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Research Article

Evaluation of dissolved carbon dioxide to stimulate emergence of red swamp crayfish *Procambarus clarkii* (Decapoda: Cambaridae) from infested ponds

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Abstract

Invasive crayfish have adverse effects on habitats and native species. Control of invasive crayfish populations is a major challenge facing natural resource managers. This study evaluated the effectiveness and optimal conditions for the control agent carbon dioxide (CO_2), which can be diffused into water to facilitate capture of red swamp crayfish (*Procambarus clarkii*; RSC). The efficacy of CO_2 shows promise in its use for a variety of invasive aquatic species. Here, we evaluate CO_2's ability to stimulate movements towards the shoreline and/or induce complete terrestrial emergence from outdoor ponds. Twelve pond trials were conducted using three, 0.02-ha experimental ponds at Auburn University, Alabama, USA. Silt fencing was installed on dry land around the perimeter of each pond with the lower 0.3 m of fencing accordion-folded to provide shelter and a collection point for emerging crayfish. Each pond was stocked with 100 RSC before testing. Experimental treatment ponds were then injected with gaseous CO_2 using porous air diffusers, whereas control ponds (C ponds) received no CO_2. Multiple water quality parameters were monitored hourly. Three independent treatment scenarios with CO_2 diffusion were crayfish captured at the end of trial only (F: final), crayfish captured hourly (H: hourly), and incorporation of continuous inflow of fresh water at a flow rate of 0.2 L/s into the central catch basin to serve as a refuge with crayfish captured hourly (R: refuge). In control ponds, crayfish were captured at the end of trial only. In F ponds, CO_2 diffusion for approximately five hours caused a mean of 12% of total crayfish to emerge from the water. However, capture efficiency was increased to a mean of 45% of total crayfish by increasing collection frequency to every hour and netting submerged crayfish near the water edge in addition to capturing terrestrially emerged crayfish. Presence of a freshwater inflow reduced capture efficiency in R ponds relative to H ponds. Odds of capturing crayfish increased with increasing water temperature, CO_2 concentration, crayfish mass, and with decreasing pH. Based on results, we provide a set of predictive equations as well as interactive calculators to help natural resource managers explore several environmental and treatment-related scenarios that predict changes in capture probability in small research ponds. Carbon dioxide shows promises as a tool to increase capture rate of RSC. It is not likely to be 100% effective by itself, but could be a useful component of an integrated management strategy.

Key words: chemical control, invasive crayfish infestation, invaded ponds, integrated pest management programs, invasive species control, crayfish capture frequency

Introduction

As of 2021, red swamp crayfish (*Procambarus clarkii* [Girard, 1852]; RSC) is one of the most invasive crayfish species worldwide. Native to the southern United States and northeastern Mexico, it is now found on all continents except Antarctica and Australia (Gherardi 2006; Loureiro et al. 2015). Impacts on invaded systems primarily stem from its burrowing activity, propensity to graze on macrophytes, and status as a vector of crayfish plague. Invaded waterbodies may exhibit increased turbidity as a result of increases in phytoplankton and loss of macrophytes, or increases in suspended sediments as a result of crayfish burrowing activity (Anastácio and Marques 1997; Geiger et al. 2005; Matsuzaki et al. 2009; Rodríguez et al. 2003). Blooms of cyanobacteria may also be triggered as a result of these changes (Yamamoto 2010). Crayfish plague can have devastating effects on crayfish species outside of North America that have not evolved resistance to this disease (Longshaw 2011; Souty-Grosset et al. 2006).

Treatment options for invasive crayfish populations after establishment in new environments fall within five broad categories: mechanical removal, physical methods, biological control methods, biocides, and autocidal methods. None of these serves as a single, universal strategy to successfully control invasive crayfish. Some treatment strategies are often biased by crayfish size (Gherardi et al. 2011) and/or characterized by sex specificity such as sex pheromone baited traps only attracting males (Stebbing et al. 2003). The best option may be to use integrated pest management (IPM) approach – combining multiple strategies (Gherardi et al. 2011). For an IPM approach to be successful, it is important to understand the strengths and limitations of individual control techniques within each category to determine how they might be best integrated into a comprehensive management strategy (Sepulveda et al. 2012).

Chemical controls (ranging from natural products to synthesized chemicals) are widely used in aquatic IPM activities to lure organisms to traps, repel organisms, or to cause direct mortality (Fredricks et al. 2019). Chemicals that affect environmental conditions (pH or dissolved oxygen) surrounding target organisms could be used to control crayfish populations (Freeman et al. 2010). For example, carbon dioxide (CO_2) is receiving increasing attention as a management option for the control of invasive species because it is naturally occurring, readily neutralized, and commercially available (Treanor et al. 2017). At high concentrations, it can be used to induce lethal effects (Cupp et al. 2017b). At lower concentrations, it may be used to induce desirable behavioral movement patterns related to site avoidance or conversely, movement towards netting/trapping areas (Cupp et al. 2018, 2017a; Treanor et al. 2017). Carbon dioxide was registered in April 2019 with the U.S. Environmental Protection Agency (EPA) as a pesticide for use to control aquatic invasive species (name of pesticide product: Carbon Dioxide — Carp; EPA Registration Number: 6704-95;

Suski 2020) such as silver carp (*Hypophrhalrnichthys molitrix*), bigheaded carp (*H. nobilis*), black carp (*Mylopharyngodon piceus*), and grass carp (*Ctenopharyngodon idella*).

Carbon dioxide has potential as a control strategy to facilitate removal of RSC from invaded ponds. In a laboratory study, RSC were repelled by CO_2 as it increased to saturations above 5% to 100% CO_2 (Bierbower and Cooper 2010). Similar effects were not observed during reductions in oxygen or pH, indicating that the avoidance was principally driven by CO_2 concentrations (Bierbower and Cooper 2010). In addition to chemical avoidance, CO_2 induced crayfish to emerge from water in other laboratory trials (Fredricks et al. 2020), indicating that diffusion of CO_2 may facilitate removal of RSC from invaded ponds via emergence and subsequent capture and provide managers with an option to better control this prolific invasive species. Although crayfish sex and size did not affect CO_2 avoidance and emergence behavior of RSC in a previous laboratory study, evaluating the differences in CO_2 effectiveness between crayfish sizes and sexes in natural settings is still recommended (Fredricks et al. 2020).

We conducted experiments to determine the degree to which behavioral changes induced by CO_2 could be used to increase capture of RSC in the field. Experimental ponds were intended to represent simulated management or control situations where RSC infestations could occur. Our three main questions addressed here were:

1. Can CO_2 be used to induce crayfish emergence from ponds?
2. Does increased effort associated with hourly collections and collection of crayfish at the water edge result in a significant increase in the number of crayfish captures?
3. What environmental (water temperature, CO_2 concentration, and water pH) or biotic conditions (crayfish wet mass, crayfish texture, and sex-reproductive status) enhance or suppress the effectiveness of CO_2 as a control strategy?

Materials and methods

Study crayfish

Red swamp crayfish were trapped from resident populations in ponds at the E.W. Shell Fisheries Research Station (SFRS), Auburn University, and at Troy University, Alabama, USA using Frabill® crayfish traps (vinyl-coated, 42 cm × 23 cm; Frabill, Plano, Illinois, USA) baited with canned wet cat food (9Lives® seafood and poultry favorites variety pack, Big Heart Pet, Inc., Walnut Creek, California, USA). Crayfish were subsequently held in three indoor, flow-through rectangular tanks at the SFRS at ambient water temperatures and fed commercial shrimp feed pellets *ad libitum* every other day until initiation of pond studies. Holding tanks were lighted from 0700 h to 1900 h, resulting in a 12:12 h light: dark cycle. Newly collected crayfish were continually added to holding tanks as trapping continued throughout the study period. Trapped crayfish were not culled for a particular size range or sexed before pond stocking. Following each experiment, crayfish were returned to holding tanks. Thus, crayfish stocked into each experimental pond represented a random mix of sizes, sexes, and naïve and previously used crayfish. If a holding tank contained any previously used crayfish, animals from that tank were not used until at least two weeks had passed since the previous trial.

Study ponds and gas

Pond trials were conducted in three, 0.02-ha (~ 14 m × 14 m) earthen-bottom research ponds with vertical concrete sidewalls and a small wooden central catch basin (2.1 m × 1.1 m × 0.3 m). Bottoms of the three ponds were similar with no rocks or weedy areas of refuge that may have provided isolated areas of lower CO_2. Ponds were located side by side at SFRS (32°39′00.5″N; 85°29′06.9″W). Each pond was partially filled with water from an 8.1-ha supply reservoir with approximately 1–2 m of exposed shore between the water edge and the concrete sidewalls. Hourly recorded air temperature, relative humidity, wind speed, and precipitation data were summarized from the Auburn-Opelika weather station at about 10 km southeast of the study ponds.

Silt fence (0.9-m height; TerraTex® SF-D, Hanes Geo Components, Winston-Salem, North Carolina, USA) was installed around the perimeter of each pond ~ 1 m from the water edge (Figure 1) before each trial. The lower 0.3 m of fencing was accordion-folded along the soil surface to provide potential shelter for emerging crayfish. The height of installed fence after folding the lower part is 0.6 m. To control variation related to spatial effects, a randomized complete block design was used to assign ponds into control or treatment groups. Carbon dioxide was diffused into treatment ponds but not into control ponds. At the side of each treatment pond, a compressed medical-grade CO_2-gas (9.3 m^3) tank (Air gas® Part Number CGA-940; Airgas Inc., Radnor, Pennsylvania, USA) was held on a cart in the shade. Clear vinyl tubing (Inner diameter = 0.95 cm; Model TV60; Pentair Aquatic Ecosystems Inc., Apopka, Florida, USA) led from a single-stage CO_2 regulator (Victor® Model: 0781-3615; Victor Technologies, Denton, TX, USA) to a terminal plastic low-pressure air diffuser (Model: SB-50; A-MI Corporation, Incheon, South Korea) placed in the central catch basin. The flow of CO_2 was adjusted to a rate of 25 L/min using an inline CO_2 pressure-reducing flowmeter (Product ID: 1056040; Banggood Technology Co. Ltd., Fo Tan, Sha Tin, Hong Kong). In preliminary trials, CO_2 concentrations in the ponds reached and exceeded our target of 200 mg/L as pH declined below ~ 5.6. Therefore, CO_2 gas was continuously diffused into treatment ponds until pH was between 5.4 and 5.6, at which point the gas cylinder was turned off. Addition of CO_2 was resumed when pH had

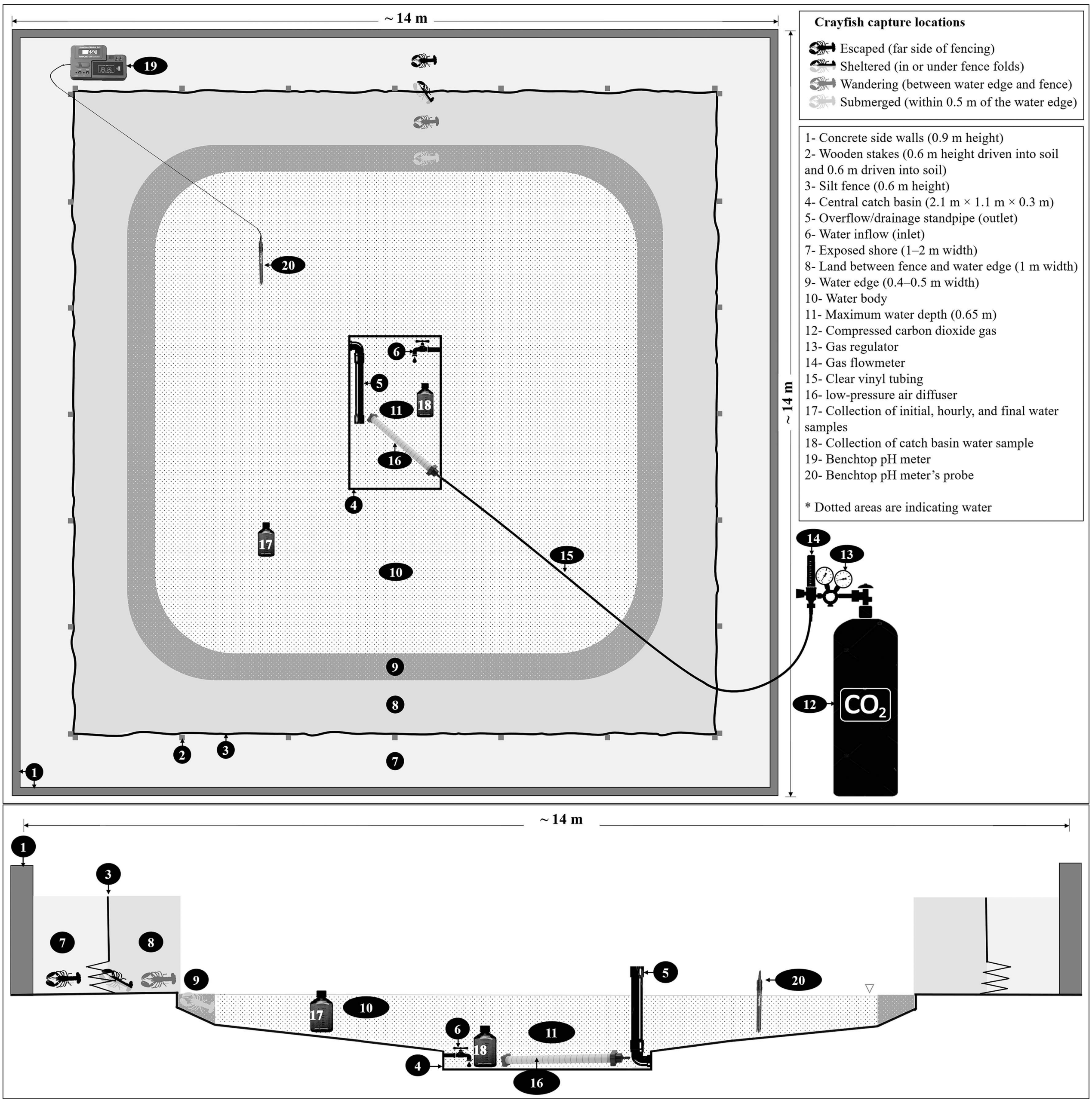

Figure 1. Schematic diagram showing top view (top figure) and cross-sectional view (bottom figure) of the experimental pond design, water samples collection areas, and crayfish capture locations.

increased to 5.6. The instantaneous monitoring of water pH was achieved by using a calibrated benchtop pH meter (PINPOINT® pH Controller; American Marine Inc., Ridgefield, Connecticut, USA) to determine when to turn the CO_2 gas on or off, rather than waiting for pH results from the hourly water samples. Before terminating any trial (draining study ponds), the length and width of water surface and the maximum water depth at the central catch basin were recorded for each pond.

Experimental design

We conducted 12 pond trials between July 30, 2018, and June 25, 2019. For each trial, 100 crayfish were randomly selected and stocked in each experimental pond. We placed them in ponds for ~ 1 h before any application of CO_2. Crayfish were always stocked in the morning and trials ran until dusk (~ 1700 h) on the same day for a duration ranging from 5.25 to 7 h (6.66 ± 0.21 h, mean ± SE). Trial information is summarized in Table 1. The first three trials were preliminary trials (P_1 to P_3) used to develop the experimental design and were not used in any subsequent analyses, whereas data obtained from the remaining nine trials (T_1 to T_9) were used for analyses.

The first non-preliminary trials (T_1 and T_2) were conducted to test whether the diffusion of CO_2 into ponds would cause crayfish to emerge from ponds and whether emerged crayfish would shelter under fence folds for easy collection (question 1). Crayfish were captured only at the end of the trial (final collection) and scored into three capture categories according to their location as between water edge and fence (wandering), in or under fence folds (sheltered), or far side of fencing (escaped). Both trials included a control pond (no CO_2 diffusion and final crayfish capture; C ponds) and a treatment pond (CO_2 diffusion and final crayfish capture; F ponds).

The next three trials (T_3 to T_5) expanded upon trials T_1 and T_2 to determine whether crayfish capture could be significantly increased by capturing crayfish every hour and by capturing submerged crayfish at the water edge (question 2). In each trial, a third pond was used from which crayfish were captured hourly (CO_2 diffusion and hourly crayfish capture; H ponds). In addition to capturing wandering, sheltered, and escaped crayfish, we also captured crayfish in each pond from a fourth category by using a dip net to capture submerged crayfish within 0.4–0.5 m of the water edge and scored them as "submerged". Capturing submerged crayfish from ponds was done either at the end of the trial only, or hourly, depending on the assigned treatment (F pond or H pond). Crayfish from C ponds were captured only at the end of the trial and thus only directly comparable to F ponds. In summary, T_1 and T_2 contained F and C ponds from which only emerged crayfish were collected, whereas T_3, T_4, and T_5 included F, C, and H ponds from which emerged + submerged crayfish were collected. All C pond trials (T_1 to T_5) were completed during 2018 (Table 1).

The final four trials (T_6 toT_9) were conducted to test the effects of freshwater inflows on crayfish capture, simulating the presence of an underground spring or seep in a water body, which might provide a refuge from CO_2 (question 3). In these trials, we replaced the C pond with a refuge treatment (CO_2 diffusion, with refuge, and hourly crayfish capture; hereafter denoted as R) where the submerged water inlet in the central catch basin was adjusted partially open such that water flowed into the catch basin at a flow rate of 0.2 L/s. This provided a small refuge of low CO_2 water at the bottom of the catch basin. Crayfish were captured from refuge ponds in the same manner as in the hourly ponds. In R ponds, to avoid the increase in water

Table 1. Date, pond assignment, and capture categories of preliminary trials (P) and study trials (T). Ponds were assigned either to control (C: no CO_2 diffusion, crayfish captured at the end of trial) or to one of the following three treatments with CO_2 diffusion: final (F: crayfish captured at the end of trial), hourly (H: crayfish captured hourly), refuge (R: continuous water inflow at a flow rate of 0.2 L/s into the central catch basin and crayfish captured hourly).

Trial	Date		Ponds				Capture Categories			
			C	CO_2 Treatment						
				F	H	R	Escaped	Sheltered	Wandering	Submerged
P_1	2018	30-Jul	x	–	x	–	–	–	x	–
P_2		7-Aug	x	–	x	–	–	–	x	–
P_3		14-Aug	–	x	–	x	x	x	x	–
T_1	2018	9-Oct	x	x	–	–	x	x	x	–
T_2		22-Oct	x	x	–	–	x	x	x	–
T_3		30-Oct	x	x	x	–	x	x	x	x
T_4		19-Nov	x	x	x	–	x	x	x	x
T_5		27-Nov	x	x	x	–	x	x	x	x
T_6	2019	29-May	–	x	x	x	x	x	x	x
T_7		13-Jun	–	x	x	x	x	x	x	x
T_8		20-Jun	–	x	x	x	x	x	x	x
T_9		25-Jun	–	x	x	x	x	x	x	x

x = available; – = not available

volume, the central overflow standpipe was adjusted such that water would exit the pond at the same rate as the inlet flow rate. In these trials, CO_2 gas was bubbled into all three treatment ponds: R, H, and F; and crayfish were captured from all four capture categories: wandering, sheltered, escaped, and submerged.

In all crayfish capture events – either final or hourly, crayfish were captured in the following order: escaped, wandering, submerged (except in T_1 and T_2), and then sheltered. In each trial, after draining ponds, crayfish remaining in each pond (not captured during the trial) were also collected. Wet mass (g), mortality (live or dead), body texture (hard or soft/fresh molt), and sex and reproductive state (male, non-berried female, or berried female) of each crayfish captured or remaining in study ponds were recorded at the end of each trial. To avoid confusion between dead and narcotized crayfish, crayfish mortality was recorded 1–2 h after draining ponds and transferring crayfish into low CO_2 water. The term "berried female" refers to ovigerous female bearing eggs attached to the abdomen.

Water chemistry

From each pond, water samples were collected in a 1-L screw-cap plastic container just before CO_2 injection, every hour during the experiment, and just before terminating the trial (draining the pond). Samples were collected at 0.05–0.1 m below water surface from mid-distance between water edge and center of the pond. In the collected water samples, water temperature (°C) and dissolved oxygen (DO; mg/L) were immediately measured using a calibrated handheld meter (YSI Pro-ODO®; YSI Inc., Yellow Springs, Ohio, USA); CO_2 (mg/L) was immediately measured with a HACH digital titrator using a pH 8.3 endpoint with 0.3636 N or 3.636 N NaOH titrant (HACH Method 8205; HACH Inc., Loveland, Colorado, USA), and/or a calibrated portable CO_2 gas meter (CGP-31; DKK-TOA Co. Ltd., Tokyo, Japan); and pH was immediately measured using a handheld Oakton pHTestr® 30 (Oakton Instruments, Vernon Hills, Illinois, USA). In each treatment pond, the benchtop pH meter's probe was submerged in pond water at a mid-distance between water edge and center of the pond. The benchtop pH meter of each pond and the handheld pH meter were calibrated before each trial with standard pH 4.01, 7.01, and 10.01 buffers (Milwaukee Instruments Inc., Rocky Mount, North Carolina, USA).

In addition to hourly collected water samples, from T_2 onward, initial (just before starting CO_2 diffusion in treatment pond/ponds) and final (just before draining ponds) water samples were collected from each pond. Within 2 h after draining ponds, total alkalinity (mg $CaCO_3$/L) and total hardness (mg $CaCO_3$/L) of pond water were measured photometrically in initial and final water samples using commercial YSI reagent kits and a YSI 9300 photometer (YSI Inc., Yellow Springs, Ohio, USA). In trials with refuge treatment, to check if the freshwater inflow was affecting CO_2 concentration in the catch basin, CO_2 was also measured in water samples collected just before draining from the catch basin in each pond using a horizontal water sampler (LaMotte® Model: 1087; LaMotte, Chestertown, Maryland, USA). As a result of technical/calibration error, all CO_2 measurements from T_6 were deemed not reliable and were not used in any data analyses involving CO_2 data.

Data analyses

To test correlations between water quality variables, results from each variable (CO_2, DO, water temperature, and pH) were analyzed for normality through Shapiro-Wilk's test. When bivariate normality was verified, data were analyzed through a Pearson correlation. Results not following this assumption were analyzed through a Spearman rank correlation. All multiple testing *p*-values for correlation analyses have been adjusted to control the false discovery rate (FDR) using the Benjamini-Hochberg procedure (Benjamini and Hochberg 1995).

To determine the effect of CO_2 diffusion on other water quality parameters, generalized linear models (GLMs) were used to compare water quality parameters (question 1) between C and CO_2 treatment groups (F and H) in the 2018 trials (T_1 to T_5) and to compare CO_2 concentrations (in catch basin and hourly samples) among the three CO_2 treatment ponds (F, H, and R; T_7 to T_9). Based on normality testing, the conducted GLMs were either parametric [analysis of covariance (ANCOVA) and repeated-measures ANCOVA (RANCOVA)] or non-parametric [ranked repeated measures analysis of variance (Friedman's test) and ranked RANCOVA (rRANCOVA)]. Repeated measures analyses were blocked by trial-pond ID.

To test whether CO_2 treatments increased the number of crayfish exiting the ponds (question 1), we analyzed capture data obtained from C and F ponds of T_1 to T_5, using a Poisson generalized linear mixed-effects model (PGLMM). The response variable was the total number of emerged crayfish (wandering + sheltered + escaped) and the grouping variable was treatment (F and C). To test for the effect of crayfish capture frequency (hourly versus final) on numbers of submerged, emerged, and total (submerged + emerged) crayfish captured (question 2), we analyzed capture data obtained from F and H ponds of T_3 to T_9 using PGLMMs. The response variable was the number of captured crayfish (either submerged, emerged, or total) and the grouping variable was treatment (F and H). To test for effects of the presence of a low CO_2 refuge (i.e., inflowing water) on numbers of submerged, emerged, and total crayfish captured (question 3), we analyzed data from H and R ponds of T_6 to T_9 using PGLMMs. The response variable was the number of captured crayfish (submerged, emerged, or total) and the grouping variable was treatment (H and R). In all PGLMMs, data were blocked by the pond ID to control for random variation encountered by the spatial effect.

Our dichotomous response variable (i.e. captured versus not captured crayfish) required the use of binary logistic regression (BLR) models, a GLM that extends linear regressions to non-continuous outcomes using logit link functions and a Maximum Likelihood estimator (Aldrich and Nelson 1984). In each BLR model, crayfish capture were assigned the integer zero when crayfish were not captured during the trial and was assigned the integer one when crayfish was captured. All BLR models were fit with maximum-likelihood estimation to develop estimates of crayfish capture:

$$\mathrm{OR}(C) = \mathrm{e}^{\mathrm{u}}$$

$$P(C) = \mathrm{e}^{\mathrm{u}}/(1+\mathrm{e}^{\mathrm{u}});$$

where OR(C) is the odds ratio of crayfish capture; $P(C)$ is the probability of crayfish capture; u is a linear combination of the predictor variables; and e is the natural exponential function.

A simple BLR (with one predictor) was used to ascertain the effect of each categorical predictor variable on the probability of crayfish capture across all treatments. The tested categorical predictor variables were crayfish sex-reproductive state groups (males, non-berried females, and berried females), crayfish texture (soft and hard), and pond treatments (C, F, H, and R). Similarly, one simple BLR model was used to test for the effect of each continuous predictor variable (water temperature, water pH, water CO_2 concentration, and crayfish wet mass) on the probability of crayfish capture across all treatments. To test for the effects of continuous variables on the probability of crayfish capture among treatments, four multiple BLR models (with more than one predictor) were used. Each multiple BLR tested for effects of treatment, one of the continuous variables, and the interaction between the continuous variable and treatment.

Finally, a multiple BLR model was developed using a backward elimination technique to select the set of predictors that could best predict the probability of crayfish capture. In this model, we included four continuous variables (crayfish mass, water temperature, pH, and CO_2 concentration), one categorical variable (treatment), and their interactions up to two-factor interaction (i.e., three-factor interactions or higher were not included). The model was developed using data from all study trials except T_6. The model adequacy was assessed using the goodness-of-fit test (Hosmer-Lemeshow test). Four interactive, self-explanatory Microsoft Excel spreadsheet-based calculator files (one for each treatment) were developed to perform the crayfish capture predictive models described in this paper. Data management and all statistical analyses were performed with SAS® version 9.4 (SAS 2013). Statistical significance was set at $p < 0.05$, and all data were presented as the mean ± standard error of the mean (*SE*).

Results

During the entire study, the hourly recorded weather data ($n = 196$) ranged from 2.00–33.00 °C (23.39 ± 0.58) for air temperature, 39.0–96.0% (53.23 ± 0.87) for relative humidity, 0.0–8.33 m/s (3.66 ± 0.12) for wind speed, and 0.0–4.30 mm (0.04 ± 0.02) for precipitation.

Study crayfish, ponds, and gas

Throughout the nine trials, the three ponds were stocked with a total of 2,500 crayfish (25 trial-pond combinations × 100 crayfish/pond). Of these, a total of 2,201 crayfish (88.04%) were successfully recaptured (captured during trials + collected after draining). The number of crayfish missing per pond after trial termination ranged from 8–21 (15.40 ± 2.18) crayfish for C ponds and from 0–34 (11.34 ± 2.17) crayfish for CO_2 diffusion ponds, with an overall mean of 12.16 ± 1.80 crayfish/pond. Out of the retrieved crayfish, 53.66% were males, 45.29% were non-berried females, and 1.05% were berried females; the carapace texture was hard in 97.72% crayfish, and soft in 2.28% crayfish (indicating freshly molted individuals); mortality rate was 1.41% in CO_2 diffusion ponds and 1.89% in control ponds, with an overall mortality of 1.50%. Wet mass of retrieved crayfish ranged from 1.5–59 g (18.77 ± 0.20 g). Crayfish counts for each category within C and CO_2 diffusion ponds across all trials are summarized in Table S1.

Across all trials, pond water surface area was 84.95 ± 2.21 m^2 while the maximum pond water depth was 0.65 ± 0.01 m. A mean of 11.61 ± 1.45 Kg of CO_2 was diffused per CO_2 diffusion pond as determined from gas tank weights before and after diffusion.

Water chemistry

The descriptive results (*n*, mean, minimum, and maximum) of different water quality parameters measured in C and CO_2 treatment ponds across

Table 2. Results from correlation analysis tests between CO_2 concentrations (mg/L) and other water quality variables. Correlations were performed for control ponds only (C), CO_2 treatment ponds only (F + H), or for control and treatment ponds (C + F + H). Numbers of measurement pairs were 35 for C, 103 for F + H, and 138 for C + F + H. Based on bivariate normality testing, tests used were either Pearson correlation (P; coefficient = *r*) or Spearman rank correlation (S; coefficient = *ρ*). Significant results at $p < 0.05$ if (**bold***). From all trials (except trial 6), data obtained from all ponds without refuge were used in correlation analyses.

Variable	Pond	Test	*r* or *ρ*	*p*-value [a]
Water temperature (°C)	C	S	0.73*	**0.0002**
	F + H	S	0.37*	**0.0002**
	C + F + H	S	0.46*	**0.0001**
Dissolved oxygen (mg/L)	C	P	−0.64*	**0.0002**
	F + H	S	−0.26*	**0.0080**
	C + F + H	S	−0.43*	**0.0001**
pH	C	P	−0.54*	**0.0010**
	F + H	S	−0.49*	**0.0002**
	C + F + H	S	−0.77*	**0.0001**

[a] All *p*-values were adjusted to control the false discovery rate using the Benjamini-Hochberg procedure.

all trials are summarized in Table S2. Mean water temperature was highly variable across trials ranging from 6.10 to 37.80 °C. Within pond diel variation (difference between maximum and minimum temperatures recorded in the same pond during a given trial) in water temperature ranged between 1.60 °C and 8.30 °C. In all ponds without a refuge, CO_2 concentration was positively correlated with water temperature (Spearman: $\rho = 0.37$, $p = 0.0001$) and inversely correlated with DO (Spearman: $\rho = -0.26$, $p = 0.0080$) and pH (Spearman: $\rho = -0.49$, $p = 0.0001$; Supplementary material Appendix 1, Figure S1). Water temperature was inversely correlated with DO (Spearman: $\rho = -0.95$, $p = 0.0001$). In C ponds, CO_2 concentrations were also inversely correlated with DO and pH (Table 2). The mean of DO measured in CO_2 treatment ponds was 1.68 to 3.29 mg/L lower than that in C ponds. In CO_2 treatment ponds, the lowest recorded DO reading was 1.43–1.52 mg/L lower than that in C ponds while the lowest recorded pH reading was 1.21–1.36 lower than that in C ponds. Across all trial-pond combinations, the lowest recorded DO reading was 5.86 mg/L while the lowest pH measurement was 5.08.

Diffusing CO_2 into treatment ponds (F + H) resulted in a significant increase in CO_2 concentrations (Table S3). There was a significant inverse relationship between water temperature and water pH (Table S3). After controlling for water temperature, treatment ponds had significantly lower pH values as compared to control ponds (Table S3). In general, the higher the alkalinity and hardness in initial water samples the higher they were in final water samples. After controlling for initial hardness, control ponds had significantly higher final hardness concentrations than CO_2 diffusion ponds (Table S3). There was a significant interaction between initial hardness and treatment (Table S3) with initial hardness significantly affecting final hardness in control ponds ($t_7 = 5.19$, $p = 0.0013$) but not in CO_2 diffusion ponds ($t_7 = 0.43$, $p = 0.6819$). Total alkalinity in final samples and DO in hourly samples were not different between C and CO_2 ponds after controlling for the covariate (initial alkalinity and water temperature, respectively; Table S3). In trials that included R ponds (T_6 to T_9), CO_2 diffusion ponds did not have different CO_2 concentrations in samples collected from catch basin (ANOVA: $F_{2,6} = 0.03$, $p = 0.9665$; Table S2) or in hourly-collected samples collected from mid-distance between water edge and center of the pond (rRANCOVA: $\chi^2_{(2)} = 0.99$, $p = 0.6083$; covariate = water temperature).

Crayfish emergence and capture

Diffusion of CO_2 into ponds significantly increased the number of crayfish that emerged from ponds. Across all trials that included C ponds (T_1 to T_5), the number of emerged (wandering + sheltered + escaped) crayfish captured from F ponds (12.00 ± 2.12 crayfish, $n = 5$) was significantly greater than from control ponds (0.80 ± 0.37 crayfish, $n = 5$; PGLMM: $\chi^2_{(1)} = 8.15$, $p = 0.0033$; Figure 2a). In those trials, numbers of sheltered crayfish were 6.60 ± 3.17 and 0.40 ± 0.24 crayfish per F and C ponds, respectively; numbers of wandering crayfish were 3.6 ± 1.12 and 0.20 ± 0.20 crayfish per F and C ponds, respectively; and numbers of escaped crayfish were 1.8 ± 0.73 and 0.20 ± 0.20 crayfish per F and C ponds, respectively.

In the F ponds, we noticed large numbers of crayfish that were still submerged but near the water edge that could be easily captured. Number of submerged crayfish collected at the end of trials $T_{3,4,5}$ was highly variable but was significantly higher in F ponds (20.0 ± 10.02) than in C ponds (3.0 ± 3.0; PGLMM: $\chi^2_{(4)} = 5.31$, $p = 0.0061$). We, therefore, continued collecting submerged crayfish in subsequent trials.

Increasing the frequency of capture events from final-only to hourly captures had a strong effect on increasing the total number of crayfish captured from CO_2 diffusion ponds. Count of submerged crayfish captured at the pond edge was significantly higher in H ponds as compared to F ponds (PGLMM: $\chi^2_{(7)} = 91.56$, $p = 0.0001$; Figure 2b). However, there was no evidence of differences in count of emerged crayfish from ponds in H ponds as compared to F ponds (PGLMM: $\chi^2_{(5)} = 70.95$, $p = 0.8431$; Figure 2b). When all captured crayfish categories were pooled, mean count of crayfish captured from H ponds (44.71 ± 6.31 crayfish) was significantly higher than those from F ponds (23.29 ± 5.13 crayfish; PGLMM: $\chi^2_{(5)} = 64.67$, $p = 0.0003$; Figure 2b).

In ponds where crayfish were captured hourly, the presence of submerged water inflow in the central catch basin in R ponds significantly decreased the numbers of crayfish captured either submerged (PGLMM: $\chi^2_{(10)} = 65.08$, $p = 0.0089$; Figure 2c), or emerged (PGLMM: $\chi^2_{(7)} = 42.60$, $p = 0.0184$; Figure 2c) as compared to H ponds. Similarly, mean count of pooled crayfish captured from R ponds (22.50 ± 7.82 crayfish) was significantly lower than those from H ponds (48.25 ± 8.51 crayfish; PGLMM: $\chi^2_{(3)} = 23.59$, $p = 0.0115$; Figure 2c).

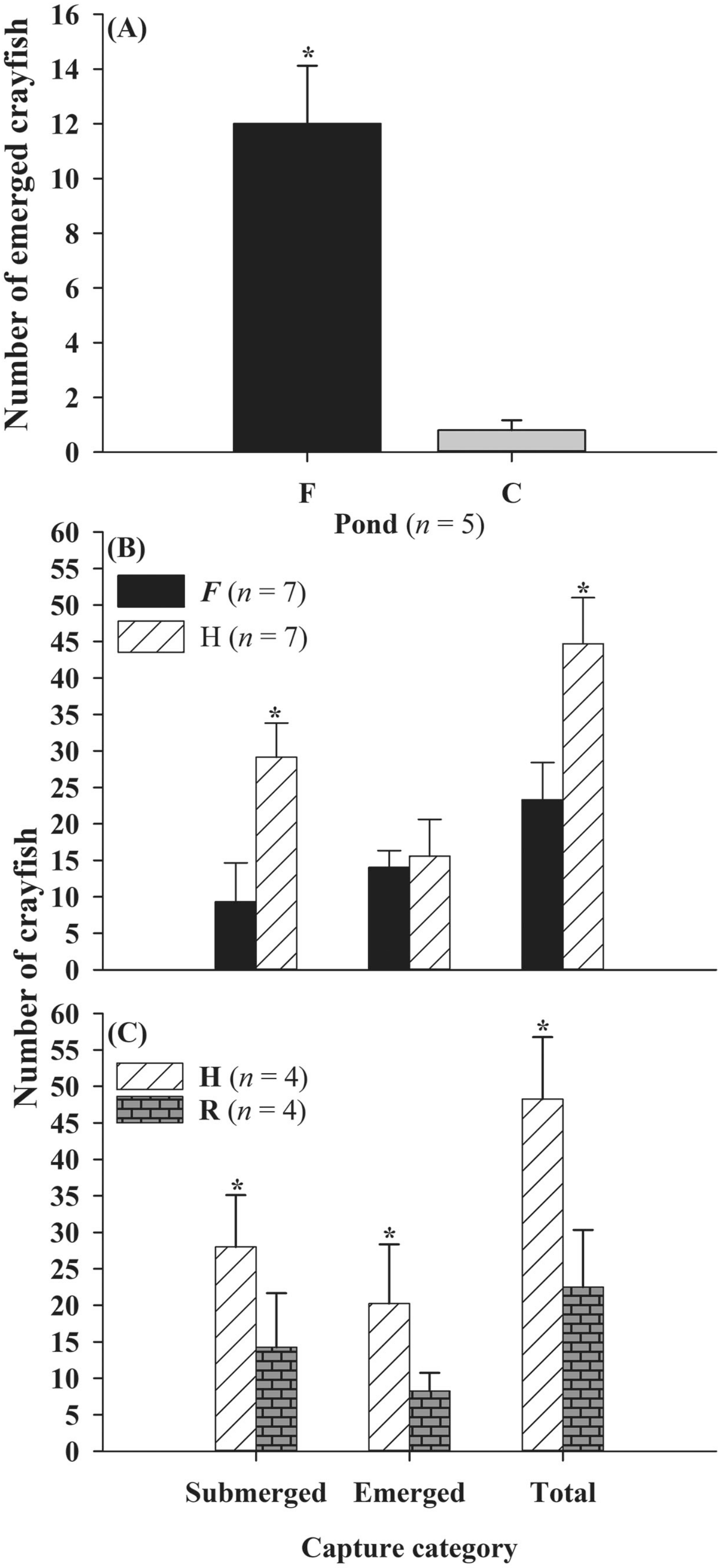

Figure 2. (A) Mean number of emerged crayfish captured from F and C ponds, and mean number of submerged (near water edge), fully emerged, or total (submerged + emerged) crayfish captured from (B) F and H ponds or (C) H and R ponds. Error bars represent ± 1 standard error. C ponds = control: no CO_2 diffusion and final crayfish capture; F ponds = final: CO_2 diffusion, final crayfish capture; H ponds = hourly: CO_2 diffusion, hourly crayfish capture; and R ponds = refuge: CO_2 diffusion, hourly crayfish capture, continuous water inflow at a flow rate of 0.2 L/s into the central catch basin. Within each capture category, ponds with (*) were significantly higher than the other ponds at $p < 0.05$.

Table 3. Odds ratio (OR), 95% confidence intervals (95% CI) for OR, crayfish capture probability ± 95% CI, and statistics of the binary logistic regression models for crayfish capture probability in presence of categorical confounding variables. Odds ratio was statistically significant if the null value (OR = 1.0) is not contained within the 95% CI. Regression models were statistically significant at $p < 0.05$ if (**bold***). Levels of the variable treatment were C (control; no CO_2 diffusion, crayfish captured at the end of trial) and three CO_2 diffusion treatments: F (final; crayfish captured at the end of trial), H (hourly; crayfish captured hourly), and R (refuge; freshwater inflow at a flow rate of 0.2 L/s into the central catch basin, crayfish captured hourly).

Categorical Predictor Variable		Odds Ratio			Probability (± 95% CI)	χ^2 (df), *p*-value
Variable	Categories	Estimate	95% CI			
			Lower	Upper		
Treatment	C [a]	–	–	–	0.03 (0.02)	300.91 (3), **< 0.0001***
	F	9.617	5.406	17.108	0.23 (0.03)	
	H	32.260	18.167	57.283	0.51 (0.04)	
	R	10.397	5.698	18.971	0.25 (0.04)	
Sex-reproductive status	Male [a]	–	–	–	0.29 (0.03)	10.57 (2), **0.0051***
	Non-berried female	0.836	0.691	1.011	0.25 (0.03)	
	Berried female	2.706	1.183	6.193	0.52 (0.19)	
Texture/molting	Soft [a]	–	–	–	0.24 (0.11)	0.3968 (1), 0.5287
	Hard	1.224	0.652	2.296	0.27 (0.02)	

[a] Reference levels.

Results of simple BLR models on categorical predictor variables revealed that crayfish capture probabilities were affected by the type of pond treatment and sex-reproduction status but not by crayfish texture (Table 3). The estimated odds (captured ÷ not captured) of crayfish capture in CO_2 diffusion treatments were higher than these in C ponds by 3,126% for H ponds, 940% for R ponds, and 862% for F ponds (Table 3). In H ponds, capture odds were 235% (167–321%) higher than that in F ponds (simple BLR: Wald $\chi^2_{(1)} = 108.68$, $p < 0.0001$) and 210% (133–313%) higher than that in R ponds (simple BLR: Wald $\chi^2_{(1)} = 60.37$, $p < 0.0001$). However, R and F ponds were not different in their capture probability (simple BLR: Wald $\chi^2_{(1)} = 0.28$, $p = 0.597$). Berried females showed significantly higher capture probabilities than males (simple BLR: Wald $\chi^2_{(1)} = 5.56$, $p = 0.0184$) and non-berried females (simple BLR: Wald $\chi^2_{(1)} = 7.69$, $p = 0.0056$). The estimated odds of capturing berried female crayfish were 171% (at least 18%, at most 519%) higher than that of males, and 242% (at least 41%, at most 643%) higher than that of non-berried females (Table 3). Capture probabilities were not different between non-berried females and males (simple BLR: Wald $\chi^2_{(1)} = 3.40$, $p = 0.0653$) or between females (berried + non-berried) and males (simple BLR: Wald $\chi^2_{(1)} = 3.40$, $p = 0.0653$) and the 95% confidence interval for the odds ratio (0.97, 2.32), which contained 1.

Results of simple BLR models on continuous predictor variables—either across all treatments or among treatments—revealed that crayfish capture probabilities were significantly affected by mean water temperature, mean CO_2 concentration, mean pH, and crayfish wet mass (Table S4). Across all treatments, the estimated overall odds of crayfish capture were increased by 1.3% (0.3–2.4%; Figure 3a) for every 1-°C increase in mean pond water temperature, 0.9 (0.6–1.2%; Figure 3c) for every 10-ppm increase in mean CO_2 concentration, 2.9% (2.0–3.9%; Figure 3d) for every 1-gram increase

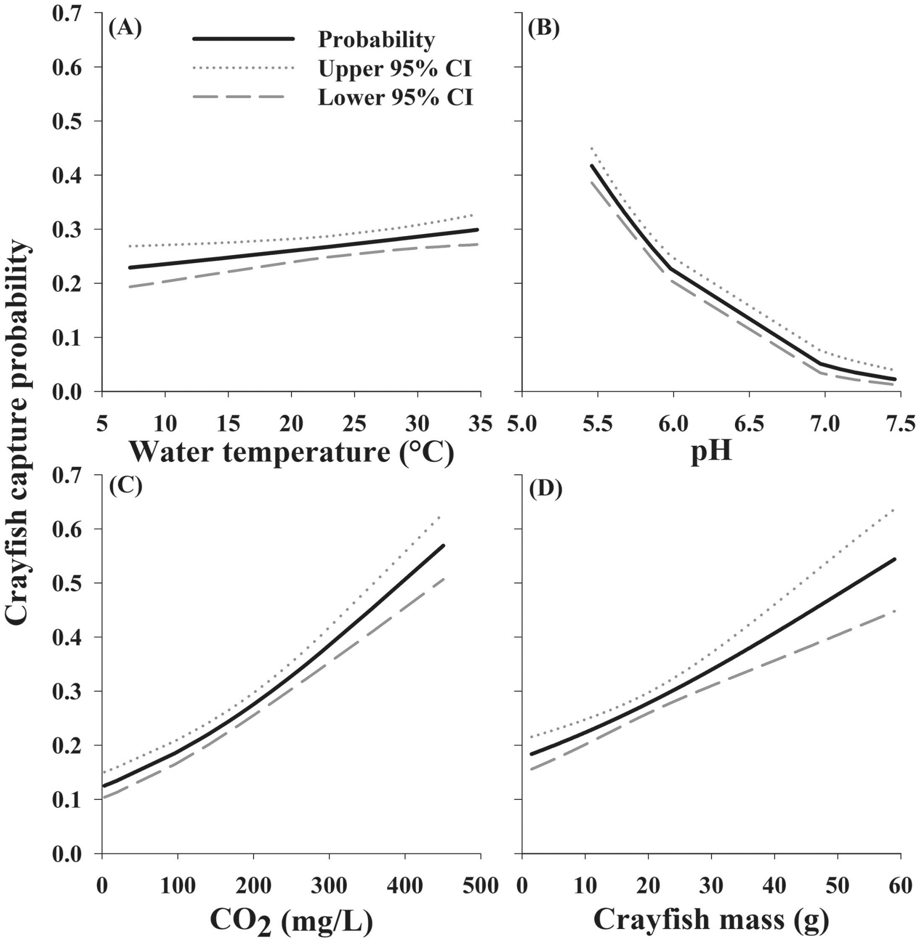

Figure 3. Predicted crayfish capture probabilities and 95% confidence intervals from statistically significant ($p < 0.05$) simple binary logistic regression models investigating the effects of water temperature (A), water pH (B), carbon dioxide concentration (CO_2; C), and crayfish wet masses (D) on capture probabilities across all treatments.

in crayfish wet mass. Odds of crayfish capture were decreased by 81.9% (75–86.9%; Figure 3b) for every 1-unit increase in mean pond water pH (Table S4).

Four multiple BLR models were used to test for the effect of continuous predictor variables, treatment, and interactions between a continuous variable and the treatment on capture probability. The interaction term (homogeneity of slopes) was significant in all models except the model for wet mass (Table S4). Heterogeneity of slopes explains the differences in relationship trends between capture probability and changes in continuous variables among treatments. For every 1 °C increase in mean pond water temperature, capture probabilities were increased in H ponds but decreased in C, F, and R ponds (Table S4). For every 10-ppm increase in mean CO_2 concentration, capture probabilities were increased in H ponds, decreased in R ponds, and did not change significantly in C and F ponds (Table S4). For every 1-unit increase in mean pond water pH, capture probability did not change significantly in C, F, nor R ponds, however, it was increased in H ponds. The values of mean pond water pH in H ponds ranged between

Table 4. Statistical results from the multiple binary logistic regression test using a backward elimination technique to select the set of predictors that best predict the probability of crayfish capture. Selected effects were statistically significant at $p < 0.05$ (**bold***).

	Effects	Wald $\chi^2_{(df)}$	p-value
Selected	Water temperature	23.02 $_{(1)}$	**< 0.0001***
	Carbon dioxide (CO_2)	8.09 $_{(1)}$	**0.0044***
	Treatment	23.33 $_{(3)}$	**< 0.0001***
	pH × Water temperature	22.24 $_{(1)}$	**< 0.0001***
	pH × CO_2	9.51 $_{(1)}$	**0.0020***
	pH × Treatment	22.49 $_{(2)}$	**< 0.0001***
	Water temperature × Treatment	41.02 $_{(3)}$	**< 0.0001***
	CO_2 × Treatment	37.54 $_{(3)}$	**< 0.0001***
	Mass × treatment	34.21 $_{(3)}$	**< 0.0001***
Removed	pH	0.03 $_{(1)}$	0.8724
	Mass	3.37 $_{(1)}$	0.0666
	Water temperature × CO_2	0.04 $_{(1)}$	0.8448
	Mass × Water temperature	0.70 $_{(1)}$	0.4038
	Mass × CO_2	0.002 $_{(1)}$	0.9686
	Mass × pH	1.21 $_{(1)}$	0.2723

5.57 and 5.80. For every 1-gram increase in crayfish wet mass, capture probabilities were increased in C and F but did not change significantly in H and R (Table S4).

Multiple logistic regression was performed—using a backward selection method—to ascertain the effects of pond water temperature, pH, CO_2, crayfish wet mass, treatment, and their interaction on the likelihood of crayfish capture (Table 4). The selected model was statistically significant ($\chi^2_{(18)} = 375.99$, $p < 0.0001$). The adequacy of the selected model was assessed using the goodness-of-fit test "Hosmer-Lemeshow test." The test was not significant ($\chi^2_{(8)} = 11.18$, $p = 0.1917$) which indicated that the model fits the data well. The following linear combinations of predictor variables (u) can be used to predict crayfish capture odds and probabilities for each treatment using equations described in the *Methods* section.

$$C = -3.5038 - 8.8093\,T + 0.806\,Cd + 0.0823\,CdP + 1.1048\,TP$$

$$F = 267.1962 - 6.3126\,T - 0.4721\,Cd - 47.1448\,P + 0.0462\,M + 0.0823\,CdP + 1.1048\,TP$$

$$H = 371.9962 - 5.9963\,T - 0.4665\,Cd - 67.0181\,P + 0.0117\,M + 0.0823\,CdP + 1.1048\,TP$$

$$R = 24.2512 - 6.916\,T - 0.5072\,Cd + 0.0507\,M + 0.0823\,CdP + 1.1048\,TP$$

where T = mean water temperature (°C) in pond during the trial (~ 8 hours); Cd = mean CO_2 concentration (ppm) in pond during the trial (~ 8 hours); P = mean pH in pond during the trial (~ 8 hours); and M = crayfish wet mass in grams.

Interactive model calculators were created to predict crayfish capture probability and odds for each treatment pond. Calculators require data entry of explanatory variables in metric units (mg/L, g, and °C). The interactive calculator files are publicly available on the author's Github repository at https://github.com/Hisham-Abdelrahman/crayfish-CO2 and directly from the corresponding author.

Discussion

Efficacy of CO_2 diffusion as a management strategy

Diffusion of CO_2 into ponds shows promise as a management strategy to enhance capture of exotic RSC from small waterbodies, but the relative success of this approach is dependent on collection protocols, environmental conditions, and characteristics of individual crayfish. In our study, increasing CO_2 concentrations from ambient concentrations (< 20 mg/L) to > 200 mg/L caused a portion of the crayfish population to emerge from ponds over several hours. On average, only ~ 12% of the 100 crayfish initially stocked into the ponds were found fully emerged near or under shelters after ~ 6 h of CO_2 diffusion (F ponds). Thus, simply providing terrestrial shelters for crayfish and coming back several hours later for collection is not likely, by itself, to be highly effective. However, CO_2 can be made much more effective simply by increasing collection frequency and by adopting techniques to collect submerged crayfish near the shoreline. Crayfish did not remain emerged over time, but frequently returned to pondwaters despite the high CO_2 concentrations. Observations indicated submerged crayfish aggregated near the water edge of CO_2 diffusion ponds in much higher numbers than in control ponds. Modifying collection techniques by increasing collection frequency to once per hour (H ponds) over the ~ 6-hour study period, and netting submerged crayfish near the water edge in addition to collecting fully emerged crayfish, increased mean capture efficiency from 12% to ~ 45% per treatment pond compared to ~ 3% per control pond. In heavily infested ponds, a possible IPM approach would be to use CO_2 gas along with the modified collection techniques to capture around 45% of individuals followed by the use of baited traps. This approach would avoid the negative effect of trap saturation – animals inside traps preventing those outsides from entering (Green et al. 2018; Miller 1979, 1990).

Effect of freshwater inflow on crayfish emergence

Carbon dioxide may not be a particularly effective tool to control RSC in infested waterbodies with underwater springs, or other sources of freshwater inflow located away from the shoreline – even if these sources are relatively small. In our study, capture of emerged and nearshore crayfish was significantly lower in R ponds compared to H ponds even though the discharge of freshwater inflow was too small to create a detectable refuge in R ponds. There was no significant decrease in CO_2 concentrations within the catch basins of R ponds where the inflow was located, nor at the regular sampling site(s) located between the shoreline and catch-basin. Despite the similar CO_2 concentrations, crayfish did not aggregate at the pond edges of R ponds as they did in the H ponds. This indicates crayfish are attracted to submerged freshwater inflows, even when flows are too small to create a large refuge from CO_2. Although freshwater inflows decreased crayfish captures in our study, it may be advantageous in other systems. If an inflow is located at the shoreline (i.e., drainage ditch leading to an infested retention pond) rather than in deeper waters, CO_2 could be used not only to push crayfish towards the shoreline, but to further concentrate crayfish in relatively small, nearshore area(s) dominated by freshwater inflows. This would be similar to the "push" strategy suggested by Fredricks et al. (2020).

Effect of water temperature on crayfish emergence

Use of CO_2 to enhance RSC capture in infested waterbodies is likely to be most effective at warm temperatures. Across all trials and treatments combined, RSC captures were highest during warmer days, with odds of capturing crayfish increasing by ~ 1% with every 1 °C increase in water temperature. This relationship was primarily driven by increased captures in the H ponds. These results can be explained in part by the findings of Claussen et al. (2000) that showed that locomotor performance of crayfish is affected by ambient temperature as a result of thermally dependent adjustments in stride frequency with the greatest walking speeds recorded at 25 and 30 °C as compared to 15, 20, and 35 °C. In contrast, captures in the F ponds showed a negative relationship with temperature. The F pond pattern was driven by an extremely high number (40) of submerged crayfish collected during our coldest trial (T_5: 7.2 °C). After removing T_5 data from analysis, F pond captures no longer showed a negative relationship with temperature. It is not clear why T_5 produced an unusually high number of submerged crayfish collected, but it does show that under some circumstances, CO_2 can be highly effective even at cold temperatures.

The overall increase in RSC captures with increasing temperature was likely due in part to the positive relationship between CO_2 and temperature – mean CO_2 concentrations in pond water increased with temperature. Odds of crayfish capture increased by ~ 3% for every 10 ppm increase in CO_2 concentration in the H ponds. Mean pH in pond water also decreased with increasing CO_2 and crayfish capture was negatively correlated with pH. However, increased RSC captures were more likely related to increasing CO_2 than to declining pH. In laboratory studies under controlled conditions, RSC avoided high CO_2 concentrations (e.g. Fredricks et al. 2020; Paietta and Hartzler 2017) but avoidance behavior was not triggered by decreasing pH alone (Bierbower and Cooper 2010). This species is nocturnal – more active during night time (Gherardi et al. 2000; Loureiro et al. 2015) which might increase capture probabilities if CO_2 is applied during night time.

Effects of crayfish size, sex, and reproductive status on crayfish emergence

Many control techniques such as trapping and chemical control may be biased towards crayfish size and sex (Loureiro et al. 2018; Paillisson et al.

2011), requiring an array of techniques to target an entire population. Laboratory studies examining effect of CO_2 on avoidance and emergence behavior of RSC found no evidence of size or sex bias but cautioned that additional trials needed to be conducted in more natural settings (Fredricks et al. 2020). In earthen-bottom research ponds, we found strong evidence of size-bias when using CO_2 to collect RSC by net and hand. Odds of capture increased by ~ 3% for every 1-gram increase in crayfish mass. These results can be explained in part by the findings of Claussen et al. (2000) which showed that crayfish walking speed increases with body mass with a scaling exponent of 0.18 as a result of the size-related increase in stride length. Additional management techniques could be evaluated to increase capture rates of smaller crayfish in combination with CO_2. Conversely, we found no evidence for sex bias in RSC captures. However, reproductive state was important, with berried females exhibiting a higher capture probability than males or non-berried females. Use of CO_2 to drive crayfish towards shore and emerge from the water is likely to be most effective for mature, reproductive individuals—especially berried females—as opposed to juveniles. The small sample size of the berried females may be influencing the capture probabilities to a greater extent than the non-berried females and males. Interestingly, we occasionally collected freshly molted individuals that were still soft. Odds of collecting freshly molted crayfish were not significantly different from collection odds of fully hardened individuals. Due to small sample sizes of fresh molts, these results warrant interpreting with caution, but molting did not appear to inhibit movement towards shore as CO_2 concentrations increased.

Bubbling of CO_2 and crayfish emergence

It is not entirely clear why increasing CO_2 concentration in a pond environment caused crayfish to move towards the shoreline and occasionally emerge – particularly as previous lab studies show crayfish may reduce activity and seek shelter in response to elevated CO_2 (Robertson et al. 2018). Crayfish will often move towards an air-water interface and even emerge completely in response to hypoxia (Bonvillain et al. 2012), and DO did decline with increasing CO_2 concentrations in our experimental ponds. However, DO never dropped below 5.86 mg/L whereas hypoxia is generally defined as a concentration of < 2–3 mg O_2/L (Bonvillain et al. 2012; Demers et al. 2006; Diaz 2001). Red swamp crayfish are particularly well adapted to hypoxia (Bonvillain et al. 2012; Chapman 1996; McMahon 2002; Nyström 2002). It is unlikely that a decrease in oxygen was the primary driver behind RSC migration and emergence. Similarly, pH also dropped to as low as 5.08 with increasing CO_2 concentrations but, as discussed previously, avoidance behavior of RSC seems to be driven by increasing CO_2 rather than by decreasing pH (Bierbower and Cooper

2010). Bubbling of CO_2 through the water surrounding the crayfish results in photic stimulation of their optic nerve which initiates locomotion (Bennitt and Merrick 1932; Meyer-Rochow and Tiang 1982; Steven 1963). As a result of the tonic effect of light, the amplitude of stride and frequency of leg movements are functions of light intensity in certain arthropods (Welsh 1932) and crayfish (Welsh 1934). It is possible that the avoidance is driven by a photomechanical response to CO_2. Increasing concentration of CO_2 can affect the heart rate of crayfish – resulting in systolic arrest (Ashby and Larimer 1964; Chapman 1996). It is possible that the avoidance is driven by cardiac response to CO_2. Additional research linking behavioral and physiological responses to increasing CO_2 concentrations would be valuable as CO_2 continues to be refined as a control technique.

Conclusions

Carbon dioxide can be utilized to induce crayfish to move toward water edges and/or completely emerge from ponds for capture. The diffusion of CO_2 into pond water does not result in hypoxia and does not leave residues. However, it can trigger avoidance behavior and loss of equilibrium in other taxa, such as fish, at the concentrations applied in this study (Cupp et al. 2017a; Tix et al. 2018) and may be lethal to taxa such as bullfrog tadpoles (Abbey-Lambertz et al. 2014).

The efficiency of using CO_2 as a control agent is dependent on multiple factors such as the capture protocol, environmental conditions, and characteristics of resident crayfish. Efficiency is improved by increasing the crayfish capture frequency to an hourly basis, by capturing submerged crayfish from water edges as well as emerged crayfish, and by diffusing CO_2 during warm days. While efficiency is reduced in the presence of a continuous inflow of low-CO_2 water into an infested pond, this weakness might be used as a strength when CO_2 is used to "push" crayfish towards a low-CO_2 refuge for easy collection. This is a strategy that could be examined further.

Results of this study provide a set of predictive equations to help managers to decide which capture protocol (hourly versus final collection) would be best to apply, as well as what environmental and biotic conditions (i.e., air temperature, water quality parameters, and the mean weight of the crayfish population) will optimize the odds of crayfish capture from infested ponds. To explore several what-if scenarios, natural resource managers can use interactive calculators developed from this study (see Results). Because predictions are based on results of small, experimental-pond trials, they may be used to explore relative changes in capture probability rather than to predict actual capture rates from invaded waterbodies.

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References

Abbey-Lambertz M, Ray A, Layhee M, Densmore C, Sepulveda A, Gross J, Watten B (2014) Suppressing bullfrog larvae with carbon dioxide. *Journal of Herpetology* 48: 59–66, https://doi.org/10.1670/12-126

Aldrich JH, Nelson FD (1984) Linear probability, logit, and probit models. Sage publications, Newbury Park, CA, https://doi.org/10.4135/9781412984744

Anastácio P, Marques J (1997) Crayfish, *Procambarus clarkii*, effects on initial stages of rice growth in the lower Mondego River valley (Portugal). *Freshwater Crayfish* 11: 608–617, https://doi.org/10.14195/978-989-26-0336-0_23

Ashby EA, Larimer JL (1964) Cardiac responses of the crayfish, *Procambarus simulans*, to external and internal carbon dioxide stress. *Physiological Zoology* 37: 21–32, https://doi.org/10.1086/physzool.37.1.30152317

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)* 57: 289–300, https://doi.org/10.1111/j.2517-6161.1995.tb02031.x

Bennitt R, Merrick AD (1932) Migration of the proximal retinal pigment in the crayfish in relation to oxygen deficiency. *The Biological Bulletin* 62: 168–177, https://doi.org/10.2307/1537548

Bierbower S, Cooper R (2010) The effects of acute carbon dioxide on behavior and physiology in *Procambarus clarkii*. *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology* 313: 484–497, https://doi.org/10.1002/jez.620

Bonvillain CP, Rutherford DA, Kelso WE, Green CC (2012) Physiological biomarkers of hypoxic stress in red swamp crayfish *Procambarus clarkii* from field and laboratory experiments. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 163: 15–21, https://doi.org/10.1016/j.cbpa.2012.04.015

Chapman SL (1996) Developmental physiology of the red swamp crayfish *Procambarus clarkii*. Master of Science Thesis, University of Nevada, Las Vegas, Nevada, USA, https://doi.org/10.25669/safa-sh6f

Claussen DL, Hopper RA, Sanker AM (2000) The effects of temperature, body size, and hydration state on the terrestrial locomotion of the crayfish *Orconectes rusticus*. *Journal of Crustacean Biology* 20: 218–223, https://doi.org/10.1163/20021975-99990033

Cupp AR, Smerud JR, Tix JA, Rivera JM, Kageyama SA, Merkes CM, Erickson RA, Amberg JJ, Gaikowski MP (2018) Assessment of carbon dioxide piscicide treatments. *North American Journal of Fisheries Management* 38: 1241–1250, https://doi.org/10.1002/nafm.10227

Cupp AR, Tix JA, Smerud JR, Erickson RA, Fredricks KT, Amberg JJ, Suski CD, Wakeman R (2017a) Using dissolved carbon dioxide to alter the behavior of invasive round goby. *Management of Biological Invasions* 8: 567–574, https://doi.org/10.3391/mbi.2017.8.4.12

Cupp AR, Woiak Z, Erickson RA, Amberg JJ, Gaikowski MP (2017b) Carbon dioxide as an under-ice lethal control for invasive fishes. *Biological Invasions* 19: 2543–2552, https://doi.org/10.1007/s10530-017-1462-9

Demers A, Souty-Grosset C, Trouilhé M-C, Füreder L, Renai B, Gherardi F (2006) Tolerance of three European native species of crayfish to hypoxia. *Hydrobiologia* 560: 425–432, https://doi.org/10.1007/s10750-005-1466-9

Diaz RJ (2001) Overview of hypoxia around the world. *Journal of Environmental Quality* 30: 275–281, https://doi.org/10.2134/jeq2001.302275x

Fredricks KT, Hubert TD, Amberg JJ, Cupp AR, Dawson VK (2019) Chemical controls for an integrated pest management program. *North American Journal of Fisheries Management* 41: 289–300, https://doi.org/10.1002/nafm.10339

Fredricks KT, Tix JA, Smerud JR, Cupp AR (2020) Laboratory trials to evaluate carbon dioxide as a potential behavioral control method for invasive red swamp (*Procambarus clarkii*) and rusty crayfish (*Faxonius rusticus*). *Management of Biological Invasions* 11: 259–278, https://doi.org/10.3391/mbi.2020.11.2.06

Freeman MA, Turnbull J, Yeomans WE, Bean CW (2010) Prospects for management strategies of invasive crayfish populations with an emphasis on biological control. *Aquatic Conservation: Marine and Freshwater Ecosystems* 20: 211–223, https://doi.org/10.1002/aqc.1065

Geiger W, Alcorlo P, Baltanas A, Montes C (2005) Impact of an introduced Crustacean on the trophic webs of Mediterranean wetlands. *Biological Invasions* 7: 49–73, https://doi.org/10.1007/s10530-004-9635-8

Gherardi F (2006) Crayfish invading Europe: the case study of *Procambarus clarkii*. *Marine and Freshwater Behaviour and Physiology* 39: 175–191, https://doi.org/10.1080/10236240600869702

Gherardi F, Aquiloni L, Diéguez-Uribeondo J, Tricarico E (2011) Managing invasive crayfish: is there a hope? *Aquatic Sciences* 73: 185–200, https://doi.org/10.1007/s00027-011-0181-z

Gherardi F, Barbaresi S, Salvi G (2000) Spatial and temporal patterns in the movement of *Procambarus clarkii*, an invasive crayfish. *Aquatic Sciences* 62: 179–193, https://doi.org/10.1007/PL00001330

Green N, Bentley M, Stebbing P, Andreou D, Britton R (2018) Trapping for invasive crayfish: comparisons of efficacy and selectivity of baited traps versus novel artificial refuge traps. *Knowledge Management of Aquatic Ecosystems* 419: 1–9, https://doi.org/10.1051/kmae/2018007

Longshaw M (2011) Diseases of crayfish: a review. *Journal of Invertebrate Pathology* 106: 54–70, https://doi.org/10.1016/j.jip.2010.09.013

Loureiro TG, Anastácio PM, de Siqueira Bueno SL, Araujo PB (2018) Management of invasive populations of the freshwater crayfish *Procambarus clarkii* (Decapoda, Cambaridae): test of a population-control method and proposal of a standard monitoring approach. *Environmental Monitoring and Assessment* 190: 1–11, https://doi.org/10.1007/s10661-018-6942-6

Loureiro TG, Anastácio PMSG, Araujo PB, Souty-Grosset C, Almerão MP (2015) Red swamp crayfish: biology, ecology and invasion-an overview. *Nauplius* 23: 1–19, https://doi.org/10.1590/S0104-64972014002214

Matsuzaki S-iS, Usio N, Takamura N, Washitani I (2009) Contrasting impacts of invasive engineers on freshwater ecosystems: an experiment and meta-analysis. *Oecologia* 158: 673–686, https://doi.org/10.1007/s00442-008-1180-1

McMahon B (2002) Physiological adaptation to environment. In: Holdich DM (ed), Biology of Freshwater Crayfish. Blackwell Science, Oxford, UK, pp 327–376, https://doi.org/10.1086/382432

Meyer-Rochow V, Tiang K (1982) Comparison between temperature-induced changes and effects caused by dark/light adaptation in the eyes of two species of Antarctic crustaceans. *Cell and Tissue Research* 221: 625–632, https://doi.org/10.1007/BF00215706

Miller R (1979) Saturation of crab traps: reduced entry and escapement. *ICES Journal of Marine Science* 38: 338–345, https://doi.org/10.1093/icesjms/38.3.338

Miller R (1990) Effectiveness of crab and lobster traps. *Canadian Journal of Fisheries Aquatic Sciences* 47: 1228–1251, https://doi.org/10.1139/f90-143

Nyström P (2002) Ecology. In: Holdich DM (ed), Biology of Freshwater Crayfish. Blackwell Science, Oxford, UK, pp 192–235, https://doi.org/10.1086/382432

Paietta EN, Hartzler LK (2017) Impact of a CO_2 gradient on the behavior of the red crayfish, *Procambarus clarkii*. Biological Sciences Student Publications, https://corescholar.libraries.wright.edu/bio_student/1

Paillisson J-M, Soudieux A, Damien J-P (2011) Capture efficiency and size selectivity of sampling gears targeting red-swamp crayfish in several freshwater habitats. *Knowledge and Management of Aquatic Ecosystems* 401: 1–12, https://doi.org/10.1051/kmae/2011015

Robertson M, Hernandez M, Midway S, Hasler C, Suski CD (2018) Shelter-seeking behavior of crayfish, *Procambarus clarkii*, in elevated carbon dioxide. *Aquatic Ecology* 52: 225–233, https://doi.org/10.1007/s10452-018-9657-4

Rodríguez CF, Bécares E, Fernández-Aláez M (2003) Shift from clear to turbid phase in Lake Chozas (NW Spain) due to the introduction of American red swamp crayfish (*Procambarus clarkii*). *Hydrobiologia* 506: 421–426, https://doi.org/10.1023/B:HYDR.0000008626.07042.87

SAS (2013) Statistical analysis system for Windows: release 9.4, SAS Institute Inc., Cary, North Carolina, USA

Sepulveda A, Ray A, Al-Chokhachy R, Muhlfeld C, Gresswell R, Gross J, Kershner J (2012) Aquatic invasive species: lessons from cancer research. *American Scientist* 100: 234–242, https://doi.org/10.1511/2012.96.234

Souty-Grosset C, Holdich D, Noël P, Reynolds J, Haffner P (2006) Atlas of crayfish in Europe. Muséum national d'histoire naturelle, Paris, 187 pp

Stebbing P, Watson G, Bentley M, Fraser D, Jennings R, Rushton S, Sibley P (2003) Reducing the threat: the potential use of pheromones to control invasives signal crayfish. *Bulletin Francais de la Peche et de la Pisciculture* 370–371: 219–224, https://doi.org/10.1051/kmae:2003017

Steven D (1963) The dermal light sense. *Biological Reviews* 38: 204–240, https://doi.org/10.1111/j.1469-185X.1963.tb00783.x

Suski CD (2020) Development of carbon dioxide barriers to deter invasive fishes: insights and lessons learned from bigheaded carp. *Fishes* 5: 25, https://doi.org/10.3390/fishes5030025

Tix JA, Cupp AR, Smerud JR, Erickson RA, Fredricks KT, Amberg JJ, Suski CD (2018) Temperature dependent effects of carbon dioxide on avoidance behaviors in bigheaded carps. *Biological Invasions* 20: 3095–3105, https://doi.org/10.1007/s10530-018-1761-9

Treanor HB, Ray AM, Layhee M, Watten BJ, Gross JA, Gresswell RE, Webb MA (2017) Using carbon dioxide in fisheries and aquatic invasive species management. *Fisheries* 42: 621–628, https://doi.org/10.1080/03632415.2017.1383903

Welsh J (1932) Photokinesis and tonic effect of light in *Unionicola*. *The Journal of General Physiology* 16: 349–355, https://doi.org/10.1085/jgp.16.2.349

Welsh J (1934) The caudal photoreceptor and responses of the crayfish to light. *Journal of Cellular and Comparative Physiology* 4: 379–388, https://doi.org/10.1002/jcp.1030040308

Yamamoto Y (2010) Contribution of bioturbation by the red swamp crayfish *Procambarus clarkii* to the recruitment of bloom-forming cyanobacteria from sediment. *Journal of Limnology* 69: 102–111, https://doi.org/10.4081/jlimnol.2010.102

Supplementary material

The following supplementary material is available for this article:

Table S1. Crayfish counts for each category within Control (C) and CO_2 diffusion ponds across all trials.
Table S2. Descriptive results of different water quality parameters measured in Control (C) and CO_2 treatment ponds across all trials.
Table S3. Statistical comparisons of different water quality parameters between Control (C) and treatment (H+F) ponds from trials T1-T5.
Table S4. Statistical results of the binary logistic regression models of the crayfish capture probability.

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