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ARTICLE

Avoidance of carbon dioxide in flowing water by bighead carp

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Abstract: Carbon dioxide (CO_2) in water has been explored for use as an invasive species deterrent system. To date, studies have not determined CO_2 avoidance by fish in flowing water, and this is a necessary step before an operational deterrent system can be implemented. The objective of the study was to define how flowing water influences the response of bighead carp (*Hypophthalmichthys nobilis*) to continuous plugs of CO_2. A choice experiment by which CO_2 was injected into channels of an annular flowing water flume was completed. In trials when CO_2 was injected into the inner vein, fish spent less time in the vein when compared with control conditions. An increased amount of lateral movements and reduced performance were also observed when fish were exposed to elevated CO_2. The study demonstrates that bighead carp in flowing water enriched with CO_2 move away, a finding consistent with static water experiments.

Résumé : Le dioxyde de carbone (CO_2) dans l'eau a été étudié en vue de son utilisation comme système de dissuasion d'espèces envahissantes. À ce jour, les études n'ont pas établi s'il y a évitement du CO_2 par les poissons dans l'eau mouvante, bien qu'il s'agisse d'une étape préalable nécessaire à la mise en œuvre de tout système de dissuasion. L'objectif de la présente étude était d'établir comment l'eau mouvante influence la réaction de carpes à grosse tête (*Hypophthalmichthys nobilis*) à des bouffées continues de CO_2. Une expérience de choix dans laquelle du CO_2 est injecté dans les chenaux d'un canal annulaire d'eau mouvante a été réalisée. Dans les essais, quand du CO_2 était injecté dans la veine intérieure, les poissons y passaient moins de temps que dans les conditions de référence. Plus de déplacements latéraux et une performance plus faible étaient aussi observés quand les poissons étaient exposés à des concentrations élevées de CO_2. L'étude démontre que les carpes à grosse tête dans de l'eau mouvante enrichie en CO_2 s'en éloignent, une constatation qui concorde avec les résultats d'expériences en eau stationnaire. [Traduit par la Rédaction]

Introduction

Aquatic invasive species (AIS) are prevalent across the planet, and management tools are often used to control their spread (Vander Zanden and Olden 2008). Specifically, for freshwater fish, unfettered movement between water bodies is common (Rahel 2007), allowing AIS to distribute themselves across aquatic landscapes with relative ease (Ricciardi and Rasmussen 1998). Because most freshwater species rely on corridors for species dispersal, barriers that block off corridors, or deterrent systems that reduce access to specific habitats, can decrease the likelihood that a species will invade a waterbody (Kolar and Lodge 2002; Rahel 2007). For example, to reduce the probability that Asian carp (e.g., bighead carp (*Hypophthalmichthys nobilis*), silver carp (*Hypophthalmichthys molitrix*), grass carp (*Ctenopharyngodon idella*), and black carp (*Mylopharyngodon piceus*)) will enter the Great Lakes, electrical deterrent systems have been operational in the Chicago Area Waterway System since 2002 (Moy et al. 2011). Nonphysical barriers, such as the Chicago Area Waterway System's electrical deterrent system, are not 100% effective at stopping all fishes (Davis et al. 2017); therefore, it is essential to continue to explore other novel deterrent systems to effectively prevent fish movements (Noatch and Suski 2012). Ideally, new deterrent systems will be used with current systems to prevent the movement of AIS and ensure Asian carp do not enter the Great Lakes (Noatch and Suski 2012).

Recently, carbon dioxide (CO_2) has shown promise as a deterrent system to reduce the movements of invasive species, including Asian carp (see review by Treanor et al. 2017). Fish have CO_2-sensitive receptors on their gills (Perry and Gilmour 2002), which enable them to detect elevated concentrations of CO_2 in water. Concomitantly, elevated CO_2 concentrations can induce an array of physiological responses in fish, including physiological stress, irregular behaviors, and loss of equilibrium (Kates et al. 2012; Dennis et al. 2015; Hasler et al. 2017). Further, fish in both laboratory and field settings have demonstrated avoidance of areas with elevated CO_2 (Kates et al. 2012; Donaldson et al. 2016; Cupp et al. 2017). However, a limitation of past studies on behavioral avoidance of elevated CO_2 is that studies have been carried out in relatively static water (i.e., limited flow). Fish may exhibit different behavioral responses to elevated CO_2 in flowing water because of reduced contact times, directionality of flow, the presences of competing sensory stimuli, exhaustion from swimming against flow, and altered sensory functions (Larrick et al. 1978; Montgomery et al. 1995; Tierney 2016). Speculatively, water chemistry and fish

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motivation may also alter how fish respond to elevated CO_2 in flowing water. Overall, understanding the responses of fish in flowing water exposed to elevated CO_2 is a necessary step before actions can be taken to implement an operational CO_2 deterrent system to control the movement of Asian carp in naturally flowing water.

To address how fish might respond differently to elevated CO_2 in flowing water as compared with static water, we had to define how a flowing water environment impacts the response of a group of bighead carp to continuous plugs of CO_2. To accomplish this goal, we undertook a choice experiment using an annular flowing water flume with near-rectilinear flow, and CO_2 was injected into either side of the flume. If fish spend less time on the side of the flume with elevated CO_2, and if fish swim away from the enriched CO_2 side, the conclusion could be made that bighead carp were avoiding areas of high CO_2. Furthermore, because fish were exposed to the parcel of CO_2 multiple times as it moved around the flume, we quantified how the parcel of CO_2 dissipated and mixed with fresh water and how this dissipation might influence avoidance behaviors. Observed irregular behaviors by individual fish were also quantified (i.e., equilibrium loss, reduced swimming ability) (Kates et al. 2012) and used to assess how a bighead carp might respond to elevated CO_2, which, as described above, had not been done previously.

Materials and methods

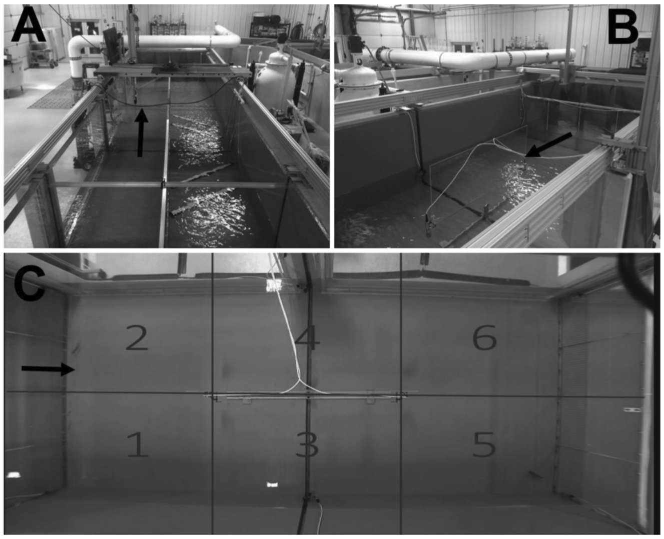
Study animals

Bighead carp ($n = 114$; 145 ± 16.8 mm; 63.8 ± 12.0 g) were obtained from J.M. Malone & Sons fish hatchery in Lonoke, Arkansas. Fish were held for several weeks prior to the start of the experiment at the Cognitive Ecology and Ecohydraulics Research Facility at the US Army Engineer Research and Development Center in Vicksburg, Mississippi. Fish were held in 1060 L circular holding tanks, and water was treated and recirculated on site. Each day, fish were fed approximately 2% of their body weight of a 50–50 mix of #1 and #2 Crumble (Zeigler Finfish Starter Feed, Gardners, Pennsylvania). The tank flow field was changed for a few hours each day to exercise fish.

Flume

To quantify whether elevated partial pressure of CO_2 (pCO_2) could induce avoidance by bighead carp, an annular swim flume was used (12.19 m in length). The "arena" where fish were placed during each experimental trial was a 4.88 m × 2.44 m × 0.61 m section of one channel of the flume (Figs. 1 and 2). Fish were restricted to the arena by a coated metal screen (1.5 cm mesh size) placed at the upstream and downstream ends of the arena. The opposite channel from the arena had an acrylic sheet installed so that the entire channel of the flume was halved into two veins (Figs. 1 and 2). Removable acrylic sheets were also installed at either end of the channel so that CO_2 plugs could be created (see below). Before the experiment, dye tests were undertaken to ensure near-rectilinear flow could be achieved and that the mixing of water across each half of the flume was minimal.

Fig. 1. (A) Photo of the channel of the flume where air or CO_2 was injected into either the outer or inner vein. (B) Photo of the channel of the flume where the arena was present. (C) Overhead photo of the arena divided into six sextants. In this study, sextants 1, 3, and 5 represent the inner vein, while 2, 4, and 6 are the outer vein. Black arrows represent direction of water flow when flume was activated.



Treatments and trials

Five treatments were undertaken: (*i*) compressed air in the inner vein, no CO_2 in the outer vein ($n = 8$); (*ii*) compressed air in the outer vein, no CO_2 in the inner vein ($n = 8$); (*iii*) CO_2 in the inner vein, no air or CO_2 in the outer vein ($n = 7$); (*iv*) CO_2 in the outer vein, no air or CO_2 in the inner vein ($n = 7$); and (*v*) no air or CO_2 in either vein ($n = 8$). Depending on the treatment, either air or CO_2 was injected into the target vein using regenerative air blowers (Sweetwater, Pentair Aquatic Ecosystems, Apopka, Florida, USA) or bubbling compressed CO_2 gas into the water using microbubble diffusers (1 m × 0.08 m, Point Four Inc., Coquitlam, British Columbia, Canada), respectively. The target pCO_2 for all trials when CO_2 was needed was approximately 240 000 μatm (1 atm = 101.325 kPa), and it took 20–30 min to reach this level. A modified infrared CO_2 probe (GMT221, 0%–20%, Vaisala, Vantaa, Finland; Johnson et al. 2010) was used to monitor pCO_2 at the injection site. A high amount of CO_2 was used to ensure that as the plug of CO_2 moved around the flume, the fish in the arena received a sufficiently high amount of CO_2 to potentially cause avoidance prior to the dissipation of CO_2. Water temperature (°C), dissolved oxygen (DO), conductivity (μS·cm^{-1}), and pH (Orion Star A3295, Thermo Fisher Scientific) were collected at the injection site, as well as hardness (ppm; GH titration kit, API, Mars Fish Care, North America, Inc.) and alkalinity (Model AL-AP, Hach Company, Loveland, Colorado). pCO_2 was calculated using the "CO2calc" app (Robbins et al. 2010); target pCO_2 varied by less than 10%. While CO_2 gas was being injected into the target vein, three bighead carp were removed from their holding tank and placed into a water-filled 19 L plastic pail and released into the arena for a 15 min acclimation period (pretrials indicated fish reduced exploratory activity in the flume after being in the flume for 15 min and is consistent with other studies investigating behavioural avoidance of elevated CO_2 by fish; Schneider et al. 2018). After the target gas level was reached in the appropriate vein in the channel opposite the arena and the acclimation period complete, the flume was activated and water velocity ramped up to 15 cm·s^{-1} (approximately 1 body length per second). It took 15 s for the flume to reach the target water velocity. Concurrently, gates holding the gas plug were removed, thus allowing the gas plug (either CO_2, air, or no gas) to move through the arena in the appropriate vein. The technique was conducted with controls as well to ensure that water movement and sounds created by the gate lifting were equal among treatments. The pH and timing of the plug of CO_2 or air was assessed using a multiparameter probe equipped with a pH electrode placed upstream of the upstream boundary of the arena (Orion Star A3295, Thermo Fisher Scientific; Fig. 1). During each trial, pH was monitored throughout the arena using eight gel-filled pH electrodes (IntelliCAL PHC101, Hach Company, Loveland, Colorado, USA) connected to data loggers (HQ40d, Hach Company). Mean alkalinity of the flume was held stable at 104.5 ± 12.4 mg $CaCO_3$·L^{-1} by the addition of sea salt (Instant Ocean Spectrum Brands, Blacksburg, Virginia, USA). Temperature of the flume throughout the study was externally controlled to remain

Fig. 2. A schematic representing the overhead view of the flume. Black dots in the domain depict approximate location of pH probes. The drawing is not to scale.

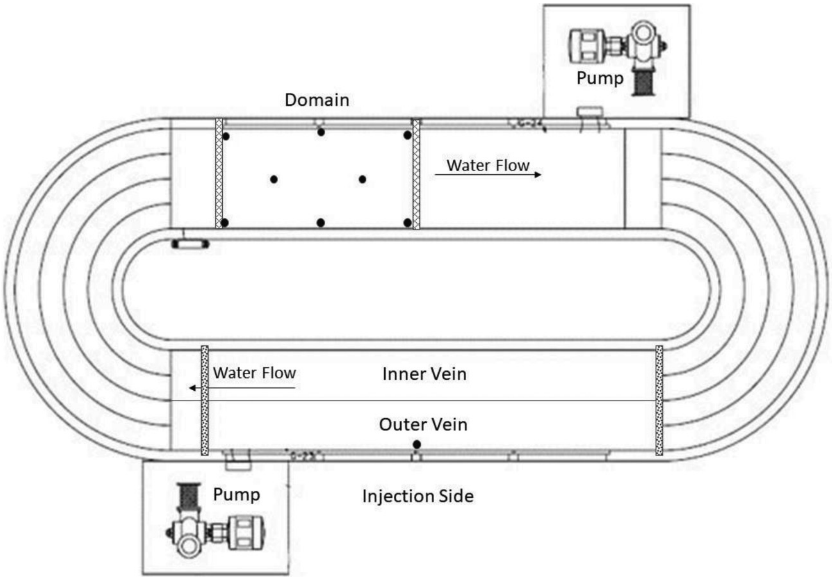


Table 1. Mean and standard deviation of water quality parameters sampled at the gas injection point located in the channel opposite the arena where fish were located, in the arena at start of the trial, and in the arena at the end of the trial.

Treatment	Location	DO (mg·L^{-1})	pH	DCO$_2$ (mg·L^{-1})	pCO$_2$ (μatm)
AIRIN	Injection	8.6±0.2	8.3±0.1	9.3±2.7	602±100
	Arena (start)	8.6±0.2	8.3±0.1	9.3±2.7	602±100
	Arena (end)	8.6±0.1	8.4±0.1	10.2±4.0	602±98
AIROUT	Injection	8.6±0.1	8.2±0.2	13.0±4.2	600±200
	Arena (start)	8.6±0.1	8.2±0.2	13.0±4.2	600±200
	Arena (end)	8.5±0.1	8.2±0.2	13.5±2.9	622±439
CO2IN	Injection	7.7±0.3	5.8±0.1	297.1±40.1	162 358±18 263
	Arena (start)	8.6±0.1	7.9±0.5	16.3±6.7	2 278±3 000
	Arena (end)	8.5±0.1	6.9±0.2	33.2±10.2	12 459±5 029
CO2OUT	Injection	7.4±0.3	5.7±0.1	306.8±95.6	186 140±16 758
	Arena (start)	8.5±0.5	8.0±0.4	18.6±9.5	2 113±2 000
	Arena (end)	8.5±0.1	7.0±0.1	40.0±15.8	36 542±26 423
FLOCON	Injection	7.7±2.7	8.3±0.1	12.4±1.0	600±200
	Arena (start)	7.6±2.7	8.3±0.1	12.4±1.0	600±200
	Arena (end)	7.6±2.7	8.3±0.1	11.5±2.5	557±229

Note: DO = dissolved oxygen (mg·L^{-1}); DCO$_2$ = dissolved carbon dioxide (mg·L^{-1}); pCO$_2$ = partial pressure of carbon dioxide (μatm; 1 atm = 101.325 kPa); AIRIN = air injected into inner vein; AIROUT = air injected into outer vein; CO2IN = CO$_2$ injected into inner vein; CO2OUT = CO$_2$ injected into outer vein; FLOCON = flow control treatment; no gas injected into either vein.

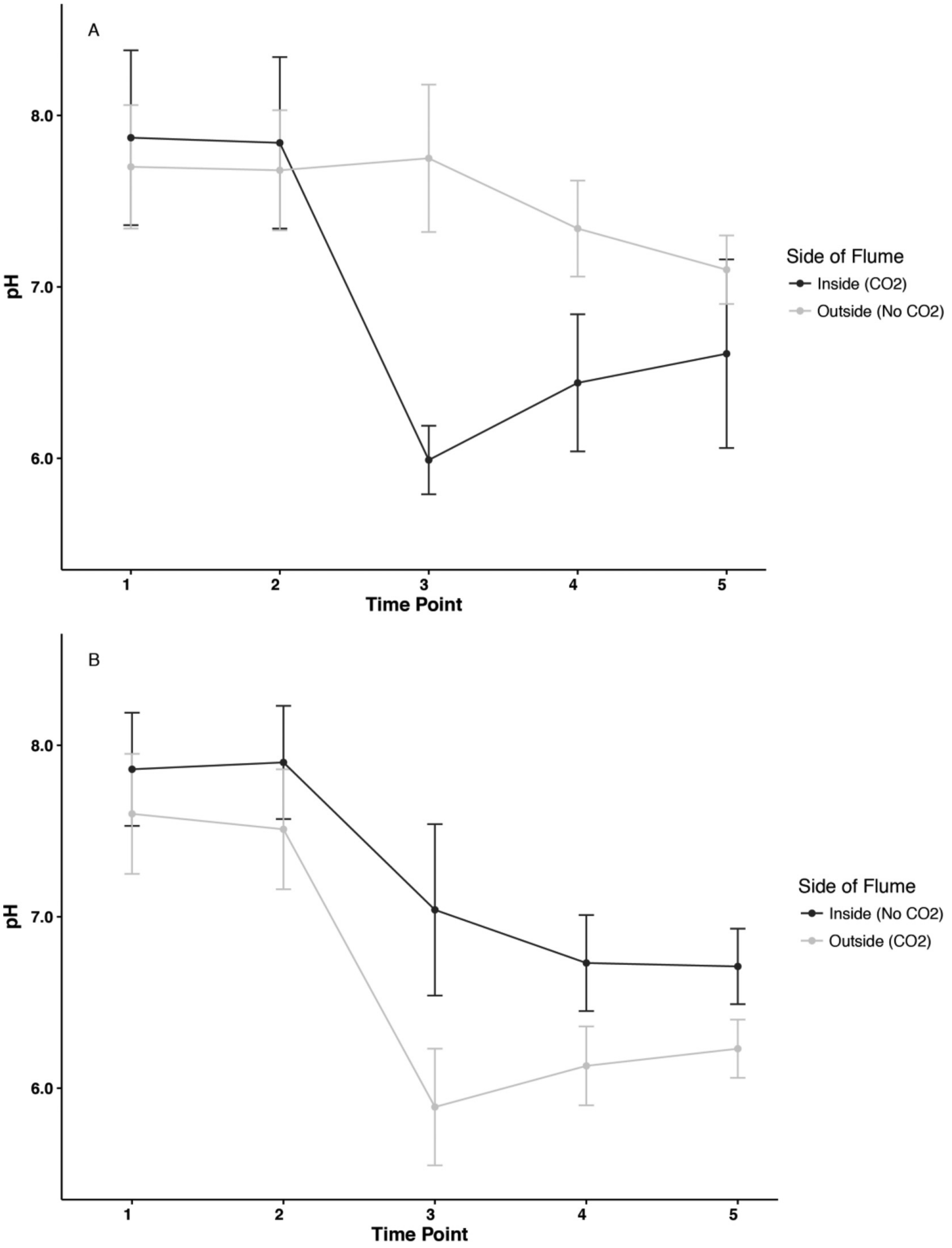
at 21.4 ± 0.1 °C. Other relevant water quality parameters are presented in Table 1. Video recordings encompassing both the 15 min acclimation and experimental periods of each trial were collected using a typical camcorder (Sony FDR-AX100 4K Professional Digital Camcorder, San Diego, California, USA) suspended overhead of the arena (Fig. 1).

Data analysis

Video recordings were used to quantify fish location during both the acclimation and experimental periods (a single observer was used for all videos). The arena was divided into sextants (Fig. 1C), and each time the group of bighead carp left a sextant, the time was recorded and the pH relative to the initial pH at the start of the trial was recorded. Other behaviors, such as fish touching the downstream screen, surface breaches, and loss of equilibrium, were also counted. From the data manually entered into a spreadsheet, we calculated time spent in each sextant, time spent in each vein, and lateral movements (i.e., moved from one vein to the other) using simple addition and logic functions in Microsoft Excel (version 15.32, Microsoft Corporation, Redmond, Washington, USA).

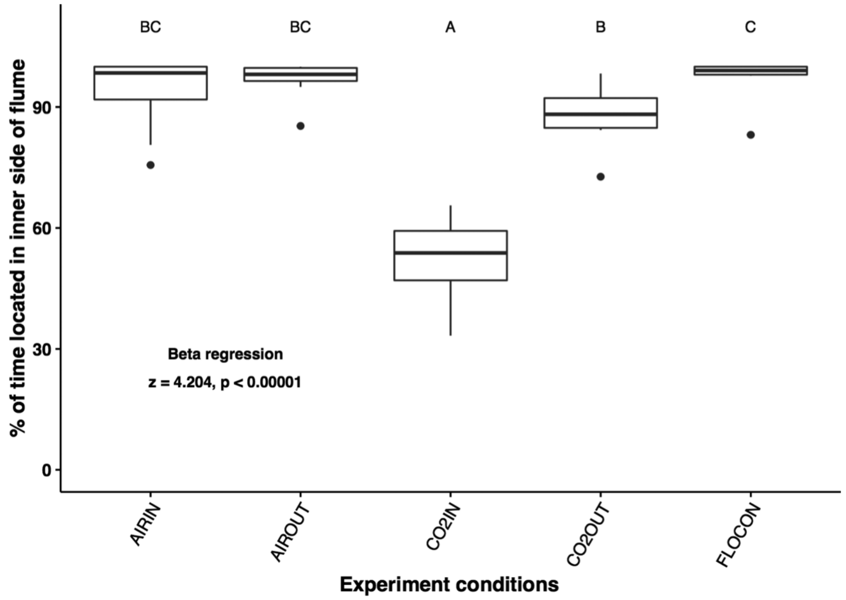
Given the nature of the data collected, several statistical analyses were undertaken. Differences between the pH of the inner and outer veins of the flume during trials when CO$_2$ was used were determined using 95% confidence limits assuming a t distribution. Owing to low variation in pH across all other treatments, pH was only analyzed for trials when CO$_2$ was added to the flume. The proportion of time that a group of fish spent in the inner vein of the flume during the experiment was analyzed across treatments using a beta regression model in the R package "betareg" (Cribari-Neto and Zeileis 2010). Total number of lateral movements made by the group of fish during each treatment was analyzed using a generalized linear model, assuming a Poisson error distribution. If the group of fish became separated, the "lead" fish was monitored until the group reformed, which was usually

Fig. 3. Mean pH recorded at the upstream end of the arena at five time points (1: pretrial, 2: acclimation, 3: first pass of CO_2 plug, 4: second pass of CO_2 plug, and 5: third pass of CO_2 plug). Panel A represents trials when CO_2 was added to the inner vein of the flume, and panel B represents trials when CO_2 was added to the outer vein of the flume. In both panels, the black line represents pH recorded in the inner vein and the gray line represents pH recorded in the outer vein. Whiskers represent 95% confidence limits using a *t* distribution.



within several seconds. The number of lateral movements during a trial were rank-transformed to meet the assumptions of the test, and a Tukey post hoc test from the R package "multcomp" (Hothorn et al. 2008) was used to distinguish significance across treatments. The relative change in pH when the group of fish vacated a sextant was determined for the first four lateral moves of each trial, rank-transformed, and compared using a linear mixed effects model in the package "lme4" (Bates et al. 2015). The first four lateral moves were used because four was the minimum number of lateral moves that each trial had. The total number of fallbacks (i.e., downstream screen touches) by treatment during the acclimation and experimental portions of each trial were analyzed using a penalized generalized linear mixed model using a quasi-Poisson error distribution in the R package "MASS" (Venables and Ripley 2002; Bolker et al. 2009). Residual plots were visually assessed to ensure the assumptions of each test were not violated. All statistical analyses were completed using R (R Development Core Team 2010), and statistical significance was tested at a 95% confidence level.

Fig. 4. Boxplots of percentage of time the group of three bighead carp were located in the inner vein of the flume during each treatment (AIRIN = air injected into inner vein; AIROUT = air injected into outer vein; CO2IN = CO_2 injected into inner vein; CO2OUT = CO_2 injected into outer vein; FLOCON = flow control treatment; no gas injected into either vein). Thick black lines represent medians (50% quantiles), the box represents the interquartile ranges (IQR; the 25th and 75th quantiles), vertical lines are whiskers and represent the higher and lower 1.5 of the IQR, and black dots represent outlying points. Letters indicate statistical differences across treatments determined using a beta regression model ($z = 4.204$, $p < 0.00001$) and a least squares Tukey post hoc test.



Results

When CO_2 was injected into either side of the flume (Table 1), a significant reduction in pH occurred (Fig. 3). On the first pass of the plug of CO_2, when CO_2 was injected into the inner vein of the flume, the pH in the arena dropped to 5.99 ± 0.22 from 7.86 ± 0.51 (Fig. 3A). The pH in the inner vein then increased to 6.44 ± 0.43 and 6.61 ± 0.60 during the second and third passes of the plug of CO_2, respectively. On the third pass of the plug when CO_2 was injected into the inner vein of the flume, the two veins did not differ in pH, and therefore the arena was uniform (Fig. 3A). When CO_2 was injected into the outer vein of the flume, pH during the eight trials decreased to 5.89 ± 0.41 from 7.60 ± 0.42 during the first pass of the CO_2 plug and then nonsignificantly increased to 6.13 ± 0.28 and 6.23 ± 0.20 during the second and third passes, respectively (Fig. 3B). During each pass of the CO_2 plug when CO_2 was injected in the outer vein, the outer vein had significantly lower pH than the inner vein, though the inner vein did also experience a significant reduction in pH during the second and third pass (Fig. 3B). Quantitatively, a pH change greater than one pH point in comparison with the starting pH of the appropriate vein occurred for on average 69.3% ± 9.3% of the 15 min exposure period.

Bighead carp had an affinity for the inner vein of the flume, as indicated by the percentage of time spent in the inner vein during all trials, except for trials where CO_2 was injected into the inner vein (Fig. 4). More specifically, during the trials when no air or CO_2 was injected into the flume, fish spent on average 97% ± 6% of the trial time in the inner vein (Fig. 4). Likewise, when air was injected into the inner and outer veins, fish spent on average 94% ± 10% and 97% ± 5% in the inner vein, respectively (Fig. 4). However, in trials when CO_2 was injected into the inner vein, fish spent significantly less time in the inner vein, spending 53% ± 10% of time in the inner vein (Fig. 4). Because fish had an affinity for the inner vein, when CO_2 was injected in the outer vein, fish would have rarely encountered the CO_2 plug. During these trials, fish spent less time in the inner vein in comparison with the treatment when no gases were added to the flume (i.e., the flow control treatment; Fig. 4).

Table 2. Output of generalized linear model using Poisson error distribution on lateral moves (rank-transformed) made by bighead carp during each treatment.

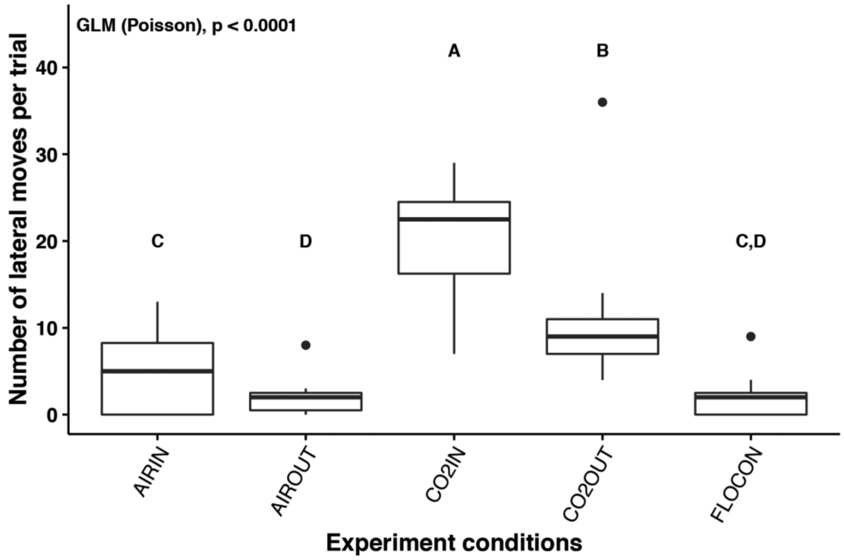
Fixed effect	Estimate	Standard error	*Z* value	*P* value
Intercept (AIRIN)	2.351c	0.109	21.551	<0.0001
AIROUT	–0.560d	0.189	–2.961	0.003
CO2IN	1.266a	0.124	10.251	<0.0001
CO2OUT	1.037b	0.127	8.169	<0.0001
FLOCON	–0.226cd	0.164	–1.381	0.167

Note: Treatments are defined in Table 1. Treatments with different letters are statistically different from each other (Tukey contrasts).

When CO_2 was injected into either vein of the flume, bighead carp had a greater number of lateral moves compared with when no CO_2 was injected into the flume (Table 2; Fig. 5). Lateral movements between veins of the flume increased fourfold when CO_2 was injected into the inner vein compared with when air was injected into the same vein and over fivefold when CO_2 was injected into the outer vein relative to when air was injected into the outer vein (Fig. 5). For the first four lateral moves in each trial, when the carp group moved from the CO_2 injected vein to the other vein, the mean pH change from the baseline pH to when the group moved was 0.87 ± 0.61 for the inner vein CO_2 treatment (Fig. 6A) and 0.54 ± 0.31 for the outer vein CO_2 treatment (Fig. 6B). The change in pH did not statistically differ across the subsequent lateral movements for when CO_2 was injected into either the inner or outer treatments (Fig. 5).

When bighead carp were exposed to the CO_2 plug, they fell back to the screen of the arena more often than during trials with air (Fig. 7). On average, during the trials when CO_2 was injected to either side of the flume, carp fell back 0 times during the 15 min acclimation period when no CO_2 was present in the arena. Once the plug of CO_2 began to move around the flume, carp fell back to the screen on average 24 times ± 48 times (Fig. 7). During trials when air was injected into the flume, no change in the number of

Fig. 5. Boxplots of the number of lateral moves per trial made by groups of three bighead carps during each treatment (AIRIN = air injected into inner vein; AIROUT = air injected into outer vein; CO2IN = CO_2 injected into inner vein; CO2OUT = CO_2 injected into outer vein; and FLOCON = flow control treatment; no gas injected into either vein). Thick black lines represent medians (50% quantiles), the box represents the interquartile ranges (IQR; the 25th and 75th quantiles), vertical lines are whiskers and represent the higher and lower 1.5 of the IQR, and black dots represent outlying points. Lateral moves were defined as movements from the inner vein to the outer vein or from the outer vein to the inner vein. Boxplots not connected by the same letter are statistically significant determined with a generalized linear model using a Poisson error distribution (link = log). See Table 2 for further statistical information.



fallbacks to the screen were observed (Fig. 7). Other known responses to exposure to elevated pCO_2, such as equilibrium loss and surfacing, were also monitored, but did not occur often enough to warrant statistical analyses (i.e., observed in less than 5% of the trials and fewer than five times in any given trial).

Discussion

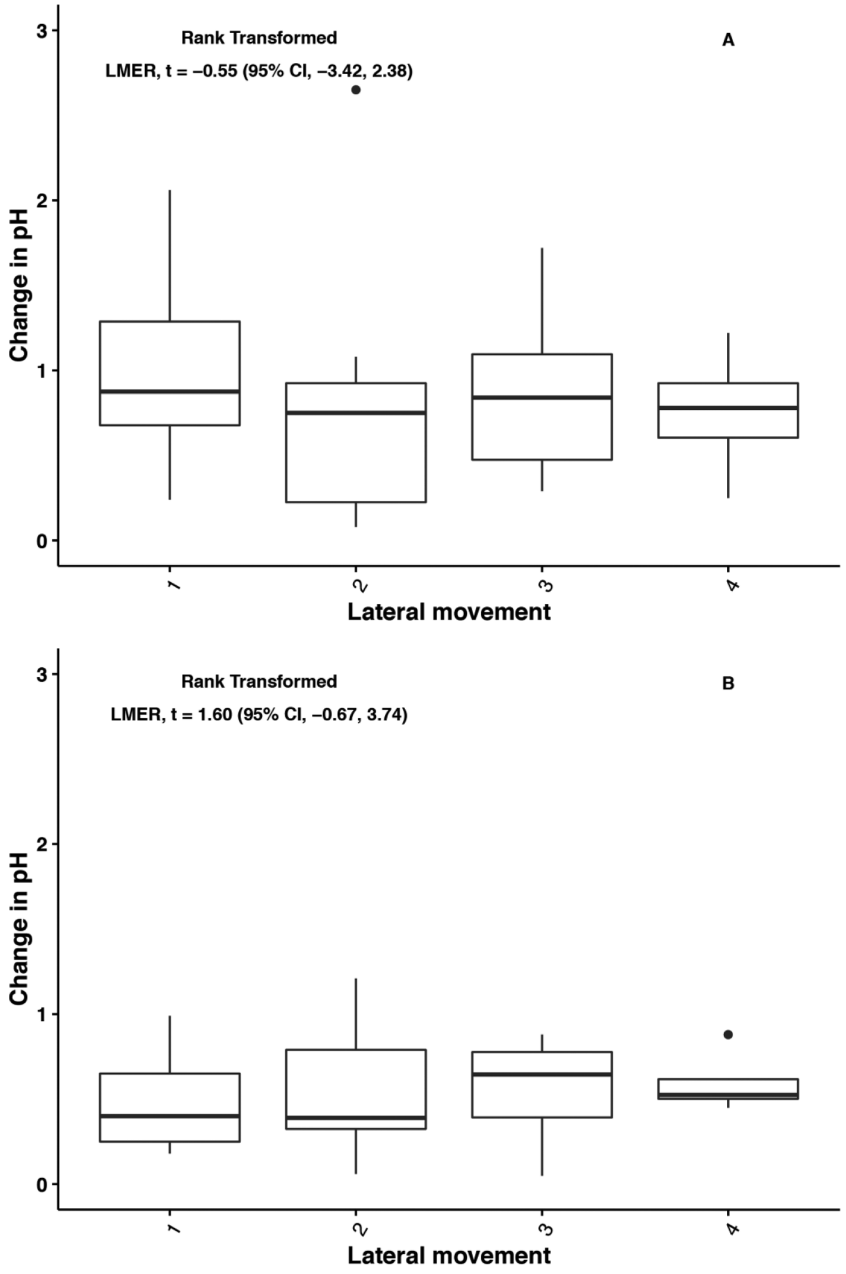
When CO_2 was injected into either side of the flume, a significant reduction in pH occurred because of the close association of CO_2 and pH, which is demonstrated by the bicarbonate buffering system (Stumm and Morgan 1996). The injection of CO_2 into only one channel at a time resulted in heterogeneous water quality between the two veins with respect to pCO_2, pH, and alkalinity (temperature and conductivity were homogeneous). Because the CO_2 was injected in only one channel of the flume, the reduction in pH mainly occurred in the corresponding channel of the arena as the flowing water moved the plug of CO_2 around the flume. This “CO_2” separation of the channels in the arena lasted for three passes of the plug in the outer vein and two passes in the inner vain. It is also important to note that during the trials when CO_2 was injected into the outer vein, some CO_2 must have “bled” into the inner vein during the second and third pass, as pH was significantly reduced in comparison with the period prior to the injection. However, there was still significantly more CO_2 in the outer vein. The separation between the veins with respect to pCO_2 permitted an analysis of whether bighead carp avoided elevated CO_2 in the flume.

Behavioral avoidance of elevated CO_2 was observed by bighead carp when in flowing water conditions. During trials when pH was reduced in the inner vein of the flume by the addition of CO_2 (i.e., pCO_2 increased), the group of bighead carp spent 53% of the time in the inner vein and thus spent 47% of time in the vein where CO_2 was not injected. The percentage of time that pH was reduced by over one pH point in the inner vein was ~69%, and so, over the entire 15 min exposure period, carp avoided elevated pCO_2 for most of the time that CO_2 was present in the inner vein. The avoidance of the inner vein when pH was reduced suggests that fish avoided areas where CO_2 had been injected, which was previously shown in both pond and linear raceway experiments (Donaldson et al. 2016; Cupp et al. 2017). Lateral moves also increased fourfold when pCO_2 was elevated, which, in past studies, has been used to indicate that fish are seeking to locate improved water quality (Kates et al. 2012; Dennis et al. 2016*a*, 2016*b*). Further, as noted above, when CO_2 was added to the outer vein, CO_2 bled into the inner vein and time spent and lateral movements in the inner vein during these trials also significantly differed from the flow control. Overall, these findings indicate that bighead carp exhibit avoidance behaviors to elevated pCO_2 in flowing water.

Not only did groups of fish avoid elevated pCO_2 for nearly half the time during the trials where CO_2 was injected into the inner vein, but individual fish exhibited signs of reduced swimming performance. For example, during trials when CO_2 was injected, fish touched the downstream screen only during the period of time when CO_2 was elevated, which is likely due to elevated pCO_2 reducing the ability of fish to swim against the current. Reduced swimming performance in high pCO_2 conditions has been considered an avoidance behavior, as fish have limited ability to move forward or away from nonoptimal conditions (Dennis et al. 2016*b*). Reduced swimming performance in the presence of high pCO_2 has been noted in an experiment on largemouth bass (*Micropterus salmoides*), where equilibrium loss during swimming trials at high pCO_2 were frequently observed (Schneider et al. 2018). Physiologically, reduced swimming performance may occur due to respiratory acidosis, caused by the inability to excrete CO_2 to the environment, and subsequently reduced oxygen uptake (Bohr effect; Cruz-Neto and Steffensen 1997), or may be due to the high pCO_2 environment inducing lower brain pH, which can result in loss of coordination and eventually loss of equilibrium and mortality (Yoshikawa et al. 1991, 1994). The observation that fish lose swimming ability during exposure to high pCO_2 may increase the efficiency of a CO_2 deterrent system, because, should fish be present in an area of high pCO_2, it is unlikely that they would have the capability to move upstream, particularly in high-flow environments, such as a large river.

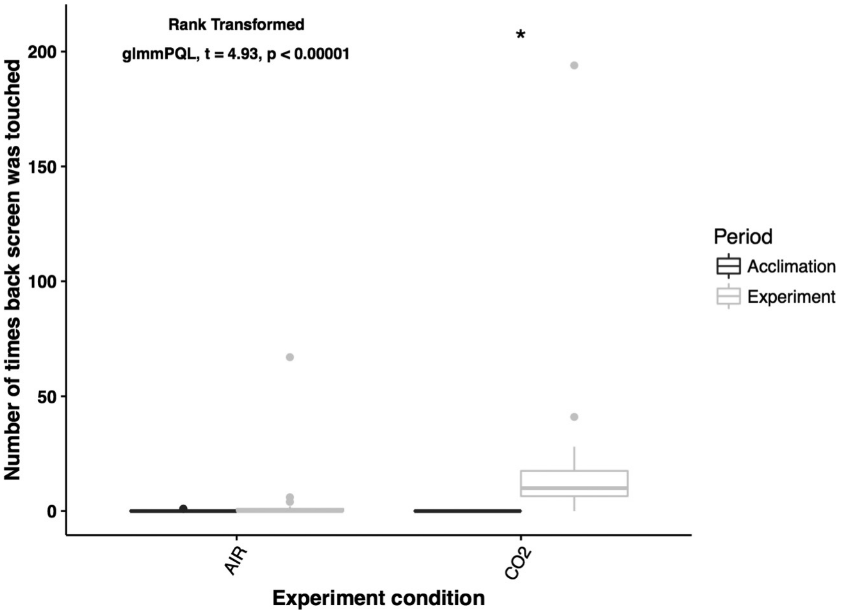
The efficiency of our laboratory-created CO_2 deterrent system to stimulate a behavioral change (in this case, CO_2 avoidance and

Fig. 6. Boxplots of the change in pH during the first four lateral movements (CO_2 vein to no CO_2 vein) made during trials when a CO_2 plug was added to the flume (panel A: CO_2 was added to the inner vein of the flume; panel B: CO_2 was added to the outer vein of the flume). Thick black lines represent medians (50% quantiles), the box represents the interquartile ranges (IQR; the 25th and 75th quantiles), vertical lines are whiskers and represent the higher and lower 1.5 of the IQR, and black dots represent outlying points. No statistical differences were calculated with a linear mixed effects model using a Gaussian error distribution when change in pH was rank-transformed.



impaired swimming) in flowing water was moderate. When CO_2 was injected into the inner vein of the flume, CO_2 was elevated for 69% of the time and fish avoided the inner vein 48% of the time, representing an efficiency of approximately 70%. Therefore, fish did spend time in the elevated pCO_2 plug, and, clearly, bighead carp can tolerate high pCO_2 for brief periods of time, an observation also made by Cupp et al. (2017). Avoidance responses occur due to sensory changes that induce neural responses related to fish movement. Tolerating high pCO_2 levels might be due to the artificial environments the above experiments were carried out in, as fish in this experiment demonstrated an affinity for the inner vein, and therefore the conditions present in the inner vein may have been more controlling of fish location than rising pCO_2 until a threshold was met (in our study, fish moved when pH change was as high as a two-point decrease; thus, the pCO_2 threshold could be high in some individuals). Fish might prefer the inner vein of our flume for a variety reasons, including laboratory lighting conditions, flow dynamics in the flume, or a combination of environmental variables. Another potential reason why 100% avoidance was not observed may be the source and holding conditions of the fish. Fish were obtained from a hatchery, and it is possible that hatchery fish have higher tolerances to elevated pCO_2 due to stocking densities and holding conditions, which can include elevated pCO_2 (e.g., Good et al. 2010). Overall, improvements to CO_2 deterrent systems such as higher pCO_2 targets or integration of CO_2 deterrents with other deterrents or attractant

Fig. 7. Boxplots of the number of times the back screen of the arena was touched by a bighead carp during trials when air and CO_2 were used in the treatment. Black boxplots were calculated from the 15 min acclimation period, and gray boxplots were calculated from the 15 min period when air or CO_2 was flowing through either the outer or inner veins. Thick black or gray lines represent medians (50% quantiles), the box represents the interquartile ranges (IQR; the 25th and 75th quantiles), vertical lines are whiskers and represent the higher and lower 1.5 of the IQR, and black or gray dots represent outlying points. The asterisk indicates statistical difference between acclimation and experimental periods calculated with a penalized generalized linear mixed model using a quasi-Poisson error distribution.



technologies (e.g., electrical barriers, food supplementation) might be necessary to ensure 100% efficiency of reducing the invasion of Asian carp into connected waterways. Furthermore, a higher pCO_2 target would not only increase behavioral avoidance, but also likely would limit physiological performance. Despite fish demonstrating avoidance of elevated pCO_2, fish did tolerate it for periods of time, and this tolerance is important to understand in the context of a barrier to control the spread of AIS because it does offer insight into the effectiveness of a CO_2 deterrent system. Future studies should attempt to understand behavioural avoidance of CO_2 in flowing water in field settings and attempt to investigate how different species might respond.

It is likely that behavioral factors need to be considered when designing an effective nonphysical barrier in flowing water. For example, bighead carp are a shoaling species, and therefore motivation of a single fish to move may depend on how the “leader” of the school perceives conditions (Reebs 2000). In the current study, we used three fish in each trial in attempt to account for the schooling behavior of bighead carp. Generally, fish during the trials stayed within the groups of three, with few exceptions (data not presented). Studies by Cupp et al. (2017) and Donaldson et al. (2016) also noted that bighead carp tended to move as a group. It is possible that the group of fish moved based on the CO_2 tolerance of the “leader” fish. For this reason, future work should aim to identify leader fish or behavioral phenotypes associated with leadership (Nakayama et al. 2012) and determine their tolerance thresholds for CO_2 and other candidate nonphysical barriers (e.g., noise; Vetter et al. 2015).

A high level of CO_2 was used in the study, and the pCO_2 level used in this study represents a high amount of CO_2 in comparison with natural levels found in freshwater lakes and rivers (e.g., Cole et al. 1994). A high level of CO_2 was necessary because the goal of any fish barrier should be to seek 100% avoidance by the species in question (Noatch and Suski 2012). Past studies using bighead carp have shown that fish in static water conditions (i.e., no flow) typically avoid CO_2 concentrations of approximately 150 $mg \cdot L^{-1}$ (pCO_2 not presented in paper; Kates et al. 2012), which is about half the concentration of dissolved CO_2 used in this study. Cupp et al. (2017) used a maximum pCO_2 of approximately 41 000 μatm and similarly found that fish exhibited an avoidance response. Likewise, acoustically tagged bighead and silver carp in a pond avoided areas of the pond where pCO_2 was ~29 000 μatm. In the current study, despite the use of a superfluous level of pCO_2 (i.e., ~240 000 μatm) in comparison with other studies, 100% avoidance of the CO_2 enriched inner vein was not observed, and poor swimming performance as demonstrated by striking the downstream screen was highly variable. Taken together, though past studies and the current study demonstrate that elevated CO_2 plugs induce avoidance in bighead carp, a higher level of pCO_2 than 240 000 μatm might be needed in natural, flowing environments to achieve 100% avoidance.

Overall, our study demonstrated that groups of bighead carp will avoid high levels of CO_2 in flowing water conditions, but high CO_2 was not 100% effective at inducing behavioral avoidance. In past studies, flowing water conditions have mostly been ignored, so our study will help to develop further the idea that CO_2 could be an effective nonphysical barrier for Asian carp, particularly in river environments where the current threat of invasion is at a critical point. Furthermore, our study monitored the movement of a group of fish, rather than single fish, and it might be that to understand fully which fish respond to nonphysical barriers, group leaders will need to be identified and specifically studied for behavioral and physiological tolerance thresholds to elevated CO_2. Lastly, the fact that 100% effectiveness was not observed suggests that other variables may also be important for barrier development.

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