Illinois-Indiana Sea Grant Final Report for Funded Research Project

Section A. Summary.

• Title of Project

The effect of electric and carbon dioxide barriers on the risk of aquatic invasive species passage through the Chicago Area Waterway System

• Completion Date (If no-cost extension was approved, use the extension end date.)

11/30/2022

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• Abstract

The Chicago Area Waterway System (CAWS) connects the Great Lakes and Mississippi River Basins. This allows for boat passage, but also for the spread of invasive species between these Basins. Over recent decades the main efforts to stop invasive species spread have been directed at the silver (*Hypophthalmichthys molitrix*) and bighead (*H. nobilis*) carps (aka "Asian carp"). These efforts revolve around a set of electrical barriers, and there are proposals to also implement carbon dioxide barriers. Many invertebrate species pose a high risk of moving through the CAWS but the effect of these barriers at preventing their spread is unknown. For this project we constructed electrical and carbon dioxide barriers and tested their effects on mortality and behavior of a range of invertebrates. We tested the barriers on crayfish, snails and mussels, zooplankton, shrimp, and amphipods. Our results show that the current electrical barriers are unlikely to affect the passage of invertebrate species. Barriers operating with a much stronger electrical field would be more effective, but are probably impractical. Invertebrates are also less affected than fishes by water with elevated levels of carbon dioxide, with the exception of the bloody red shrimp (*Hemimysis anomala*) which showed high mortality. In combination, these results show that although current efforts may be effective for silver and bighead carp, they are unlikely to slow the much more numerous species of invertebrates that pose risks of spreading through the CAWS. Additional measures will be needed if the spread of these invertebrates is to be prevented.

• Keywords

Electric Barrier, Carbon dioxide barrier, Chicago Area Waterway System, Chicago Sanitary and Ship Canal, Apocorophium lacustre, Hemimysis anomala, bloody red shrimp, invasive species.

• Lay Summary

Aquatic invasive species pose a large and well-known risk of expanding their ranges through the Chicago Area Waterway System (CAWS). The CAWS is an artificial waterway that connects the Great Lakes and Mississippi River Basins. It has facilitated the spread of many species in the past – including zebra mussel and round goby – and is likely to be an important route for invasive species spread in the future. Recent efforts to prevent spread have been focused on preventing silver and bighead carp (aka "Asian carps") from moving into the Great Lakes from the Mississippi where they are already established. These efforts include the construction and operation of electric barriers, and the proposal to develop and implement carbon dioxide as an additional barrier. The operation of the electric barriers, and testing for the carbon dioxide barriers, is targeted specifically at these species of carp. Despite the large risk posed by silver and bighead carp, dozens of other invasive species pose a risk of moving through the CAWS. Most of these are invertebrates and to date there has been no research to determine whether the electric barriers and carbon dioxide may prevent their spread. In this project we have used lab-scale electric and carbon dioxide barriers to determine the risk that a range of invertebrate species may move through the CAWS.

Our electric barrier was constructed so that we could observe the reactions of a range of invertebrates in electrical fields the same as in the barrier in the CAWS, and at higher electrical field strengths. Our results show that invertebrates are much less susceptible to the electric field than fishes, and that much higher electrical field strengths would likely be required to deter the spread of these species. The field strengths required are probably not practical due to issues of cost and the danger of having boats moving through the electrical fields. Our results for carbon dioxide are similar; with one notable exception we found that invertebrates are much less susceptible than fishes to water with elevated carbon dioxide levels. The exception is the bloody red shrimp, a relatively recent invader to the Great Lakes that poses a risk of moving through the CAWS and into the Mississippi. This species had high mortality in relatively low concentrations of carbon dioxide. In total our research shows that the electrical and carbon dioxide barriers are unlikely to prevent the spread of most invasive invertebrates. If the spread of these species is to prevented then additional measures will be necessary.

Section B. Accomplishments

• Introduction

Aquatic invasive species pose a large and well-known risk of expanding their ranges through the Chicago Area Waterway System (CAWS). The CAWS is an artificial waterway that connects the Great Lakes and Mississippi River Basins. It has facilitated the spread of many species in the past – including zebra mussel and round goby – and is likely to be an important route for invasive species spread in the future. Recent efforts to prevent spread have been focused on preventing silver and bighead carp (aka "Asian carps") from moving into the Great Lakes from the Mississippi where they are already established. These efforts include the construction and operation of electric barriers, and the proposal to develop and implement carbon dioxide as an additional barrier. The operation of the electric barriers, and testing for the carbon dioxide barriers, has so far been targeted specifically at silver and bighead carp. While these are undoubtably high risk species, dozens of other invasive species pose a risk of moving through the CAWS. Most of these are invertebrates and to date there has been no research to determine whether the electric barriers and carbon dioxide may prevent their spread. The overall goals of this project were to a) determine whether electric and carbon dioxide barriers will prevent the spread of invasive invertebrates when those barriers are operated at levels parameterized to prevent the spread of invasive carps, and b) determine whether changing the strength (i.e., electric field strength, concentration of carbon dioxide) of these barriers may be effective for preventing the spread of invertebrates.

To address these goals we developed four objectives:

1) To determine the effectiveness of the electric barriers in the Chicago Area Waterway System at deterring the passage of invertebrate species from a wide range of taxonomic groups.
2) To determine the effectiveness of the proposed carbon dioxide barriers in the Chicago Area Waterway System at deterring the passage of invertebrate species from a wide range of taxonomic groups.
3) To determine whether different voltages and frequencies of electricity affect the effectiveness of the electric barriers in the Chicago Area Waterway System at deterring species passage.
4) To determine whether different concentrations of carbon dioxide affect the effectiveness of proposed carbon dioxide barriers in the Chicago Area Waterway System at deterring species passage.

To meet these objectives we modified an existing lab-scale electric barrier, and we developed new equipment and protocols for testing the effects of carbon dioxide on invertebrates. This allowed us to experimentally test the effects of these barriers on aquatic invertebrates at a range of barrier strengths.

• Project Narrative

Because the electric barrier was developed and operated separately to the carbon dioxide barrier we explain the methods and results of each separately in this section. The implications of our results are discussed in a single section at the end of the Project Narrative.

Electric Barrier Methods:

Development of Electric Barrier

We constructed an electric field within a rectangular glass tank (122 cm long, 32 cm wide, 34 cm deep) and calibrated it to produce the same electric field strength and waveform as the electric barriers in the CAWS. Our equipment consisted of three elements: a modified backpack electrofishing unit (ETS PK-C; https://www.ets.org), a power supply (Volteq HY3010EX) which supplies a direct current to the electrofishing unit and replaces its battery, and two Type 316 stainless steel plate electrodes (38 cm x 27 cm) placed at opposite ends of the tank (Fig. 1). The electrofishing unit was rewired so that the anode and cathode lines were attached with jumper cables to the electrodes. These were placed at each end of the tank and produced a uniform electric field throughout the tank.

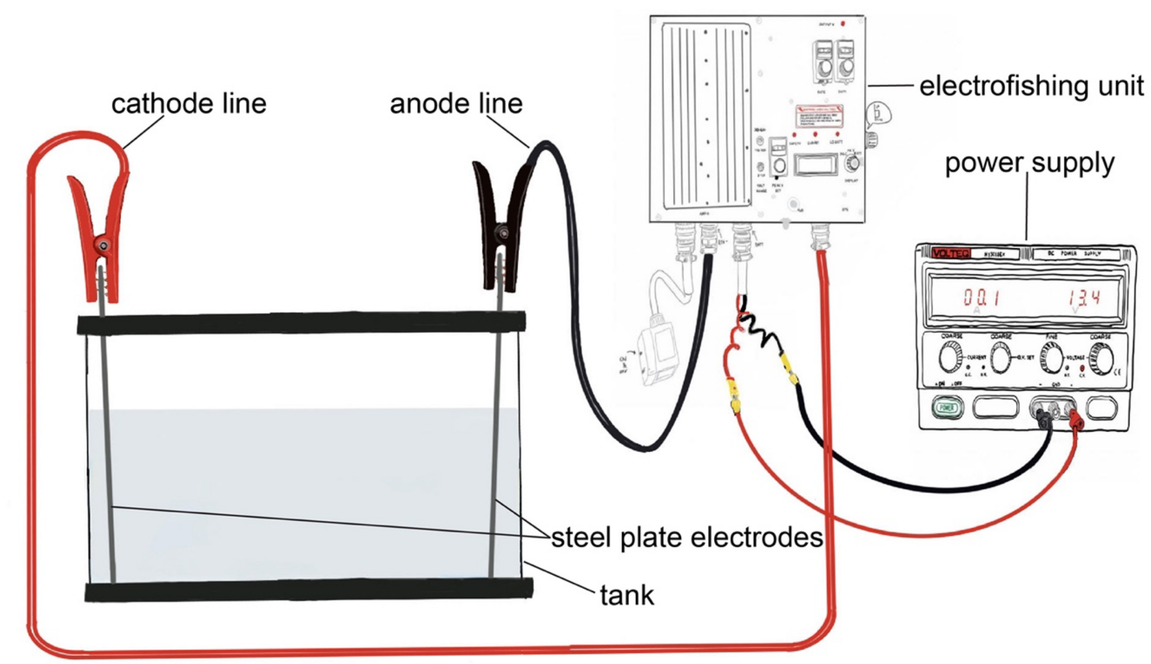

Fig. 1. Diagram of experimental electric barrier setup, including power supply, electrofishing unit, tank, steel plate electrodes, and cathode and anode lines.

To confirm that our system produced the desired electric field we compared the true output of the electric field to the expected output based on the backpack readings. In particular, we measured the voltage, current, frequency, duty cycle, and waveform integrity in the tank using a Fluke 87V Industrial Multimeter, Fluke 124B Industrial Scopemeter, and Fluke 80i-119s AC/DC Current Clamp. We used both the multimeter and the scopemeter to test voltage. The average difference of the true output voltage compared to the expected output was 1.2% as measured by the multimeter and 3.3% as measured by the scopemeter. The multimeter measured current between 0.04-0.10A higher than the expected output when measuring a range of currents from 0-1.1A. We also used the scopemeter to measure frequency and duty cycle. The scopemeter confirmed that the waveform of the output was as expected. The average difference was 2.1% for frequency and 6.4% for duty cycle. These differences are minor considering the range over which we ran the electric field and confirm that the electric field produced was similar to that of the barrier in the CAWS.

Electric Barrier Experimental Protocols

All experiments were conducted using lab water at both ambient water temperature (mean ± standard deviation = 20.3 ± 1.4 ℃) and ambient specific conductivity (mean ± standard deviation = 321.6 ± 56.8 µS/cm). To recreate the parameters of the electric barrier in the CAWS the initial settings on the electrofishing unit were 106 V, 34 Hz, pulse length = 2.3 ms, and duty cycle = 7.2%, creating an electric field of 2.3 V/in.

Each trial began with five individual organisms (haphazardly selected from a tank containing the available pool of individuals) and placed in a non-conductive nylon mesh container in the center of the tank. Trials consisted of three consecutive phases of 5 minutes: pre-stimulus, stimulus, and post-stimulus. The electric field was off for the pre-stimulus phase, on for the stimulus phase, and off again for the post-stimulus phase. Trials were recorded with a video recorder (GoPro, HERO4) placed overhead at the center of the tank. During each trial we recorded behavior at the end of each minute and response to a physical prodding with non-conductive rod every 2.5 minutes. All organisms were kept for 24 hours after each trial to check for delayed mortality. It was not possible to record the behavior of each individual organism over time. Instead, we recorded the number of organisms in each behavioral state at each time. All personnel who recorded behaviors were trained by Colette Copic and frequently coordinated with each other in an effort to limit observer bias (e.g., two observers viewing the same organism but describing behavior differently).

Three trials were conducted for each combination of field strength and species, and each trial included five individuals. For juvenile crayfish, 18 trials were conducted using a total of 90 individuals (mean carapace length ± standard deviation = 20.2 ± 7.2 mm, n = 90; Table S1). There were inevitable differences in sizes of individuals available for the different trials. The only trial where this may have been important is the 25% trial which had somewhat smaller organisms than the other trials (see Results). For amphipods, 12 trials were conducted using a total of 60 individuals (mean length ± standard deviation = 4.6 ± 1.0 mm, n = 60; Table S2). Individual organisms were not reused in any trials.

Behavioral Analysis

Observations and video were used to score behaviors during each trial. During the 5 minute stimulus phase organism behavior was recorded and classified every minute for each individual as one of 5 categories: **no change in behavior** – individual exhibits normal behavior; **altered movement** – individual exhibits difficulty in moving or swimming; **rigid and maintaining equilibrium** – body is rigid but stays upright and organism maintains equilibrium; **rigid and lost equilibrium** – body is rigid with no motor functions and organism is not maintaining itself in upright position; **mortality –** a loss of equilibrium and motor functions with no recovery, death.

Electric Barrier Results:

At 100% of the existing electric barrier field strength most *P. clarkii* individuals experienced altered movement (46%) or rigidity with maintained equilibrium (36%; Figure 2A). Fewer *P. clarkii* experienced rigidity and lost equilibrium (10%), and none died either during the experiment or the 24 hours following. For *H. azteca*, behavioral responses at 100% of the existing barrier strength were similar (Figure 2B), with a majority of individuals (57%) remaining responsive but experiencing altered movement. *H. azteca* individuals also displayed rigidity with maintained equilibrium (31%) and rigidity with lost equilibrium (12%). No *H. azteca* died during the experiment at 100% of existing barrier field strength or during the 24 hours following. Results in Figure 2 are aggregated across time to show overall behavior during the stimulus phase at different electric field strengths because reactions across the five organisms in each trial did not change consistently throughout the 5 minute stimulus phase for either species.

At electric field strengths ≥200% of the existing electric barrier field strength, the number of strong behavioral responses increased for *P. clarkii*, with the majority of individuals experiencing rigidity and lost equilibrium (64% at 200% barrier strength, 77% at 300% barrier strength, 91% at 400% barrier strength; Fig. 2A). Again, none were killed during the experiments and all recovered within 24 hours after the experiments. At electric barrier strengths lower than the existing barrier (25% and 50%), behavioral effects on *P. clarkii* were less extreme. At 25% of the existing barrier's electric field strength *P. clarkii* did not exhibit any behavioral response, but at 50% of the existing barrier's electric field strength a majority of individuals displayed altered movement (72%). We note that juvenile crayfish used in the trials for 25% of the existing barrier's strength were slightly smaller. This was due to the availability of individuals and may have affected the behavioral response seen in the 25% trial.

For the amphipod *H. azteca* an increasing number of individuals experienced rigidity and lost equilibrium at electric field strengths ≥200% of the existing barrier, with 51% of individuals experiencing rigidity and lost equilibrium at 400% (Fig. 2B). None were killed during the experiments and all except three individuals survived for 24 hours after the experiment. Two *H. azteca* from the 200% trial died, and one from the 400% trial.

The same experiments have been conducted on scud (*Apocorophium lacustre*), rusty crayfish (*Faxonius rusticus*), marmokrebs crayfish (*Procambarus fallax*), Daphnia (*Daphnia magna*), bloody red shrimp (*Hemimysis anomala*), and Chinese mystery snail (*Bellamya chinensis*). Analysis of these results is ongoing, but we note here that the results broadly mirror those presented above. To be clear, no invertebrates that we tested experienced significant mortality at 100% of barrier strength, and all showed increasingly strong responses at higher electric field strengths.

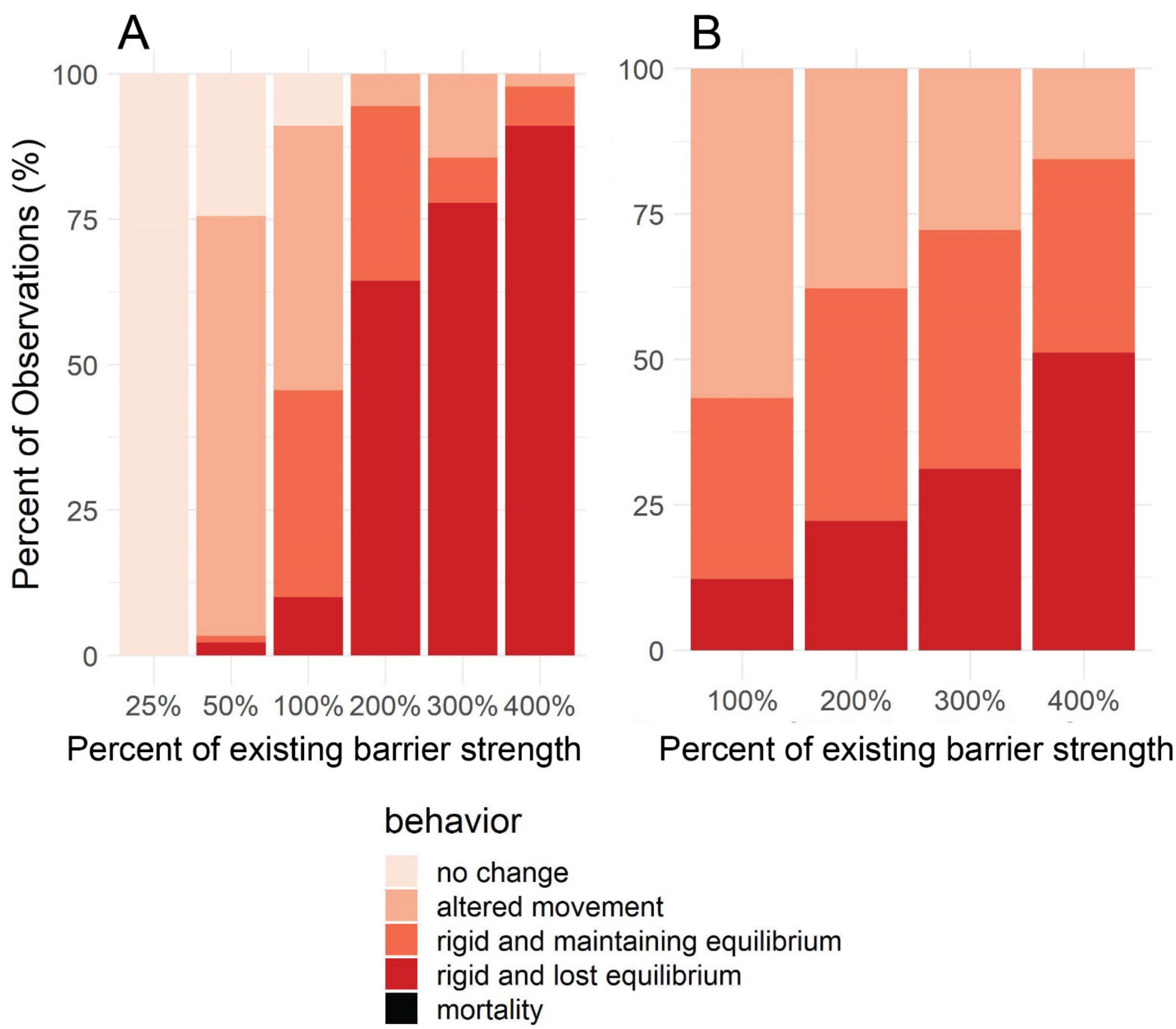

Fig. 2. Aggregated behavior across time during 5 minutes of stimulus phase for A) juvenile *Procambarus clarkii* (n = 15 for each barrier strength) for 25%, 50%, 100%, 200%, 300%, and 400% of existing barrier strength, and B) *Hyalella azteca* (n = 15 for each barrier strength) for 100%, 200%, 300%, and 400% of existing barrier strength.

Carbon Dioxide Barrier Methods:

The purpose of this study was to understand the short-term behavioral and avoidance responses of invasive invertebrates when exposed to a range of concentrations of dissolved Carbon dioxide (CO_2). This was examined in two ways: exposure and avoidance experiments. Invertebrate species identified by managers as potential concerns were chosen for testing (Table 1). Organisms were allowed to acclimate to lab conditions for at least 24 hours before testing. Collection, transport, and possession of organisms was conducted under permits from the ILDNR (SCP-NH 206403, Permit #0113 RSTP20-110) and the Chicago Park District (Permit # LY0).

Species	**Description**	**Mean size (mm) ± standard deviation**
Red swamp crayfish (*Procambarus clarkii)*	Native to southern US as far north as southern IL (Taylor et al. 2007), invasive populations of red swamp crayfish decrease macroinvertebrate density and diversity, alter fish communities, and displace native crayfish through competition for food and shelter.	Adults: 52.72 ± 4.5 (n=120) Juveniles: 9.72 ± 1.4 (n=210)
Rusty crayfish *(Faxonius rusticus)*	The rusty crayfish is native to the Ohio river basin and has established invasive populations throughout the Midwest US where they displace native crayfish.	Adults: 25.5 ± 1.6 (n=120) Juveniles: 9.3 ± 1.3 (n=200)
Marbled crayfish *(Procambarus virginalis)*	The marbled crayfish is a parthenogenetic crayfish primarily raised for the aquarium pet trade. It is invasive in Europe and is on the Great Lakes St. Lawrence Governors and Premiers' List of Least Wanted Aquatic Invasive Species.	15.49 ± 3.8 (n=70)
Hyalella azteca	Amphipod common in North America; chosen for its similarity to invasive amphipods that were not available for testing.	3.8 ± 0.67 (n=215)
Bloody red shrimp *(Hemimysis anomala)*	This mysid shrimp is native to freshwaters of the Ponto-Caspian region. It is widely established as an invader in Europe and has been established in the Great Lakes since at least 2006.	8.78 ± 0.4 (n=45)

Daphnia magna	*D. Manga* are a broadly distributed zooplankton species and serve as a proxy for non-native zooplankton that were not available for testing.	2.78 ± 0.61 (n=100)
Bladder snail *(Physella acuta)*	Bladder snails are popular in the aquarium trade because they reproduce prolifically. Although the native range of this species is unknown, it is abundant throughout the eastern US and Europe. This species serves as a proxy for other invasive snails.	7.1 ± 1.4 (n=180)
Chinese mystery snail *(Bellamya chinensis)*	This snail was likely introduced to North America via pet release, as it is a popular aquarium species. This species poses risks to native snails because it has fewer natural predators and can outcompete native grazers including other snails and some insects for food, especially when they grow at high densities.	47.95 ± 5.99 (n=136)
Dreissenidae spp.	The establishment of invasive zebra (*Dreissena polymorpha*) and quagga (*Dreissena bugensis*) mussels causes adverse ecological changes to systems including major trophic structure shifts. Dresseneids continue to expand their range throughout the US and Canada.	19.81 ± 1.3 (n=204)

Table 1- Description of species used in CO_2 experiments and mean size ± standard deviation of all individuals tested. Crayfish size is carapace length from the tip of the rostrum to end of the carapace. *H. azteca*, bloody red shrimp, and *D. magna* size is full body length. Snail and mussel size is shell length.

Carbon Dioxide Concentration Curve

Changing concentrations of CO_2 in water lead to predictable changes in pH. To determine CO_2 concentrations in real time, we developed a curve that relates CO_2 concentration to pH (Figure 3). This curve was created by bubbling CO_2 gas into 100 individual water samples, recording pH with a digital sensor (Vernier LabQuest 2), and measuring corresponding CO_2 concentration using a pH 8.3 endpoint titration with 0.3636N NaOH titrant (HACH Method #8205). To ensure that this curve could be applied throughout the study, we used the same water source (lab tap water) at the same temperature (19°C - 21.9°C) for developing the curve and all experiments

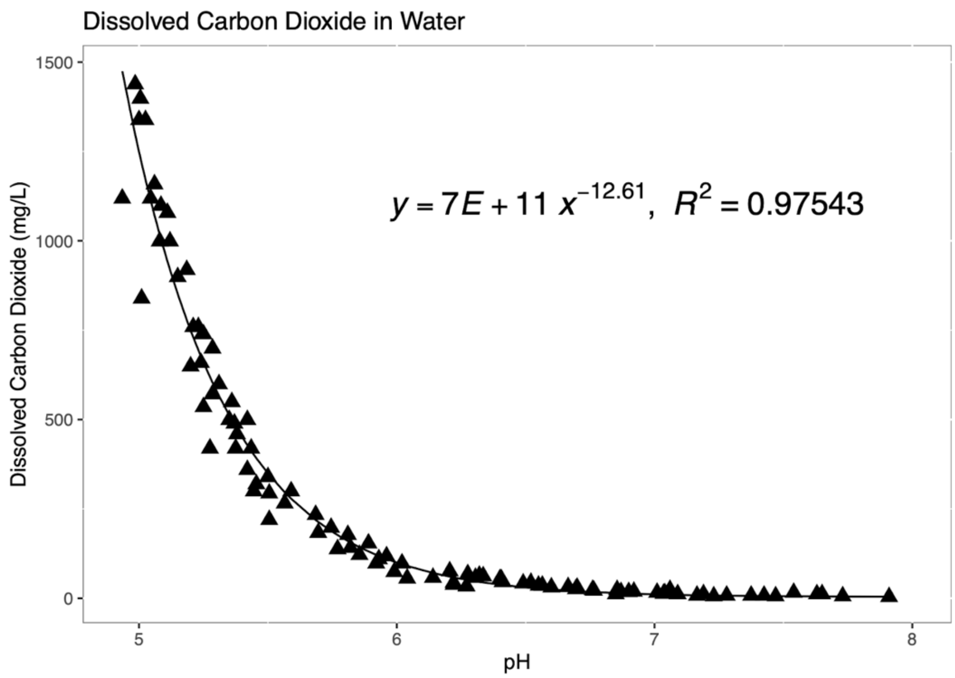

Figure 3- Relationship between pH and dissolved CO_2 concentration in lab water. Equation is given in the figure.

Exposure Experiments

The first step in the exposure experiments was to develop a scale of organism responses to elevated CO_2. The goal was to identify organism behaviors that accurately reflected the organism's condition in response to elevated CO_2. To do this, we placed ten individuals of each species in separate containers and exposed them to CO_2 concentrations that were increased from 7 to ~500 mg/L over the course of two hours by dripping in water that had a CO_2 concentration of ~600 mg/L. Two researchers independently recorded behaviors of each organism every minute based on visual observation and poking with a plastic rod every five minutes. For each species, we observed behaviors on a continuum from unaffected behavior to narcosis. For each species, the researchers compared notes and established behavior categories for each species that represented a scale of responses from low to high (Table 3). Species had two to five behavior categories, each based on readily observable differences in their responses.

In full trials, individual organisms were placed in separate plastic containers (0.5 L for all organisms except adult crayfish, which were placed in 3.3 L containers) for a five-minute acclimation and observation period. Clumps of 5-10 dresseneids, rather than individuals, were placed in each container. Organisms were then transferred to another open container with the target concentration of CO_2 for a 30 or 60-minute exposure period. Finally, organisms were removed from the test container and placed in a container with ambient CO_2 concentrations for a 30-minute recovery period.

CO_2 target concentrations during the exposure period were 7 mg/L (control), 70 mg/L, 120 mg/L, 300 mg/L, and 500 mg/L (Table 2). The lowest concentration was the ambient control with no CO_2 added. The lowest treatment concentration (70 mg/L) was selected because it is documented to be the lower threshold that elicits physiological responses such as loss of equilibrium and avoidance in fish. The next level, 120 mg/L, was chosen because it is near the high end of what is allowed by the US Environmental Protection Agency. The higher concentration levels (300 and 500 mg/L) were chosen because we observed limited responses of most species at the lower levels and wanted to determine whether higher levels would cause more extreme responses. Water conditions were stable across all replicates.

The behavior category of each organism was recorded every minute during the acclimation, exposure, and recovery phases. Additionally, every 5 minutes during the exposure period the organisms were poked with a zip tie (stimulus) and their reaction recorded according to the relevant behavioral scale (Table 3). After observing the recovery period, individuals were left in ambient CO_2 water for 24 hours after which any dead organisms were counted. Organisms were euthanized after the 24-hour checkpoint by freezing (crayfish, snails) or submersion in ethanol (amphipods, zooplankton, shrimp, mussels). Individual sex was recorded, and the size of each organism was measured using a digital caliper. Narcotization thresholds, or the CO_2 concentration in which a given species became narcotized, were determined by selecting the CO_2 treatment in which ≥ 50% of individuals tested were completely unresponsive for at least 10 minutes.

Taxa	Behavior Category	Description
Red swamp, rusty, and marbled crayfishes, *H. azteca*, bloody red shrimp	No change	Crawling or swimming, upright, responds to stimulus
	Altered movement	Twitching at surface of water or tail flipping, responds to stimulus
	Impeded movement	Loss of equilibrium, legs twitching while on back, responds to stimulus
	Narcotization	Motionless, loss of equilibrium, no response to stimulus
D. magna	No change	Hopping, swimming, appendages are moving
	Narcotization	Motionless, loss of equilibrium, no response to stimulus
Bladder snail	Motion	Crawling, swimming, flicking shell from side-to-side
	Semi-motion	Observing, outside of shell, not crawling
	Motionless	Inside shell or floating
Chinese mystery snail	Upright	Upright, attached to substrate, out of shell
	Folded foot	Upright, folded foot that looks like a funnel
	Closed operculum	Closed operculum
	On shell	On back of shell, operculum open, body extended, responding to stimulus
	Narcotization	On back of shell, operculum open, not moving, not responding to stimulus
Dreissenidae spp.	Open shell	Valve open, extended foot, responds to stimulus
	Closed shell	Valve closed
	Narcotization	Valve open, extended foot, no response to stimulus

Table 2- Behavioral categories for organisms exposed to water with elevated CO_2 concentrations.

Avoidance Experiment- Crayfish and amphipods

To test whether invertebrates are capable of avoiding areas with high levels of CO_2, we used a modified shuttle box tank arena. A 10-gallon tank was divided into two chambers where individual organisms were exposed to increasing levels of dissolved CO_2 in one chamber and given the option to "shuttle" to the adjoining freshwater chamber through a narrow tunnel. pH probes (Vernier LabQuest 2) placed in the freshwater chamber, the shuttling tunnel, and the CO2 chamber provided live readings that were converted to CO2 concentrations (Figure 4).

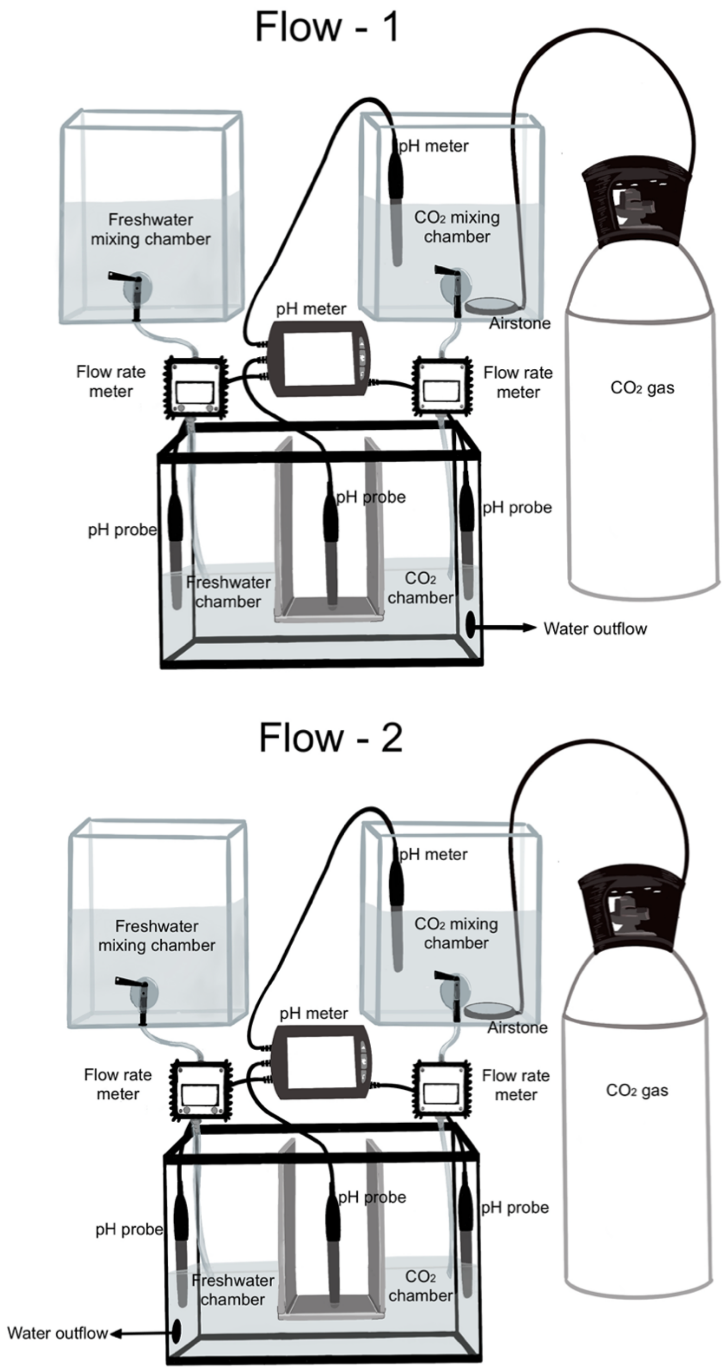

Figure 4- Shuttle Box Tank Area with flow-1 and flow-2 configurations. In flow-1, the water outlet was in the CO_2 chamber, creating a net flow toward the CO_2 inlet. In flow-2, the water outlet was in the freshwater chamber, creating a net flow in the direction of the freshwater inlet.

Avoidance Experiment- Snails

To determine whether snails show avoidance behavior we exposed them to elevated levels of CO_2 and recorded if and when they crawled to or above the water surface.

Statistical methods

Statistical analysis employed generalized linear mixed models (GLMM) using the "lme4" package in R v. 3.4.1.. GLMM models with crayfish and amphipods were fitted for avoidance responses with a binomial distribution (organism is in CO_2 treated water vs in freshwater refuge) with group (control or treatment) and time as fixed effects and replicate as a random effect. Each initial GLMM model included the variables size, sex, replicate, group (control or treatment), and time (0-30 minutes). Models were fit using backwards stepwise deletion of variables and AIC values, then validated using the DHARMa package. Models with the lowest AIC values were selected for analysis, and the final GLMM examining crayfish and amphipod avoidance did not include size or sex of the organism.

A GLMM model was also fit to assess avoidance behavior in snails with a binomial distribution (above vs. below waterline) with group (CO_2 treatment level) and time (0-6 hours) as fixed effects and the identity of individuals and replicate as random effects. Original models included size, but this variable was deleted because a comparison of AIC values revealed that the model fit better without this variable. A GLMM model was also fit specifically to assess the behavioral response of Chinese mystery snails (closed operculum vs open operculum) with group (CO_2 treatment level) and time (0-6 hours) as fixed effects and individuals and replicate as random effects.

Finally, a log likelihood test was employed to compare models with and without treatment as a fixed effect (alternate vs. null) to test for significant avoidance responses for all species tested.

Carbon Dioxide Barrier Results

Exposure Experiments

All species showed behaviors indicating higher levels of sedation as CO_2 concentrations increased. Crayfish species responded to elevated dissolved CO_2 with altered movement (tail flipping), lost equilibrium (floating or on back with appendages twitching) or narcotization (not moving, no response to stimulus). Although both adults and juveniles showed a decline in locomotor activity, adult red swamp crayfish and adult rusty crayfish were more resistant to narcotization at levels up to and including my maximum test concentration of 500mg/L. Juvenile crayfish were more susceptible to narcotization and lethal effects than adults of the same species. Of the juvenile crayfish tested, marbled crayfish were the least sensitive behaviorally, though their survival rates were almost identical to juvenile rusty crayfish. Adult crayfish did not exhibit behavior different from that observed in controls until concentrations reached at least 120 mg/L. Adult crayfish recovered from narcotization quicker than juveniles of the same species. Time for recovery was similar for all juvenile crayfish species. For example, after exposure to 300 mg/L of CO_2 for 60 minutes, average recovery time for juvenile red swamp, rusty, and marbled crayfish was 17.25, 14.25, and 12.55 minutes respectively. Average recovery time for adult crayfish was lower. After exposure to 300mg/L for 60 minutes, recovery time average was 8.75 minutes for adult red swamp and 9 minutes for adult rusty crayfish.

H. azteca showed signs of stress through altered movement (twitching aggressively at the surface of the water), rigidity and maintaining equilibrium, and rigidity and lost equilibrium (narcotization). Similar to crayfish, altered movement and rigidity were rarely shown by *H. azteca* until concentrations reached 120 mg/L. Of those that survived, recovery of *H. azteca* took longer in 60-minute exposure trials than in 30-minute trials. For example, *H. azteca* exposed to 300 mg/L for 30 minutes recovered in an average of 15.28 minutes whereas those in the 60-minute exposure of the same concentration recovered after an average of 27.8 minutes.

Bloody red shrimp was the most severely affected by elevated CO_2 levels. All individuals died during the 30- and 60-minute trials at 70 and 120 mg/L. Based on this high mortality we tested this species at a lower CO_2 level of 50mg/L for a 30-minute trial. At this level we observed 85% mortality and narcotization of >50% of individuals.

D. magna showed effects of CO_2 through movement (hopping, swimming, appendages are moving) and narcotization (remaining motionless on the bottom of the container with no response to stimulus, loss of equilibrium). Counts of narcotization increased with CO_2 concentration, with the majority (>60%) of observations were narcotization at 300mg/L. Mortality of *D. magna* was high in levels ≥ 300 mg/L. Among replicates with *D. magna,* only four individuals recovered from narcotization; the rest died before the 24-hour observation period was over.

Snails and mussels had varied behavioral responses (Figure 4). For example, bladder snails displayed a range of motion behaviors including crawling, swimming, and flicking the shell. Additionally, bladder snails were observed motionless in different states, including stagnant but out of shell, floating, and in shell. Bladder snails decreased motion behaviors with increasing levels of CO_2. Counts of stress behaviors in Chinese mystery snails increased with higher CO_2 levels, but narcotization was not observed at levels below 500 mg/L and all Chinese mystery snails that were narcotized recovered after an average of 6.29 minutes in freshwater.

Dreissenidae responded to treatment by either closing their shell or by showing narcotization (i.e., open valves, extended foot, and no response to poking with stimulus for the duration of exposure). This narcotization effect was only observed in CO_2 treatments of 300 mg/L and higher. Among the species, Dreissenidae had the longest recovery times with an average of 19.82 minutes and 26.89 minutes to recovery of normal behaviors after exposure to 300mg/L for 30 and 60 minutes respectively.

Avoidance Experiments

Results from flow-1 and flow-2 shuttle box treatments were similar. Because of this, we primarily report results here for the flow-1 trials, while comparing results from flow-2 trials in GLMM models below. In flow-1, CO_2 concentration increased from an average of ~5 mg/L to ~324mg/L over the

30-minute period while conditions in the freshwater chamber were unaltered and conditions within the shuttling tunnel increased much more slowly to ~18mg/L.

CO_2 treatment was significantly associated with avoidance behavior in all crayfish (adult and juvenile rusty, adult red swamp, and juvenile marbled crayfish) except for juvenile red swamp. Individuals of the taxa that avoided CO_2 were significantly more likely to be recorded in the freshwater chamber over time as CO_2 levels in the CO_2 chamber were rising. Although treatment had a significant effect on these crayfish, we did observe organisms cross back and forth between freshwater and CO_2 chambers during the experiment. For adult crayfish, these occurrences were infrequent. For example, there were only four counts of adult rusty crayfish re-entering the CO_2 chamber after exiting, whereas juvenile rusty crayfish crossed back and forth between chambers more frequently.

Over the course of the experiment, juvenile red swamp and *H. azteca* individuals crossed back and forth between chambers the most frequently, despite showing signs of stress during the treatment (altered movement, lost equilibrium, and narcotization).

CO_2 treatment had a significant effect on avoidance behavior in both 'flow 1' and 'flow 2' shuttle box tank arenas for juvenile rusty crayfish and marbled crayfish. Juvenile red swamp was the only crayfish group where treatment did not have a significant effect on vacating the CO_2 chamber in neither flow-1 nor flow-2. Similarly, treatment did not have a significant effect on avoidance of CO_2 within the shuttle box for *H. azteca* in either flow direction.

Avoidance- Snails crawling above waterline

CO_2 treatment did not significantly affect avoidance behavior (crawling above waterline) in Chinese mystery snails. However, there was a significant effect of treatment on closed opercula (GLMM, χ^2 = 52.86 , p = **<** 0.001) suggesting that rather than leaving water with high CO_2 concentrations Chinese mystery snails respond by closing their opercula. Treatment did have a significant effect on bladder snails crawling above the water surface, and this effect was strongest in the 300 mg/L treatment where 95% of individuals moved above the waterline after 60 minutes.

Accomplishments

As described above, all of the objectives were met during this project, enabling us to achieve our goals. Across a broad taxonomic range we have shown that the electric barrier in the CAWS – operating at 100% of its current strength – does not cause large changes to the behavior of invertebrates. We believe that any species transiting the CAWS from the Great Lakes to the Mississippi (i.e., downstream) have potential to be moved through the barriers in river flow and are likely to be alive on the other side. In contrast, invertebrates may not be able to transit the barriers in the other direction (i.e., upstream) by swimming alone. However, most of the species that we tested are most likely to move attached to the hulls of boats, and our results indicate that although they may be affected while within the barriers they are likely to survive to the other side.

Higher electric field strengths elicit stronger responses from invertebrates, and it is likely that the higher strengths we tested would provide a greater deterrent to the spread of invasive invertebrates. We note, however, that these field strengths are likely impossible due to cost, and due to the danger of moving boats through water with such a strong electric field.

Results for carbon dioxide were broadly similar. With just one exception all of the invertebrate species tested were minimally affected by concentrations of carbon dioxide that are currently permitted, and these species all showed full and rapid recovery. The exception is the bloody red shrimp which had very high mortality even at the lowest treatment levels carbon dioxides. This is a promising result because this species is currently established in Lake Michigan and is considered a high risk of moving into the Mississippi River Basin through the CAWS. Our result show that permitted levels of carbon dioxide may be sufficient to kill this individuals of this species.

• Potential Applications, Benefits, and Impacts

Our results have direct and immediate application to the management of invasive species in the Chicago Areas Waterway System. We show that the current efforts to prevent the spread of invasive species, and one of the proposed methods, are unlikely to be effective for slowing the spread of invasive invertebrates. This is an important result because it illustrates the need for additional measures if invasive invertebrates are going to be prevented from moving between the Great Lakes and Mississippi River Basins.

While our results are not promising for preventing the spread of invasive invertebrates, they do provide useful information for the use of electrical and carbon dioxide barriers in areas with native invertebrates. Specifically, our results show that the application of electrical and carbon dioxide barriers to prevent the spread of fishes is unlikely to significantly affect native invertebrates.

• International Implications If applicable to your report.

The Chicago Area Waterway System (CAWS) is a system of canals that artificially connects two previously separated aquatic ecosystems. Canals have been constructed across the globe, and in many regions these canals are of concern for the passage of invasive species. The US Army Corps of Engineers and its partners are at the forefront of attempts to prevent the spread of invasive species through canals, and there is much international attention being paid to the electric barriers in the CAWS. We project that the same will be true if the carbon dioxide barriers are implemented. Our work is the only (of which we are aware) that explicitly investigates how these barriers affect invertebrates. Thus, our work will be relevant to any other regions – both in the U.S. and internationally – that are considering these barrier technologies.

• Data Management Plan

We foresee that two main types of data from our work will be relevant to other researchers and the wider community. First, we overcame a lot of logistical and equipment hurdles to develop our electric and carbon dioxide barriers. Other researchers interested in similar questions will likely benefit from our experience. To facilitate this, we have already published full details of our electric barrier equipment. This includes model numbers and suppliers of all equipment, and the ways that different components were wired together. These data are included in Egly et al. 2021. Full details of our development of the carbon dioxide barrier experimental setup – including the shuttle tanks – are included in Colette Copic's thesis which is publicly available. These details will also be published in upcoming manuscripts.

Second, the results from our experiments will be interesting to professionals and the public interested in the passage of invasive species through the Chicago region, and through other freshwater systems worldwide. Full data from all trials will be published. Much of this is already publicly available in Copic's thesis and in Egly et al. 2021. We anticipate submitting the final electric barrier results to a peer-reviewed journal in early summer, 2023.

Section C. Outputs

• Media Coverage

Sea Grant has published some shorter pieces about our work.

• Publications, Theses, Dissertations

Copic, C. 2021. The Efficacy of abiotic barriers at preventing the spread of invasive invertebrates in the Chicago Area Waterway System. School of Environmental Sustainability, Loyola University Chicago. Masters Thesis.

Egly RM, RD Polak, ZA Cook, HD Moy, JT Staunton & **RP Keller**. 2021. Development and first tests of a lab-scale electric field for investigating potential effects of electric barriers on aquatic invasive invertebrates. *Frontiers in Ecology and Evolution, section Biogeography and Macroecology* 9:631732. https://doi.org/10.3389/fevo.2021.631762

Two additional papers are in preparation and will be submitted to peer-reviewed journals during 2023. These are a paper reporting on the full carbon dioxide results (lead author Colette Copic) and a paper reporting the full results from our electric barrier testing (lead author Rachel Egly).

The following presentations have been based on work conducted for this project:

Szklaruk N, R Egly RP Keller. August 25, 2022. Effects of Electric, Carbon Dioxide, and Water Quality Barriers on Invasive Invertebrates. Presentation in the session *Detection, Control and Eradication of Invasive Crayfishes* at the Annual Meeting of the *American Fisheries Society* (Spokane, Washington).

Keller RP, C Copic & R Egly. January 27, 2021.The effect of electric and carbon dioxide barriers on invasive invertebrates. Invited presentation to the *Asian Carp Regional Coordinating Committee.*

Cook Z*, R Egly, J Staunton, H Moy, R Polak and RP Keller. January 2021. Effectiveness of Electric Barriers in Preventing Spread of the Invasive Invertebrates *Apocorophium lacustre* and *Procambarus clarkii*. Oral presentation at annual Midwest Fish and Wildlife Conference.

Copic C^, N Szklaruk and RP Keller. May 2021. The efficacy of dissolved carbon dioxide for preventing spread of aquatic invasive invertebrates. Oral presentation at annual meeting of the Society for Freshwater Science.

• Undergraduate/Graduate Names and Degrees

The carbon dioxide barrier work was led by Colette Copic. Colette earned their Masters of Environmental Science through this work. Much of Colette's Masters degree was funded by this work.

The following undergraduates at Loyola University Chicago also conducted research as part of this project. Almost all of these students received Loyola funding to support their work:

Megan Barrera, Julianna Scivinsky, Rose Mohammadi, Zalia Cook, Harrison Moy, Jonathon Staunton, Sarah Sukanen, Justin Hammeline, Tommy Sisk, Turner Mullarkey, Lauren Hammett, Natalia Szklaruk, Kannon Larison, Emma Donnelly, Dylan Herald, Mikayla Ballard, Sydney Ware, and Madeline Misicka.

• Other Outputs

None.

• Patents/Licenses

None.

• Project Partnerships

Throughout this project we worked closely with Dr. Robert (Bo) Polak and his students. He was a lecturer at Loyola and recently moved to Kent State University in Ohio, where he is continuing to collaborate with us.

This project strengthened the relationship between the Keller lab at Loyola University Chicago and Cory Suski's lab at University of Illinois at Urbana-Champaign. This has led to new projects (see next section).

• Related Projects

Funding from Sea Grant has stimulated several additional projects in the Keller lab. These projects have received funding from other sources, including internal funding from Loyola, US Fish and Wildlife Service, and the Illinois Department of Natural Resources.

Additional projects that have emerged from the research reported here are:

- The extent to which boats transiting the electric barriers affect the strength of the electrical field. While this is known at a coarse scale, it is not yet known at the finer scale, which is the scale at which invertebrates would experience the field.
- An additional barrier in the CAWS may be water quality. Water moving out of the Chicago region contains many pollutants and these may deter the spread of invertebrates. We are working to determine how the behavior and survival of a range of species is affected by being placed in CAWS water. This work is being conducted in collaboration with scientists at USGS and University of Illinois.
- We have received funding to determine the lethal limits of carbon dioxide for a range of invertebrates. This will help the USGS, USEPA and others to determine whether carbon dioxide can be a lethal control for invertebrates.

• Awards and Honors

None.