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Research Article

Field evaluation of carbon dioxide as a fish deterrent at a water management structure along the Illinois River

Aaron R. Cupp[1,*], Justin R. Smerud[1], John A. Tix[1], Susan M. Schleis[1], Kim T. Fredricks[1], Richard A. Erickson[1], Jon J. Amberg[1], William S. Morrow[2], Carolyn Koebel[2], Elizabeth A. Murphy[2,5], Chad Vishy[3] and K. Douglas Blodgett[4]

[1]*U.S. Geological Survey, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Rd. La Crosse, WI 54603, USA*
[2]*U.S. Geological Survey, Illinois-Iowa Water Science Center, 405 N. Goodwin Avenue Urbana, IL 61801, USA*
[3]*U.S. Geological Survey, Columbia Environmental Research Center, 6200 New Haven Road, Columbia, MO 65201, USA*
[4]*The Nature Conservancy, Emiquon Preserve, 11304 N. Prairie Road, Lewistown, IL 61542, USA*
[5]*Current address: Center for Advanced Bioenergy and Bioproducts Innovation, University of Illinois, 1206 W. Gregory Dr., Urbana, IL 61801, USA*

Author e-mails: acupp@usgs.gov (ARC), jrsmerud@usgs.gov (JRS), jtix@usgs.gov (JAT), sschleis@usgs.gov (SMS), kfredricks@usgs.gov (KTF), rerickson@usgs.gov (RAE), jamberg@usgs.gov (JJA), wsmorrow@usgs.gov (WSM), ckoebel@usgs.gov (CK), emurphy@usgs.gov (EAM), cvishy@usgs.gov (CV), dblodgett@tnc.org (KDB)

**Corresponding author*

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Abstract

Construction of a water management structure (WMS) in the levee surrounding The Nature Conservancy's Emiquon Preserve (Havana, Illinois, USA) created a new hydrological connection and potential aquatic invasive species pathway between the Illinois River and a large conservation wetland complex. Site managers need a control tool that deters the upstream passage of non-native fishes into the wetland lakes, but does not interfere with normal gate operation and water discharge. This short field study evaluated carbon dioxide (CO_2) injected into water as a non-obstructive method to reduce fish abundance near the WMS culverts. We quantified relative fish abundance using underwater sonar with and without injection of CO_2 into culverts during three discharge events: no flow (0 m^3/s), restricted flow (0.9 m^3/s), and unrestricted flow (3.2 m^3/s). Overall, CO_2 reached or exceeded our target concentration of 100 mg/L during no flow and restricted flow, and fish abundance was 70–95% lower at culvert entrances relative to untreated control days. The target CO_2 level was not reached during unrestricted flow and fish abundance was not reduced during CO_2 injection. Atmospheric CO_2 concentrations were inconsequential and unaffected by CO_2 treatments throughout testing. Results from this initial field study provide several considerations for CO_2 as a fish deterrent in natural environments.

Key words: Asian carps, bigheaded carps, behavior, barrier

Introduction

Invasive bigheaded carps (*Hypophthalmichthys* spp. Bleeker, 1860) are established throughout several large river basins in North America (Chapman and Hoff 2011) and have caused considerable economic and ecological damage (Pimentel et al. 2005). Bigheaded carps have altered zooplankton assemblages, reduced native fish condition and contributed to a loss of recreational sport fisheries (Pimentel et al. 2005; Irons et al. 2007; Sass et al. 2014). Once established, management efforts to eradicate bigheaded carps have been largely unsuccessful. Thus, considerable effort has been focused on preventing their introduction into new areas (Kolar and Lodge 2002; Lodge et al. 2006; Vander Zanden and Olden 2008; Vetter et al. 2017).

Infusion of carbon dioxide (CO_2) into water has shown promise as a chemical fish deterrent that could be useful to as a non-physical means to block the movements and passage of bigheaded carps and other invasive fishes. Results from laboratory experiments showed that fish consistently avoided CO_2-enriched water when given access to ambient freshwater areas (Kates et al. 2012; Dennis et al. 2016; Cupp et al. 2017c). Subsequent studies at larger spatial scales in outdoor ponds documented similar avoidance behaviors with telemetered bigheaded carps exposed to CO_2 enriched water under flowing and static conditions (Donaldson et al. 2016; Cupp et al. 2017a). Fish exposed to elevated CO_2 undergo impairments ranging from acid-base disturbance, oxygen debt, altered response to alarm cues, narcosis and mortality depending on exposure duration and CO_2 concentration (Perry and Abdallah 2012; Cupp et al. 2017d). Modified swimming behavior exhibited by fish to avoid these physiological disturbances supports that CO_2 could be applied for management purposes to non-selectively deter or block fish movement. However, because CO_2 has yet to be studied outside of small controlled experiments, little is known about the feasibility of this deterrent method under field conditions.

Natural environments have unpredictable physical, chemical, and biological conditions that could influence CO_2 efficacy as a fish deterrent. For example, the ability to reach and sustain elevated CO_2 concentrations in water could be influenced by hydrodynamics and water temperature (Wiebe and Gaddy 1940). Furthermore, biologically available CO_2 in water is primarily a function of water chemistry related to pH, alkalinity, barometric pressure, and temperature which often varies spatially and temporally across management sites (Robbins et al. 2010). Other biological processes (i.e., photosynthesis) that uptake CO_2 within the photic layer could also stratify treatments and decrease treatment uniformity (Hollander and McKenzie 1991). Although CO_2 has been effective to alter the behavior and deter the movements of invasive fishes in controlled experiments, the influence of environmental variables is largely unknown. Hence, field studies are needed to determine if CO_2 injection into water is a feasible method to deter fish under expected management conditions.

The goal of this study was to evaluate CO_2 as a fish deterrent during field applications. A short period of water releases from a water management structure (WMS) provided an opportunity to conduct field trials during normal water discharge conditions. The recently constructed WMS has created a new hydrological connection and potential aquatic invasive species pathway between the Illinois River and a large conservation wetland complex. Site managers are concerned that invasive fishes, particularly bigheaded carps, could migrate through the WMS into the wetland lakes. Carbon dioxide was identified as a possible invasive fish deterrent strategy because the altering of water quality within the WMS would not physically interfere with gate operation and water discharge. We hypothesized that CO_2 injected into water within WMS culverts would reduce nearby fish abundance and potentially decrease the risk of upstream fish passage into the wetland lakes. To test our hypothesis, we quantified relative fish abundance using underwater sonar with and without CO_2 across three WMS water release events. Water quality and air quality were also monitored throughout testing to quantify the aquatic and terrestrial effects of CO_2 as a chemical fish deterrent. Based on a review of published literature, we expected to see a decrease in fish abundance and occupancy around the WMS culvert entrances during treatments at approximately 100 mg/L CO_2. More specifically, upstream movements of invasive bigheaded carps were reduced by approximately 50% in outdoor ponds at 70–100 mg/L CO_2 and this threshold would be a reasonable target for field testing (Kates et al. 2012; Donaldson et al. 2016; Cupp et al. 2017a). Results from this study provide several considerations for CO_2 as a fish deterrent in natural environments.

Materials and methods

Study site

The Nature Conservancy's Emiquon Preserve is a 2,723 ha floodplain restoration area in the La Grange pool of the Illinois River near Havana, Illinois (Lemke et al. 2017). Located within the Preserve is a 1,800-ha wetland complex that includes two lakes (locally known as Flag and Thompson) that are physically separated from the Illinois River by a surrounding levee. Lake levels can be modified with WMS gates in the southeast portion of the lake (40º20.066′N; 90º3.165′W). During this study, water was passively drained into the Illinois River through two parallel (2.4 m wide by 2.7 m tall) concrete culverts and reverse flow of water into the lake was prevented by complete closure of sluice gates (Figure 1). The upstream (inlet) side of the WMS is considered the wetland lake and downstream (outlet) side of WMS is the Illinois River. All trials were conducted during routine WMS draining events from 21 October 2016–28 October 2016 under The Nature Conservancy research permit TNC-2016-19. Timing and duration of this study was determined by river stage and few water releases in 2016.

CO_2 trials

Trials were conducted during three WMS operating conditions: (1) no flow, (2) restricted flow, and (3) unrestricted flow. Each discharge condition was tested

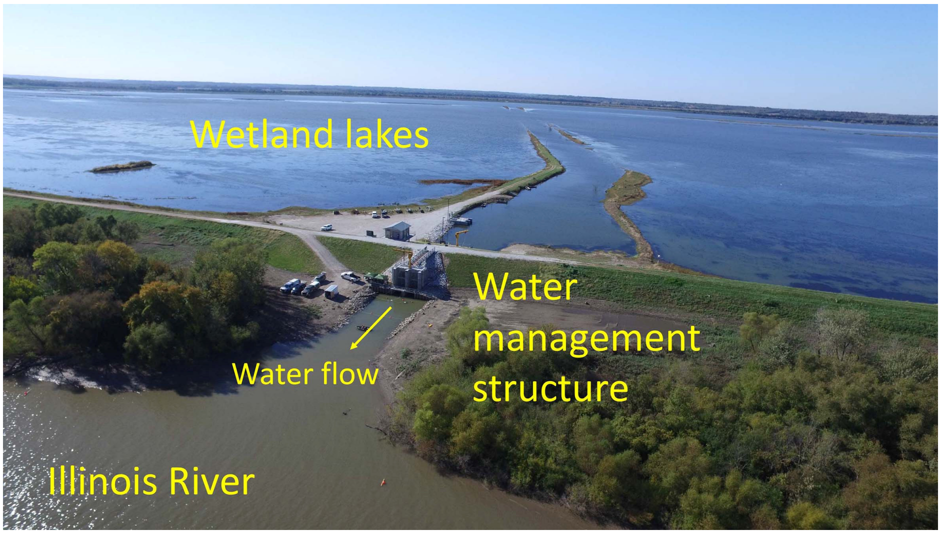

Figure 1. Aerial view of study area at the water management structure (40º20.066′N; 90º03.165′W) at the Emiquon Preserve near Havana, IL (photo credit: Doug Blodgett, The Nature Conservancy).

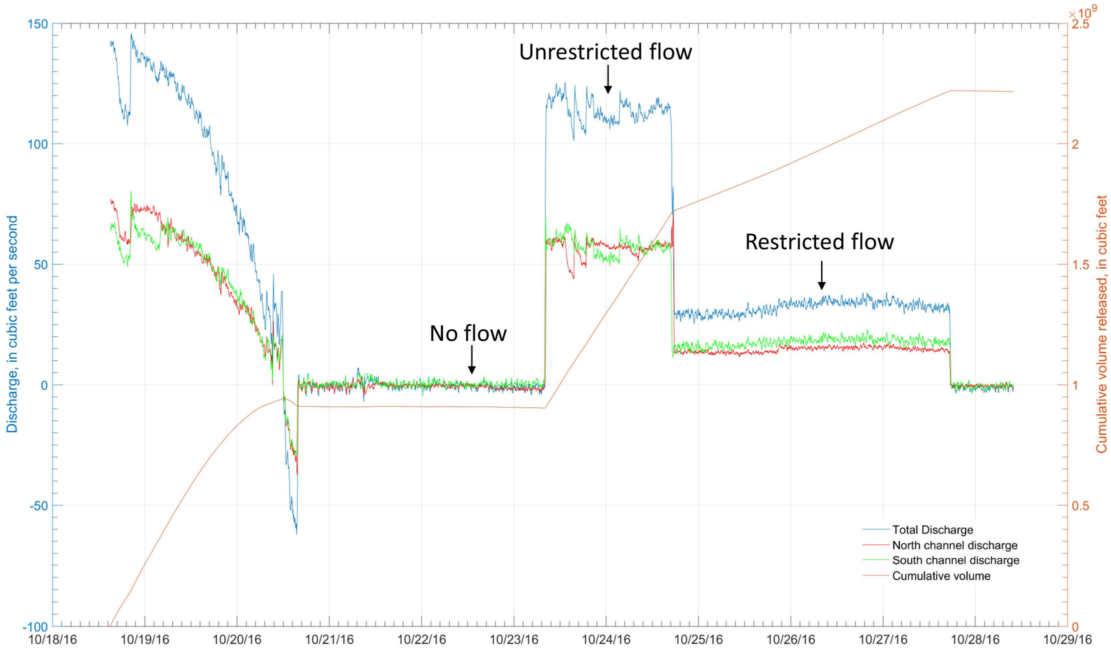

Figure 2. The Nature Conservancy's Emiquon Preserve water management structure culvert discharge during carbon dioxide testing from 21 October 2016–28 October 2016.

with and without injection of CO_2 on separate days for a total of six independent testing days. Trials with no flow were conducted with sluice gates completely closed and no water passed through WMS culverts. Restricted flow trials were conducted with sluice gates completely open and three, 45-cm tall stop-logs installed on the upstream end of each WMS culvert to reduce total discharge. Unrestricted flow trials were conducted using no stop-logs with sluice gates completely open. Mean water flow velocity through both culverts was 0.11–0.13 m/s during restricted flow trials and 0.38–0.40 m/s during unrestricted flow trials (U.S. Geological Survey 2017). Gate closure during no flow trials resulted in a nominal flow velocity of 0 m/s. Mean total discharge through both culverts during restricted and unrestricted flow trials was calculated as 0.9 m^3/s and 3.2 m^3/s, respectively (Figure 2). Testing under differing discharge events was intended to represent a range of expected conditions during normal WMS

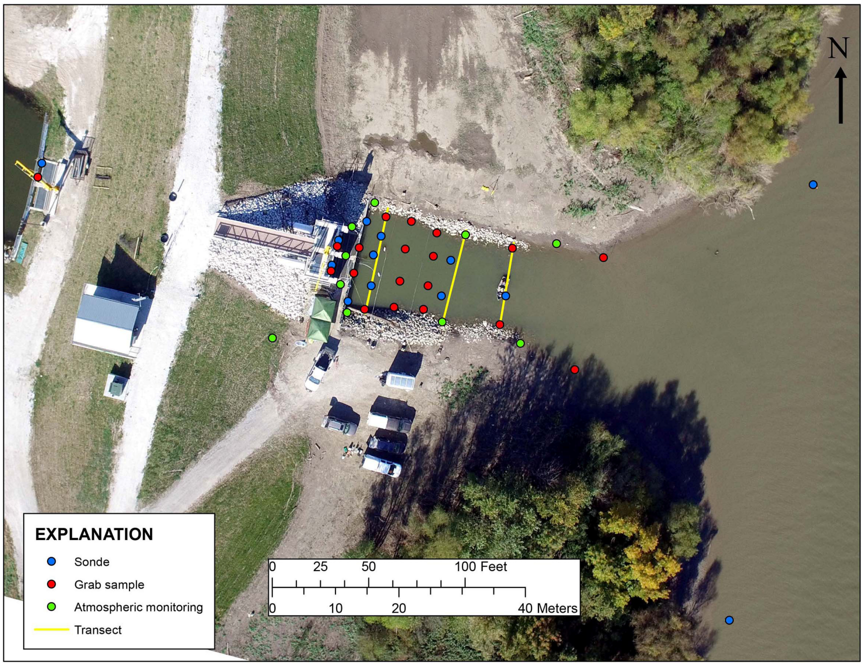

Figure 3. Aerial view showing water quality and air quality sampling locations during testing. Blue dots show locations of autonomous water quality sondes. Red dots show grab sample locations that were analyzed for pH, dissolved oxygen, alkalinity, CO_2 and temperature. Green dots show air quality monitoring locations. Yellow lines show transect locations. Data were collected from 0800 CDT to 1600 CDT over six test days from 21 October 2016–28 October 2016.

operation. Trials were conducted during daylight hours from 0800 CDT to 1600 CDT. No CO_2 was injected during control days at each flow condition for comparison purposes.

Common gas-transfer equipment was used to inject CO_2 into water. Briefly, 10 porous air diffusers, consisting of five micro diffusers (Sweetwater, Model DYFP16, Pentair, Apopka, FL), two plastic micro diffusers (Point Four, Model 1PMBD075, Pentair, Apopka, FL), and three fine pore diffusers (Sweetwater, Model ALR300CP, Pentair, Apopka, FL), were attached to an aluminum bar and placed directly into slots within culvert walls approximately 3 m inside each culvert outlet. Twenty additional diffusers (Point Four, Model 1PMBD075, Pentair, Apopka, FL) were attached to small concrete bricks and dispersed throughout the plunge pool adjacent to WMS culvert outlets. Diffusers were indiscriminately placed approximately 1–3 m from culvert outlets. Size and type of CO_2 diffusers were primarily a function of available components at the time of testing. Gas was supplied directly from 14–15 compressed 23-kg CO_2 cylinders (CD50, Airgas Inc., Peoria, IL) through individual regulators (Smith® Model: 32-80-320; Airgas Inc., La Crosse, WI) and split through multiple flow manifolds (Western Medical, Model 1FMM222, Pentair, Apopka, FL) leading to terminal air diffusers. Injection components were adapted from oxygen delivery equipment used in hauling tanks for live fish transport (Cupp et al. 2017b). Gas flow rates were set to approximately 7–10 L/min per diffuser until tanks emptied and all trials were conducted using similar injection parameters. A target CO_2 concentration for each flow regime was set at 100 mg/L as previous research has shown freshwater fish including invasive Bigheaded Carps strongly avoid CO_2 at this level in laboratory and pond experiments (Kates et al. 2012; Donaldson et al. 2016, Dennis et al. 2016).

Water quality and other hydrological parameters were monitored throughout testing. Acoustic Doppler Velocity Meters (Sontek Argonaut SW, San Diego, CA) located within each culvert measured water flow velocities to determine total discharge. Thirteen water quality sondes (YSI 600XLM, Yellow Springs, OH) recorded temperature, dissolved oxygen, and pH at fixed locations every 5 min throughout the WMS culverts, downstream plunge pool, upstream and downstream of Illinois River confluence and within the wetland lake (Figure 3). Shore-to-shore transects were intermittently used to measure water quality across the plunge pool immediately downstream of WMS during injection. Near-surface grab samples were collected by hand at several locations from shore. Clear vinyl tubing (Sweetwater® Model TVR80; Pentair Aquatic Ecosystems Inc., Apopka, FL) and peristaltic pumps (Global Water Instruments, Model SP100, Vernon Hills, IL) collected mid-depth grab samples in locations not accessible from shore. Water samples were analyzed for temperature, pH,

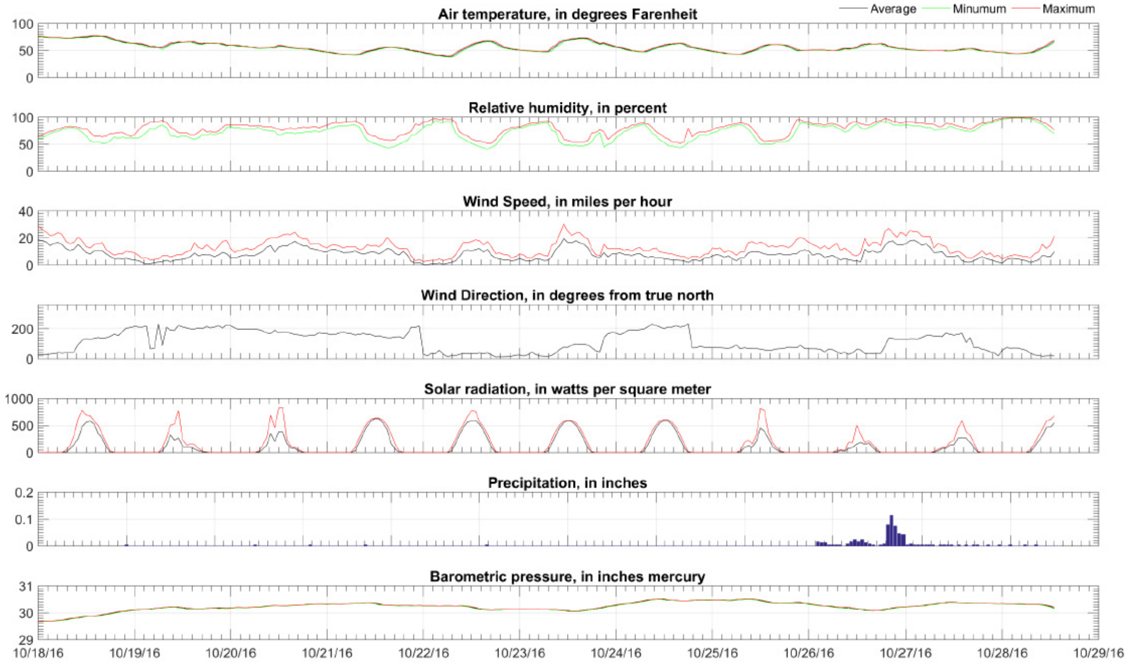

Figure 4. General weather conditions during testing at the The Nature Conservancy's Emiquon Preserve from 21 October 2016–28 October 2016.

dissolved oxygen, alkalinity, and CO_2. Temperature, pH, and dissolved oxygen were measured using a handheld meter (HACH Model HQ40d, Hach Company, Loveland, CO). Alkalinity was determined titrimetrically with 0.02 N H_2SO_4 to a pH 4.5 endpoint (APHA 1998). Carbon dioxide was determined using a pH 8.3 endpoint titration with 0.3636 or 3.636 N NaOH (HACH Method 8205). Carbon dioxide was also directly measured at a single location within WMS culverts using a CO_2 probe (Vaisala CO_2 Sensor, Model GMT2200, Vantaa, Finland). Sample locations, sonde placement, and other pertinent boundaries were surveyed (Trimble RTK, Model R10, Sunnyvale, CA) before testing.

Air quality was monitored during CO_2 injection to evaluate personnel safety. Atmospheric CO_2 concentrations were measured using a handheld meter (pSense Model AZ-0002, CO2meter.com, Ormond Beach, FL) at the water's surface and 1 m above the surface at each sampling location based on recommendations from the Illinois Environmental Protection Agency (Figure 3). Near-surface sampling represented exposure concentrations someone would experience when falling into the water, while 1 m above the surface represented concentrations someone would experience if sitting in a boat or standing on shore. Staff were cognizant to stand down wind during sampling to avoid inaccurate readings. A single atmospheric oxygen alarm (BW Clip O2 Detector, Model BWC2-X, JJS Technical Services, Schaumburg, IL) that triggered when atmospheric oxygen decreased below 19.5% O_2 was also placed within a WMS culvert for safety. The alarm was not triggered at any time during testing. General weather conditions during testing were collected from a nearby weather station by staff at the Emiquon Preserve (Figure 4).

Fish abundance was monitored remotely using underwater sonar. Adaptive Resolution Imagine Sonar (ARIS) transducers (ARIS Explorer 3000, Sound Metrics, Bellevue, WA) were positioned at the outlets of both WMS culverts pointing in opposite directions towards shorelines. Units were mounted to plates on 3.8 cm diameter aluminum poles with slip-on rail fittings. Height was set so that both mounting plates were 1.2 m off bottom. Sonar units operated at 3.0 MHz and 128 beams to give a maximum view of 30° × 14° at a frame rate of 15.0 frames per second. Units had a tilt of −15° and were pointed in opposite directions parallel to the structure to prevent cross-interference and fully monitor both culvert outlets. Raw data were processed using Echoview software (Echoview Software Pty Ltd, Hobart, Tasmania) to generate fish counts over time. A target threshold assumption of 10 cm length across beams was used to reduce false detections caused by debris and vegetation exiting the lake.

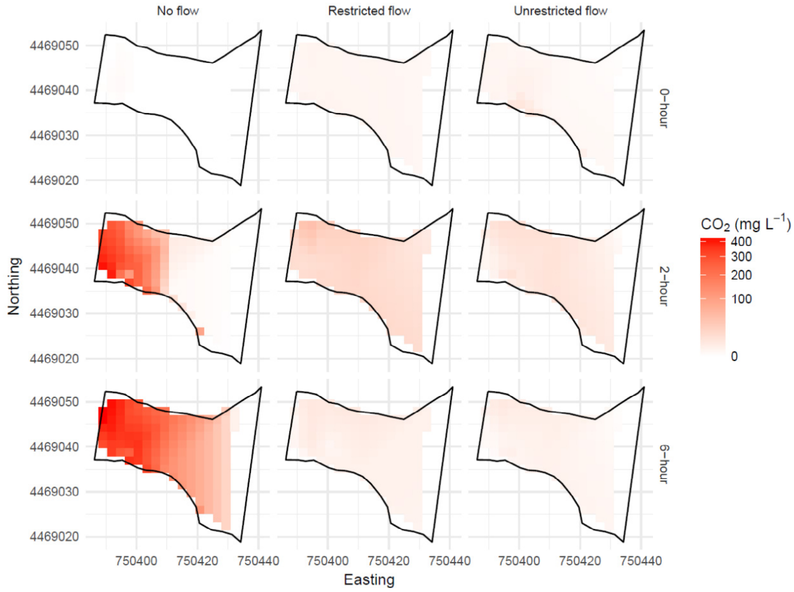

Figure 5. Carbon dioxide (CO_2) concentrations in water downstream of the water management structure (WMS) during CO_2 injection (see sampling locations in Figure 2). The WMS outlet (not shown) is located left of each figure. Concentrations were visualized across three WMS flow conditions: no flow, restricted flow, and unrestricted flow, each at three time intervals (0h, 2h, 6h) during CO_2 injection. Maximum concentrations measured in water during no flow, restricted flow and unrestricted flow were 458, 100 and 32 mg/L CO_2, respectively. Unrestricted flow had negligible CO_2 accumulation throughout most of injection due to dilution from higher volumes of influent lake water.

Statistical analyses

Water quality, air quality, and total daily fish counts were evaluated with and without injection of CO_2 during the three WMS flow conditions. Although treatment combinations were evaluated independently on separate days, single observations for each testing scenario limited appropriate inferential comparisons. Repeated observations on additional days were not possible due to receding Illinois River water levels and closure of WMS gates. Thus, water quality, air quality, and total daily fish counts were visualized or summarized using descriptive statistics during each test condition. Plots were generated in R (R Core Team 2016) using the ggplot2 package (Wickham 2009).

Results

Water quality

Increased water discharge through WMS negatively influenced CO_2 and pH levels within the treatment area (Figure 5). Highest CO_2 concentrations were recorded during CO_2 injection with no flow (Figure 6). Conversely, little to no CO_2 accumulation was observed during unrestricted flow. Acidity followed a similar pattern with lowest pH values recorded during no flow (Figure 6). Although concentrations varied by flow condition, CO_2 was generally well mixed shoreline-to-shoreline within the treatment area during all trials (Figure 5). Overall, we met or exceeded target concentrations of 100 mg/L CO_2 only during no flow and restricted flow, but were not successful at reaching target concentrations during unrestricted flow. Target concentrations of 100 mg/L were only briefly achieved at one sampling point 1 hour after injection started during restricted flow and gradually returned to ambient levels (Figure 6). Measurements in the main channel showed that some CO_2 was present in the Illinois River outside of the initial treatment area, but water from the back-water lakes also influenced pH levels in the Illinois River downstream of the confluence (Figure 7). Mean alkalinity in WMS culvert water was 177 (range: 131 – 250) mg $CaCO_3$/L during testing.

Air quality

Negligible changes in air quality were recorded during trials (Table 1). Atmospheric CO_2 levels were similar before and during CO_2 injection at the water's surface and 1 m above. All atmospheric CO_2 concentrations were well below levels causing human safety concern (NIOSH 2016).

Fish abundance

Daily fish counts varied widely across WMS flow conditions. Total daily fish counts were 70–95% lower on days when CO_2 was injected relative to control days (no CO_2) during no flow and restricted flow (Table 2). Total daily fish counts were higher on the day CO_2 was injected relative to the control day during unrestricted flow. During the no flow trial, 20–30 small (< 10 cm total length) incapacitated fish were observed after CO_2 concentrations exceeded

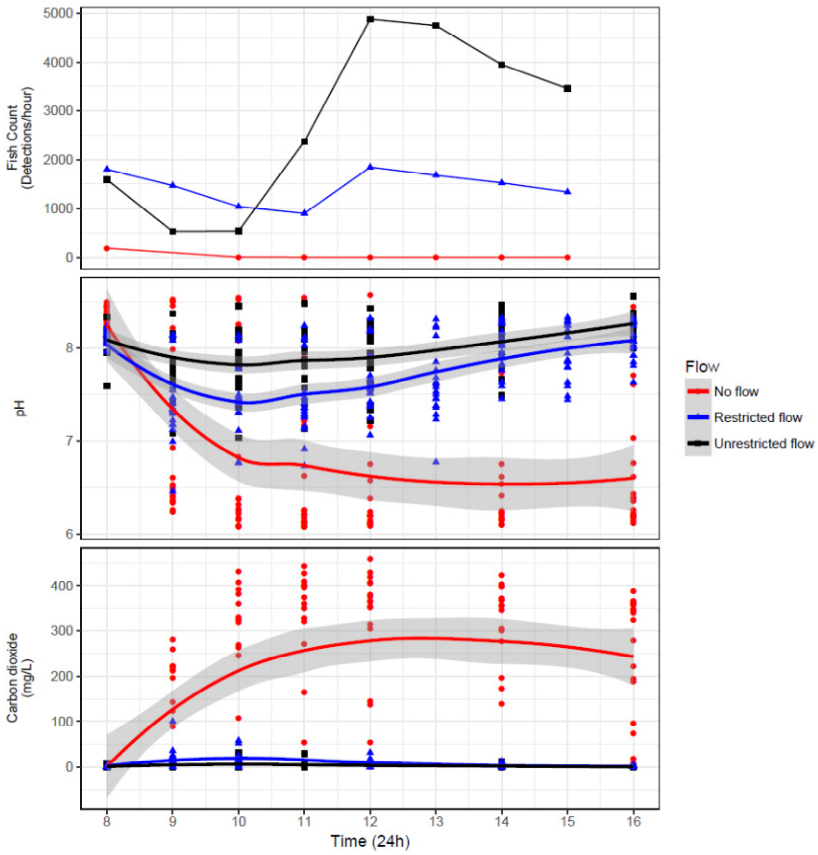

Figure 6. Fish counts, pH, and carbon dioxide (CO_2) concentrations recorded downstream of culvert outlets during CO_2 injection at three water management structure (WMS) flow conditions. Fish counts per hour were recorded using underwater sonar at culvert entrances. The no flow trial had the highest CO_2 concentrations (maximum 458 mg/L CO_2), while the restricted flow (maximum 100 mg/L CO_2) and unrestricted flow (maximum 32 mg/L CO_2) trials had substantially lower concentrations that declined over time during injection due to continuous dilution by influent lake water. Variation for CO_2 and pH illustrates spatial variation within the downstream plunge pool (see Figure 3) during each time interval and is shown as splines and 95% confidence intervals (shaded area).

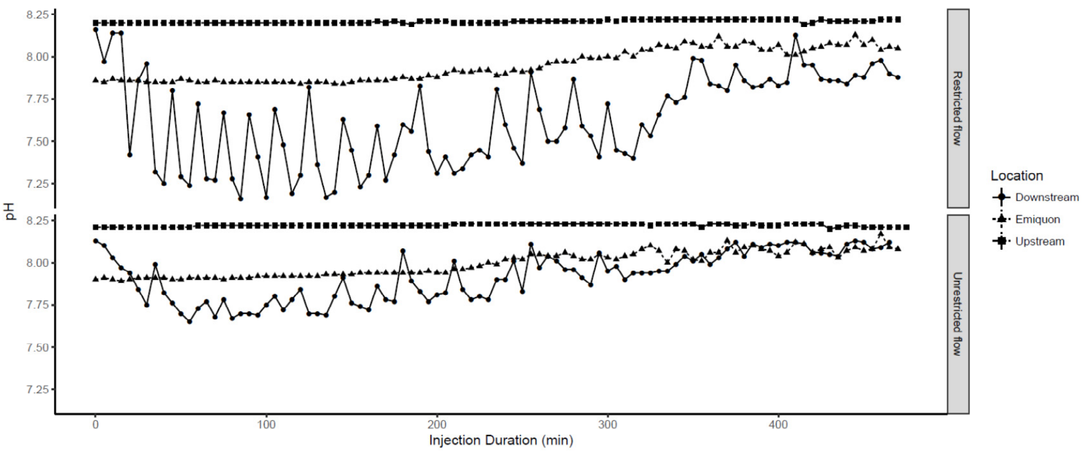

Figure 7. Measurements of pH in the Illinois River during carbon dioxide (CO_2) injection into a water management structure at the Emiquon Preserve. Sonde locations in the Emiquon wetland lakes, and upstream and downstream of the treatment area confluence are shown in Figure 2. No flow trials are not shown because water did not flow into the Illinois River during injection.

200 mg/L in the downstream plunge pool. Species identified were bluegill (*Lepomis macrochirus* Rafinesque, 1819), warmouth (*Lepomis gulosus* Cuvier, 1829), other small unidentified centrarchids (*Lepomis* sp.), black crappie (*Pomoxis nigromaculatus* Lesueur, 1829), gizzard shad (*Dorosoma cepedianum* Lesueur, 1818), and an unidentified species of madtom (*Noturus* sp.). No larger (> 10 cm) fish were observed to be incapacitated during CO_2 injection but were present before this trial as determined by visual observations with underwater sonar. Incapacitated fish near shore were alive when collected for species identification.

Table 1. Atmospheric CO_2 concentrations (atmCO_2) measured at the water's surface and 1 m above before and during CO_2 injection into water management structures (WMS) culverts across three discharge conditions: (1) no flow, (2) restricted flow, and (3) unrestricted flow. Data are shown as mean (range). For reference, OSHA's acute exposure limits for human safety are 30,000 ppm CO_2 and 8-h exposure limit is 5,000 ppm CO_2. Values recorded during this study were well below OSHA's levels of concern[1].

WMS Operation	atmCO_2 at Surface Before Injection (ppm)	atmCO_2 at 1m Before Injection (ppm)	atmCO_2 at Surface During Injection (ppm)	atmCO_2 at 1m During Injection (ppm)
No flow	423 (415–434)	422 (415–432)	430 (392–734)	424 (393–590)
Restricted flow	410 (408–415)	410 (408–413)	421 (395–573)	407 (396–435)
Unrestricted flow	418 (413–421)	419 (417-423)	425 (404–504)	424 (405–503)

[1]NIOSH 2016, www.cdc.gov/niosh/pel88/124-38.html, accessed 14 December 2017

Table 2. Total daily fish counts observed using underwater sonar with and without injection of CO_2 into the water management structure (WMS) culverts at the Emiquon Preserve near Havana, IL. Data represent total fish counts at WMS culvert entrances between 0800 to 1600 CDT. Maximum CO_2 concentration measured in WMS culvert water during injection trials are reported for reference. For more detail on water quality throughout testing, see Figure 6.

WMS Operation	Culvert Entrance	Max CO_2 (mg/L)	Total Fish Detections (Without CO_2)	Total Fish Detections (With CO_2)	Difference (%)
No flow	North	458	624	108	−82.7
No flow	South	458	1501	78	−94.8
Restricted flow	North	100	17423	3371	−80.7
Restricted flow	South	100	26801	8243	−69.2
Unrestricted flow	North	32	3180	8594	+170.3
Unrestricted flow	South	32	6303	13476	+113.8

Discussion

Results from this short field evaluation describe several considerations for CO_2 in natural environments. Most notably, fish abundance near culvert entrances was 70–95% lower during injection of CO_2 with restricted flow and no flow, respectively. In contrast, CO_2 concentrations that altered fish behavior could not be attained during unrestricted flow and resultant fish counts were higher. Fish movement away from treatment areas with elevated CO_2 is consistent with avoidance behaviors reported in laboratory and pond experiments (Kates et al. 2012; Donaldson et al. 2016; Cupp et al. 2017a; Cupp et al. 2017c). However, few replicates during our field trials has limited inferential comparisons and more robust evaluations are still needed. Interestingly, considerably higher than expected CO_2 concentrations were recorded during the no flow trial. We recommend that flow and discharge are incorporated into all subsequent calculations for field testing to avoid potentially acutely toxic CO_2 exposure (Treanor et al. 2017); although we did not observe any notable fish mortalities associated with CO_2 treatment during any trial. Finally, outcomes from water quality and air quality monitoring are also important to address knowledge gaps not captured during laboratory and pond testing with regard to flowing water, human safety, and downstream effects.

Water discharge through WMS culverts limited our ability to reach and sustain elevated CO_2 concentrations. Air diffusers were initially favorable for this short-term study because they could be easily deployed and quickly modified or removed. However, higher capacity CO_2 infusion systems should have been considered to improve gas-transfer efficiency during high discharge. For example, Donaldson et al. (2016) found that CO_2 injected directly into carrier water piping through venturi devices was efficient at mixing high volumes of CO_2 gas into solution before applying into outdoor ponds. Similarly, pressurized liquid-to-liquid CO_2 injection processes are used in wastewater treatment facilities to efficiently drive CO_2 gas into solution and neutralize the pH of effluent water before discharge (Thomas Zolper, University of Wisconsin Platteville, personal communication). This study found that high flow velocities and discharge through WMS culverts, in combination with simple gas infusion equipment, severely hindered CO_2 accumulation (Figures 5, 6). Adapting and utilizing existing gas injection technology, such as wastewater treatment designs, would offer a more sophisticated approach for field installations and would likely overcome the challenges with dilution at unrestricted WMS flow.

Atmospheric CO_2 concentrations were not altered by CO_2 injection into water and there were no human safety concerns during this study. Relative to exposure limits established by the Occupational Safety and Health Association (OSHA), maximum CO_2 levels were < 10% of OSHA's 5,000 ppm 8 h time weighted average standards and < 1% of the 30,000 ppm acute exposure limits (NIOSH 2016). We found no immediate safety concerns during CO_2 injection into water and results suggest that CO_2 could be a safer fish deterrent option relative to other control technologies (e.g., seismic water guns, electricity, chlorine, hot water, caustic chemicals) (Slater et al. 2011; Noatch and Suski 2012; Romine et al. 2015; Buley et al. 2017). Comprehensive risk-assessments are needed to validate this observation. Specifically, atmospheric CO_2 is denser than ambient air and low elevation areas (e.g., man-holes, concrete recesses) are recommended monitoring locations during future applications.

Downstream exposure to non-target organisms should be considered when applying CO_2 as a fish deterrent. Although treatments were diluted and mixed with Illinois River water, some CO_2 was present approximately 20 m downstream of study site (see Figure 7) suggesting that water quality effects from CO_2 injection covered a greater spatial area than originally expected. Treatment dispersal is one consideration and potential challenge with CO_2 relative to other more localized deterrent strategies such as underwater sound, bubbles and electricity (Vetter et al. 2015, 2017; Parker et al. 2015). Non-target organisms, particularly macroinvertebrates and other sedentary organisms, could be susceptible to unintended CO_2 exposure. For example, (Waller et al. 2017) documented physiological impairments of juvenile fatmucket mussels (*Lampsilis siliquoidea* Barnes, 1823) during continuous 28-d CO_2 exposure at concentrations > 87 mg/L CO_2. Downstream concentrations in the main channel of the Illinois River during our study were well below levels of concern identified by Waller et al. (2017). However, sufficiently characterizing nearby species assemblages to the injection area to determine the risks to non-target species would be prudent before applying CO_2 to control fish movements (Hannan et al. 2016; Jeffrey et al. 2017). Exploring other measures, such as the use of aeration to facilitate off-gassing of downstream CO_2, could also be useful to reduce dispersal and exposure-risk to non-target organisms outside of treatment areas.

Altering fish behavior with CO_2 could be a useful tool for controlling the movements of nuisance fishes. At the Emiquon Preserve, WMS operators could apply CO_2 during static or low discharge conditions to temporarily reduce fish abundance near culvert entrances or void fish from culverts during maintenance and repair. Limiting fish occupancy near WMS culverts could reduce the risk of non-native fishes migrating into the Preserve's wetland area and help protect the local recreational opportunities and conservation efforts by The Nature Conservancy. However, more research is needed to determine if lower fish abundances (i.e. reduced number of fish at-risk of passing upstream) directly corresponds with a reduced number of upstream passages. Based on our results, we were not able to make this distinction because individual fish were not tracked. Regardless, reducing the abundance and occupancy of fish in particular areas could be useful for broader management implications to inhibit invasive fish movement near navigational locks, limit occupancy near critical habitat, reduce migration through pinch points into new areas, or isolate populations to support removal efforts (USACE GLMRIS 2014; MacNamara et al. 2016). We identified several limitations with CO_2 applications in flowing water and further evaluations across broader temporal scales and discharge conditions are needed to better explain observations during this study and to aid in appropriate site selections. Additionally, different injection methods (e.g. venturi injection systems, pressurized liquid-to-liquid CO_2 injection) should be explored for increasing efficiency and effectiveness in varying field applications to address challenges with flowing water.

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