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ORIGINAL PAPER

Temperature dependent effects of carbon dioxide on avoidance behaviors in bigheaded carps

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Abstract Effective behavioral deterrents are needed to prevent aquatic invasive species from entering novel ecosystems. One deterrent strategy that shows promise is elevated carbon dioxide (CO_2) concentrations in water which can alter the behavior of freshwater fishes, including invasive bigheaded carps (*Hypophthalmichthys* spp.). However, few studies have evaluated behavioral responses to elevated CO_2 concentrations at different water temperatures. The objective of this study was to quantify CO_2 concentrations needed to achieve avoidance (voluntary response) and narcosis (involuntary response observed by loss of equilibrium) behaviors in silver carp (*H. molitrix*) and bighead carp (*H. nobilis*) at 5, 15, and 25 °C. Overall, silver carp and bighead carp displayed avoidance and narcosis behaviors to CO_2 at each water temperature, however bighead carp responded at higher CO_2 concentrations than silver carp. Behavioral avoidance and narcosis were observed at approximately 40% lower CO_2 concentrations in 5 °C water relative to 25 °C suggesting considerable influence of water temperature on a CO_2 stimulus for both species. Results indicate that fluctuating water temperature (e.g., spatial and temporal variation across management sites) can influence how fish respond to elevated CO_2, and may usefully be considered when applying CO_2 as a behavioral deterrent.

Keywords Bigheaded carps · Carbon dioxide · Deterrent · Behavior · Temperature · Invasive

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Introduction

Silver carp (*Hypophthalmichthys molitrix* [Valenciennes, 1844]) and bighead carp (*H. nobilis* [Richardson, 1845]), two species of bigheaded carps, are invasive fish that pose a serious threat to aquatic ecosystems within North America. Bigheaded carps outcompete native planktivores and the fright response of silver carp is a nuisance for recreational users (Irons et al. 2007; Kolar et al. 2007; Sampson et al. 2009). Bigheaded carps currently reside in the Mississippi River basin where they have shown to exhibit extremely high densities (Sass et al. 2010) and fast growth rates (Williamson and Garvey 2005), and have reduced the condition factors of native fishes due to their ability to efficiently and effectively filter feed on extremely small organisms (Vörös et al. 1997; Irons et al. 2007). Control efforts are often costly and challenging after bigheaded carps have become established in an ecosystem (Tsehaye et al. 2013), but investing in prevention could be more cost effective and potentially cause less damage to an ecosystem (Finnoff et al. 2007). Fisheries managers need effective tools that prevent the spread of bigheaded carps beyond their current range to avoid further negative consequences to freshwater ecosystems in North America.

The potential spread of bigheaded carps into the Laurentian Great Lakes, with an estimated $7 billion per year fishery, is a major concern of many natural resource conservation groups (American Sportfishing Association 2008). If silver and bighead carp became established in the Great Lakes, they could potentially change food web dynamics, reduce growth rates, and affect recruitment and abundance of other species, negatively impacting the multibillion dollar sport fishery (Irons et al. 2007; Cudmore et al. 2012). Introduction and establishment should be avoided even though the potential effects of bigheaded carps to the Great Lakes are difficult to forecast (Rasmussen et al. 2011). The immediate concern is that bigheaded carps may enter the Great Lakes through the Chicago Area Waterway System (CAWS) from the Mississippi River system where silver and bighead carp are currently prevalent (Kolar et al. 2007). Currently, an array of bottom-mounted electrical barriers are operated to prevent upstream migration of bigheaded carps through the CAWS (Moy et al. 2011). However, effectiveness of non-physical barriers may vary under different physical or biological scenarios (Sparks et al. 2010). For example, electrical fields are altered by flooding events, power outages, water quality fluctuations (e.g., low conductivity and temperature), and electrical parameters (i.e., current type and characteristics, distance from electrode, variation in electrical field in water column, direction of current; Noatch and Suski 2012). Preventing further range expansions of bigheaded carps could benefit from the development of additional or redundant non-physical deterrent strategies to further prevent the upstream migration of bigheaded carps.

Dissolved carbon dioxide (CO_2) has previously been shown to alter the movement of *Hypophthalmichthys* sp. (regardless of size) during laboratory (Kates et al. 2012; Dennis et al. 2015) and pond testing (Cupp et al. 2016; Donaldson et al. 2016) and could be a viable non-physical strategy to block unwanted fish movements. Acute exposure to increased CO_2 concentrations can result in behaviors such as erratic swimming (Kates et al. 2012), increased activity (Hasler et al. 2016), gill ventilations (Heisler 1989), fin beats and surface attempts (Ross et al. 2001). While prolonged exposure to elevated CO_2 concentrations may result in respiratory acidosis which will cause a disruption in cellular ionic gradients (Nilsson et al. 2012; Heuer and Grosell 2014) causing alterations to fish physiology [increased plasma glucose and altered cortisol levels (Ross et al. 2001)] and behaviors [predator avoidance and alarm cue responses (Tix et al. 2016)]. Fish use chemoreceptors within their gills to sense and avoid these environments with elevated CO_2 (Gilmour 2001; Perry and Reid 2002). If fish are resistant or unable to avoid increased CO_2, the sustained exposure to elevated CO_2 can result in hypercarbia and narcosis due to changes in brain biochemistry (Iwama et al. 1989; Yoshikawa et al. 1991; Hiromasa et al. 1994). These previous studies demonstrated that addition of CO_2 has the potential to advantageously alter bigheaded carps' movements and behavior.

Research on other fish dispersal barriers (e.g. electrical barrier and complex sound) has shown temperature-dependent effectiveness (Ross et al. 1996; Parker et al. 2015). Recent studies with invasive round goby (*Neogobius melanostomus* [Pallas, 1814]) found similar temperature related effects with CO_2 as a non-physical deterrent where warmer water temperatures required higher CO_2 concentrations to achieve behavioral endpoints relative to colder water (Cupp et al. 2017c). These findings suggest that CO_2 could be more efficient in colder water than warmer water. Determining the behavioral responses of other target species to elevated CO_2 concentrations at varying water temperatures is critical to determine the utility of this new control method. Specifically, quantifying the effects of temperature on silver carp and bighead carp avoidance behavior in response to a range of CO_2 concentrations in water will better inform management decisions on the effectiveness of CO_2 to control the movements of bigheaded carps.

The objective of this study was to quantify CO_2 concentrations that achieve avoidance (voluntary) and narcosis (involuntary) behaviors from juvenile silver and bighead carp across a range of water temperatures. We hypothesized that water temperature will influence the effectiveness of CO_2 in causing avoidance and narcosis in bigheaded carps and that CO_2 concentrations will be positively correlated with water temperature, based on results from Cupp et al. (2017c). Results are intended to inform field testing and implementation of CO_2 as a deterrent to bigheaded carps.

Methods

Study animals

Juvenile silver carp and bighead carp were cultured in flow-through tanks at the U.S. Geological Survey, Upper Midwest Environmental Sciences Center (UMESC) in La Crosse, WI, USA. Juvenile life stages were tested because electrical barriers were shown to be less effective at deterring small fishes (Reynolds 1996) and juveniles were more appropriate than adults for the size of the experimental tank. Fish were held at 12 °C and maintained on a diet of trout starter feed (Starter Crumble #0, Skretting, Tooele, Utah) until testing started on June 10, 2016. A group of 12 individuals of each species were transferred from the general holding tank (12 °C) to a separate tank where they were acclimated to the test temperature at a rate of $\leq$ 3 °C per day to improve thermal acclimation (Brauhn and Schoettger 1975). Fish were then held at the test temperature for at least 24 h before the behavioral trials. A 24-h acclimation period was chosen as previous research has shown the acid–base balance establishes equilibrium and oxygen consumption rates stabilize after 24 h in a new temperature (Heisler et al. 1980; Davison 1984). Each behavioral test group (described below) was completed within 6 days after the 24-h acclimation period and the entire study concluded on August 11, 2016.

Narcosis trials

Carbon-dioxide concentrations that narcotize silver carp and bighead carp were quantified by evaluating narcosis (observed by loss of equilibrium) as the behavioral endpoint using methods described in Cupp et al. (2017c). Briefly, a single silver carp or bighead carp was placed into a square 100 L stainless tank (597 mm × 610 mm) containing 30 L of source tank water. After a 15-min acclimation period, CO_2 (Airgas Inc., La Crosse, Wisconsin) was continuously delivered at a flow rate of 1 L/min from a compressed CO_2 tank through a single ceramic diffuser at the center of the tank. To assess narcosis, light pressure was applied to one side of the fish to set it from its side to an upright position using the end of a small aquaria net every 30–40 s during CO_2 injection. To assure this was done consistently, the two observers running the trials were the same as Cupp et al. (2017c) and they went through multiple pilot narcosis trials together to avoid subjective biases. Narcosis was deemed to have occurred when fish were unable to maintain an upright dorsal–ventral orientation for greater than 20 s (Cupp et al. 2017a). Dissolved oxygen (DO; HACH model: HQ40d meter, HACH Inc., Loveland, Colorado), pH (HACH model: HQ40d meter, HACH Inc., Loveland, Colorado), and temperature (Thermapen Mk4 thermometer, ThermoWorks, American Fork, Utah) were recorded before fish were placed into the experimental tank (Online Resource Table S1) and immediately after fish succumbed to narcosis. Relationships between pH and CO_2 in mg/L were determined using the standard curve developed in Cupp et al. (2017c). Total ammonia nitrogen was measured before and after trials using a spectrophotometer (HACH® Model: DR3900, HACH Inc., Loveland, Colorado) and were always below 0.13 mg/L which is well below the toxic level of 0.60 mg/L at our highest temperature and pH (Emerson et al. 1975). Alkalinity (titrated using 0.02 N H_2SO_4, Fisher Scientific, Hampton, New Hampshire; APHA 1998), hardness (titrated using 0.01 M Na_2EDTA, Ricca Chemical Company, Arlington, Texas; APHA 1998) and light intensity at the water's surface (Light meter MW700, Milwaukee, Rocky Mount, North Carolina) were measured once for each temperature tested (Online Resource Table S1). Ten fish were tested at each temperature (5, 15, and 25 °C) for a total of 30 individuals per species. At the conclusion of each trial, fish were euthanized in a solution of 200 mg/L MS-222 (Tricaine methanesulfonate, Western Chemical Inc, Ferndale, Washington) and total length as well as weight were recorded for each individual. Silver and bighead carp had an average length ± standard deviation of 87.2 ± 10.8 and 95.3 ± 11.4 mm, respectively. Fish size did not vary within species across temperatures for both species (one-way analysis of variance F values < 4.0, p values > 0.05).

Avoidance trials

Carbon-dioxide avoidance was evaluated at different temperatures for silver carp and bighead carp using a shuttle-box choice tank (Loligo Systems Inc., Viborg, Denmark; see figure in Kates et al. 2012) following methods described in Cupp et al. (2017c). Unlike in Cupp et al. (2017c), we did not add rock substrate to one side of the choice tank due to bigheaded carps being more pelagic swimmers than round goby. Two circular tanks (1.5 m diameter × 0.5 m deep) were connected by a narrow, rectangular passage (0.2 m wide × 0.5 m deep). Water was drawn from each circular tank using small electric pumps and delivered into a respective buffer column. Independent buffer columns were used for each circular tank to increase CO_2 levels in one tank without modifying levels in the adjacent tank (high/low gradient). Water then passed through the buffer column and was returned to the original tank by gravitational flow (3.5–3.6 L/min). Fish movement was observed remotely using a single overhead camera (Loligo® uEye USB video camera, Viborg, Denmark) and tanks were surrounded by a 2 m high wall to prevent human influence on fish behavior (Kates et al. 2012).

At the beginning of each trial, the shuttle-box was filled with 270 L of water at the pre-determined testing temperature (5, 15, or 25 °C). Temperature and DO were recorded before each trial (Online Resource Table S2). Carbon dioxide, alkalinity, light intensity and water hardness were taken at the first and last trial for each combination of species and temperature tested (Online Resource Table S2). Total ammonia nitrogen was measured at the first and last trial in the shuttle-box and never exceeded 0.13 mg/L.

A single fish was netted from the source tank that matched the experimental temperature and was randomly placed into one of the circular tanks within the shuttle-box (determined by coin flip for each fish). Fish were then allowed 60 min to acclimate in the shuttle-box (Cupp et al. 2017c). After acclimation, CO_2 was continuously bubbled into the buffer column supplying water to the tank occupied by the fish (deemed the treated tank). Carbon dioxide was delivered from a compressed cylinder at a flow rate of 1 L/min through a single ceramic diffuser located at the bottom of the buffer column. The buffer column for the non-treated adjacent tank (not occupied by the fish) received a continuous supply of room air delivered from an aquarium compressor (Sweetwater, SL94A, Pentair, Apopka, Florida) in the same manner as CO_2 delivery. Carbon dioxide was continuously injected for 30 min during which pH and time were recorded whenever the fish shuttled out of the treated tank into the non-treated tank. Fish were determined to have "shuttled" when their whole body passed from one tank to the other via the connecting channel. Carbon dioxide was injected into the same tank and did not change with fish location during the 30 min trial. Carbon dioxide levels were monitored every 10 s using pH meters at the edge of each circular shuttle tank between the outflow and inflow of the buffer columns and at mid-depth. Carbon dioxide was calculated using the relationship curve between pH and CO_2, as used in Cupp et al. (2017c).

At the conclusion of each trial, fish were euthanized in a 200 mg/L solution of MS-222 and total length and weight. Silver and bighead carp had an average total length of 88.1 ± 8.7 and 89.1 ± 6.9 mm, respectively. Fish size did not vary within species across temperatures for both species (one-way analysis of variance F values < 4.0, p values > 0.05). Temperature and DO were also recorded for each tank at the conclusion of a trial (Online Resource Table S3). Ten fish were tested (n = 10) at each temperature (5, 15, and 25 °C) for a total of 30 observations per species.

Data acquisition and analysis

Carbon-dioxide concentrations that caused avoidance and narcosis were compared separately using a two-way analysis of variance in R (Zar 2010; R Core Team 2016). Each behavioral endpoint (concentration at the last shuttle to avoid CO_2 and concentration at narcosis) was analyzed using temperature (5, 15, 25 °C), species (silver carp and bighead carp) and their interaction as fixed effects (Sokal and Rohlf 1995). One silver carp from 15 °C and three silver carp from 25 °C did not shuttle and were excluded from all analyses. Assumption of normality was assessed through visual inspection of fitted residuals using a normal probability plot (Anscombe and Tukey 1963). Assumption of homogeneity of variances was assessed across groups using a visual inspection of fitted residuals plots (Zar 2010). Pairwise comparisons of significant effects were evaluated using Tukey's honest significant difference test (Zar 2010). Relationships between temperature and narcosis or avoidance were plotted using the ggplot package in R (Wickham 2009). Separately from the ANOVA analysis, behavioral endpoints were calculated as 95th percentiles at each temperature (Helsel 2012) using the percentile function in Microsoft Office Excel 2013 (Version 15, Microsoft, Redmond, Washington). Partial pressure of carbon dioxide (pCO_2 in micro atmospheres [µatm]) for each treatment was also quantified by entering temperature, pH and alkalinity into CO2Calc (Robbins et al. 2010; http://pubs.usgs.gov/of/2010/1280/) while all other parameters were constants (Salintity = 0; K1, K2 from Millero 1979, KHSO4 = Dickson, pH scale = NBS scale, Air-sea Flux Wanninkhof 1992). Statistical significance for comparisons was declared at $\alpha < 0.05$.

Results

Across all temperatures, silver carp displayed avoidance and narcosis at a 95th percentile of 110–141 mg/L (45,283–76,582 µatm) and 115–206 mg/L (59,333–127,952 µatm), respectively (Table 1). Bighead carp displayed avoidance and narcosis at a 95 percentile of 117–162 mg/L (46,892–86,328 µatm) and 188–278 mg/L (97,497–201,858 µatm), respectively across all temperatures (Table 1).

Temperature had a significant effect on CO_2 concentrations causing narcosis in silver carp and bighead carp (Table 2; Fig. 1). Specifically, a 85% higher CO_2 concentration was required to cause narcosis in silver carp acclimated to 15 and 25 °C water compared to silver carp acclimated to 5 °C water (Fig. 1). Carbon-dioxide concentrations at narcosis in bighead carp acclimated to 15 °C increased 45% compared to bighead carp acclimated to 5 and 25 °C (Fig. 1). Carbon-dioxide concentrations at narcosis were significantly greater for bighead carp at 15 °C than all other groups (Fig. 1). Similar results were found for silver carp in 5 °C water, as narcosis occurred at significantly lower concentrations than all other groups (Fig. 1). Bighead carp tested in 15 °C required approximately 2.4 times more CO_2 at narcosis compared to silver carp in 5 °C (Fig. 1). However, during 5 °C bighead carp trials, CO_2 concentrations at narcosis did not differ between 25 °C bighead carp (Tukey's HSD, p value = 0.83) or 25 °C silver carp (Tukey's HSD, p value = 0.16; Fig. 2). The same was true for silver carp in 15 °C where concentrations did not differ between 25 °C silver carp (Tukey's HSD, p value = 0.99) as well as 25 °C bighead carp (Tukey's HSD, p value = 0.95) and 5 °C bighead carp (Tukey's HSD, p value = 0.32; Fig. 2). No difference in narcosis were found between both fish species tested at 25 °C (Tukey's HSD, p value = 0.82; Fig. 1).

Temperature had a significant effect on the concentration of CO_2 required to induce avoidance (Table 3). Specifically, the concentration required at 25 °C was about 68% higher than at 5 °C (Tukey's HSD, p value = 0.00004; Fig. 2) and 30% higher than at 15 °C (Tukey's HSD, p value = 0.02; Fig. 2). However, there was no difference in CO_2 concentrations between 15 and 5 °C (Tukey's HSD, p value = 0.10; Fig. 2). Silver carp displayed

Table 1 Carbon dioxide concentrations (mg/L) and pressure (µatm) estimated as 95th percentiles resulting in free-swimming avoidance and narcosis of silver and bighead carp at three temperatures (5, 15, and 25 °C)

Species	Temperature (°C)	Avoidance	Narcosis
Silver carp	5	110 mg/L (45,283 µatm)	115 mg/L (59,333 µatm)
	15	137 mg/L (61,489 µatm)	197 mg/L (107,920 µatm)
	25	141 mg/L (76,582 µatm)	206 mg/L (127,952 µatm)
Bighead carp	5	117 mg/L (46,892 µatm)	188 mg/L (97,497 µatm)
	15	138 mg/L (64,517 µatm)	278 mg/L (201,858 µatm)
	25	162 mg/L (86,328 µatm)	207/L (130,503 µatm)

Confidence intervals were not calculated due to low sample size (n = 10) for each treatment combination

Table 2 Results of a two-way analysis of variance (ANOVA; sum of squares [*SS*], degrees freedom [df], F-value [*F*] and *p* value [*p*]) examining the concentrations of CO_2 (mg/L) needed to induce narcosis in each species (silver and bighead carp) across three temperatures (5, 15, 25 °C)

Measured variable	Main effects	*SS*	df	*F*	*p*
CO_2 concentration (mg/L)	**Temperature**	**83,350**	**2**	**48.17**	**< 0.001**
	Species	**2068**	**1**	**23.82**	**< 0.001**
	Temperature X species	**21,596**	**2**	**12.48**	**< 0.001**
	Residuals	46,720	54		

Bold text indicates statistical significance (α = 0.05) for a main effect within the measured variable

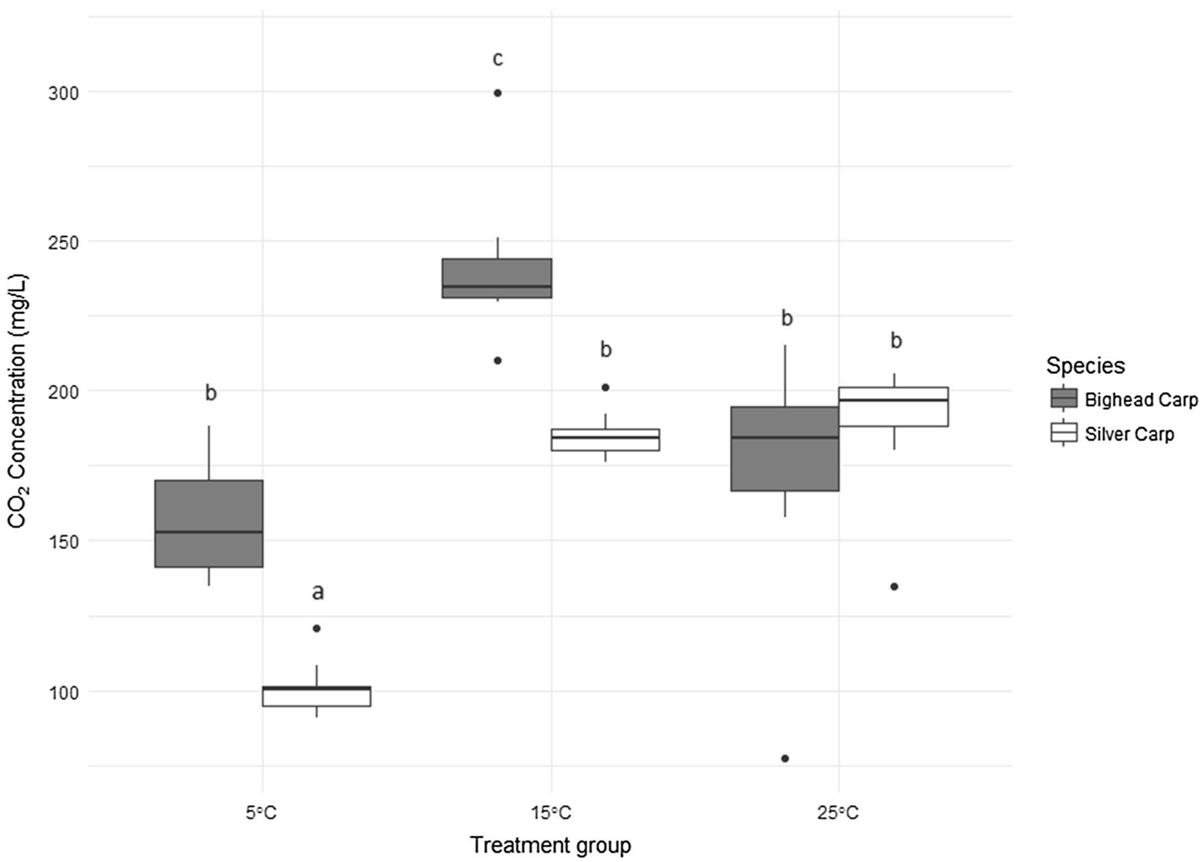

Fig. 1 Carbon dioxide concentrations (mg/L) that induced narcosis in bighead (grey plots) and silver carp (white plots) at each temperature treatment group (5, 15, and 25 °C). The boxes represent the first and third quartiles with the interquartile range and the median (black line inside the box) between them. The whiskers represent the minimum and maximum values excluding outliers (black circles). Samples size is ten fish for each of the six groups. Letters above box plots denote a $p < 0.05$ significance from the Tukey test performed

avoidance to CO_2 approximately at 85.5 mg/L while bighead carp displayed avoidance to a 28% higher CO_2 concentration compared to silver carp (109.6 mg/L; Tukey's HSD, *p* value = 0.02; Fig. 2). However, there was no significant interaction between species and temperature for the concentration of CO_2 needed to induce avoidance (Table 3).

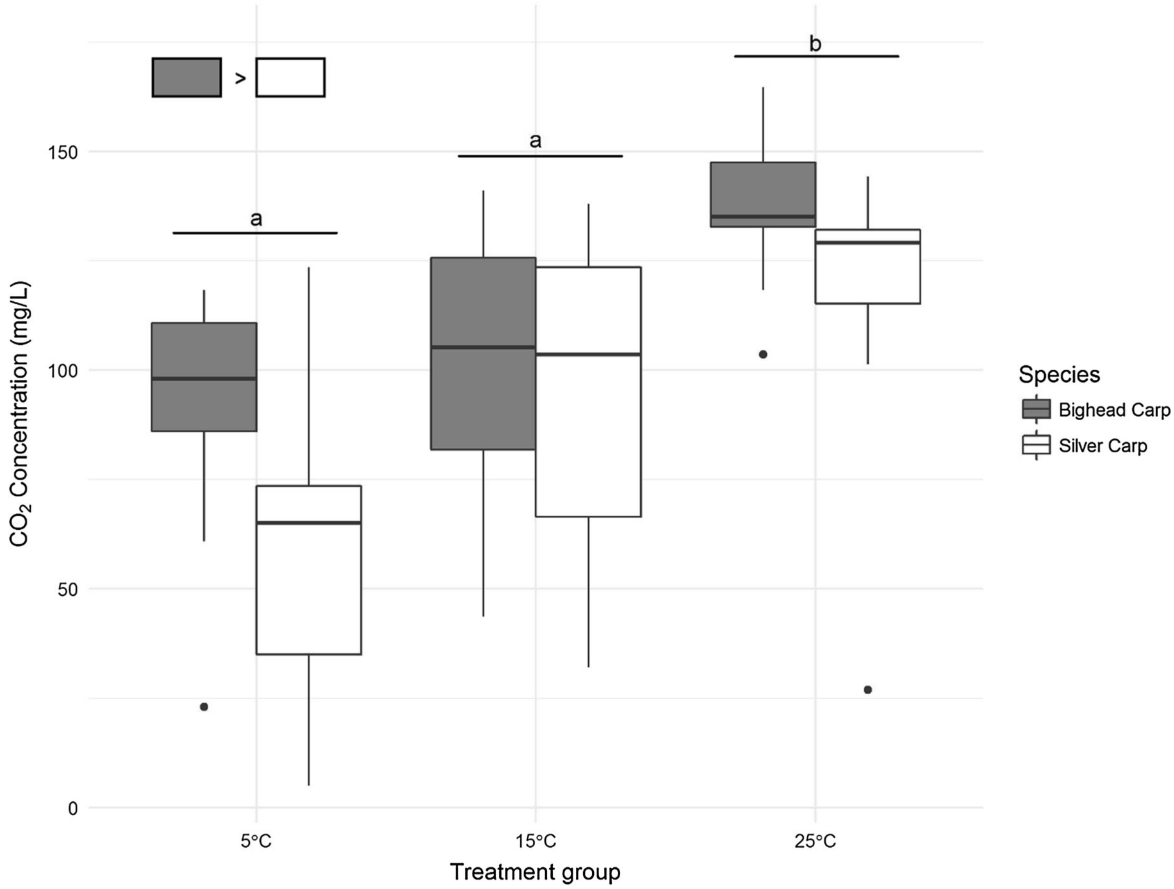

Fig. 2 Carbon dioxide concentrations (mg/L) that caused avoidance in both bighead (grey plots) and silver carp (white plots) at each temperature treatment group (5, 15, and 25 °C). The boxes represent the first and third quartiles with the interquartile range and the median (black line inside the box) between them. The whiskers represent the minimum and maximum values excluding outliers (black circles). Samples size is ten fish for each of the six groups. Horizontal line with letters above bars indicate a $p < 0.05$ significance across temperatures. The grey and white boxes with a greater than symbol represent a $p < 0.05$ significant effect of species between bighead and silver carp

Table 3 Results of a two-way analysis of variance (ANOVA; sum of squares [*SS*], degrees freedom [df], F-value [*F*] and *p* value [*p*]) examining the concentrations of CO_2 (mg/L) needed to induce avoidance in each species (silver and bighead carp) across three temperatures (5, 15, 25 °C)

Measured variable	Main effects	*SS*	df	*F*	*p*
CO_2 concentration (mg/L)	**Temperature**	**25,675**	**2**	**11.703**	**0.00007**
	Species	**6170**	**1**	**5.625**	**0.0216**
	Temperature X species	1518	2	0.692	0.5053
	Residuals	54,844	50		

Bold text indicates statistical significance (α = 0.05) for a main effect within the measured variable

Discussion

Silver carp and bighead carp responded to CO_2 with avoidance behaviors at about 41% lower concentrations in cold water ($\approx$ 74.9 mg/L) relative to warmer water ($\approx$ 127.7 mg/L). The similar temperature effect was found in a recent study by Cupp et al. (2017c) where CO_2 concentrations in 5 °C water were approximately 50% less than those needed to achieve the same behavioral endpoints in 25 °C water for invasive round goby. Together, these studies demonstrate how temperature impacts avoidance behaviors in invasive fishes when exposed to elevated CO_2 concentrations. Cooler water temperatures have been shown to decrease cellular respiration and production of CO_2 in poikilothermic animals, such as fishes (Perry and Gilmour 2006). Cupp et al. (2017c) hypothesized that the increased gradient of CO_2 between water and blood increased diffusion rates of CO_2 into fish, based on Fick's Laws of Diffusion, resulting in narcotizing levels of CO_2 in blood over shorter durations. Along with temperature impacting CO_2 solubility, fish movement may have been impacted by the varying temperatures tested. It has been well established that water temperature has a direct relationship with metabolic rates and activity in fishes (Wetzel 2001). The bigheaded carps tested at 25 °C had 192 shuttles away from the CO_2 side while the fish at 5 °C had 106 shuttles away. Fish in 25 °C seemed to be more active than in 5 °C causing them to challenge the injected side of the shuttle-box more, potentially causing our CO_2 concentration to be inflated. The similarity between our results and Cupp et al. (2017c) suggests that managers may need to use higher CO_2 concentrations in warmer water to modify fish movements.

Kates et al. (2012) demonstrated 100% of silver carp showed signs of narcosis at 70 mg/L, while only 60% of bighead carp showed signs of narcosis in the same water temperature. In this study, we observed a similar effect as higher CO_2 concentrations were required for bighead carp relative to silver carp for avoidance across all three temperatures (Fig. 2) and to cause narcosis at 5 and 15 °C water (Fig. 1). Although we did not record gill ventilation rates, the higher CO_2 concentration needed to induce narcosis and avoidance in bighead carp may be related to increased ventilation rates within the first few minutes of CO_2 exposure to expel excess CO_2 within their blood as suggested by previous studies (Gilmour et al. 2005; Kates et al. 2012). Previous studies have shown hypercarbia results if fish cannot expel CO_2 faster than it is accumulated, causing narcosis (Gilmour et al. 2005; Gilmour and Perry 2007). Bighead carp may be able to expel the CO_2 within their blood by ventilating more frequently than silver carp, allowing them to tolerate longer CO_2 exposure durations before causing agitation and narcosis. Thus managers could use this information to establish CO_2 concentrations that are effective at inducing avoidance in bighead carp while still being effective at deterring other species such as silver carp.

Both silver and bighead carp displayed narcosis following CO_2 exposure regardless of varying water temperatures. Previous studies have shown the level of sedation and recovery from narcosis using CO_2 is dependent on exposure duration and concentration (Yoshikawa et al. 1991; Cupp et al. 2017b). For example, bigheaded carps in the current study exposed to CO_2 concentrations of about 150 mg/L for 15 min experienced narcosis in contrast to Kates et al. (2012), where exposure for a longer duration using a 50% lower CO_2 concentration caused bigheaded carps to narcotize. The mechanism causing the narcosis is believed to be caused by altered blood pH and impairment of brain electrical activity from CO_2 diffusing across the blood–brain barrier suggesting why bigheaded carps narcotized at each temperature tested (Yoshikawa et al. 1991; Hiromasa et al. 1994). Another recent study by Hasler et al. (2017) found that narcosis is repeatable within the same individuals and can be predicted based on a fish's capacity for anaerobic activity. These findings by Hasler et al. (2017) suggest tolerance to high CO_2 may be heritable and influenced by natural selection, possibly suggesting why bighead carp were more tolerant of CO_2 than silver carp. Our results, together with previous studies, further demonstrate that high CO_2 concentrations, regardless of water temperature, can be used to induce narcosis to decrease movements of invasive fishes, such as bigheaded carps, from invading areas like the Great Lakes.

Interestingly, four silver carp (one from 15 °C and three from 25 °C) failed to shuttle away from the CO_2 injected side of the shuttle-box. Although it is unclear if those fish responded in an anomalous fashion, we did note behavioral agitation (coughing, darting, surface ventilations) in all four individuals. Previous research using an identical set up observed narcosis at

70 mg/L after 30 min in multiple species including bigheaded carps (Kates et al. 2012). Due to these four silver carp being exposed to increasing CO_2 concentrations over 70 mg/L for longer than 15 min and potentially being more sensitive than bighead carp as was observed in Kates et al. (2012) and this study (Fig. 1), these fish may have not shuttled due to the anesthetic properties of CO_2 (Yoshikawa et al. 1988) and the physiological changes occurring early in the trial causing them to not be able to find the channel to the ambient fresh water. We recommend that future research examines a full suite of behaviors as well as variation in individual behavior response to elevated CO_2 to better explain the failure to shuttle away from a CO_2 stimulus by these four silver carp.

As a non-selective control tool, CO_2 is expected to also affect non-target species. Due to the relatively high CO_2 concentrations needed to induce avoidance in carp, other species in the same environment may be more vulnerable to behavioral changes and to the anesthetic effects of CO_2 which may result in mortality if there is no refugia (Yoshikawa et al. 1988). For example, physiological disruptions and mortality of native mussels has been observed during periods of prolonged CO_2 exposure (Hannan et al. 2016; Waller et al. 2016). Although, high pCO_2 influences the behaviors of several native fish species such as largemouth bass (*Micropterus salmoides* [Lacépède, 1802]) and fathead minnow (*Pimephales promelas* [Rafinesque, 1820]; Hasler et al. 2016; Tix et al. 2016) the available data describing the effects of elevated CO_2 concentrations on freshwater organisms is limited including impacts on threatened and endangered species.

The results of this study demonstrate that voluntary and involuntary movements of silver carp and bighead carp can be altered in various temperatures using CO_2. However, additional research is needed in a natural setting where bigheaded carp movements in relation to CO_2 can be observed under different natural conditions (e.g., seasonal fluctuations, weather variations, solar radiation, turbidity, water depth). For example, this study suggests that the volume of CO_2 required as a deterrent will almost certainly be higher in summer than winter deployments. This study also demonstrates the silver carp species is likely more sensitive than bighead carp to elevated CO_2 concentrations, indicating different species have different tolerances to increases in CO_2. Additional studies are needed on aquatic organisms, particularly threatened and endangered species in the Great Lakes region, to determine the range of responses to CO_2 by species that inhabit suitable locations for a non-physical CO_2 barriers. Overall, this study supports that CO_2 is a valuable resource for integrated aquatic pest management for species of concern such as bigheaded carps.

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