467.45	***<0.0001***	16, 3.5×10^5	37.99	***<0.0001***	6, 3.0×10^5	11,199.17	***<0.0001***
Treatment × Time period	6, 3.5×10^5	781.89	***<0.0001***	6, 4.5×10^5	593.86	***<0.0001***	6, 3.5×10^5	29.86	***<0.0001***	6, 3.0×10^5	739.3407	***<0.0001***
						UD						
Treatment	1, 12	1.63	0.2287	1, 20.7	3.30	0.0902	1, 12	0.01	0.9797	1, 10	3.15	0.1096
Time period	6, 72	***11.05***	***<0.0001***	6, 80	54.51	***<0.0001***	6, 72	2.62	***0.0238***	6, 59	19.26	***<0.0001***
Treatment × Time period	6, 72	0.48	0.8201	6, 80	3.30	0.0902	6, 72	1.84	0.1028	6, 59	1.01	0.4282

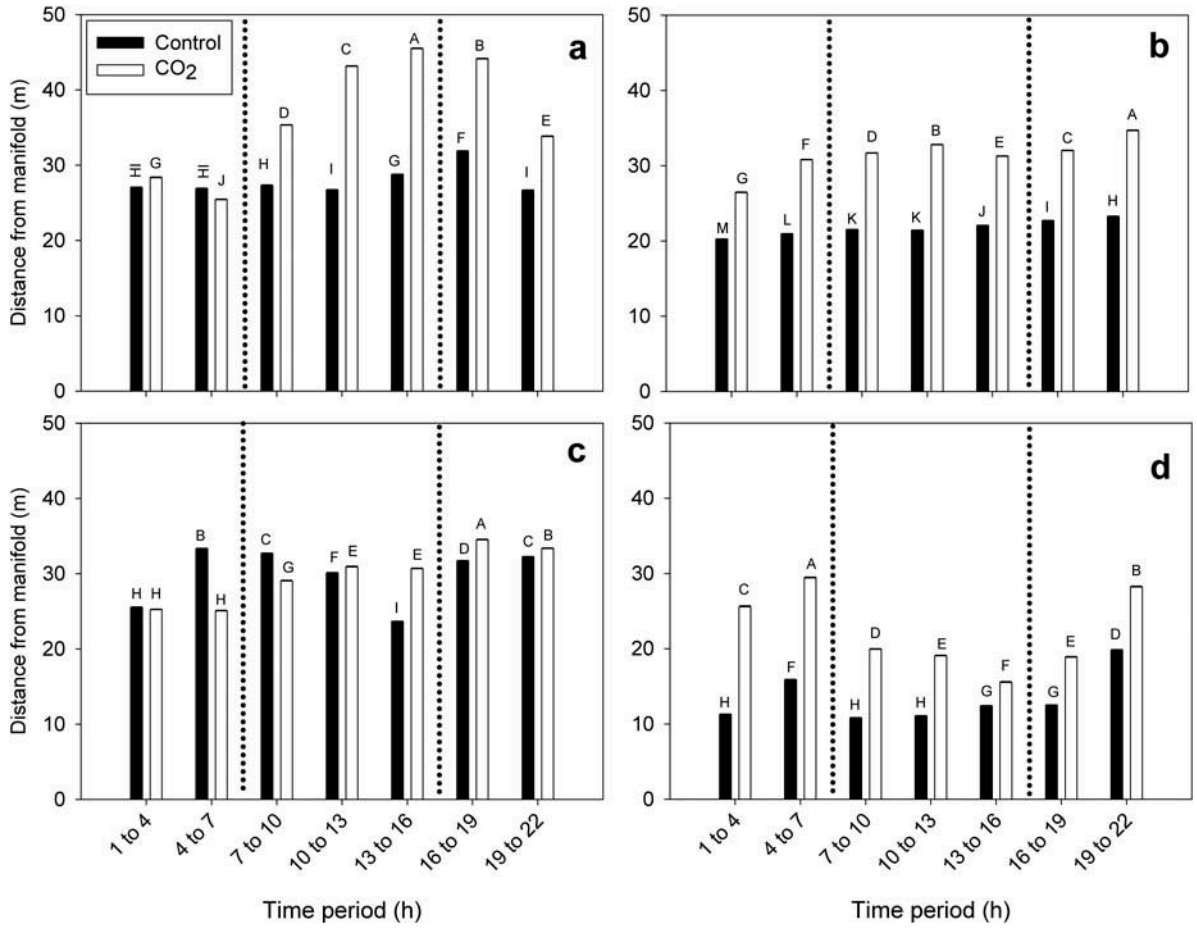

FIGURE 6. Distance (m) from the CO_2-infusion manifold over time for (**a**) Bigmouth Buffalo, (**b**) Channel Catfish, (**c**) Paddlefish, and (**d**) Yellow Perch during control (black bars) and CO_2 treatment (white bars). Vertical dotted lines represent sunrise (left line) and sunset (right line), and bins along the *x*-axis represent time since the beginning of either the control or CO_2 treatment (aligned to match time of day). Dissimilar letters represent significant differences ($P < 0.05$) between groups for the treatment × time period interaction. Values are shown as means + SE; error bars may not be apparent due to small SE.

significantly greater than in control periods during the remainder of the study (Figure 6d; Table 2). The distance from the manifold displayed by Paddlefish did not show clear trends during the study, with the distance from the manifold during CO_2 addition increasing relative to control periods only for four of the seven monitoring periods (Figure 6c; Table 2).

Persistence velocities among native species were highly variable following CO_2 infusion and also over time. During control trials, Bigmouth Buffalo showed strong diel variation in PV, with velocities at night being almost twice as high as those observed during the day. The addition of CO_2 to the pond did not alter this overall diel pattern in velocity, but did cause changes in PV within a time period. For example, in the first 4 h after the addition of CO_2 to the pond, the PV of Bigmouth Buffalo was 15% higher than during control periods. For the remainder of the trials, persistence velocities of Bigmouth Buffalo were approximately 17% to 56% lower than in associated controls (Figure 7a; Table 2). Both Channel Catfish and Yellow Perch also showed diel variation in persistence velocities during the control period, with velocities in the day approximately twofold greater than at night (Figure 7b, d; Table 2). Additions of CO_2 increased the PV of Channel Catfish relative to the control in four of the seven monitoring periods, while CO_2 increased the PV of Yellow Perch across all time periods examined (Figure 7b, d; Table 2). Paddlefish showed reduced PV following the addition of CO_2 to the pond relative to control values, but actual differences were minor (Figure 7c; Table 2).

Additions of CO_2 did not influence the UDs of native fishes (Figure 8; Table 2). The UDs for Bigmouth Buffalo, Channel Catfish, and Yellow Perch were significantly lower during day relative to night periods, but these distributions were not influenced by CO_2 (Figure 8a, b, d; Table 2). Paddlefish did not show significant variation in UDs (Figure 8c; Table 2).

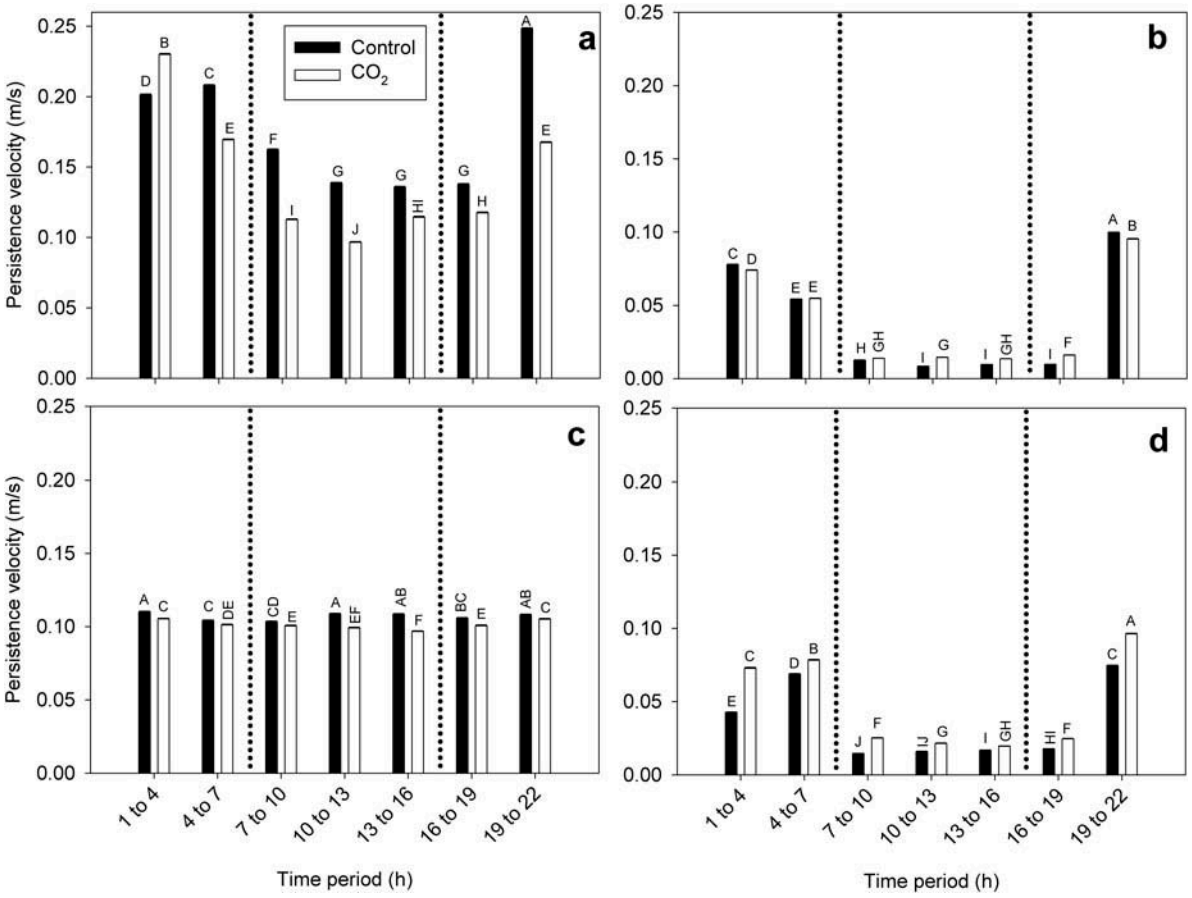

FIGURE 7. Persistence velocity (m/s) for (**a**) Bigmouth Buffalo, (**b**) Channel Catfish, (**c**) Paddlefish, and (**d**) Yellow Perch during control (black bars) and CO_2 treatment (white bars). Vertical dotted lines represent sunrise (left line) and sunset (right line), and bins along the *x*-axis represent time since the beginning of either the control or CO_2 treatment (aligned to match time of day). Dissimilar letters represent significant differences ($P < 0.05$) between groups for the treatment × time period interaction. Values are shown as means + SE; error bars may not be apparent due to small SE.

DISCUSSION

Avoidance of the infusion manifold increased over time for the bigheaded carps, suggesting that, as CO_2 spread throughout the experimental pond, individuals continually sought refuge in areas increasingly far from the manifold. Avoidance behaviors are typically the first response when fish detect areas of high CO_2, since prolonged exposure results in CO_2 passing through the blood–brain barrier and altering brain biochemistry, resulting in equilibrium loss, physiological stress, and ultimately death (Yoshikawa et al. 1991, 1994).

Laboratory investigations have revealed that adult Silver Carp avoid CO_2 concentrations that exceed 100 mg/L in experimental tanks (Kates et al. 2012), while Dennis et al. (2015a) found that juvenile Silver and Bighead carps demonstrated avoidance of CO_2 at concentrations of approximately 200 mg/L. As predicted, bigheaded carps had lower PVs following CO_2 infusion. The lower PVs were most apparent in the early time periods following CO_2 addition, indicating that fish showed a change in behavior and were more likely to move in a tortuous path. Romine et al. (2015) found that Bighead Carp and Silver Carp PVs likewise decreased during the firing of water guns, indicating a change in swimming behavior and a less direct path of movement. Bighead Carp were the only species in this study that showed changes in UD following CO_2 addition, indicating they used a smaller area of the pond after the addition of CO_2 (see Supplemental Figure S.1 available in the online version of this article). Despite Silver Carp displaying a similar pattern in PV and distance from the manifold to Bighead Carp, their UD did not differ significantly after CO_2 was added (see Figure S.2). This may be the result of Silver Carp occupying a similarly sized area of the pond before and after CO_2 addition, albeit moved farther from the manifold, a pattern also observed for the native species.

Bigmouth Buffalo, Channel Catfish, and Yellow Perch generally maintained farther distances from the manifold following CO_2 infusion throughout the observation period. In the laboratory, native adult (Kates et al. 2012) and juvenile

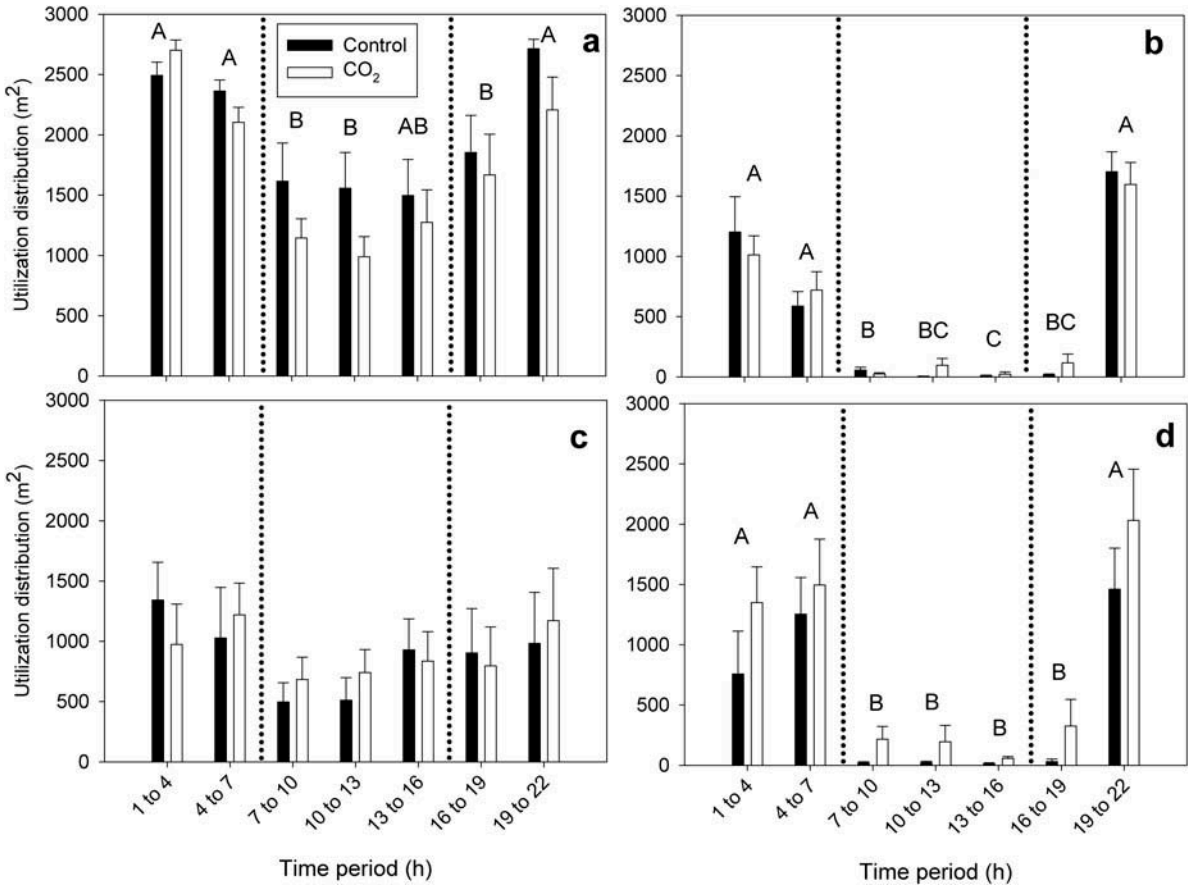

FIGURE 8. Utilization distributions (UD; m^2) for (**a**) Bigmouth Buffalo, (**b**) Channel Catfish, (**c**) Paddlefish, and (**d**) Yellow Perch during control (black bars) and CO_2 treatment (white bars). Vertical dotted lines represent sunrise (left line) and sunset (right line), and bins along the *x*-axis represent time since the beginning of either the control or CO_2 treatment (aligned to match time of day). Dissimilar letters denote significant differences ($P < 0.05$) over the main effect, time. Values are means + SE.

(Dennis et al. 2015a) Largemouth Bass *Micropterus salmoides* and Bluegill *Lepomis macrochirus* both demonstrated behavioral avoidance to CO_2 concentrations between 100 and 200 ng/mL. Bigmouth Buffalo showed a rapid increase in PV for the treatment group immediately following CO_2 infusion, perhaps due to individuals actively moving and seeking habitat with lower CO_2. Moss and McFarland (1970) found that abrupt decreases in pH (from CO_2 infusion into water) resulted in increased average swimming velocity of the schooling Northern Anchovy *Engraulis mordax*. Like the initial response by Bigmouth Buffalo, Channel Catfish and Yellow Perch had PVs that were generally higher among the treatment group relative to controls. Despite this finding, the PVs observed for Channel Catfish and Yellow Perch were much lower compared with the other species in the study, particularly during daylight hours, likely due to these species being structure-oriented and seeking refuge during the day. For Bigmouth Buffalo, after the initial increase in PV following CO_2 infusion, PV decreased for the treatment group at subsequent time periods, similar to the bigheaded carp species, possibly because individuals from these three species were observed schooling throughout the experiment. This variability of PVs for each of the native species was reflected in the finding that the UDs did not differ between treatments and controls for any of the native species. The lack of treatment effect observed for Paddlefish may be because they rely on continuous swimming for ram ventilation (Mims and Shelton 2005) and that even in low O_2 conditions, they can compensate in part by reducing their metabolic rates (Aboagye and Allen 2014).

Carbon dioxide gas spread from the distribution manifold throughout the entire pond, likely due to a combination of wind-induced mixing and passive diffusion. Concentrations of CO_2 increased by approximately 10 mg/L (5,600 µatm) across each 4-h block. The addition of CO_2 to the experimental pond resulted in a steady reduction of water pH. Upon being added to water, CO_2 dissociates and forms carbonic acid, a weak, diprotic acid that further dissociates into bicarbonate and carbonate ions, generating protons that concomitantly reduce water pH (Cole et al. 1994). The relative abundance of each of

these ion species, as well as the resulting change in pH of the water, will largely be driven by alkalinity, buffering capacity, and temperature (Bishop et al. 2000). Although there is species-specific variation, water pH ranging from 6.5 to 9.0 is considered optimal for the growth and health of most freshwater aquatic animals (Boyd and Tucker 1998), yet pH values near the extremes of this range may prompt behavioral avoidance. Increased water CO_2 concentrations and decreased pH can result in changes in blood pH and blood O_2 levels for fish, which, in turn, can stimulate increased ventilation and physiological stress as well as behavioral changes (Wood et al. 1990). Fish are able to detect regions of elevated CO_2 (Gilmour 2001) and will avoid areas that contain unfavorable CO_2 gradients (Fromm 1980).

If a zone of elevated CO_2 is to be used in situ to deter the movement of invasive fishes such as bigheaded carps, nontarget organisms (e.g., mussels, native fishes) would also be affected. For example, a reduction in water pH has the potential for direct negative consequences for both fish (Freda and McDonald 1988) and shell-forming invertebrates (Michaelidis et al. 2005), and elevated pCO2 levels also have potential to negatively impact several aspects of fish behavior including olfaction and homing (Munday et al. 2009). An additional practical consideration is that it is difficult to contain CO_2 coverage over time and under all conditions (e.g., lotic river systems), and factors such as wind, currents, and hydraulic actions could lead to the dispersal of a CO_2 zone beyond the target area or limit the locations where a barrier might be deployed. Even so, an integrated approach could be considered, in which multiple deterrents and barriers (e.g., electric, sonic, CO_2) are used in synergy to provide control and redundancy (Noatch and Suski 2012).

As predicted, the zone of elevated CO_2 influenced the movement and behavior of Bighead Carp and Silver Carp but also affected the native species. Future studies could expand on the results of this pond study by examining the behavior of bigheaded carps to CO_2 infusion in a field setting at a larger scale. Such an approach would provide a better understanding of how effective CO_2 is as a behavioral deterrent in an open system with habitat complexity. These results, along with the growing body of evidence from laboratory studies that CO_2 can be an effective deterrent for both juvenile and adult bigheaded carps (Kates et al. 2012; Dennis et al. 2015a), suggest that CO_2 can be an important tool to incorporate into integrated management strategies to control the spread of invasive bigheaded carps.

ACKNOWLEDGEMENTS

Any use of trade, product, or company name is for descriptive purposes only and does not imply endorsement by the U. S. Government. This work was supported by the Illinois Department of Natural Resources, through funds provided by the U.S. Environmental Protection Agency's Great Lakes Restoration Initiative. M.R.D. was supported by a Natural Sciences and Engineering Research Council of Canada postdoctoral fellowship. We thank Tyson Hatton and Nicholas Swyers, USGS, Western Fisheries Research Center, for telemetry array design and data processing.

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