An Evaluation of Carbon Dioxide and Water Flow as Control Techniques for the Invasive Red Swamp Crayfish

by

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Abstract

The Red Swamp Crayfish (RSC) is an invasive crayfish species that has invaded many water bodies in North America and other continents. Recent work has demonstrated the potential of carbon dioxide (CO_2) as a tool to drive crayfish out of invaded ponds, or to push them towards low CO_2 areas for increased capture. Additionally, these studies showed that there was a relationship between crayfish behavior and the presence of water flow. Due to the need to evaluate new control techniques for RSC, we estimated the number of crayfish that moved to a low CO_2 concentration refuge or emerged from the water surface in both field and lab studies. Additionally, we estimated the number of crayfish that moved towards a small area with water flow in it with field and lab studies. Results suggested that CO_2 did not cause crayfish to move to a lower concentration, however, crayfish exposed to CO_2 were more likely to emerge than crayfish in control settings. Water flow attracted RSC in a lab setting but not in a pond setting.

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List of Abbreviations

CO_2	Carbon dioxide
RSC	Red Swamp Crayfish
mg/L	Milligrams per liter
DO	Dissolved oxygen
LPM	Liters per minute
PVC	Polyvinyl chloride
Id	Inner Diameter
CV	Current velocity
Glm	generalized linear model

Chapter 1: Evaluation of carbon dioxide as a control technique for Red Swamp Crayfish

Introduction

Red Swamp Crayfish (RSC) is considered an invasive, aquatic nuisance species in many parts of the world. Three main categories of control techniques - biological, physical, and chemical - have been developed and evaluated in lab and field experiments in attempts to control their invasion. One type of biological control involves the introduction of predators to invaded waters. For example, freshwater European eels (*Anguilla anguilla*) introduced to RSC invaded waters have shown potential as a valuable supplemental tool, in addition to trapping, to decrease RSC populations (Aquiloni et al, 2010). Another type of biological control focuses on the sterilization of male crayfish using x-ray irradiation (Aquiloni et. al, 2009). Reproductive behavior is not affected by the irradiation, allowing for competition between released, sterile males and wild, non-treated males to potentially lower RSC recruitment and abundance. Potential limitations of this technique include cost and labor suggesting it may be most effective when used in combination with other control techniques (Piazza et. al, 2015). Another type of biological control involving reproduction is the creation and release of “neo females” by manipulating the insulin-like androgenic gland hormone (IAG). (Savaya et al, 2020). These “neo females” only produce male progeny and represent a way to manipulate population dynamics in the wild and that could help reduce RSC populations as part of an integrated management plan (Savaya et al, 2020).

Physical control methods include trapping, barriers, and electroshocking. Similar to biological control techniques, none of these techniques have been proven to eradicate populations of RSC on their own (Stebbing, 2014) with each technique having some bias.

Limitations include size and sex bias. Baited funnel crayfish traps tend to be biased towards the removal of large males (Gherardi, 2011). Female RSC are less susceptible to baited funnel crayfish traps and can carry more pleopodal eggs than many other crayfish species (Holdich and Lowery, 1988). Although baited trapping may reduce population numbers, eradication by trapping alone is nearly impossible. Electroshocking is less selective than trapping (Price, 2009) and has been used to shock and immobilize crayfish for easier collection and removal. However, crayfish that are immobilized while in shelters (i.e., under rocks or undercut banks) may remain in shelter and recover without being collected (Stebbing, 2014). Another physical management technique is the implementation of barriers to prevent RSC populations from moving to un-infested reaches of the stream (Stebbing, 2014). However, crayfish can emerge from the water and move past many physical barriers (Frings et. al, 2013). Additionally, crayfish may bypass barriers via transportation from invaded areas to other areas by predators or people.

Multiple chemicals have been tested for crayfish control. Pyrethrum is a natural extract from chrysanthemum family of flowers. It contains multiple toxic substances that have short persistence times in natural environments (Lidova, 2019). Another chemical applied to water bodies for crayfish control is rotenone. However, crayfish have a higher tolerance to rotenone than fish and amphibians (Peay, 2001) leading to increased cost and effort relative to control of other taxa. A major drawback of most chemical control agents is that they are not specific to the target organism and may kill large numbers of desirable, non-target species.

Carbon dioxide is receiving increasing attention as a chemical control technique for invasive species. Carbon dioxide occurs naturally in aquatic environments, does not persist long at artificially high concentrations following application to waterbodies, and it is commercially available (Treanor, 2017, Cupp, 2017). Carbon dioxide has been used to deter or repel other invasive species including invasive carp (Cupp, 2017). CO_2 concentrations that facilitate RSC behaviors such as avoidance shuttling or emergence have been identified in lab trials (Fredricks et al., 2020). In field trials, concentrations of ≥200 mg/L caused movement of RSC towards the edges of small ponds (Abdelrahman et. al, 2021). Red Swamp Crayfish experience loss of equilibrium at CO_2 concentrations ~ 1,231 mg/L (Fredricks et al., 2020). It is important to keep concentrations high enough to induce avoidance behavior and low enough to not cause loss of equilibrium. These studies support additional testing of CO_2 as a control technique on RSC in larger, more natural field settings.

Experimental ponds provide an optimal environment for testing CO_2 control techniques because they are semi-natural while allowing for replication (Treanor, 2017). It has been suggested that CO_2 should be evaluated to “push” crayfish from one end to the other in large ponds (Fredericks et al., 2020, Abdelrahman et al., 2021). Theoretically a CO_2 gradient (as opposed to uniformly high CO_2 concentrations throughout) would keep crayfish within the pond instead of causing them to emerge. Crayfish emergence requires fencing and/or pit traps to reduce chances of crayfish from spreading from one pond to another (Abdelrahman et al., 2021). Constructing fencing and having people patrol the entire pond perimeters takes time and money and requires increased allocation of resources in larger ponds. “Pushing” crayfish to a smaller refuge could reduce the amount of labor, require a smaller area for controlled chemical application, and make capture of crayfish more efficient.

In this study, we conduct a combination of field and laboratory studies to evaluate the effectiveness of CO_2 gradients to push crayfish towards a low CO_2 refuge. Main objectives were 1) development of methodology to create a CO_2 gradient in 0.1 ha ponds and 2) determining whether CO_2 gradients cause crayfish to migrate to low CO_2 refuge areas.

Methods

Experimental Animals

RSC were trapped throughout the year from wild, resident populations living in earthen ponds at the E.W. Shell Fisheries Research Station, Auburn, Al. Frabill crayfish traps (vinyl-coated, 42x23cm; Frabill, Plano, IL, USA) were baited with Nine Lives canned cat food, and crayfish collected no more than 48 hours after setting the traps. After collection, crayfish were distributed among eight indoor, flow-through raceways (3.21l, 0.87w, 0.61h in meters) at ambient water temperatures. Each tank was equipped with artificial shelters composed of Polyvinyl chloride (PVC) ribbon (Bio-Fill, Pentair Aquatic Eco-Systems, Inc.; Apopka, FL, USA) and 2.54-3.81 cm diameter PVC pipe segments to minimize aggressive behaviors and to serve as biofilters for water quality maintenance. Crayfish density was kept at < 200 crayfish / raceway. Crayfish were fed Ziegler shrimp feed once a day every other day (Shrimp Grower SI-35 3/32") ad libitum. The water used for the flow through tanks was sourced from a nearby reservoir and allowed the crayfish to also prey upon small invertebrates such as snails and blood worms that were carried in with the reservoir water. After crayfish were used in experiments, they were released in a pond planted with rice to provide natural forage and shelter and not used again in subsequent trials.

Crayfish Labeling

Crayfish were labeled with Bic WhiteOut to increase visibility in behavioral experiments (Jurcak, 2014). Prior to trials, crayfish were gently scrubbed of any ectobionts and pat dried using a paper towel. Then the appropriate symbol was painted on the crayfish’s carapace below the cervical groove with whiteout (See Table 1). Crayfish were placed in shallow water to allow the white out to dry before placing them in overnight holding tanks prior to experiments.

Water Sampling

Water samples were collected from experimental ponds or tanks using "Jar Stick Samplers". Each sampler consisted of a 16-ounce Mason jar attached to the bottom of either a 1.82m wooden plank or 1.82m 1" PVC pipe. The side of the jar was pressed against the pole such that the bottom of the jar would be adjacent to the bottom of the plank or pipe. A cork was fit snugly into a hole cut into the lid of the jar. A string ran from the eyelet screw through the cork to the top of the pole. To collect samples, a sampler was lowered into the pond or tank until the bottom of the jar reached the bottom of the habitat. The cork was then removed using the string, and water was allowed to flow into the jar. Once the jar was filled with water, the sampler was carefully brought up and gently poured into a wide mouthed 250 ml sampling bottle. Sample bottles were filled to the top, sealed with a lid, and then placed upright and securely into a bucket to prevent the samples from being disturbed and losing gas. Preliminary trials showed no significant differences between CO_2 concentrations in samples collected with the Van Dorn sampler compared to the jar stick sampler (two tailed t-test; p=0.858; Sigma Plot version 13.0, Systat Sofware Inc.) (G. Elliott, unpublished data).

CO_2 measurements

Samples were titrated for CO_2 concentration (mg/L) using the Sodium Hydroxide Method (Hach Method 8205). One pillow of Hach phenolphthalein indicator powder was placed in the water samples and then a Hach digital titrator (Digital Titrator, 0.00125 mL/digit, Hach) was used in conjunction with a sodium hydroxide cartridge to titrate samples to a 8.3 pH endpoint as indicated by color change (Hach Method 8205).

pH measurements

An Oakton™ pH Testr™ Waterproof Pocket pH Tester was calibrated using the three-point calibration technique with 4.01, 7.00, and 10.01 Oakton buffers the morning of every experiment. The pH probe was placed in each sample jar until the pH stabilized and then was recorded.

Experiment 1: Push Trials in Ponds

Pond set up

Experiments were conducted in ~0.1 hectare (55m x 15m), earthen ponds that were sloped towards one end. Approximate depth was 1.5m at the deep end. At the shallow end, seine fencing was set up at 45-degree angles to create a “W” shape across the width of the pond. Both “points” of the “W” had a ~0.6-m wide opening such that the fencing served to create two large funnel traps located ~14m from the shallow edge of the pond (Fig. 1.1). Seine material was supported with metal fence posts, with the top of each seine extending above the water surface and seine bottoms buried 0.3 meters into the pond bottom. Two days prior to each experiment, ponds were drained until the seine fencing was fully visible. The seines were repaired if there were any holes, and then the ponds were refilled the same evening using water from a nearby reservoir. Standpipes in the deep ends of the ponds were set to ensure similar depths between ponds. Inflow pipes in the shallow end were shut off the evening before the experiment.

Within each of the openings in the seine funnel-trap, four custom-made traps were placed: two on the bottom and two on the top for a total of eight traps per pond (Fig. 1.1). The openings of the traps faced the deep end of the pond. Traps were constructed using ¾ in. PVC, and Tenax hardware net with 1.27cm x 1.27cm mesh (Fig. 1.1). Several preliminary raceway and pond trials showed homemade traps effectively trapped and retained RSC (G. Elliott, unpublished data).

Each pond was organized into four zones of equal lengths. Zone 1 was at the deepest end of the pond where CO_2 was applied via diffusers. Zones 2, and 3 were the middle two quarters of the pond (Fig. 1.2). Zone 4 was at the shallow end. The seine fencing was located along the Zone 3 and 4 boundaries. The refuge was on the other side of the fencing in the shallowest part of the pond (Zone 4). Three Styrofoam floats were anchored in each zone (left side, center, and right side) to mark fixed-site locations for water sampling. The left and right-side sampling floats were placed two meters in from their respective banks, and the center sampling float was placed midway between them.

CO_2 Diffusion

Carbon dioxide was supplied to treatment ponds via compressed medical-grade CO_2-gas cylinders (Air gas® Part Number CGA-940; Airgas Inc.). Seven CO_2 tanks were placed around pond edges of Zones 1 and 2 (Fig. 2), with two of the tanks in Zone 1 serving as spares to be used if other tanks were depleted before the end of the experiment. Each tank was equipped with a Victor ESS42 Next Generation High-Capacity pressure series regulator (Edge Series 2.0). A 5.49- m length of 0.95-cm inner diameter clear vinyl airline tubing was then used to attach a 10-100 LPM air gauge flow meter (10-100 LPM Brooks- 2500 Series) to the CO_2 regulator. Preliminary trials showed 5.49 meters was the shortest length of tubing that prevented the flow meter from reading inaccurately due to freezing (G. Elliott, unpublished observations). A 6.1 m length of airline tubing ran from the flow meter to a T-splitter, which connected two, 6.1-m lengths of tubing to two, 55.8- cm plastic, low pressure air diffusers (Model SB-50: manufacturer) in Zone 1 or two 30.48 cm air stones (Pentair ASI model Sweetwater generation 2 air stones) in Zone 2.

The day before an experimental trial, 240 crayfish were haphazardly collected from the flow-through holding tanks described previously. Crayfish were then randomly split into two groups of similar sizes and assigned to either the control or CO_2 treatment. Crayfish from each group were then further divided into three groups of 40 to be released into either zone 1, 2, or 3 and then labeled with a unique symbol designating zone, treatment, and trial assignment. Visually disparate symbols were chosen so that they would not be easily mistaken for each other (Table 1.1).

Four experimental trials were completed between August 27, and October 1, 2020. Each trial was initiated at approximately 7 am and included one control pond and one CO_2 treatment pond. Initial water samples for CO_2 analysis were collected simultaneously in both the control and CO_2 treatment ponds using the “jar stick samplers” at the marked floats as described previously. Wind speed, and outside temperatures were also recorded (Table 1.2). Dissolved oxygen (DO) in mg/L and temperature in Celsius were measured in each zone of the pond using a YSI Pro O DO meter. All measurements were taken from kayaks. Water samples were collected hourly from the CO_2 treatment pond and at the beginning and end of the trial for the control pond.

Crayfish traps were placed in the gaps of the seine-funnel traps as described previously, and all CO_2 diffusers were placed in the ponds. After initial samples were collected, forty marked crayfish were gently released into the appropriate zone (1, 2, or 3) of each pond for a total of 120 crayfish per pond. This occurred synchronously for the CO_2 application pond and the control pond. Crayfish were left to acclimate in the pond for one hour to approximate the acclimation times of previous experiments (Abdelrahman et al., 2021). After 1 hour, CO_2 was diffused into zone 1 from three tanks at a rate of 48.6 LPM (60 LPM Air x 0.81 conversion factor) per tank. At the start of hour 2, the two tanks in zone 2 were turned on. All five tanks from both zones were periodically adjusted to diffuse at a rate of 48.6 L CO_2 / min (LPM). By hour 4, the tanks in zone 1 were typically close to depletion and replaced with full tanks. By hour 5, the zone 2 tanks were typically close to depletion. At this point, the tanks from the left and right bank were moved from zone 1 to zone 2 to continue pushing high CO_2 concentrations towards the shallow end. At hour 6, final water samples, DO, and temperatures were taken in both ponds and all CO_2 tanks were turned off. After turning off the CO_2 tanks, crayfish were collected from the traps in both ponds. The traps were pulled from each pond at the same time and approached from the shallow side of the seine funnel-trap to avoid scaring additional crayfish into the trap. Crayfish from each pond were kept in separate buckets and then taken to the lab to record sex, wet weight, the pond they were trapped in, and their pond symbol (i.e., whiteout marking).

Experiment 2: Emergence vs Migration in Raceways

Due to emergent behavior observed in the pond trials (see results section), raceway experiments were conducted to test whether CO_2 was more likely to cause crayfish to emerge than to migrate to a low CO_2 refuge area. Each experimental trial included a treatment raceway and a control raceway (dimensions: 6.30 x 2.65 x 0.80 m). Raceways were filled with water from a nearby ~8 ha reservoir the evening before the experiment and divided into three zones of equal length: Zone 1 at the outflow end, zone 3 at the inflow end, and zone 2 in the middle. Six emergence boxes were evenly distributed along the edges of zones 1 and 2 (Fig. 1.3). Emergence boxes were made of Tenax hardware net with 1.27cm x 1.27cm mesh attached to a 71.12x 35.56x 38.10 m pvc frame. Shelters consisting of ~10cm lengths of 1” id PVC pipes were placed into each zone (51 in zones 1 and 2, plus 51 in zone 3) to reduce stress and aggressive interactions among crayfish during trials.

Prior to a trial, 100 crayfish were numbered (1-50 for each raceway) with (White Out, Bic) on the carapace, below the cervical groove. Weight and sex were recorded. Each crayfish was randomly assigned to the control or CO_2 treatment and held in separate flow-through tanks overnight, containing water from the same source as the experimental raceways.

The morning of a trial, 50 marked crayfish were placed into zones 1 and 2 of each raceway and allowed to acclimate for 1 hour. During this time, zones 2 and 3 of the treatment and control troughs were separated by a barrier made of a metal grate covered with heavy-duty black plastic to prevent crayfish from entering zone 3. After acclimation, CO_2 was diffused into zone 2 of the treatment raceway from a CO_2 tank equipped with a Victor ESS42 Next Generation High-Capacity regulator (Edge Series 2.0). An air flow meter (10-100 LPM Brooks- 2500 Series) was used to monitor gas flow from the tank to a plastic low-pressure air diffuser (Model: SB-50; A-MI Corporation, Incheon, South Korea) submerged at the bottom of the raceway. No CO_2 was diffused into the control raceway. The barrier between zones 2 and 3 of both raceways was then lifted and held ~11 cm off the bottom with a row of bricks. The third brick from each raceway side was removed to provide two benthic corridors for crayfish to move between zones 2 and 3.

To create a CO_2 gradient in the treatment raceway and reduce the amount of CO_2 diffusing from zone 2 to zone 3, water was pumped into zone 3 at a constant rate (11 L / minute) using a submersed pump (Supreme Aqua-Mag 1200 GPH Magnetic Drive; Danner Manufacturing Inc.). Two pumps were placed in a neighboring raceway that had been filled the previous day with water from the same source as for the experimental raceways. Two, 3.62-m lengths of 1” id PVC tubing directed water from each pump into zone 2 of the treatment raceway. Water then flowed under the zone 2/3 barrier (described previously), through zone 2, and exited zone 1 via a vertical standpipe at the end of the raceway. To keep flow conditions consistent between treatment and controls, water was also pumped into control raceways using the same methodology.

Water temperature (Celsius) and DO (mg/L) were measured in each zone hourly using an YSI PRO O DO meter. Water samples from each zone were collected using a "Jar Stick Sampler" at the left, right, and center of each zone for CO_2 analysis using methodology described in the previous experiment. Crayfish were observed every 30 minutes for emergence in zones 1 and 2: id of each crayfish that had climbed up an emergence box was recorded. After six hours, the experiment was terminated by moving the zone 2/3 barrier off the bricks and lowering it to the tank bottom. Number of crayfish and id of each crayfish in Zone 3 was then recorded.

Data analysis

All one-way repeated measures ANOVAS, normality tests, equal variance tests and pairwise multiple comparison procedures were conducted in Sigma Plot 13 (2015 Systat Software Inc. version 13.1). Generalized linear models (glm) with poisson distributions were conducted with the program R version 4.02.

Pond analysis

Pond water quality variables: We used one-way repeated measures analysis of variance (ANOVA) to test for differences in CO_2 concentrations among zones for each pond trial. The pond trials on August 27th, September 26th, and October 1st passed the Shapiro-Wilk normality test and Brown-Forsythe equal variance test and the pairwise multiple comparison procedures were ran using the Holm-Sidak method on the CO_2 concentrations among zones for each of the three pond trials. The pond trial on September 10th passed the Shapiro-Wilk normality test, but failed the Brown-Forsythe Equal Variance Test. The pairwise multiple comparison procedures for September 10th were run using a Tukey test on the CO_2 concentrations among zones. Alphas were set at 0.05.

For all four trials, we used one-way repeated measures ANOVAs to test for differences in pH among zones for each pond trial. They all passed the Shapiro-Wilk normality test and passed the Brown-Forsythe equal variance test. The pairwise multiple comparison procedures were done with the Holm-Sidak method. Alphas were set at 0.05.

For the pond trials on September 10th, and September 26th, we used one-way repeated measures ANOVAs to test for differences in DO among zones for each trial. They both passed the Shapiro-Wilk normality test and the Brown-Forsythe equal variance test, all pairwise multiple comparison procedures for these two trials were done with the Holm-Sidak method. For the pond trial on October 1st, we also used a one-way repeated measures ANOVA to test for differences in DO among zones. The trial on October 1st failed the Shapiro-Wilk normality test so we ran a Friedman Repeated Measures ANOVA on ranks. The pairwise multiple comparison procedures were run using a Tukey Kramer HDS test. Our alphas were set at 0.05.

Pond captures: A paired t-test was conducted in Sigma Plot to compare the number of crayfish captures between the treatment and control ponds. A paired t-test was conducted to compare the number of crayfish captures between the North Pond and the South Pond. Our alpha was set at 0.05.

Raceway Analysis

Raceway water quality variables: we used one-way repeated measures analysis of variance (ANOVA) to test for differences in CO_2 concentrations among zones for each of the raceway trials. The raceway trials on January 5th, and January 8th passed the Shapiro-Wilk normality test and Brown-Forsythe Equal Variance Test, and the pairwise multiple comparison procedures were ran using the Holm-Sidak method on the CO_2 concentrations among zones for each of the three raceway trials. The raceway trial on January 18th failed the Shapiro-Wilk normality test and passed the Brown-Forsythe Equal Variance Test. The pairwise multiple comparison procedures were ran using a Tukey Kramer HDS test on the CO_2 concentrations among zones for the trial on January 18th. Our alphas were set at 0.05.

Raceway migration and emergence comparisons: A generalized linear model with a Poisson sampling distribution was used to compare the number of crayfish that moved into zone 3 (refuge) at the end of the experiment between CO_2 and control raceways and to test for significance of crayfish mass, and crayfish sex. A generalized linear model with a Poisson sampling distribution was used to compare the number of crayfish that emerged from the water onto the surface traps between CO_2 and control raceways and to look at significance of crayfish mass, or sex on capture. Our alpha was set at 0.05. For purposes of the analysis, individual crayfish were only counted once as emerged throughout the experiment. Some individuals that emerged during the experiment returned to the water and had migrated to the refuge by hour 6. Thus, some individuals were counted as emerged and as moving into the refuge.

RESULTS

Pond Trials

We effectively maintained a CO_2 gradient in our treatment pond when compared to the control pond. Across all trials, control pond CO_2 concentrations were ≤ 21 mg/L, pH was ≥ 6.71, and DO was > 4 mg/L in all zones (Table 1.2). In contrast, a strong CO_2 gradient was created in each treatment pond with CO_2 eventually rising above our target threshold of 200 mg/L at the deep end while remaining below 100 mg/L at the shallow end (Fig. 1.4). There was a significant difference in CO_2 among all zones in the treatment ponds on the August 27th, September 26th, and October 1st trials ($p<0.05$) (Fig. 1.4, Table 1.3). On September 10th, there was a significant difference in CO_2 between zones 1 and 4, 1 and 3, and 2 and 4 ($p<0.05$). There was no significant difference between zones 1 and 2, 2 and 3, and 3 and 4 ($p>0.05$) (Fig. 1.4).

Application of CO_2 also created a pH gradient across zones. In all four trials, pH was significantly lower in zone 1 than zone 4 of the CO_2 treatment ponds ($p<0.05$). In three of the four trials, zone 3 pH was also significantly higher than zone 1 but significantly lower than zone 4 ($p<0.05$) (Fig. 1.5, Table 1.4).

Dissolved oxygen was not recorded on August 27th. In the remaining three trials, DO did not consistently exhibit a gradient (i.e. a gradual increase across zones) as there were often no significant differences in DO observed among zones 1, 2 or 3. However, a threshold effect was consistently observed with mean DO of zones 1, 2, and 3 being significantly lower than DO of zone 4 in all three trials (Fig. 1.6, Table 1.5).

There was no significant difference in the number of marked crayfish trapped in the control ponds compared to the CO_2 application ponds ($t(6) = -1.192$, $p = 0.278$) (Fig. 1.7). An average of five marked crayfish out of the 120 that were stocked into the pond were captured in the CO_2 treated ponds whereas an average of seven marked crayfish were captured in the control ponds. There was a significant difference between the number of marked crayfish captured between the North Pond and the South Pond ($t(14) = -6.438$, $p<0.001$).

Raceway Trials

We effectively created low CO_2 concentration refuge areas in the treatment raceways and high CO_2 concentrations above 200 mg/L CO_2 in the non-refuge zones. Control raceway CO_2 concentrations were always ≤ 13.4, while pH remained ≥ 6.84. In contrast, we were able create a CO_2 threshold in treatment raceways wherein concentrations in zones 1 and 2 did not significantly differ ($p>0.05$) and consistently exceeded our target of >200 mg/L. Conversely, CO_2 concentrations in zone 3 were significantly lower ($p<0.05$), averaging ≤ 50 mg/L (Fig. 1.8, Table 1.6). Despite the creation of a strong CO_2 threshold between zones, there was no significant difference in the number of crayfish collected from zone 3 (refuge) between CO_2 treatment raceways and control raceways ($p=0.291$ (Fig. 1.9). On average, 26 crayfish out of the 50 crayfish placed in the CO_2 treatment raceways were in the refuge at the end of the experiments compared to an average of 21 crayfish in the refuge of the control raceways. Neither crayfish mass (0.0581) nor sex ($p=0.696$) had a significant effect on the number of crayfish in the refuge (Table 1.7).

High CO_2 concentrations (> 200 mg/L) had a significant effect on crayfish emergence with significantly more crayfish emerging from Zones 1 and 2 in the CO_2 treatment raceways than in the control raceways ($p<0.001$) (Fig. 1.10). An average of nine individual crayfish emerged in the CO_2 treatment raceways per trial, whereas zero crayfish emerged in the control raceways. There was no statistically significant effect of crayfish wet mass ($p=0.0668$) or sex ($p=0.7845$) on crayfish emergence (Table 1.8).

Crayfish that emerged did not remain emerged throughout the duration of the experiment, but few crayfish emerged more than once over the course of the experiment. On January 5th, one crayfish emerged twice. On January 18th, two crayfish emerged twice, and one crayfish emerged seven times.

Discussion

A first step towards using CO_2 as a control measure for crayfish is to examine the feasibility of raising CO_2 concentrations to desired concentrations and/or creating a gradient. In this study, we demonstrated that it is possible to consistently create a CO_2 gradient ranging from ≥ 200 to ≤ 50 mg/L across the length of 0.1 hectare ponds using readily available CO_2 gas cylinders. Larger, liquid CO_2 dewars are also available, but may weigh ~272 kg (~600 lbs) each, may constantly purge excess pressure in warm weather (depleting CO_2 in tanks), and typically require heated regulators to keep them from freezing up. The smaller, gas cylinders we used weighed ~70kg (~170 lbs) when full of gas and were light enough to be loaded into truck beds, could be moved manually in carts, and did not constantly off-gas in warm weather. Heated regulators were not required and issues with freezing were resolved using a ~6 m length of tubing between the cylinder and the flow meter.

There were some drawbacks to using the gas cylinders. Establishment and maintenance of a six-hour CO_2 gradient typically required seven CO_2 cylinders per pond, and CO_2 levels typically did not reach or exceed 200 mg/L until hours 5 and 6. Future experiments using larger, liquid CO_2 dewars may allow for higher concentrations of CO_2 for a longer period of time but would require that logistical challenges related to dewar transport and off-gassing issues be solved.

Creation of a CO_2 gradient also affected pH and, to a lesser extent, dissolved oxygen in ponds. However, the combination of increased CO_2, decreased pH, and decreased DO did not appear to push crayfish to the refuge end of the pond. There was no significant difference between the number of marked crayfish trapped in the refuge of the CO_2 application pond compared to the control pond. This lack of evidence for crayfish moving to a low CO_2 refuge is supported by Robertson (2018) that showed crayfish exposed to high CO_2 spent more time sheltering than wandering. In contrast, Abdelrahman et al. (2021) suggested increased activity of crayfish, with more crayfish observed wandering near pond edges and emerging from ponds when exposed to high CO_2. This discrepancy may be partially explained by vegetation. The small ponds used in Abdelrahman et al. (2021) had limited vegetation in them, and few places for crayfish to shelter. In the current study, significantly more crayfish (marked + wild combined) were trapped in the South Pond, which was much less vegetated (G. Elliott, pers. obs.) than the North Pond – suggesting greater movement in sparsely vegetated ponds.

Although we found no evidence for movement into refuge areas, we observed crayfish would move towards the pond edges and climb aquatic plants to air-breathe at the surface when CO_2 exceeded 200mg/L for over two hours in both zones 1 and 2. Crayfish can air-breathe if their gills are moist, and they often do so in low DO conditions (Morris, 1998). However, DO remained above four mg/L in all zones throughout these trials so this was not a likely explanation for air-breathing behavior. Emergence may have been related to changes in pH. CO_2 can alter the pH of the hemolymph in aquatic organisms, decreasing the oxygen transport efficiency and mimicking effects of hypoxia (Burnett, 1992, Treanor, 2017). Although we did not measure changes in the pH of crayfish hemolymph, it is possible that crayfish were emerging into the air to increase the amount of oxygen flowing across their gills due to pH issues even though the pond water was not hypoxic.

In response to the anecdotal observations of crayfish coming to the surface of the ponds to air-breathe when CO_2 concentrations were high, we conducted raceway trials to quantify the number of crayfish emerging when CO_2 concentrations are elevated or if they would move into a low concentration refuge. Raceway trials provided further evidence that high concentrations of

CO_2 were more effective at inducing emergence behaviors than “pushing” crayfish to a low CO_2 refuge. Significantly more crayfish emerged from the CO_2 treatment raceways compared to the control raceways – which had zero emerged crayfish. In the CO_2 raceways, approximately 18% (N=150) of the crayfish emerged at least once. This is very similar to the percentage (~16%) of crayfish that emerged from small ponds in a previous study (Abdelrahman et al. 2021) when pond perimeters were checked hourly for emerged crayfish. Interestingly, approximately 45-50% (N=150) of crayfish were found in zone 3 (“refuge”) at the end of our raceway trials. This was not due to CO_2 “pushing” the crayfish to zone 3 as we observed the same pattern in the control raceways. Rather we hypothesize crayfish were attracted by the water flowing out of zone 3. Positive rheotaxis has been documented in multiple species of crayfish (Jones 1994, Moore 1999, Frings et al. 2013) and is even used as a trapping technique for Australian red claw crayfish (*Cherax quadricarinatus*) in aquaculture ponds (Masser, 1997). These findings suggest the need for additional studies to determine the effectiveness of water flow used to “pull” Red Swamp Crayfish into a smaller area for easier capture.

Conclusions Regarding CO_2 as a Control Measure

Although this study did not show that we could “push” crayfish towards a low CO_2 refuge as suggested by previous lab trials (Fredericks et al., 2020), it did provide further evidence that CO_2 causes crayfish to move towards pond edges and partially emerge from water (Abdelrahman et al. 2021). More crayfish moved towards the surface in both the CO_2 treated ponds and CO_2 treated raceways compared to the controls. Future studies should investigate the design, construction, and evaluation of shallow water or edge traps to catch the crayfish that come to the pond edges and/or climb aquatic plants and emerged structures to air-breathe. Studies that monitor crayfish movement using telemetry in response to CO_2 would also be useful. For example, placing traps in the locations that crayfish are most likely to move towards when responding to CO_2, could reduce the number of traps, bait and labor needed while still targeting the RSC. Crayfish do appear to respond to increasing CO_2 concentrations. A better understanding of movement patterns in response to CO_2, will facilitate more efficient trap design and deployment strategies that can be used to control crayfish alone or in combination with other removal strategies.

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Table 1.1. Symbols assigned to crayfish in both the CO_2 treated ponds and control ponds.

Date	**Symbols**
Aug. 27, 2020	5, 7, 9
Sept. 10, 2020	J, K, L
Sept. 26, 2020	S, T, U
Oct. 01, 2020	G, H, I

Table 1.2. Water temperatures, air temperatures, and wind speeds of CO_2 treatment pond trials, averaged across all hours. Mean ± *SE*.

Date	Water Temperature °C	Air Temperature °C	Wind Speed (mph)
8/27/2020	30.25+/-0.81	31.48+/-2.21	8.28 +/- 0.56
9/10/2020	28.13+/-0.70	28.43+/-4.56	5.65 +/- 0.35
9/26/2020	25.4+/-1.26	25.83+/-4.68	3.30 +/- 0.23
10/1/2020	23.28+/-1.12	23.98+/-4.26	7.82 +/- 1.21

Table 1.3. Summary statistics for one-way repeated measures ANOVA used to test for differences in CO_2 among zones in experimental ponds. Note that data failed the Brown-Forsythe test for equal variance in the 9/10 trial. Data was therefore analyzed by a Friedman Repeated Measures ANOVA on Ranks for this trial.

Trial Date	Source of variation	DF	SS	MS	F	Chi^2	P
8/27/2020	Between Time (hours)	5	34803.62	6960.724			
8/27/2020	Between Zones	3	75158.81	25052.94	30.7		<0.001
9/10/2022	Between Zones	3				18	<0.001
9/26/2020	Between Time (hours)	5	30239.7	6047.939			
9/26/2020	Between Zones	3	70986.58	23662.19	67.653		<0.001
10/1/2020	Between Time (hours)	5	42611.11	8522.221			
10/1/2020	Between Zones	3	60905.19	20301.73	52.633		<0.001

Table 1.4. Summary statistics for one-way repeated measures ANOVA used to test for differences in pH among zones of experimental ponds.

Trial Date	Source of variation	DF	SS	MS	F	P
8/27/2020	Between Time (hours)	5	3.153	0.631		
8/27/2020	Between Zones	3	9.233	3.073	47.251	<0.001
9/10/2020	Between Time (hours)	5	1.298	0.26		
9/10/2020	Between Zones	3	7.398	2.466	42.576	<0.001
9/26/2020	Between Time (hours)	5	2.734	0.547		
9/26/2020	Between Zones	3	2.784	0.928	16.621	<0.001
10/1/2020	Between Time (hours)	5	3.528	0.706		
10/1/2020	Between Zones	3	2.272	0.757	23.306	<0.001

Table 1.5. Summary statistics for one-way repeated measures ANOVA used to test for differences in dissolved oxygen among zones of experimental ponds. Note that data failed the Brown-Forsythe test for equal variance in the 10/1 trial. Data was therefore analyzed by a Friedman Repeated Measures ANOVA on Ranks for this trial.

Trial Date	Source of variation	DF	SS	MS	F	Chi^2	P
9/10/2020	Between Time (hours)	5	16.825	3.365			
9/10/2020	Between Zones	3	6.103	2.034	8.837		0.001
9/26/2020	Between Time (hours)	5	8.702	1.74			
9/26/2020	Between Zones	3	4.536	1.512	29.392		<0.001
10/1/2020	Between Zones	3				15	0.002

Table 1.6. Summary statistics for one-way repeated measures ANOVA used to test for differences in CO_2 between zones in the raceways.

Trial Date	Source of variation	DF	SS	MS	F	P
1/5/2021	Between Time (hours)	4	2484.52	621.13		
1/5/2021	Between Zones	2	112887.063	56443.53	97.351	<0.001
1/8/2021	Between Time (hours)	4	8633.157	2158.289		
1/8/2021	Between Zones	2	129790.445	64895.22	449.108	<0.001
1/18/2021	Between Time (hours)	4	2367.951	591.988		
1/18/2021	Between Zones	2	170806.827	85403.41	237.867	<0.001

Table 1.7. Summary statistics for generalized linear model with a Poisson distribution used to test for differences between CO_2 and control raceways in the number of crayfish that moved into the refuge area.

Coefficients	Estimate	Std. Error	t value	Pr(>|t|
(Intercept)	0.70618	0.12003	5.884	1.05E-08
TreatmentControl	-0.0596	0.05635	-1.058	0.2908
SexM	-0.0237	0.06061	-0.391	0.6963
Weight	-0.0097	0.00511	-1.902	0.0581

Table 1.8. Summary statistics for generalized linear model with a Poisson distribution used to test for differences between CO_2 and control raceways in the number of crayfish that emerged.

Coefficients	Estimate	Std. Error	t value	Pr(>|t|
(Intercept)	0.110348	0.080697	1.367	0.1725
TreatmentControl	-0.197653	0.037887	-5.217	3.35E-07
SexM	-0.011155	0.040749	-0.274	0.7845
Weight	0.006317	0.003434	1.839	0.0668

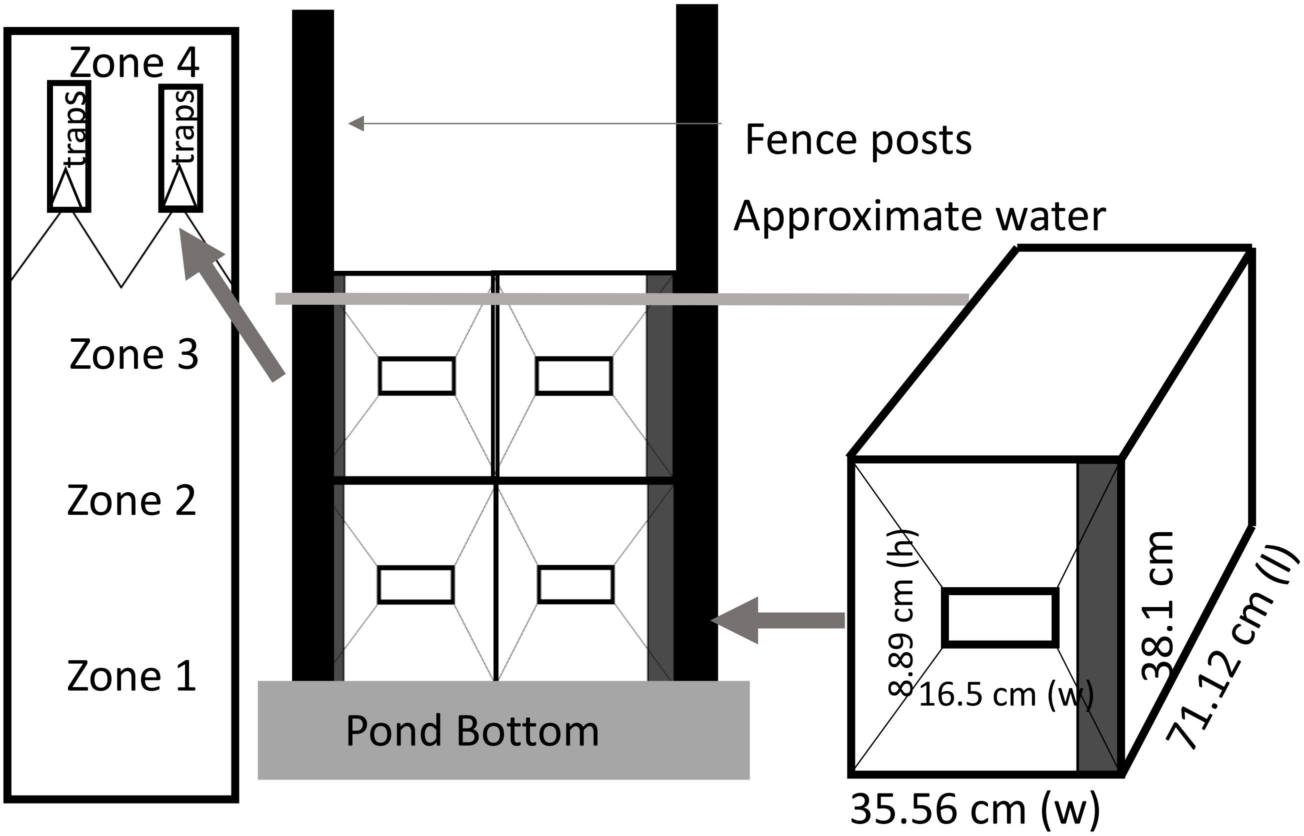

Figure 1.1. Left: Overhead schematic of pond showing location of pond zones, seine fencing used to create two large funnels, and crayfish traps. Center: Cross section schematic showing how ponds were stacked across seine openings. Right: Trap dimensions.

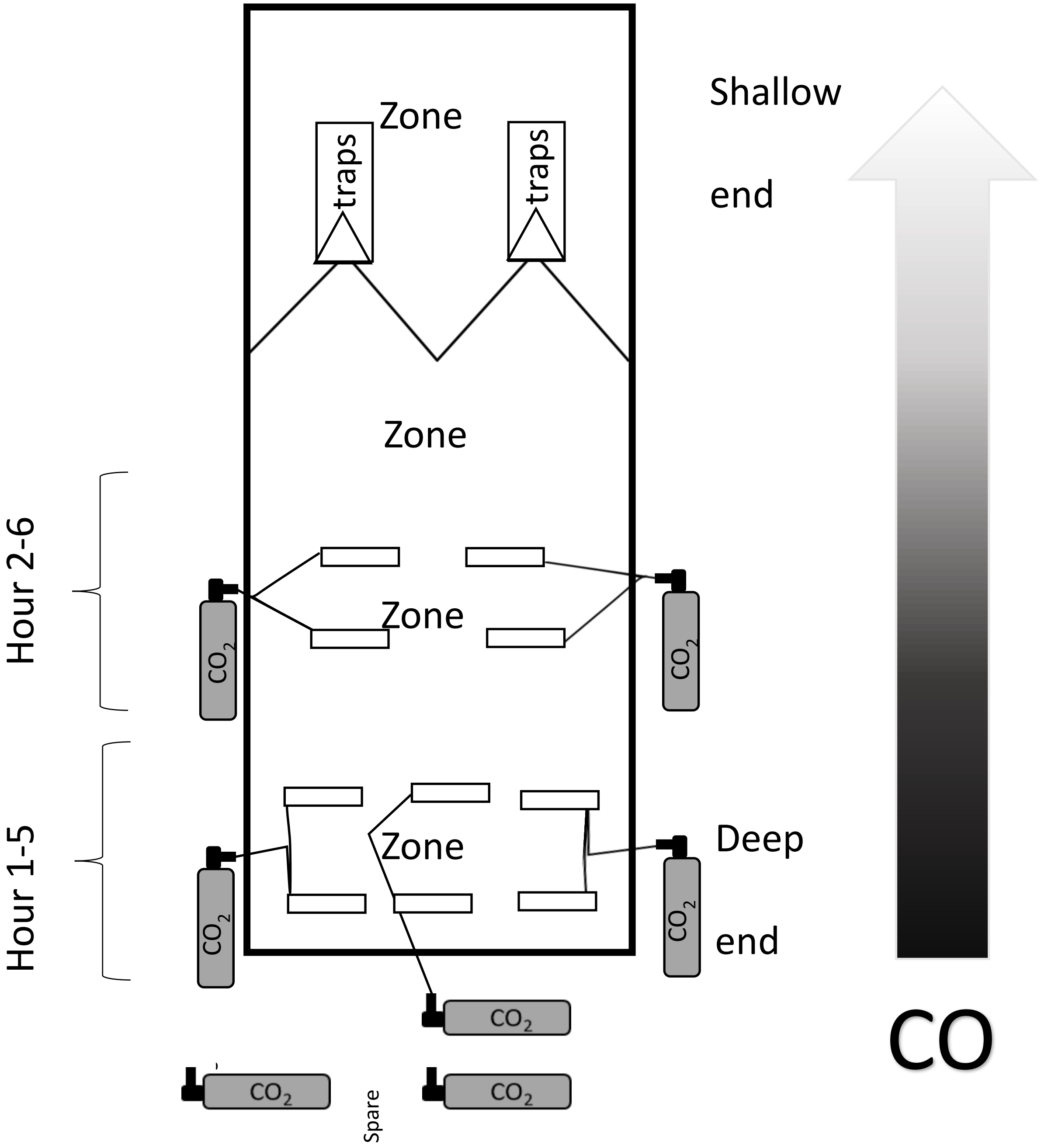

Figure 1.2. Birdseye schematic of treatment pond. Small rectangles connected to CO_2 tanks depict placement of diffusers. Tanks and diffusers within the Hour 1-5 bracket depict the tanks within zone 1 that were diffusing CO_2 for 5 continuous hours after crayfish acclimation. The two tanks within the Hour 2-6 bracket are in zone 2 and were diffusing CO_2 starting 2 hours after crayfish acclimation and continued to diffuse CO_2 until the end of the experiment.

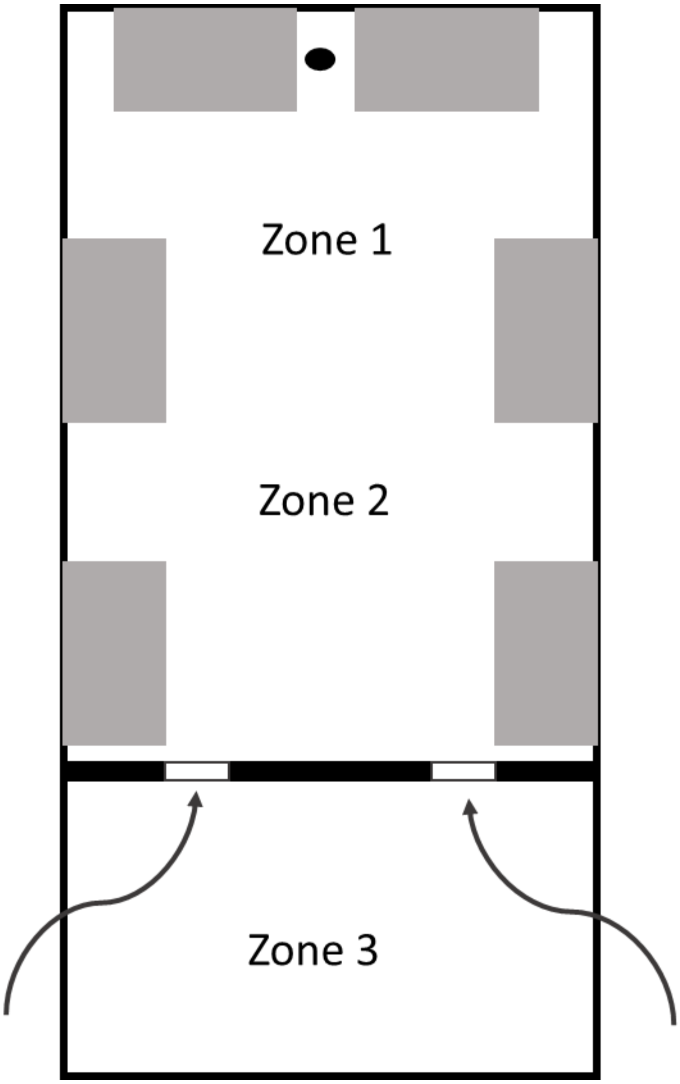

Figure 1.3. Overhead schematic of raceways. Zones 1 and 2 contained emergence boxes represented by grey squares and were separated from Zone 3 by a barrier which could be lifted onto a line of bricks to create two corridors connecting Zone 3 with Zone 2. Water was pumped into Zone 3 and exited the raceway via a standpipe in Zone 1.

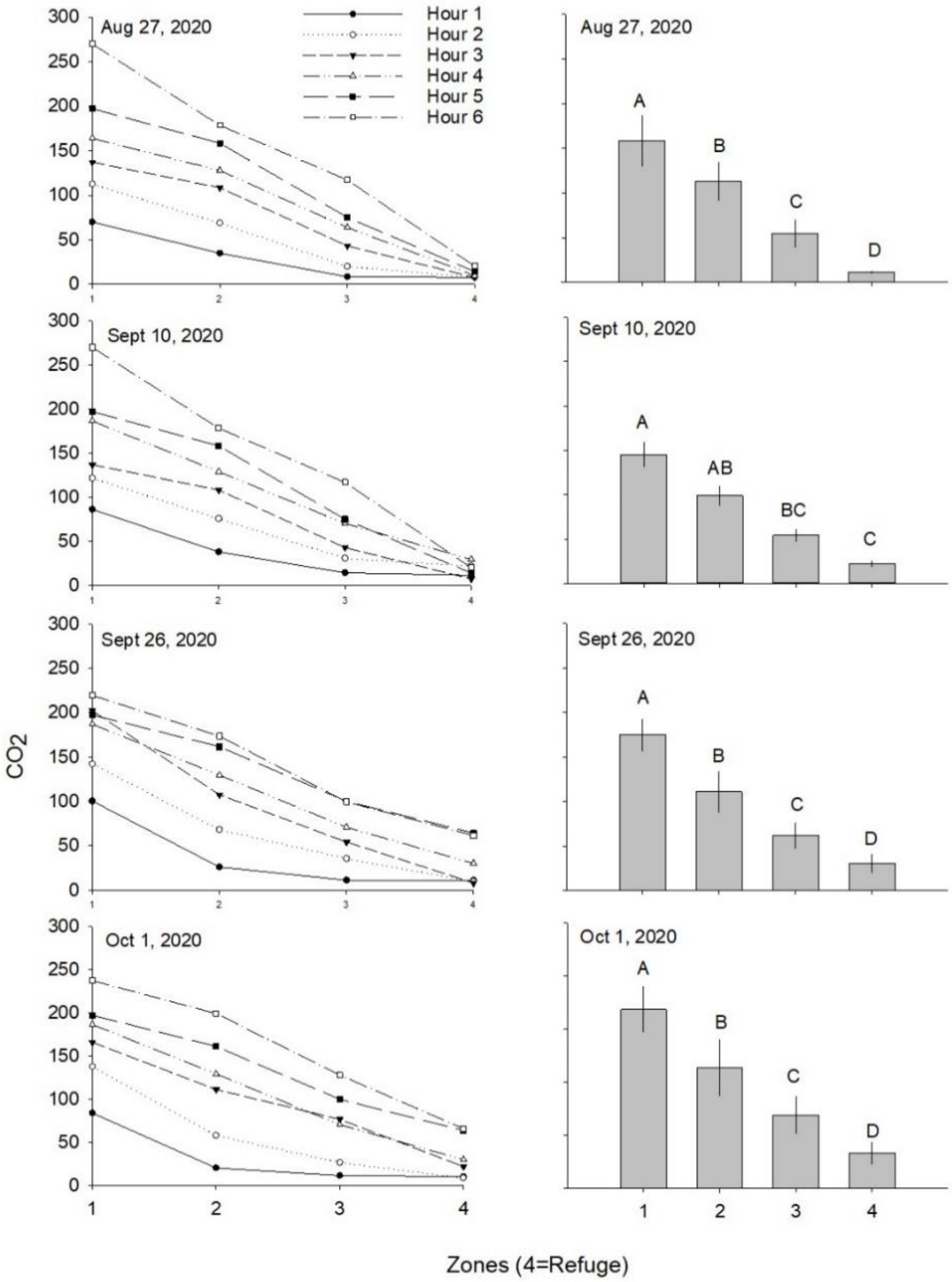

Figure 1.4. Left panels: Carbon dioxide concentrations in each treatment pond zone during hours 1-6 of each trial. Right panels: Mean carbon dioxide concentrations across all hours in each zone of treatment pond in each trial. Error bars represent ±*SE*. Letters above bars indicate significant differences in CO_2 concentrations among zones.

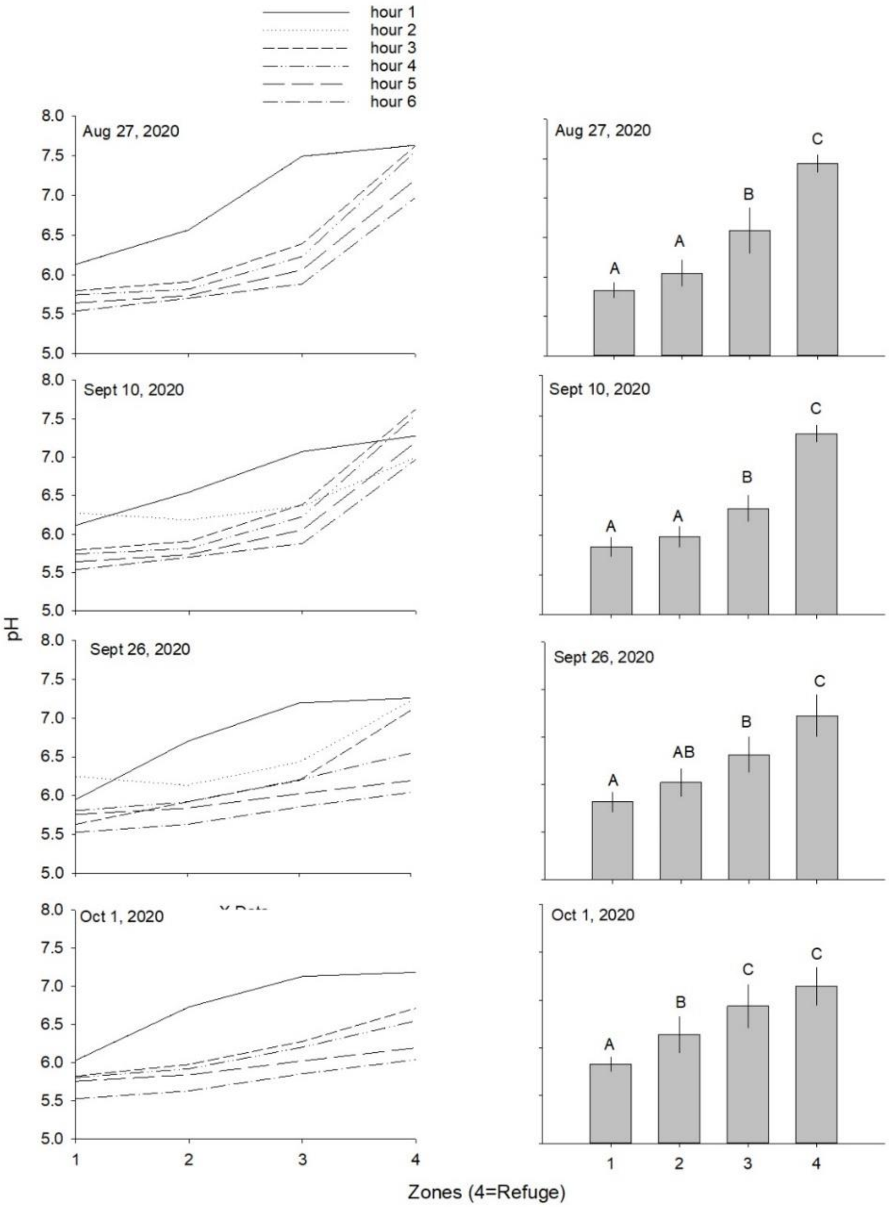

Figure 1.5. Left panels: pH of each zone of the treatment pond during hours 1-6 of each trial. Right panels: Mean pH across all hours in each zone of the treatment pond in each trial. Error bars represent ±*SE*. Letters above bars indicate significant differences in CO_2 concentrations among zones.

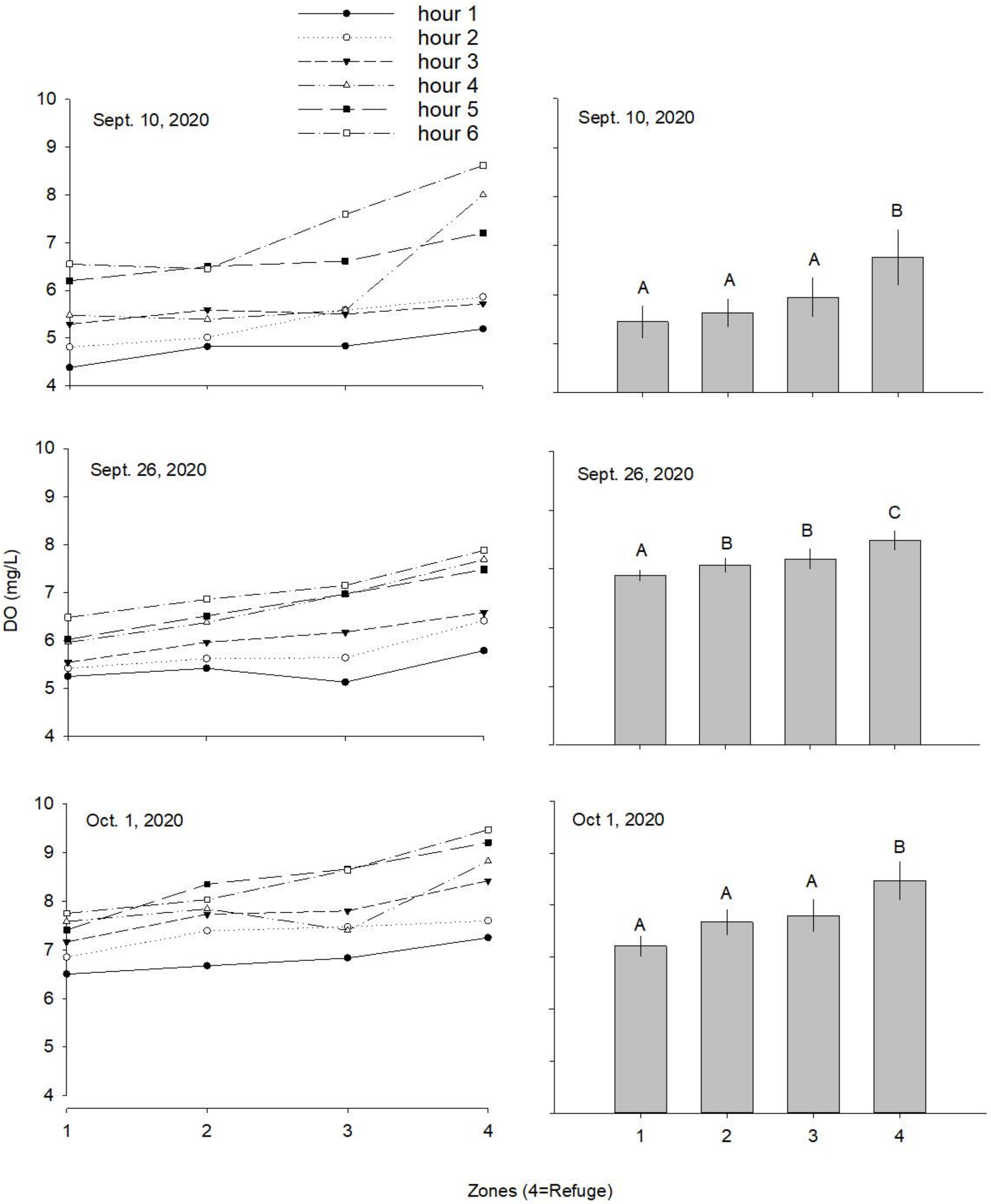

Figure 1.6. Left panels: Dissolved oxygen (DO) concentrations in each treatment pond zone during hours 1-6 of each trial. Right panels: Mean DO concentrations across all hours in each zone of treatment pond in each trial. Error bars represent *SE*. Letters above bars indicate significant differences in CO_2 concentrations among zones.

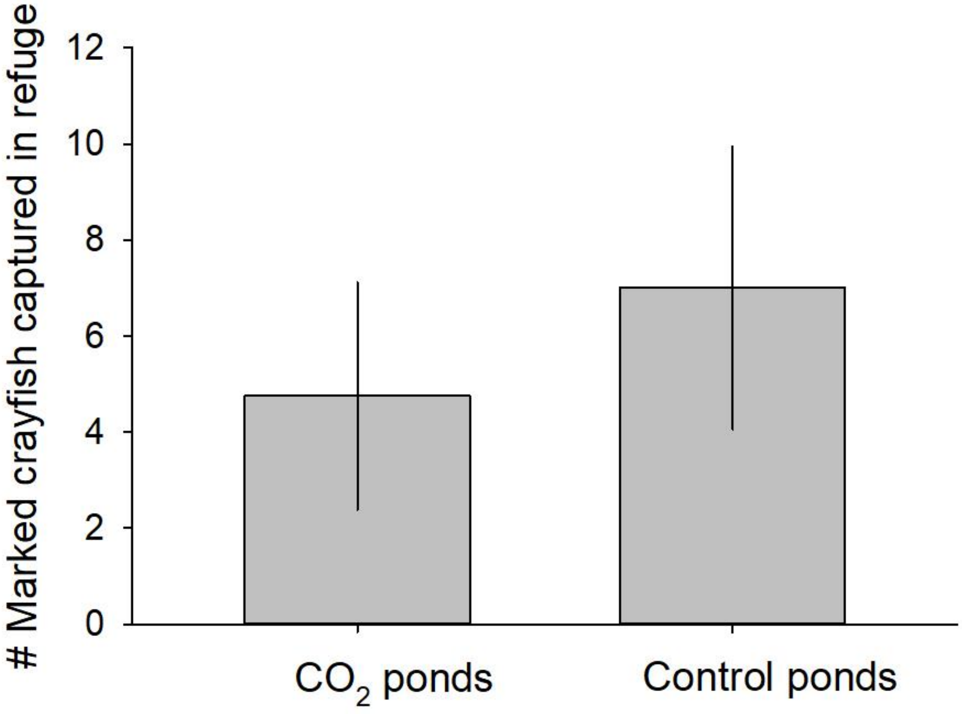

Figure 1.7. Mean number of marked crayfish captured in the refuge (Zone 4) of the CO_2 treated ponds and control ponds. Error bars represent *SE*.

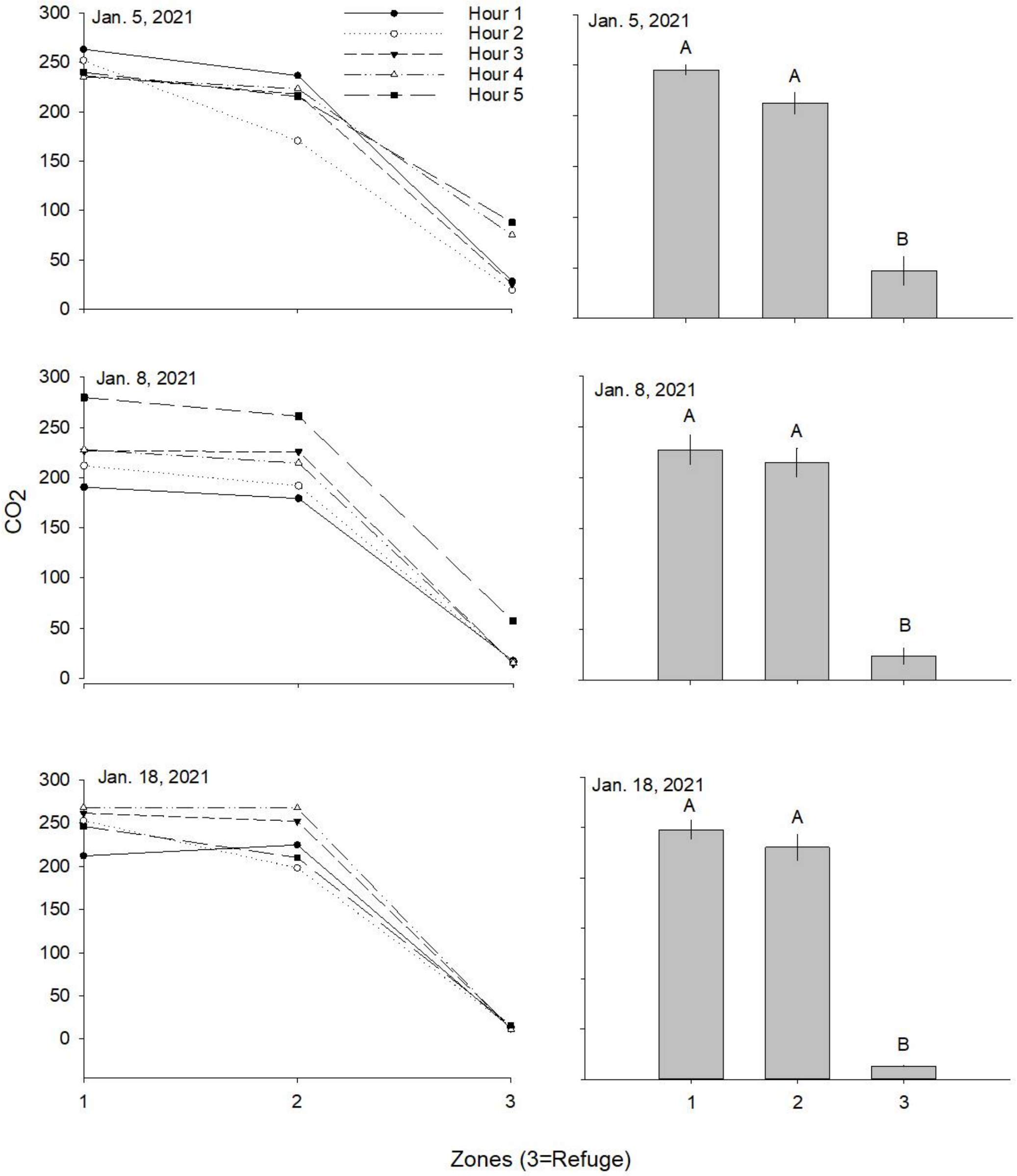

Figure 1.8. Left panels: Carbon dioxide concentrations in each zone during hours 1-5 of the treatment raceway in each trial. Right panels: Mean CO_2 concentrations across all hours in each zone of the treatment raceway in each trial. Error bars represent *SE*. Letters above bars indicate significant differences in CO_2 concentrations among zones.

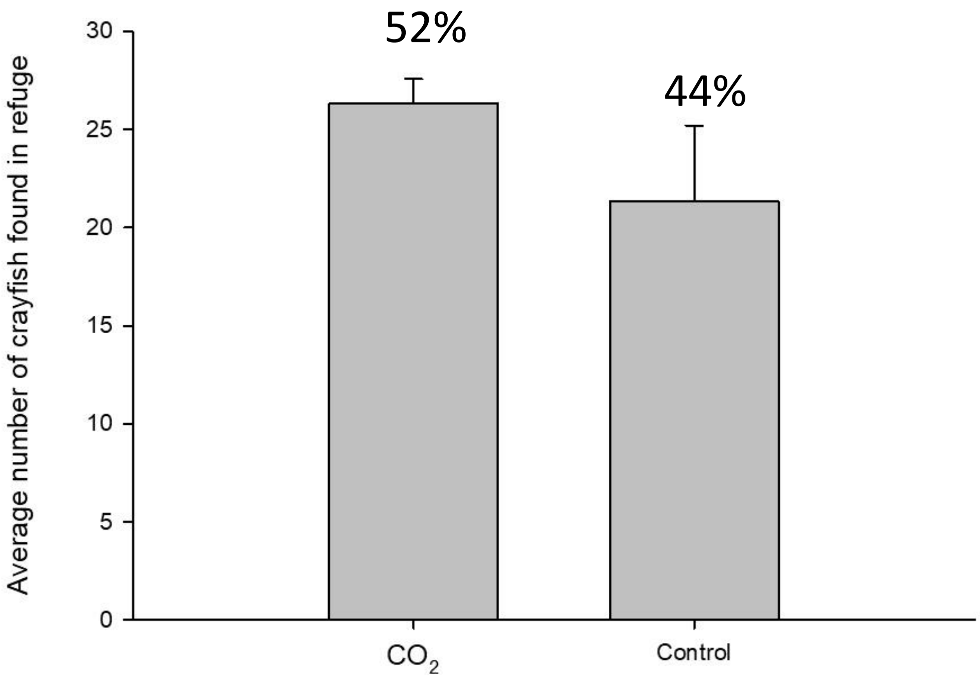

Figure 1.9. Mean number of crayfish found in the refuges (Zone 3) of the treatment and control raceways at the trial end. Percentages represent percent of crayfish in the refuge. Error bars represent*SE*.

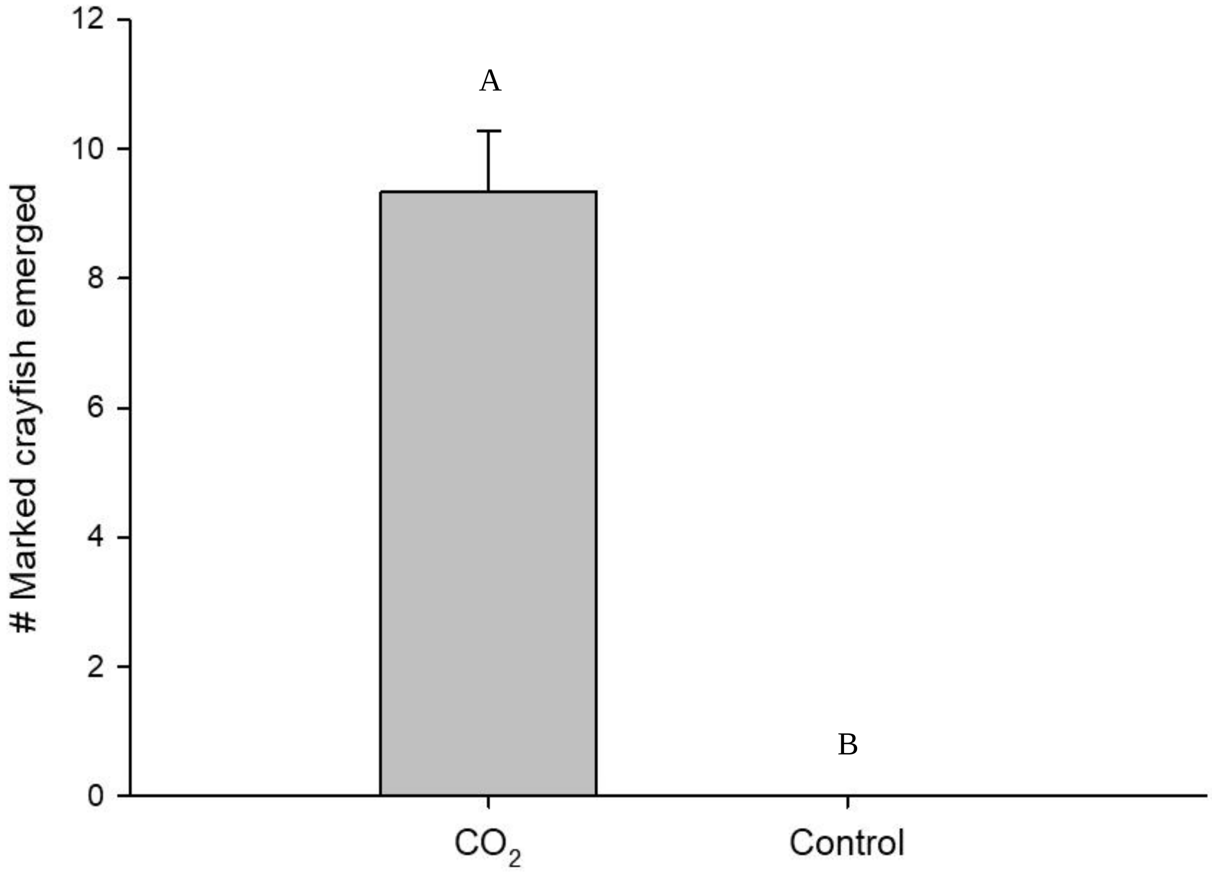

Figure 1.10. Mean number of marked crayfish that emerged at least once in the treatment and control raceways. Error bars represent*SE*. Letters designate a significant difference between treatment and control emergence.

Chapter 2: Evaluation of water flow as a control technique for Red Swamp Crayfish

Introduction

Red Swamp Crayfish (RSC) are a highly invasive species that have invaded many water bodies in North America and throughout the world. Invasive RSC have been found in a wide variety of aquatic systems. School retention ponds and golf course ponds are particularly troubling invasion pathways of RSC (Larson, 2008). Many schools use live RSC in labs, and there have been documented cases of teachers sending children home with live RSC (Larson, 2008; Smith, 2018). RSC are also sold as pets and can be sold for a higher price than other crayfish because of their different color morphs (Faulks, 2015a, b). This is problematic if the students or pet owners do not properly dispose of the crayfish and live release them, allowing for invasion of local waterways near schools. Despite many countries and states prohibiting the sale of crayfish, research has shown that the sales of crayfish continue to occur online even in places with the strictest guidelines (Faulks, 2015b; Faulks, 2018). Golf courses are also an invasive pathway as some golf courses intentionally stock invasive crayfish for aquatic plant control (Smayda and Packard, 1994). The proximity of multiple ponds in golf courses allows crayfish to move from one pond to another and provides potential steppingstones for them to invade nearby natural systems. This makes small ponds an important ecosystem to target when controlling invasive RSC.

A common method of removing this nuisance species is baited crayfish traps. Crayfish traps are often baited with cans of wet and/or dry pet food or cut fish. The costs of bait and labor hours add up quickly when trapping large ponds. In addition, not all areas of ponds are easily accessible to managers for placing and managing traps. These issues that managers face call for a solution that would attract crayfish to a smaller area for easier application of control techniques, including baited traps.

Carbon dioxide is a control technique that has been shown to alter crayfish movement patterns in laboratory and small pond studies and has been proposed as a methodology to “push” crayfish to one end of an infested pond for more efficient collection (Abdelrahman, 2021 and Fredericks 2020). In Chapter 1 of this thesis, we showed that creation of a CO_2 gradient in ponds did not appear to “push” crayfish to a low CO_2 refuge and did not increase trapping success. However, observations during subsequent raceway trials provided some evidence that RSC were attracted to water flow. These observations were supported by previous small pond trials where crayfish remained aggregated around areas of freshwater inflow even in the presence of high CO_2 (Abdelrahman et al. 2021).

Flow is commonly used to attract and trap some species of crayfish such as the Australian Redclaw (*Cherax quadricarinatus*), wherein crayfish move “upstream” and into traps with water flowing out of them (Jones, 1994; Masser, 1997). Positive rheotaxis has also been shown in other crayfish such as Signal Crayfish (*Pacifastacus leniusculus*; Frings et al. 2013). This behavior has been hypothesized to be related to seeking out deeper waters during low flow conditions (Jones, 1994) and increased ability of crayfish to detect food in lotic, compared to lentic conditions. For example, Virile crayfish transferred from lentic conditions exhibited increased walking speeds and decreased angles while orienting towards food sources in lotic conditions (Moore, 2015). This suggests that crayfish that are typically found in ponds or lakes have an increased ability to locate food in lotic conditions. The increased walking speeds of lentic crayfish towards food sources in lotic conditions could mean that invasive crayfish within a pond would be more effective at orienting and moving into baited traps if water flow was artificially created within the pond. It is possible that RSC also exhibit positive rheotaxis.

In this study, we conducted a series of raceway and pond experiments to examine whether flow could be used to “pull” crayfish to a specific region and concentrate them for easier collection. We hypothesized that flowing water would induce positive rheotaxis in experimental raceways and would increase trapping rates in experimental ponds.

Methods

Experimental Animals

RSC were trapped throughout the year from wild, resident populations living in earthen ponds at the E.W. Shell Fisheries Research Station, Auburn, Al. Frabill crayfish traps (vinyl-coated, 42x23cm; Frabill, Plano, IL, USA) were baited with Nine Lives canned cat food, and crayfish collected no more than 48 hours after setting the traps. After collection, crayfish were distributed among eight indoor, flow-through raceways (3.21l, 0.87w, 0.61h in meters) at ambient water temperatures. Each tank was equipped with artificial shelters composed of PVC ribbon (Bio-Fill, Pentair Aquatic Eco-Systems, Inc.; Apopka, FL, USA) and 2.54-3.81 cm diameter PVC pipe segments to minimize aggressive behaviors and to serve as biofilters for water quality maintenance. Crayfish density was kept at < 200 crayfish / raceway. Crayfish were fed Ziegler shrimp feed once a day every other day (Shrimp Grower SI-35 3/32") ad libitum. The water used for the flow-through tanks was sourced from a nearby reservoir and allowed the crayfish to also prey upon small invertebrates such as snails and blood worms that were carried in with the reservoir water. After crayfish were used in the experiments, they were released in a pond planted with rice to provide natural forage and shelter and were not used again in the subsequent trials.

Crayfish Labeling

Crayfish were labeled with Bic White Out to increase visibility in behavioral experiments (Jurcak and Moore, 2014). Prior to trials, crayfish were gently scrubbed of any ectobionts and pat dried using a paper towel. The appropriate symbol (Table 1) or number ID (1-50) was then painted on the crayfish's carapace below the cervical groove with whiteout. The crayfish were placed in shallow water to allow the white out to dry before placing them in their respective tanks.

Water Sampling

Water samples were collected from experimental ponds or tanks using "Jar Stick Samplers." Each sampler consisted of a 16-ounce Mason jar attached to the bottom of either a 1.82m wooden plank or 1.82m 1" PVC pipe. The side of the jar was pressed against the pole such that the bottom of the jar would be adjacent to the bottom of the plank or pipe. A cork was fit snugly into a hole cut into the lid of the jar. A string ran from the eyelet screw through the cork to the top of the pole. To collect samples, a sampler was lowered into the pond or tank until the bottom of the jar reached the bottom of the habitat. The cork was then removed using the string, and water was allowed to flow into the jar. Once the jar was filled with water, the sampler was carefully brought up and gently poured into a wide mouthed 250 ml sampling bottle. Sample bottles were filled to the top, sealed with a lid, and then placed upright and securely into a bucket.

Flow Raceway Experiment

Each raceway experimental trial included a treatment raceway and a control raceway (dimensions: 6.30 x 2.65 x 0.80 m). Each raceway was filled with water from a nearby ~8 ha reservoir the evening before the experiment. Raceways were divided into three zones of equal length: Zone 1 at the outflow end, zone 3 at the inflow end, and zone 2 in the middle. Six emergence boxes were evenly distributed along the edges of zones 1 and 2 (Fig. 1). Emergence boxes were made of 1.27 cm Tenax hardware net attached to a 71.12x 35.56x 38.10 m pvc frame; the same as the traps but without any openings (Fig. 2). Shelters consisting of ~10cm lengths of 1" id PVC pipes were placed into each zone (51 in zones 1 and 2, plus 51 in zone 3) to reduce stress and aggressive interactions among the crayfish during trials.

Crayfish were prepared for the experiment the day before the experiment. Prior to a trial, 100 crayfish were numbered (White Out, Bic) on the carapace, below the cervical groove. Weight and sex were recorded. Each crayfish was randomly assigned to the control or flow treatment and held in separate flow-through tanks overnight, containing water from the same source as the experimental raceways. Each crayfish was labeled a number between one and fifty for each trial for individual identification. The morning of a trial, 50 marked crayfish were placed into zones 1 and 2 of treatment and control raceways and allowed to acclimate for 1 hour. During this time, zones 2 and 3 of each raceway was separated by a barrier made of a metal grate covered with heavy-duty black plastic to prevent crayfish from entering zone 3.

After the 1-hour acclimation period, flow was generated in the treatment raceway, by pumping water from an adjacent, "source" raceway into Zone 3 of the treatment raceway via two Supreme Aqua-Mag 1200 GPH Magnetic Drive submersible water pumps (Danner manufacturing inc. 02712) fitted with PVC tubing (1" inner diameter and 1-19/64" outer diameter). The source raceway was initially filled from the same source and at the same time as the treatment raceway. No water was pumped into the control raceway. The barrier between zones 2 and 3 of both raceways was lifted and held ~11 cm off the bottom with a row of bricks. The third brick from each raceway side was removed to provide two benthic corridors for crayfish to move between zones 2 and 3.

Water temperature (°C) and dissolved oxygen (DO: mg/L) were measured in each zone hourly using an YSI PRO O DO meter. Water samples from each zone were collected at the initial and final hour using a "Jar Stick Sampler" at the left, right, and center of each zone. Crayfish were observed every 30 minutes for emergence in zones 1 and 2: the ID of each crayfish that was on an emergence box at the surface was recorded. After six hours, the experiment was terminated by moving the zone 2-3 barrier off the bricks and lowering it to the tank bottom. The number of crayfish and ID of each crayfish in Zone 3 was then recorded.

Pond Experiments

Pond set up

Experiments were conducted in ~0.1 ha (55m x 15m), earthen ponds that were sloped to a depth of 1.5m at the deep end. In the shallow end (~0.5m), seine fencing was set up at 45-degree angles to create a "W" shape across the width of the pond. Both "points" of the "W" had a ~0.6m wide opening such that the fencing served to create two large funnel traps located ~14m from the shallow edge (Fig. 2). Seine material was supported with metal fence posts, with the top of each seine extending above the water surface and seine bottoms buried 0.3 meters into the pond bottom. Two days prior to each experiment, ponds were drained until the funnel traps were fully visible. The seines were repaired if there were any holes, and then the ponds were refilled the same evening using water from a nearby reservoir. The standpipes in the deep ends of the ponds were set to ensure similar depths between ponds. The inflow pipes in the shallow end were shut off the evening before the experiment.

Four custom-made traps were placed within each of the openings in the seine funnel-trap: two on the bottom and two on the top for a total of eight traps per pond (Fig. 2). The openings of the traps faced the deep end of the pond. The traps were constructed using ¾ in. PVC, and Tenax hardware net with 1.27cm x 1.27cm mesh (Fig. 2).

Each pond was organized into four zones of equal lengths. Zone 1 was at the deepest end of the pond. Zones 2, and 3 were the middle two quarters of the pond. Zone 4 was at the shallow end. The seine fencing was located along the Zone 3 and 4 boundaries. Flow was generated in the treatment pond via two airlifts made of 40 schedule PVC pipe on a metal frame. Airlifts were placed directly behind the traps in zone 4 (Fig. 3) and kept in place with rope attached to the seine fencing. In the first three trials, two airlifts were connected to a single blower (S31 Sweetwater Regenerative Blower ½ HP, Pentair Aquatic Eco-Systems) with 5.08 cm diameter Hi-tech duravent industrial ducting hose split with a PVC T piece. In the second three trials, we attempted to increase current velocity (CV) by powering each airlift with a separate blower.

Pond Experiment

The morning of each experimental trial, the 240 marked crayfish were taken from the flow-through holding raceways. The marked crayfish were separated into six buckets, three colored ones for the flow treatment pond and six white buckets for the control pond, with each bucket having forty crayfish of the same symbol in it. At ~ 8:00 am CT initial air temperatures (°C), DO (mg/L) and pH were recorded. After initial samples were taken, crayfish were gently tossed from the pond sides on both sides of the sampling buoys into zones 1, 2, and 3. This occurred synchronously for both the flow treatment pond and the control pond. Traps were then placed in the gaps of the funnel traps. The crayfish were allowed to acclimate to the pond for one hour. After the hour of acclimation, traps were baited with 9 Lives canned cat food, pate formula and the airlifts were turned on in the treatment pond. Every hour thereafter, wind speed, wind direction and air temperatures were recorded. Water temperature (°C) and DO (mg/L) were recorded hourly in each zone of both ponds using an YSI Pro O DO meter. pH was recorded using an Oakton™ pH Testr™ Waterproof Pocket meter. The pH meter was calibrated before every run using the three-point calibration technique using 4.01, 7.00, and 10.01 Oakton buffers. At hour six, final water quality measurements for the day were taken and the traps checked for crayfish. No crayfish were removed from traps at this time, only the number and the symbol of the crayfish in the traps were recorded. Traps were left in their original location, and the experiment continued to run until hour 24 (i.e., ~9:00 the following morning), at which time all the traps were removed from the ponds. Crayfish were collected and the sex, weight, and symbol recorded. Traps were then placed back into the pond at the original location and current velocity in a given pond was measured in front traps at one of the seine openings (between Zone 3 and Zone 4), and in Zones 3, 2, and 1. Measurements were taken using a portable flow meter (Flo-Mate Model 2000, Marsh-McBirney inc) and were recorded by walking into the pond, standing still, and allowing the CV readings to stabilize. Nine readings were taken in each location with three readings each at the surface, at three-fourths water depth, and at ~5cm off the bottom of the ponds.

Data Analysis

Raceway Experiment

Poisson regressions were conducted using the program *R* (Version 4.0.2) to test for significant effects of treatment (flow or no flow) and crayfish sex and weight on the number of individuals found in Zone 3 of raceways at experiment end. Poisson regressions were also conducted to test for significant effects of treatment (flow or no flow) and crayfish sex and weight on the number of crayfish that emerged throughout the raceway experiments. Our alpha was set at 0.05.

Pond Experiment

I used T-tests (Sigma Plot 13) to test for significant effects of treatment (flow, no flow) on the mean number of crayfish captured in traps at hour 6 and at hour 24 of experiments. We used a one-way repeated measures ANOVA to test for effects of pond ID and average surface CV at the traps, on the mean number of captured crayfish after 24 hours. It passed the Shapiro-Wilk's Normality test, failed the Equal Variance test, and passed the Equal Slopes test. Our alpha was set at 0.05.

Results

Raceway Experiment

Significantly more crayfish had migrated to zone 3 of flow raceways than control raceways ($p<0.001$) (Fig. 4). Sex did not have a significant effect on the crayfish movement into zone 3 ($p=0.997$). Weight had a significant effect on crayfish movements into zone 3 ($p=.0466$) (Table 2.2). Larger crayfish were more likely to move into zone 3 than smaller crayfish. No crayfish emerged from the water in either treatment (Table 2.3). The DO was typically higher in the flow raceways than in the control raceways ($p<0.05$), but the difference between treatment and control was consistently < 1 mg/L (Fig. 6).

Pond Experiment

When both airlifts were powered by a shared ½ hp air blower, current velocities generally remained below 0.06 m/s at all sites and depth. Longitudinal patterns in CV were variable, with highest velocities observed nearest the traps in some trials, but not in others. Vertical patterns in CV were also variable with surface velocities higher than subsurface velocities at some sites but not at others (Fig. 7; left panels).

When each airlift was powered by a separate ½ hp air blower, CV at the surface followed a more consistent pattern with CV near the traps consistently exceeding 0.1 m/s and then declining exponentially with increasing distance from the traps. Current velocity patterns at mid depth and bottom did not show a consistent decline with increasing distance from traps but remained below 0.06 m/s at all sites (Fig. 7; right panels).

Dissolved oxygen increased in both control and flow treatment ponds over the hours of the experiment. However, there was not a consistent pattern of one treatment having higher dissolved oxygen than the other. (Fig. 8). The water temperatures ranged from 23 °C to 33.7 °C, air temperatures from 21.1 °C to 33.89 °C and wind speed from 1 mph to 15mph. (Table 2.4).

There was no significant difference between the number of trapped crayfish in flow ponds compared to control ponds after 6 hours (t(7)=-0.892, p=0.402), or after 24 hours t(7)=0.227, p=0.827) (Fig. 9). There were significantly more crayfish caught in the South Pond than the North Pond regardless of CV ($p<0.001$) but no significant effect of surface CV at traps on number of crayfish trapped, regardless of pond ID (p=0.358). There was no significant interaction between pond ID and surface CV at traps (p=0.553) (Fig. 10).

Discussion

The invasive Red Swamp Crayfish is a problematic species that can be very difficult and costly to control. There is a great need for control techniques that are effective, easy to implement, and do not result in the harm of non-target species. Many crayfish species exhibit positive rheotaxis, suggesting that water flow might be harnessed as a valuable tool for RSC control – avoiding many of the problems associated with toxic chemicals (i.e., lethality to non-target organisms) and potentially increasing the efficiency of traditional control techniques such as trapping.

Raceway experiments provided strong evidence that RSC adults are positively rheotactic. The presence of flow resulted in a mean migration of ~ 36% (N=150) of crayfish to the inflow end of the raceway within 5 hours compared to only ~12% (N=150) when flow was absent. Unlike CO_2 (see Chapter 1), the presence of flow did not induce crayfish to emerge from the water. Like the CO_2 trials, the sex of the crayfish did not influence capture. Unlike the CO_2 treated raceway trials, larger crayfish were more likely to move into zone 3. These results supported that flow may be a useful non-chemical control technique that could be implemented with other management techniques in ponds while avoiding problems associated with potential emergence and dispersal of crayfish and the increased manpower needed to patrol pond edges to capture emerging crayfish.

A significant challenge when using flow to control RSC in ponds is scaling up methodologies to create flow across a larger distance and in a larger volume of water. In this study, we opted to try airlifts rather than water pumps because power sources may be limited at infested waterbody sites, and airlifts are efficient in moving large amounts of water. Airlifts of various designs are commercially available through aquaculture supply companies and are easy to transport. A single air blower can potentially be used to power multiple airlifts within an infested pond. However, a disadvantage of airlifts is that they may not be able to generate localized current velocities as high as what can be generated by pumps.

We successfully used air lifts to generate current velocities in 0.1 ha earthen ponds. Use of a ½ hp air blower to power two airlifts in a single pond resulted in the generation of water current for 45 m past the opening of a given stack of crayfish traps, without an obvious decrease in subsurface velocity along that distance. However current velocities were low, generally remaining below 0.06 m/s. Current velocities just under the surface were substantially increased when each airlift was powered by a separate air blower, but surface velocities quickly decreased with increasing distance from traps and subsurface velocities were generally not elevated above 0.40 m/s.

Although we successfully generated currents in experimental ponds using airlifts, the presence of these currents did not increase our catch rates in baited "upstream" traps compared to controls. Catch rates remained low in flow ponds with only ~6% of marked crayfish captured within 6 hours and ~15% captured in 24 hours, on average. The low current velocities we generated with airlifts may not have been sufficient to attract crayfish via positive rheotaxis and/or enhanced ability to sense baited traps. Current velocity decreases boundary layer thickness (Vogel, 1994). The boundary layer around an object acts as a small "shield" from chemical cues. The thicker the shield the harder it is to process the chemical cues. By creating higher velocity water, crayfish would be able to process more chemical cues and be more likely to forage and find baited traps. However, crayfish can decrease the boundary layer around their antennas by the behavior of "flicking" by doing this they are creating a higher velocity around themselves and decreasing the boundary layers around their antennas (Kraus Epley, 2003). Crayfish do not have to flick their antennas as often in higher flow velocities, which may allow them to increase walking speeds and locate food sources more quickly. Therefore, in future studies it will be important to create higher current velocities, especially at the bottom of the pond where the crayfish reside. This could reduce the amount of effort crayfish use to detect baited traps and thus increase crayfish catches.

It is also possible that differences between raceway and pond trials were affected by DO. In the raceway experiments, we saw significantly higher DO in the flow treatment compared to the control. Conversely, in the pond experiments, DO was not consistently higher in one treatment than the other. However, this is not likely because DO remained high in both raceway trials and the differences between treatment and control DO were small (<1 mg/L). In the pond trials, DO was often higher in the South Pond than in the North Pond, regardless of treatment. This was probably due to differences in aquatic vegetation and nighttime respiration of vegetation in the highly vegetated North pond causing the North pond's DO to drastically drop at night and have lower DO first thing in the morning when experiments started. It was evident there was more vegetation in the North Pond than in the South Pond. There was no vegetation in the South Pond, while in the North Pond there was submerged and surface vegetation in all four zones. The vegetation was present all the way across the pond in zones 1 and 4. Visually, the North Pond vegetation was very thick and there appeared to be more vegetation in the fourth flow experiment than in the first flow experiment.

In conclusion, we found that RSC were attracted to flow in a raceway setting, but not in a semi-natural pond setting. This was likely due to the low flow velocities generated by airlifts in the pond experiments. Additional laboratory studies to determine the threshold current velocities required to stimulate positive rheotaxis and migration of RSC, followed by trials to develop methodology to replicate these velocities over long distances in ponds would be very useful.

Additionally, the significant differences in catch rates between our ponds, regardless of treatment, created a high degree of variation in catch. We alternated assignments of ponds to treatments between trials, to account for potential differences in catch between ponds, but did not anticipate the high degree of pond bias that we observed. Results suggest factors such as vegetation might affect trapping rates and differences in vegetation among experimental ponds need to be minimized for future studies. A better understanding of crayfish responses to a range of current velocities as well as factors driving pond biases in catch rates will facilitate more efficient trap designs and strategies to further investigate the potential role of flow as a tool to assist in the control of invasive crayfish populations.

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Table 2.1. Symbols assigned to crayfish in both the flow treated ponds and control ponds.

Date	Pond treatment	Pond ID	Crayfish Symbol
May. 20, 2021	Flow	North	O
May. 20, 2021	Control	South	↑
May. 27, 2021	Flow	South	↓
May. 27, 2021	Control	North	-
Jun. 01, 2021	Flow	North	/
Jun. 01, 2021	Control	South	^
Jun. 05, 2021	Control	North	>
Jun. 05, 2021	Flow	South	+
Jul. 29, 2021	Flow	South	:
Jul. 29, 2021	Control	North	ɔ
Aug. 04, 2021	Control	South	~
Aug. 04, 2021	Flow	North	∴
Aug. 11, 2021	Control	North	[
Aug. 11, 2021	Flow	South	:)
Aug. 19, 2021	Flow	South	?
Aug. 19, 2021	Control	North	⊓

Table 2.2. Summary statistics for generalized linear model with a Poisson distribution used to test for differences between flow and control raceways in the number of crayfish that moved to refuge.

Coefficients	Estimate	Std. Error	t value	Pr(>|t|
(Intercept)	5.10E-03	6.03E-03	0.846	0.398
TreatmentFlow	7.73E-03	6.73E-03	1.15	0.251
SexM	-9.31E-03	6.87E-03	-1.355	0.176
Weight	-6.51E-07	2.52E-05	-0.026	0.979

Table 2.3. Summary statistics for generalized linear model with a Poisson distribution used to test for differences between flow and control raceways in the number of crayfish that emerged.

Coefficients	Estimate	Std. Error	t value	Pr(>|t|
(Intercept)	0.005099	6.029-e03	0.846	0.398
TreatmentFlow	0.007734	6.73E-03	1.15	0.251
SexM	-0.009309	6.87E-03	-1.355	0.176
Weight	-6.51E-07	2.52E-05	-0.026	0.979

Table 2.4. Water temperatures, outside temperatures, and wind speeds of flow treatment pond trials averaged from hourly samples. Mean ± *SE*.

Date	Water Temperature °C	Air Temperature °C	Wind Speed (mph)
5/20/2021	26.28 ±0.24	25.72 ±0.76	13.0 ±0.36
5/27/2021	29.80 ±0.23	27.14 ±0.66	5.25 ±0.59
6/1/2021	27.40 ±0.30	26.03 ±0.19	7.86 ±0.19
6/4/2021	28.09 ±0.25	26.59 ±0.34	3.23 ±0.31
7/29/2021	31.96 ±0.19	31.19 ±1.19	3.57 ±0.38
8/4/2021	30.03 ±0.25	25.72 ±0.95	5.86 ±0.12
8/11/2021	29.57 ±0.24	28.81 ±1.14	3.43 ±0.32
8/19/2021	30.18 ±0.18	29.52 ±1.02	5.14 ±0.55

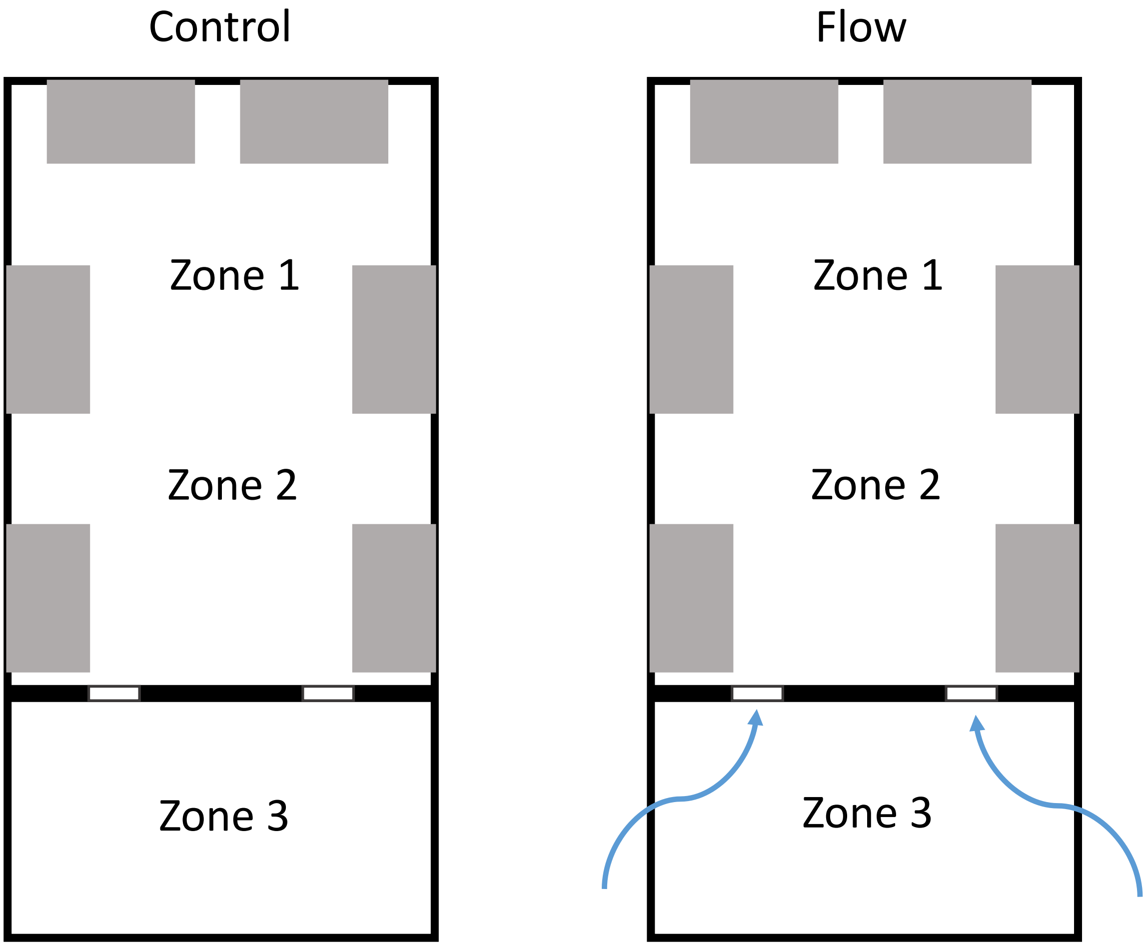

Figure 2.1. Left: Overhead schematic of control raceway showing location of raceway zones, emergence boxes and barrier with corridors between zone 2 and zone 3. Right: Overhead schematic of flow treated raceway showing location of raceway zones, emergence boxes and barrier with corridors between zone 2 and zone 3. Arrows represent the tubing delivering water flow through the corridors of the flow raceway.

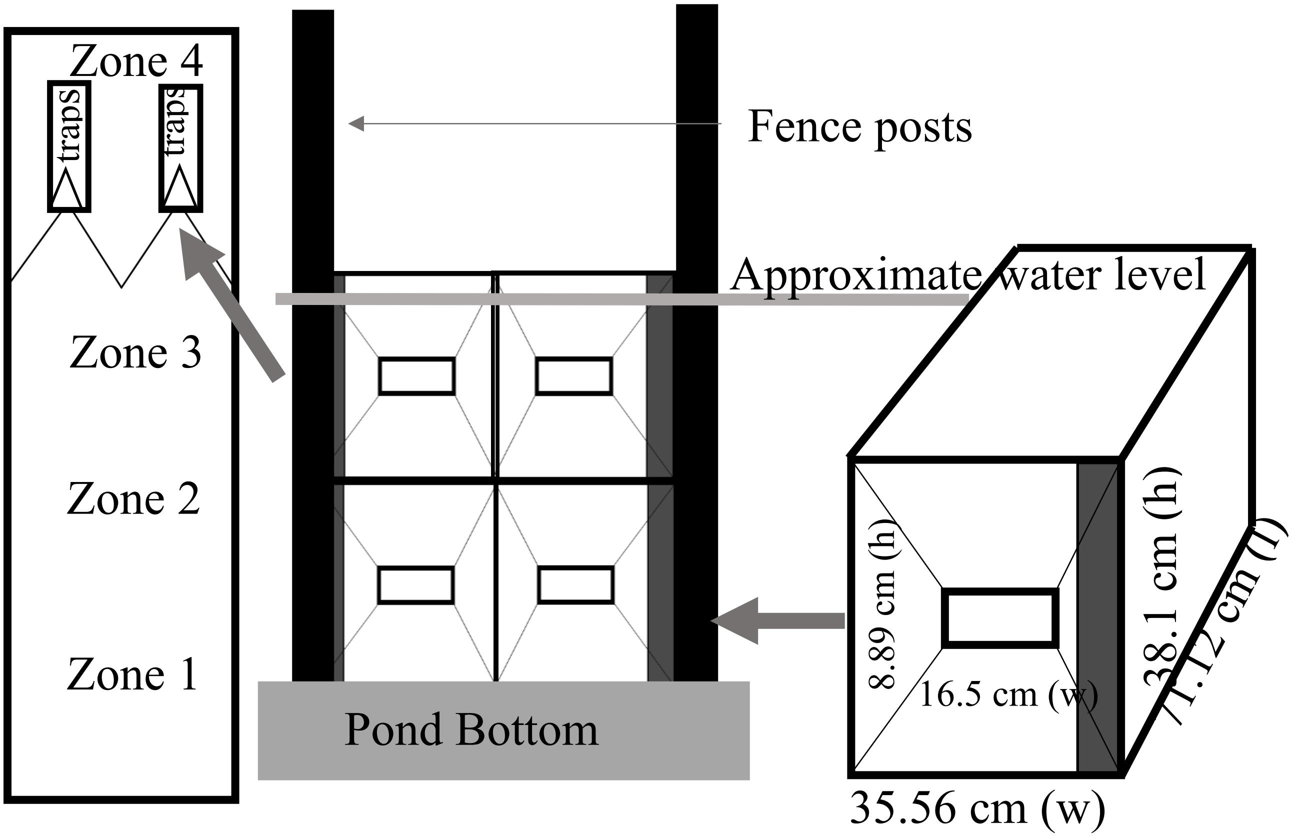

Figure 2.2. Left: Overhead schematic of pond showing location of pond zones, seine fencing used to create two large funnels, and crayfish traps. Center: Cross section schematic showing how ponds were stacked across seine openings. Right: Trap dimensions.

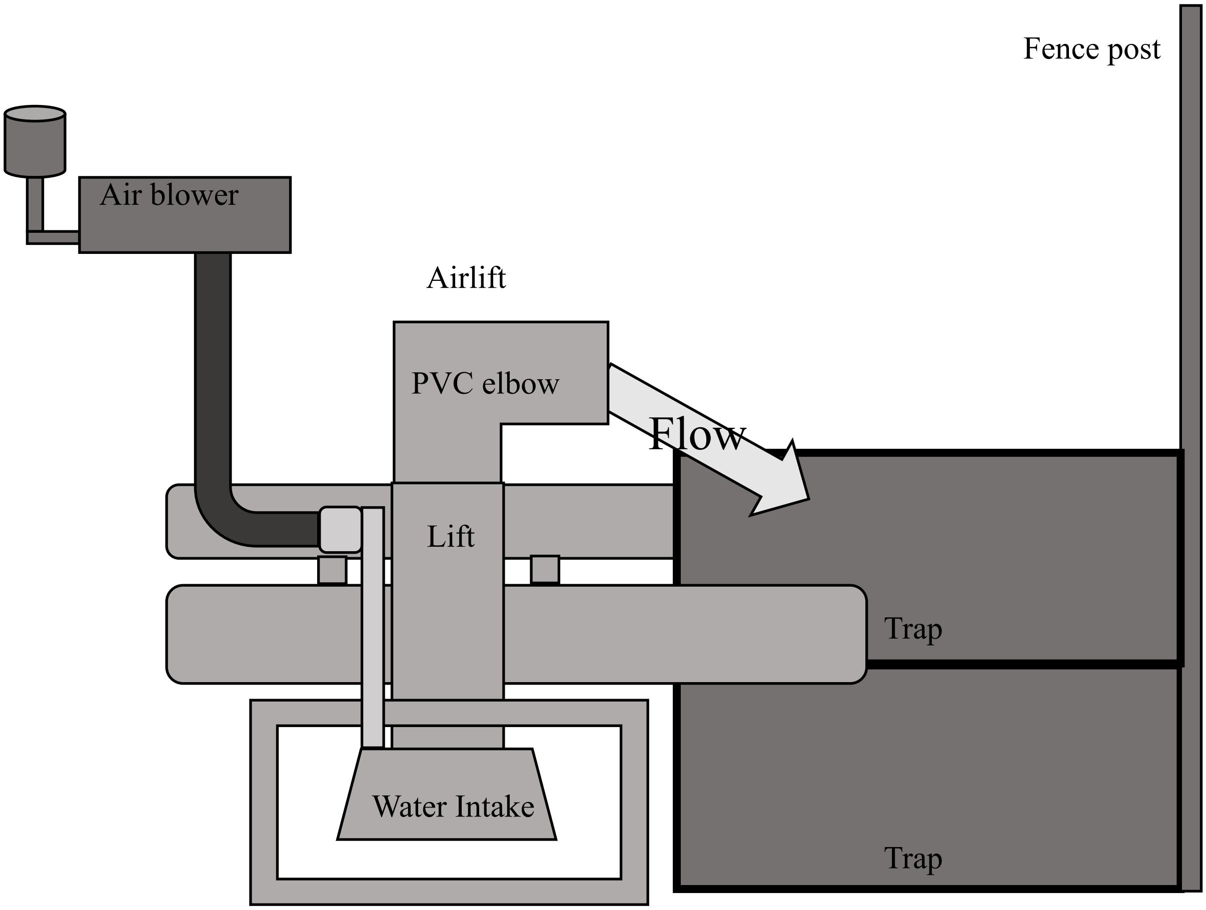

Figure 2.3. A schematic of the airlift set up that was placed behind the traps in the flow treatment ponds.

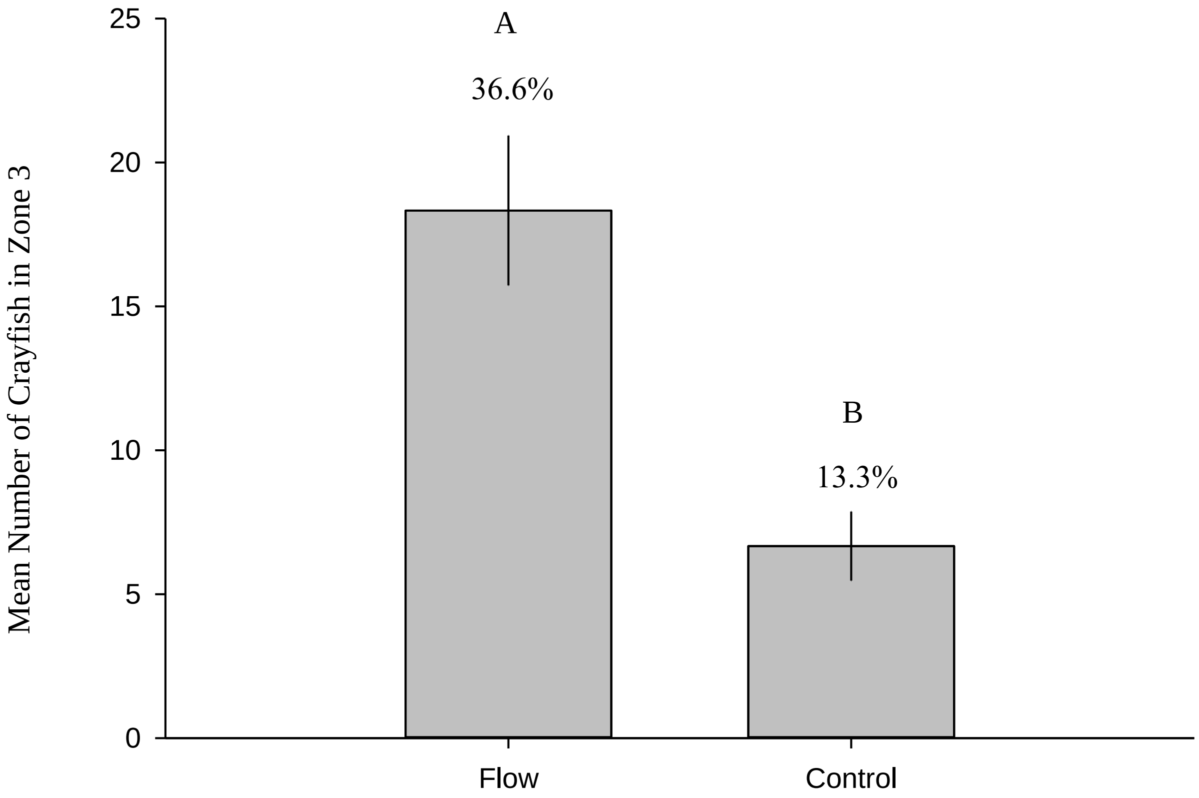

Figure 2.4. Mean number of marked crayfish captured in Zone 3 of the flow and control raceways at the end of each trial. Error bars represent ± *SE*. Letters above bars indicate significant differences between treatments. Percentage indicates the percent of marked crayfish captured.

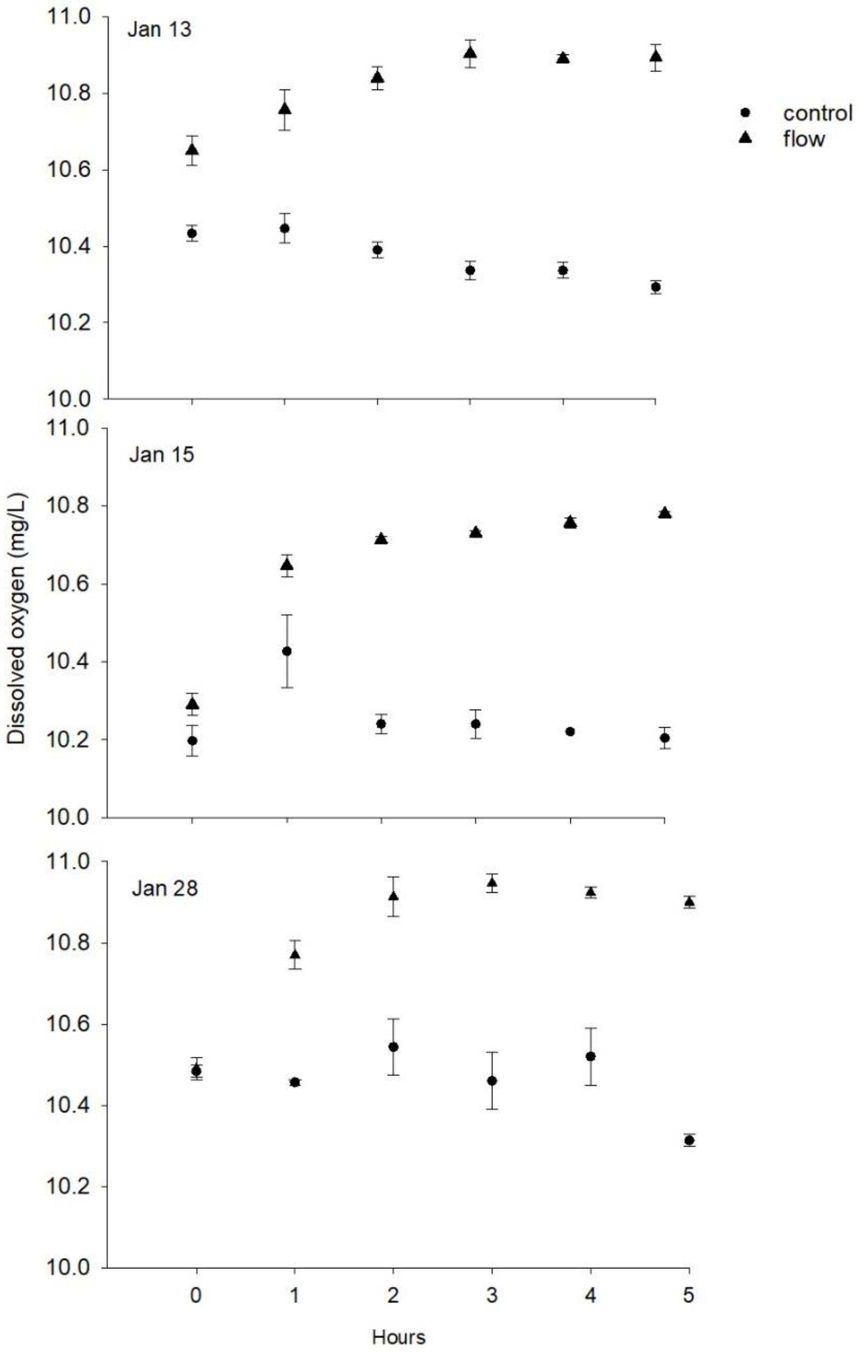

Figure 2.5. Dissolved oxygen in the flow and control raceways at each experimental hour for each of three trials. Error bars represent ± *SE*.

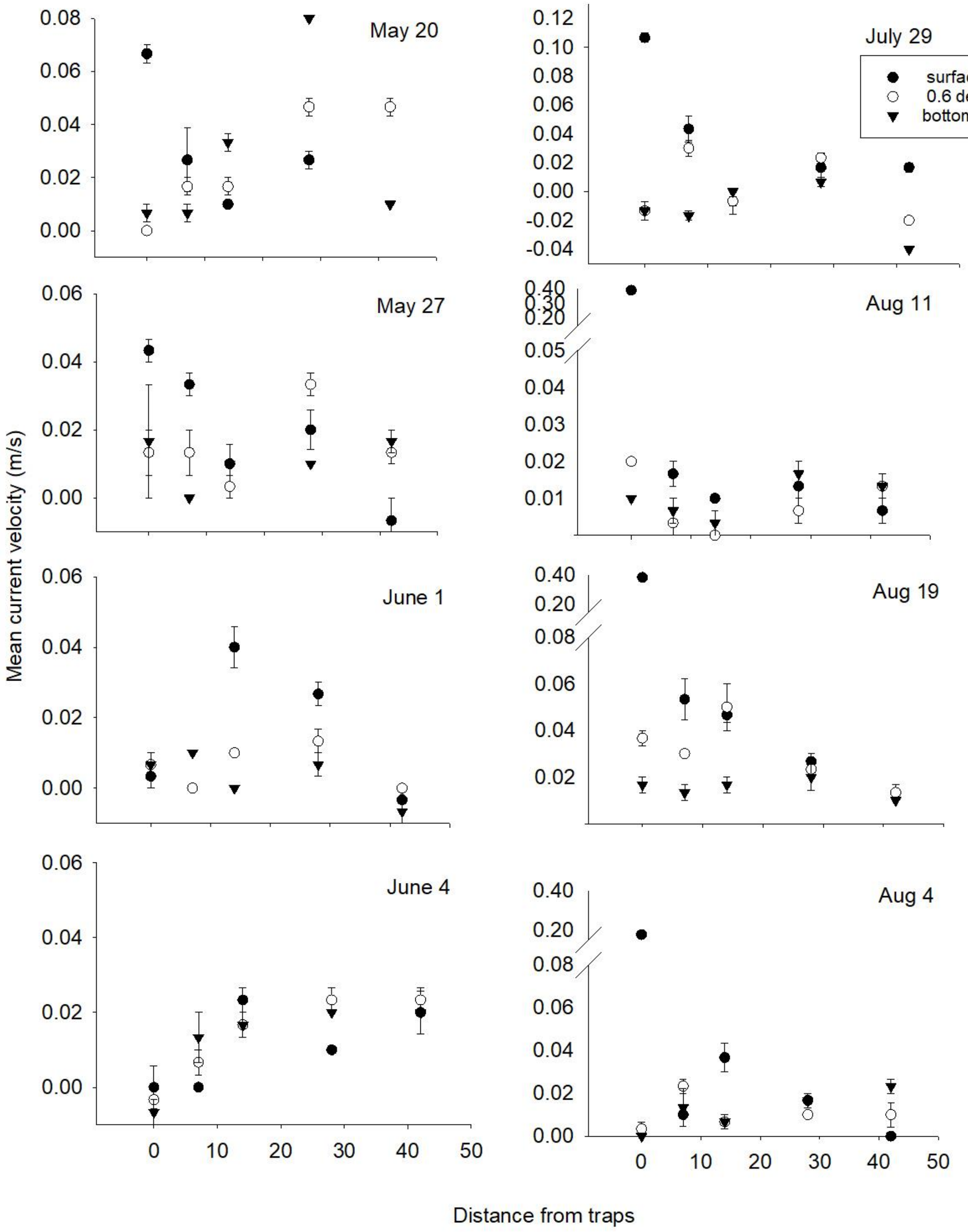

Figure 2.6. Relationships between current velocity and distance-from-traps at three depths for each of eight trials. Dates represent the start date of each trial. Left panels show trials with a single air blower powering two airlifts. Right panels show trials with a single air blower powering only a single airlift. Error bars represent ± *SE*.

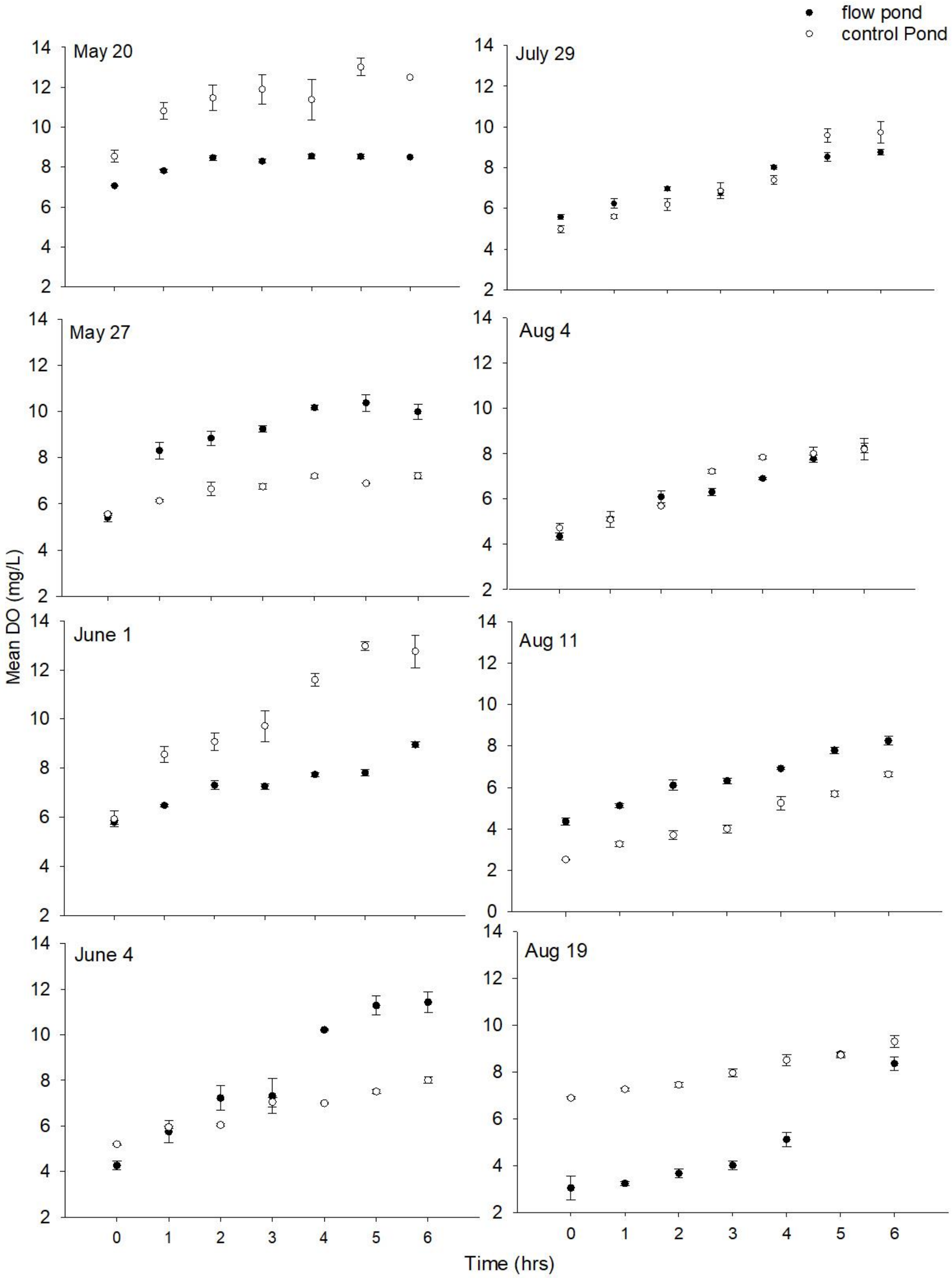

Figure 2.7. Dissolved oxygen concentrations during hours 0-6 of each pond trial. Error bars represent ± *SE*.

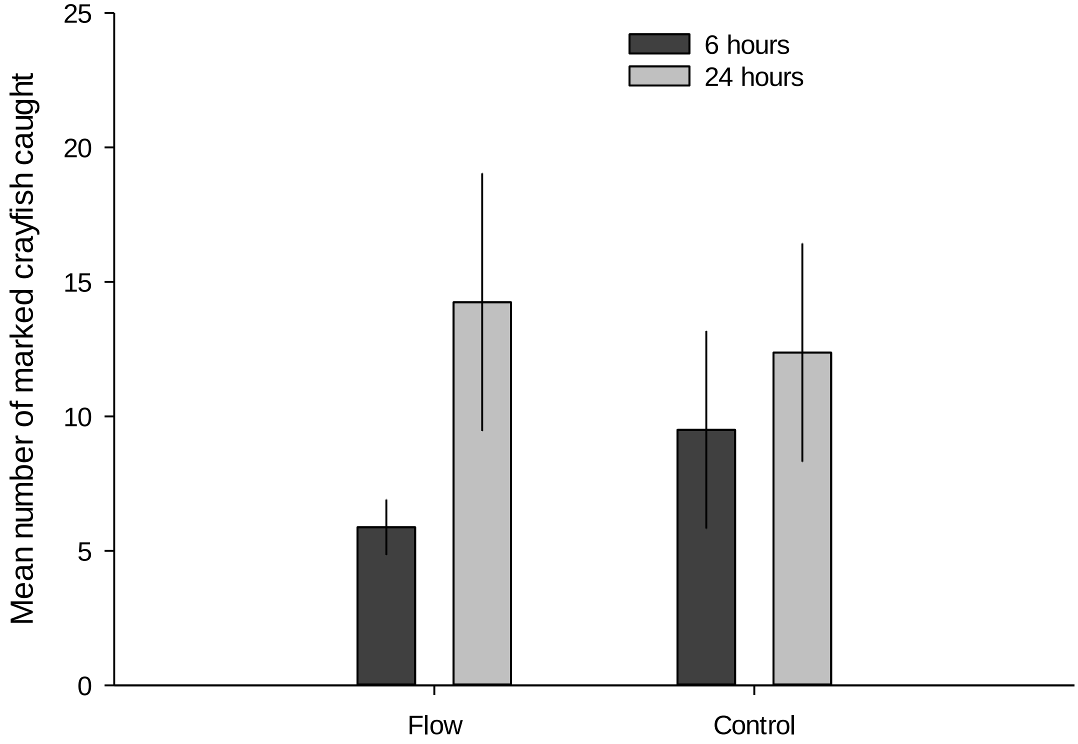

Figure 2.8. Mean number of marked crayfish caught in the flow treatment ponds and control ponds at 6 hours and 24 hours. Error bars represent ± *SE*.

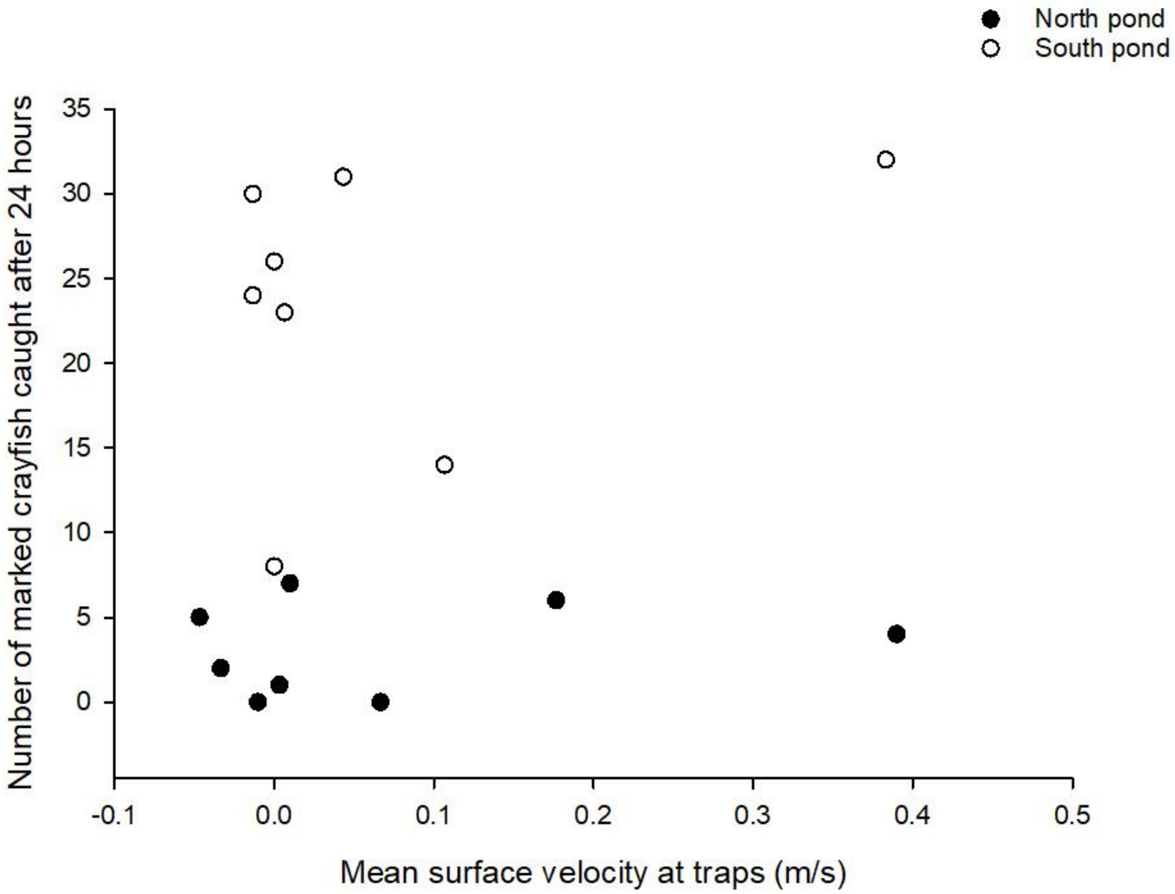

Figure 2.9. Relationship between number of crayfish captured at 24 hours and the mean current velocity (CV) measured at the pond surface just in front of the traps in the North pond and the South pond. Treatments (airlifts, control) were alternated between ponds for each successive trial. Data includes both treatments.