



Notes

Potential for carbon dioxide to act as a non-physical barrier for invasive sea lamprey movement



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ABSTRACT

Invasive sea lampreys have had substantial negative ecological and economic impacts on the Laurentian Great Lakes region. Control efforts, such as lampricide application and barriers, have resulted in a reduction in number of sea lampreys in the Great Lakes. Due to environmental and non-target impacts of existing control mechanisms, coupled with the fact that no non-physical barrier is completely effective at stopping fish movement, there is a critical need to develop novel control technologies to assist with the control and suppression of sea lamprey populations. Recent work has indicated that carbon dioxide gas (CO₂) applied to water will influence the movement and behavior of fishes, providing the potential for CO₂ to act as a non-physical barrier that can exclude fish from a target area. To date, the effectiveness of CO₂ at influencing the movement and behavior of sea lampreys has not been explored. The current study showed that CO₂ applied to water will result in behavioral agitation for both adult and transformer sea lampreys, and will eventually result in equilibrium loss. More importantly, both adult and transformer sea lampreys will 'choose' to avoid water with CO₂ concentrations of 85 and 160 mg/L (respectively). Together, results from this study suggest that CO₂ applied to water has the potential to act as a non-physical barrier to deter the movement of free-swimming lamprey in the wild. Carbon dioxide gas can be integrated with existing control technologies to act as a novel barrier technology and augment existing control strategies for sea lampreys.

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Introduction

Invasive species can cause tremendous ecological, economical, and social damages (e.g., recreational, ecosystem services) on the receiving environment (Pimentel et al., 2005; Ricciardi and Maclsaac, 2011). In the Laurentian Great Lakes, biologists and managers have struggled to contain the spread and minimize the impacts of biological invaders (e.g., sea lamprey (*Petromyzon marinus*), alewife (*Alosa pseudoharengus*), zebra mussels (*Dreissena polymorpha*)) over the past 50 years (Rasmussen et al., 2011). Invasive species have been shown to have direct (e.g., competition, predation) and indirect (e.g., habitat modification, disease transfer, hybridization) impacts on native species that can result in population declines and the eventual reduction of species biodiversity within the invaded landscape (Clavero and García-Berthou, 2005; Ricciardi and Maclsaac, 2011). The establishment of invasive species can also result in negative economic consequences due to declines in the abundance of native species (Pimentel et al., 2005). As management strategies to extirpate already established invasive species have tremendous monetary costs and are often

ineffective, one cost-effective and efficient management strategy for invasive species control is preventing the initial colonization event through the use of prevention technologies (Finnoff et al., 2007; Lodge et al., 2006).

Since the 1930s, sea lampreys have been an invasive species of particular concern in the Great Lakes region (Smith and Tibbles, 1980). The introduction and establishment of sea lampreys within the Great Lakes remain one of the most potent examples of how invasive species can impart negative biological and economic impacts on an ecosystem level. The biological impacts of sea lamprey invasion were evident relatively soon after introduction (~5 years) with declines in native fish populations, especially lake trout (*Salvelinus namaycush*) and burbot (*Lota lota*), which resulted in the restructuring of aquatic communities in these lakes (Smith and Tibbles, 1980). Sea lampreys are obligate migrants, with adults migrating into streams to spawn, and transformers (juveniles) outmigrating after a variable period of stream residency. This life history makes sea lampreys vulnerable to a variety of control measures. With the discovery and application of lampricides, along with other control measures such as traps and barriers, sea lamprey populations have been suppressed relative to historical levels (Christie and Goddard, 2003; McLaughlin et al., 2007; Smith and Tibbles, 1980). However, lampricide treatments must be applied annually to control sea lampreys within the Great Lakes (Irwin et al., 2012). A recent study by Irwin et al. (2012) suggests that current lampricide

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treatments would need to be increased 25–50% to maximize the economic benefit of fisheries within the Great Lakes (i.e., lower sea lamprey abundances yield greater fish harvest), resulting in substantial additional cost to apply lampricide. Other control mechanisms exist to prevent fish movement; however, physical, in-stream barriers vary in effectiveness across sites and can have negative impacts on native communities by impairing movements (McLaughlin et al., 2007). Thus, there is a critical need to develop additional techniques to facilitate the control and removal of sea lampreys that would supplement or enhance existing control efforts, particularly during juvenile migration events from spawning grounds and adult migration events to spawning grounds.

Carbon dioxide gas applied to water has recently shown promise as a physiological and behavioral deterrent to fish movement (Clingerman et al., 2007; Kates et al., 2012; Ross et al., 2001). An elevated carbon dioxide barrier initially acts as a behavioral modifier, with fishes actively avoiding hypercarbic water (Clingerman et al., 2007; Kates et al., 2012). If fish are resistant to the deterrence aspect of the carbon dioxide barrier, individuals will eventually succumb to the anesthetic effects of hypercarbia resulting in loss of consciousness and equilibrium (Iwama et al., 1989). Previous research on two species of invasive cyprinids (silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Hypophthalmichthys nobilis*)), along with two centrarchids native to North America (largemouth bass (*Micropterus salmoides*) and bluegill (*Lepomis macrochirus*)) has shown that CO₂ concentration of 70 mg/L will result in behavioral modifications (changes in ventilation frequency, loss of equilibrium, and irregular behaviors), while CO₂ concentrations of 120 mg/L will induce active avoidance of hypercarbic waters (Kates et al., 2012). These results suggest that zones of carbon dioxide gas applied to water have the potential to act as a non-physical barrier and deter movements for these species. Unfortunately, only a few studies have investigated behavioral responses of fishes to hypercarbia, and none have examined the behavioral impact of elevated carbon dioxide on sea lampreys. As such, it is presently not known if zones of elevated carbon dioxide gas have potential to act as a non-physical barrier for lamprey movement; and, if feasible, what concentrations of carbon dioxide should be targeted.

Based on this background, the overall goal of this study was to quantify the influence of elevated CO₂ on the behavior of sea lamprey transformers (juveniles) and adults. This goal was achieved by completing two complementary series of experiments. The first experiment quantified the behavioral consequences of elevated CO₂ exposure to sea lampreys by determining the concentration of hypercarbia needed for these fishes to elicit agitation responses (i.e., elevated activity levels, twitching, erratic swimming) and eventual equilibrium loss, while the second experiment used a behavioral choice arena to define the concentration of carbon dioxide necessary to induce behavioral avoidance. The comparison of two distinct life stages was important to ensure that any target concentration of CO₂ identified as a barrier be effective against both adults and transformers to ensure maximum effectiveness. When combined, results from these two experiments will define the target concentrations that would be required if carbon dioxide gas were to be considered as a non-physical barrier to influence the movement of naturally migrating sea lampreys, potentially providing managers and invasive species biologists with a novel 'tool' to help control the abundance, distribution and spread of invasive sea lampreys (McLaughlin et al., 2007).

Materials and methods

Experimental animals

Sea lamprey transformers and adults were collected by U.S. Geological Survey (USGS) biologists and transported to the Hammond Bay Biological Station (HBBS, Millersburg, MI). Lamprey transformers were housed indoors in 200 L plastic holding tanks continuously supplied

with fresh water from Lake Huron, while adult sea lampreys were housed outdoors in 1000 L plastic holding tanks, also continuously supplied with fresh water from Lake Huron. Lampreys did not receive supplemental food while being held at HBBS prior to experiments.

Agitation response and equilibrium loss

To quantify the concentration of carbon dioxide that induced agitation and equilibrium loss, sea lampreys were subjected to incremental increases in CO₂ concentrations. Studies with incremental increases in CO₂ were conducted with transformers at HBBS on March 25–27, 2014, while experiments with adult sea lampreys occurred at HBBS on July 10–13, 2014. Trials were performed in complete darkness, and behavioral responses were recorded by a single observer using a headlamp producing a low-intensity red light, as previous research has shown this light source has a minimal impact on lamprey behavior (Hárosi and Kleinschmidt, 1993). Experiments began by placing an individual lamprey into a 15 L cooler that contained an air stone and water from Lake Huron. Lampreys were allowed 30 min to acclimate to the cooler, and the following water quality parameters were measured: water temperature and dissolved oxygen using a portable dissolved oxygen meter (YSI, 550A Yellow Springs Instruments, Irvine, California); pH using a WTW pH 3310 meter with a SenTix 41 probe (Germany); dissolved carbon dioxide and total alkalinity using a digital titration kit (Hach Company, titrator model 16900, kit 2272700 for CO₂ and kit 2271900 for total alkalinity). The sensitivity of the pH meter was ± 0.01 pH units, and the sensitivity of the digital titration kits was $\pm 1\%$. Temperature, pH, and total alkalinity were subsequently combined to generate values for pCO₂ (μatm) using CO₂Calc (version 1.2.0, U.S. Geological Survey, Reston, VA, USA) (Robbins et al., 2010). Initial water quality measurements for all experiments are provided in Table 1. Following this acclimation period, carbon dioxide levels were increased using the common technique of bubbling CO₂ gas into the water until a target pH was reached (Munday et al., 2009; Dixon et al., 2010), which, in the current study, was a pH decrease of 0.25 units (corresponding to an increase in carbon dioxide of approximately 25 mg/L, or 30,000 μatm). Once this target pH was achieved, lampreys were observed over a 5 min period for indications of 'agitation' that included erratic swimming, elevated activity levels or twitching (Gattuso et al., 2010; Kates et al., 2012). Following this 5 min observation period, CO₂ gas was added to the cooler to generate a second reduction in pH of 0.25 units, and animals were again observed over a 5 min period for behavioral responses. This process of CO₂ addition coupled with behavioral observations continued until lampreys lost equilibrium, at which point lampreys were removed from the cooler, weighed to the nearest tenth of a gram (g), measured to the nearest millimeter (mm) and placed in a separate holding tank. Lamprey sizes were 152 ± 5 mm standard error (SE) and $4.5 \text{ g} \pm 0.5 \text{ g}$ for transformers, and $489 \text{ mm} \pm 16 \text{ mm}$ and $215 \text{ g} \pm 18 \text{ g}$ for adults (N = 8 animals of each life stage for all experiments).

Hypercarbia avoidance

Hypercarbia avoidance was quantified using a "shuttle box" choice arena (Loligo Inc., Hobro, Denmark), which consists of two circular chambers (1.5 m diameter, 0.5 m depth) connected by a narrow tunnel (0.2 m wide \times 0.5 m deep) (described in detail in Kates et al., 2012). Briefly, external pumps moved water from one circular chamber into an external buffer column where it could be treated and returned to the choice arena via gravitational force. One buffer column was dedicated to one of the circular chambers of the arena, and the system allowed water chemistry in one circular chamber to be independently manipulated without affecting water chemistry in the other chamber. Water quality in each circular chamber was manipulated using a computer and software package (ShuttleSoft 2.6.0, Loligo Inc., Hobro, Denmark), and two pH probes, connected to two portable pH meters, were placed

Table 1
Initial water quality measurements taken prior to starting experiments to define the concentration of carbon dioxide gas (CO₂) necessary to cause either (a) an agitation response and loss of equilibrium, or (b) avoidance of hypercarbic water for sea lamprey transformers or adults.

	Temp (°C)	Dissolved oxygen (mg/L)	pH	Total alkalinity (mg/L)	Dissolved CO ₂ (mg/L)	pCO ₂ (µatm)
<i>Agitation response and equilibrium loss</i>						
Transformers	3.8 ± 0.1	13.7 ± 1.1	8.1 ± 0.02	76.8 ± 2.1	15.1 ± 1.0	666 ± 36
Adults	11.5 ± 0.2	10.6 ± 0.1	7.9 ± 0.1	77.1 ± 1.3	11.7 ± 0.5	1362 ± 248
<i>Hypercarbia avoidance</i>						
Transformers	5.2 ± 0.4	12.5 ± 0.1	6.5 ± 0.1	81 ± 3.3	36.1 ± 5.3	37,116 ± 7148
Adults	11.4 ± 0.2	10.9 ± 0.1	7.2 ± 0.01	78 ± 1	18.9 ± 1.6	6404 ± 1818

in each circular chamber to allow for real-time monitoring of pH (Kates et al., 2012). Black plastic sheets attached to a frame surrounding the entire arena ensured that external activity did not influence lamprey behavior. Lamprey movements were monitored in real-time using an infrared video camera above the arena connected to a computer monitor.

The procedure for the hypercarbia avoidance challenge followed techniques summarized in Kates et al. (2012), with a few minor modifications to account for the use of lampreys. For example, avoidance trials with sea lampreys were performed in complete darkness (from 2100 h–0500 h) when sea lampreys are most active (Dauble et al., 2006). In addition, the arena was lined with plastic mesh (XV1672-48X50, Industrial Netting Inc., Minneapolis, MN) to prevent lampreys from attaching to the sides or bottom of the arena, and to promote swimming. For the avoidance trial, animals were first carefully netted from their holding tank, and a coin-flip was used to designate which one of the two choice arenas that would receive the animal; lampreys were allowed 1 h to acclimate to the arena. Initial water quality measurements were collected from the chamber where the lampreys were placed using methods described above (Table 1).

Immediately after the acclimation period, the buffer column associated with the circular chamber into which the lamprey had settled received a continuous addition of carbon dioxide gas, while the opposite buffer column was supplied with compressed air through the use of a small blower (Sweetwater Linear II, Aquatic Eco-systems, Apopka, FL, USA). Carbon dioxide gas continued to be added slowly to the buffer column, and the time was recorded when the lamprey ‘shuttled’ through the tunnel and into the opposite chamber (typically > 30 s), or when the individual lost equilibrium. When the lamprey shuttled to the opposite chamber, both external buffer columns received compressed air for 10 min to reduce CO₂ in the water. Immediately following this 10 minute period, CO₂ gas was again supplied to the external buffer column associated with the circular chamber where the lamprey was located. This was repeated until the lamprey shuttled six times, or when it lost equilibrium. Following each shuttling event, or at the time of equilibrium loss, the water quality measurements listed above were again taken. At the conclusion of the hypercarbia avoidance challenge, each individual was removed from the choice arena, measured, weighed, and placed in a separate holding tank. Lamprey transformers averaged 150 mm ± 4 mm and 4.2 g ± 0.3 g, while adults were 516 mm ± 17 mm and 276 g ± 27 g (N = 8 for each life stage). All procedures used in this study were in compliance with the University of Illinois Institutional Animal Care and Use Committee, protocol 13123.

At this point, it should be noted that pCO₂ values generated during this entire study should be interpreted cautiously because, due to the rapid addition of large quantities of CO₂ coupled with short time frames involved in many trials, CO₂ pressures may not have equilibrated at the time of measurements (Riebesell et al., 2010). In addition, dissolved CO₂ was effectively stripped from the shuttle box system between shuttles, but, typically, pH remained low due to the unavoidable formation of carbonic acid in the water; this reduced pH will influence formulae used to generate CO₂ pressures in CO₂Calc, and may also result in the subsequent release of CO₂ into the water over extended periods of

time. Therefore, the most consistent and reliable metric to define CO₂ levels during this study was concentration of CO₂ in mg/L, generated with the digital titrator; carbon dioxide pressures in µatm are provided for reference, however.

Statistical methods

Comparison of CO₂ concentrations that induced agitation behaviors and equilibrium loss were made across life stages using a two-way analysis of variance (ANOVA), with main effects being behavior (agitation, equilibrium loss), life stage (juvenile, adult) and their interaction (behavior × life stage) (Sokal and Rohlf, 1995). Prior to performing the ANOVA, data were rank transformed to meet assumptions of normality and homogeneity of variances (Conover and Iman, 1981; Iman et al., 1984; Potvin and Roff, 1993). Normality was verified through visual analysis of fitted residuals using a normal probability plot (Anscombe and Tukey, 1963), while homogeneity of variances was confirmed using a Hartley's F_{max} test (Hartley, 1950), coupled with visual analysis of fitted residuals using a residual by predicted plot. If at least one of the main effects in the model was significant, or if the interaction was significant, a Tukey–Kramer honestly significant differences (HSD) post hoc test was used to separate means (Sokal and Rohlf, 1995).

Comparisons of CO₂ concentrations that induced avoidance responses were compared across life stages using a one-way, mixed-model ANOVA (Sokal and Rohlf, 1995), with individual identification number added to the model as a random effect. The use of a random effect (essentially a repeated measures design) was necessary because multiple measurements were taken from each animal during a trial, meaning that each measurement was not independent and potentially correlated within an individual (Laird and Ware, 1982; Lindstrom and Bates, 1990). Prior to analysis of the hypercarbia avoidance data, a rank transformation was applied so that data met assumptions of normality and homogeneity of variances, verified with tests listed above.

All statistical analyses were performed using JMP version 9.0.3 (SAS Institute Inc., Cary, North Carolina), except for Hartley's F_{max} tests, which were run using a spreadsheet on a personal computer. All means are reported ± SE where appropriate, and the level of significance (α) for all analyses was 0.05.

Results

Sea lamprey transformers began to display behaviors associated with agitation (e.g., twitching, erratic swimming, elevated activity levels) after being exposed to water at approximately 38 mg/L CO₂ (6761 µatm), while sea lamprey adults displayed agitation behaviors at concentrations of CO₂ that were almost 50% lower than that of the transformers (23 mg/L; 11,470 µatm; Fig. 1A; Table 2). Similarly, sea lamprey transformers lost equilibrium at CO₂ concentrations of approximately 170 mg/L (96,596 µatm), while adults lost equilibrium at approximately 113 mg/L (157,068 µatm) (Fig. 1A; Table 2).

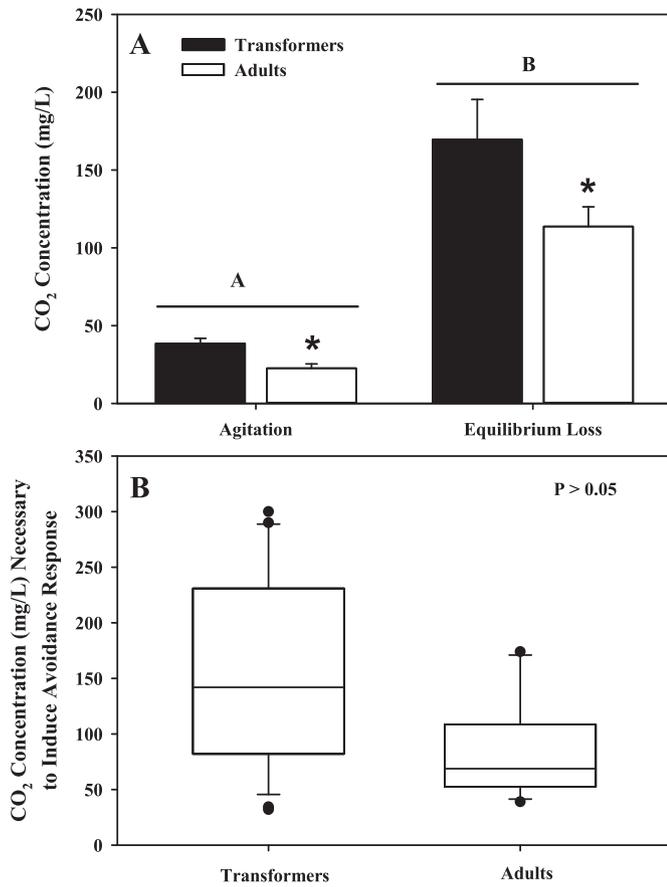


Fig. 1. Concentrations of carbon dioxide in water that induced agitation or equilibrium loss in sea lamprey transformers (black bars) or sea lamprey adults (open bars), or avoidance. Capital letters above bars in panel A refer to significant differences in carbon dioxide concentrations across behaviors, while an asterisk (*) refers to significant differences in CO₂ concentrations within a behavior across life stages. For panel B, differences in carbon dioxide concentrations necessary to induce avoidance did not differ significantly across life stages. For box plots, the top and bottom of the box represent the 75th and 25th percentiles (respectively), while the horizontal line within the box represents the median; whiskers (error bars) above and below the box represent the 90th and 10th percentiles. Sample sizes are N = 8 animals for each life stage across all trials.

Exposure of both sea lamprey transformers and adults to elevated CO₂ resulted in voluntary shuttling to water with lower CO₂. Sea lamprey transformers displayed active avoidance of CO₂ at concentrations of approximately 160 mg/L (206,843 μ atm) (range for all transformers from 32–300 mg/L, 24,303 to 540,968 μ atm), while adults avoided water with elevated CO₂ starting at approximately 85 mg/L (46,204 μ atm) (range for all adults from 39–174 mg/L, 4308–94,812 μ atm) (Fig. 1B). Patterns in individual responses to CO₂ across successive shuttling attempts were challenging to define, particularly for transformers. Adults typically showed an increase in the CO₂ concentration required to induce avoidance with successive shuttle attempts, but the magnitude of concentration of increase was small, increasing approximately 1.75-fold across attempts. Some transformers showed a

Table 2

Results of two-way analysis of variance (ANOVA) comparing the occurrence of behaviors associated with discomfort (agitation and equilibrium loss) following exposure to carbon dioxide gas by two life stages of sea lamprey (transformers and adults). Data were rank-transformed prior to analyses to meet assumptions of normality and homogeneity of variances. Data are shown in Fig. 1A.

Effect	df	F	P
Life stage	1	15.9	0.0004
Behavior	1	134.8	<0.0001
Life stage \times behavior	1	0.8	0.37

decrease in the concentration of CO₂ required to induce shuttling across successive shuttling attempts, while others showed an increase. Differences in the CO₂ concentration (mg/L) that induced avoidance did not differ significantly across life stages (Mixed-model ANOVA; $F_1 = 5.9$; $P > 0.05$).

Discussion

Following exposure to water with elevated carbon dioxide gas, both sea lamprey transformers, as well as sea lamprey adults, displayed behaviors related to agitation (erratic swimming, elevated activity levels or twitching (Kates et al., 2012)), and ultimately lost equilibrium. Upon being added to water, a portion of carbon dioxide gas will remain in solution, and a portion will hydrate and form carbonic acid (H₂CO₃), which can dissociate to bicarbonate (HCO₃⁻) and/or carbonate (CO₃²⁻) anions, along with associated protons (H⁺), to reduce the pH of water. The relative abundance of each of these species of carbon dioxide will vary depending on water chemistry related to hardness, alkalinity and temperature (Wetzel, 2001). In fishes, exposure to carbon dioxide gas has been documented to result in a suite of physiological and behavioral responses including an elevation of the general stress response (Dennis et al., 2014; Kates et al., 2012), a reduction in blood pH (Iwama et al., 1989), ion loss (Brauner et al., 2000) and, ultimately, equilibrium loss and anesthesia (Stage 2 or Stage 3) (Bernier and Randall, 1998; Iwama et al., 1989). At present, the exact mechanism(s) responsible for the loss of equilibrium and anesthetic impacts of carbon dioxide on fishes have not been well defined, but are believed to occur due to the movement of carbon dioxide across the blood/brain barrier, which results in altered brain pH and an impairment of brain electrical activity (Yoshikawa et al., 1991; Yoshikawa et al., 1994); additions of H⁺ or HCO₃⁻ alone will not result in anesthesia for fish (Yoshikawa et al., 1991). In the present study, the concentrations of carbon dioxide gas that resulted in agitation (approximately 40 mg/L) and equilibrium loss (approximately 120 mg/L) for sea lamprey was similar to work by Kates et al. (2012) who showed that adult silver carp, largemouth bass and bluegill displayed agitation behaviors at approximately 70 mg/L, and lost equilibrium at 120 mg/L. Interestingly, for both the agitation response and loss of equilibrium, sea lamprey transformers appeared to be more tolerant of carbon dioxide additions than were adults, as transformers required elevated CO₂ concentrations to elicit a response relative to adults. Dennis et al. (2015) showed that, within species, juvenile largemouth bass, bluegill and silver carp were more tolerant of CO₂ than were adults of these species (Kates et al., 2012), suggesting ontogenetic variation in carbon dioxide tolerances within fishes. However, work with transformers in the current study occurred at water temperatures that were approximately 6–7 °C colder than work with adults, so differences in tolerances may be related to metabolic differences across trials, reduction in the speed of diffusive processes (such as movement of CO₂ into the blood stream and around the body), and should be investigated further to increase confidence in the comparisons across life stages. Together, results from the current study clearly show that extended exposure of sea lamprey to elevated concentrations of carbon dioxide in water will result in behavioral modifications related to agitation, followed by equilibrium loss.

Both sea lamprey transformers and sea lamprey adults 'chose' to swim away from areas of elevated carbon dioxide in water. In the present study, transformers swam away from CO₂ concentrations of approximately 160 mg/L (maximum concentration experienced by transformers was 300 mg/L), while adults swam away from approximately 85 mg/L CO₂ (maximum concentration experienced was 174 mg/L). Upon encountering poor-quality water, fishes have a number of potential responses to avoid degraded conditions, and movement to water with more favorable conditions is one of the first options typically employed (Kramer, 1987; Magnuson et al., 1979). The ability of organisms to sense environmental carbon dioxide is believed to help animals avoid sub-optimal environmental conditions, and exists in

prokaryotes, eukaryotes, plants, and animals (Cummins et al., 2014). Fishes appear to have external and/or internal CO₂ receptors, allowing them to detect environmental hypercarbia, presumably for the purpose of avoiding CO₂ and moving to more favorable water (Hedrick et al., 1991; Gilmour, 2001; Perry and Reid, 2002). Recent work has shown that several species of fish will actively avoid areas of elevated carbon dioxide in a laboratory setting (Clingerman et al., 2007; Ross et al., 2001), including work by Kates et al. (2012) who used equipment identical to that of the current study and showed that silver carp, largemouth bass and bluegill all actively avoided CO₂ concentrations of approximately 120 mg/L. Together, results from the current series of experiments clearly demonstrate that both juvenile and adult sea lampreys will 'choose' to actively avoid areas of elevated carbon dioxide in water, and will swim to water with lower carbon dioxide concentrations.

Results from this study provide support for the use of zones of elevated carbon dioxide to act as a non-physical barrier to deter the movements of free-swimming sea lampreys in the wild, although, several caveats should be considered along with this support. Non-physical barriers to deter fish movements can typically be grouped into two broad categories: stimuli that 'pull' organisms into an area, and stimuli that 'push' individuals in an effort to exclude them from an area (Noatch and Suski, 2012), also referred to as 'attractors' and 'distractors' (McLaughlin et al., 2007). Carbon dioxide added to water has the potential to act as a 'push'-type non-physical barrier (i.e., distractor) that can be deployed in a field setting to prevent either the upstream or downstream movement of lamprey, or encourage animals to avoid target areas. While no non-physical barrier is 100% effective against the movement of fishes (Noatch and Suski, 2012), there are a number of advantages that would support the use of carbon dioxide as a barrier to deter the movement of lampreys. For example, carbon dioxide is relatively easy to apply to water and can be performed in remote locations with little infrastructure as it simply requires cylinders of gas and an airstone. Similarly, due to the 'low-tech' approach to carbon dioxide applications, barriers of CO₂ could be easily deployed only at key times of the year (i.e., peak migration), can be installed at small scales, and can easily be coupled with other barrier technologies (i.e., traps, pheromones). More importantly, CO₂ barriers could be deployed in concert with other lamprey control technologies, such as traps, to increase the concentration of animals in an area and improve trap efficiency. However, there are a number of potential concerns that would hamper the deployment of CO₂ barriers for lamprey that include cost/application logistics, potential negative impacts on non-target organisms, potential impacts to the downstream receiving environment from unavoidable reductions in pH, and potential regulatory obstacles preventing the use of carbon dioxide gas as a pesticide (i.e., registration of carbon dioxide gas with the US Environmental Protection Agency for pesticide applications). For example, recent work in the marine literature concerned with effects of ocean acidification has indicated that exposure of fishes to elevated, but still low, concentrations of carbon dioxide for relatively short exposure durations (11 days) can negatively impact several different behaviors such as the response to predators and homing (Munday et al., 2009; Dixon et al., 2010); the responses of freshwater fishes to similar exposures to carbon dioxide are not known, but should be quantified prior to the large-scale deployment of a carbon dioxide barrier. Despite these challenges, however, carbon dioxide gas has the potential to act as a novel, non-physical barrier to influence the movement of sea lamprey adults and transformers, which could work to enhance existing efforts to control sea lamprey populations.

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