

Exposure to elevated $p\text{CO}_2$ alters post-treatment diel movement patterns of largemouth bass over short time scales

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SUMMARY

1. Studies with marine fishes indicate that exposure to elevated partial pressures of CO_2 ($p\text{CO}_2$) related to climate change have negative consequences for fish behaviour. Freshwater fishes may experience similar increases in $p\text{CO}_2$ due to a number of different mechanisms, but there is a paucity of information on how freshwater fishes may respond to exposure to elevated $p\text{CO}_2$.
2. To define the effects of elevated $p\text{CO}_2$ on a free-swimming freshwater fish, 19 adult largemouth bass (*Micropterus salmoides*) were tagged with acoustic transmitters, held in water with $p\text{CO}_2$ levels of either $\sim 10\,000\ \mu\text{atm}$ or ambient $p\text{CO}_2$ ($<100\ \mu\text{atm}$) for 5 days and released into a naturalised, earthen-bottom pond outfitted with an acoustic telemetry array.
3. Findings indicate that largemouth bass not exposed to elevated levels of $p\text{CO}_2$ decreased movement over 35% during the daylight periods; however, fish exposed to elevated levels of $p\text{CO}_2$ did not exhibit this pattern. This difference in diel movement patterns between fish exposed to elevated $p\text{CO}_2$ and fish not exposed was not detectable after 11 days.
4. Changes in home range size and daily distance travelled were not observed. However, based on an assessment of position estimates after the completion of the telemetry monitoring portion of the study, space use differed for fish exposed to elevated $p\text{CO}_2$.
5. Exposure to elevated $p\text{CO}_2$ therefore can have consequences for some movement behaviours of freshwater fish and this may influence a variety of ecological processes including energetics, foraging and predator–prey dynamics. CO_2 -induced alterations to behaviour should recover upon a return to ambient water.

Keywords: activity, carbon dioxide, climate change, *Micropterus salmoides*, space use

Introduction

Wild freshwater fishes are exposed to a variety of naturally occurring and anthropogenic stressors that can impact a range of behaviours. For instance, natural stressors, such as winter (e.g. cold temperature, low dissolved oxygen), result in a reduction in swimming speeds and habitat selection away from anoxic areas (Hanson *et al.*, 2007; Hasler *et al.*, 2009b), while anthropogenic stressors, such as thermal effluent, can cause increased activity when temperatures rise (Cooke &

Schreer, 2003). Quantifying the behavioural and physiological impacts of stressors in wild fishes in a field setting is challenging, but monitoring the behavioural responses of wild fishes using electronic tags (i.e. telemetry) can offer insight into the behavioural consequences of stressors (Cooke *et al.*, 2004). Following exposure to an environmental stressor, fishes presumably will exhibit avoidance behaviours (Beitinger, 1990) or experience compensatory changes (e.g. acclimatisation) (Thorstad *et al.*, 2007; Hasler *et al.*, 2009a). Furthermore, short-term exposures to stressors that are sub-lethal can

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result in a return to typical behaviours in fishes once the stressor is eliminated (Bauer & Schlott, 2006).

A natural stressor that has the potential to impact fish behaviour is elevated carbon dioxide (CO_2). Over the past several decades, CO_2 in the atmosphere has increased, which, in turn, has led to a rise in the amount of CO_2 in the ocean (IPCC, 2007). This increase in oceanic CO_2 partial pressure ($p\text{CO}_2$) has been shown to have a number of negative consequences for fish in marine systems, including increased activity and heightened aerobic scope (Jutfelt *et al.*, 2013; Rummer *et al.*, 2013; Green & Jutfelt, 2014; Ou *et al.*, 2015; Sundin & Jutfelt, 2015) that can translate into population-level impacts, particularly if reproductive output or survival of juvenile fish are impacted (Munday *et al.*, 2010). Future $p\text{CO}_2$ levels in fresh water have not been well defined (Hasler *et al.*, 2016), but evidence to date suggests some freshwater systems could experience a rise in $p\text{CO}_2$ similar to that of the marine environment (Phillips *et al.*, 2015). For example, $p\text{CO}_2$ can vary naturally (Maberly, 1996), and fish may also be exposed to high $p\text{CO}_2$ due to non-physical fish barriers (Noatch & Suski, 2012), or in hatcheries (Carmichael *et al.*, 1984). To date, few ecologically relevant studies have focused on freshwater fishes exposed to elevated $p\text{CO}_2$, making it difficult to predict the biological consequences of a projected rise in freshwater $p\text{CO}_2$ on either individuals or populations (reviewed by Hasler *et al.*, 2016). Should the observed changes in marine fishes exposed to elevated $p\text{CO}_2$ also occur in freshwater fishes, freshwater fish populations might be similarly at risk for reduced recruitment and high mortality (Munday *et al.*, 2010). Therefore, fully understanding how elevated $p\text{CO}_2$ may influence freshwater fishes is important for ensuring productive fish populations.

In this study, we sought to quantify the effect of exposure to elevated $p\text{CO}_2$ on the movement patterns of largemouth bass (*Micropterus salmoides*: Centrarchidae), an ambush and pursuit predator (Howick & O'Brien, 1983) that will form groups (Hasler *et al.*, 2007) and territories (Hasler & Wisby, 1958). Specifically, we aimed to quantify differences in movement behaviours (e.g. minimum distance travelled across days and within days) and spatial distribution (e.g. home range) of largemouth bass exposed to either elevated $p\text{CO}_2$, or not, and released into a pond equipped with an acoustic telemetry array. Given that elevated $p\text{CO}_2$ has been shown to alter typical behavioural patterns in marine fish species, we hypothesised that upon release into the pond, fish earlier exposed to elevated $p\text{CO}_2$ may demonstrate a period of increased activity and larger home range size

like has been found in marine studies (Devine, Munday & Jones, 2012), and that movement patterns would differ between the two treatment groups.

Methods

Study animals

Nineteen wild-caught largemouth bass, habituated to experimental ponds for over a year and then collected during pond draining (total length = 404 ± 33 mm; mean \pm SD), had an acoustic transmitter surgically implanted into their body cavity (120 s burst rate, 8.5×47 mm [d \times l], 6.0 g, M-Series, Lotek Wireless Inc., Newmarket, ON). The surgery followed procedures outlined in Bridger & Booth (2003). Briefly, fish were anaesthetised using a solution of 150 mg L^{-1} of tricaine mesylate (MS-222) buffered with 300 mg L^{-1} of sodium bicarbonate. Once fish loss equilibrium, they were removed from the solution and maintained during surgery using a mixture of 10 mg L^{-1} MS-222 and 20 mg L^{-1} sodium bicarbonate. The surgery occurred on a wetted v-slot trough and consisted of an approximately 15 mm incision slightly off centre from the ventral mid-line behind the pelvic girdle, where the transmitter was inserted. The incision was closed using 2–3 sutures (3/0 PDS II, absorbable monofilament sutures, Ethicon Inc., Somerville, NJ). Surgeries lasted less than 5 min, and postoperative fish were held in one of two 300 L tanks of recirculating pond water for 24 h to monitor recovery and ensure they were active and swimming upright prior to separation into the treatment tanks.

Twenty-four hour post-surgery, fish were haphazardly placed into one of two 300 L tanks to ensure similar sized fish were used in both treatments. Both tanks received water from an adjacent, earthen-bottom pond with natural vegetation. One tank exposed fish ($N = 9$) to elevated $p\text{CO}_2$ levels using the common method of bubbling CO_2 gas into the water through an airstone, using water pH to maintain a target $p\text{CO}_2$ level (Pinpoint pH controller, American Marine Inc., Ridgefield, CT) (e.g. Riebesell *et al.*, 2010; Kates *et al.*, 2012). A modified infrared CO_2 probe was used to monitor $p\text{CO}_2$ [GMT221, 0–20%, Vaisala, Vantaa (Johnson *et al.*, 2009)]. The second tank ($N = 10$) received no supplementary CO_2 addition, and remained at ambient $p\text{CO}_2$ levels. Water quality in both tanks was monitored twice daily over the treatment period (see Table 1, for mean values and equipment used to make measurements), and the tank with supplemental CO_2 had a $p\text{CO}_2$ level of

Table 1 Water quality of the 300 L tanks recorded during the 5-day exposure period. Each parameter was sampled twice daily (morning and afternoon) in each tank. For each value, means ± standard deviations have been listed.

Tank	NH ₃ * (ppm)	pH [†]	Alkalinity [‡] (mg L ⁻¹ CaCO ₃)	Dissolved oxygen [§] (mg L ⁻¹)	Temperature [§] (°C)	CO ₂ measured using titration [¶] (mg L ⁻¹)	CO ₂ measured using infrared probe** (µatm)
Ambient	<0.1	8.8 ± 0.2	171 ± 16	7.8 ± 0.3	21.0 ± 1.4	7.46 ± 1.4	<100
CO ₂ treated	<0.1	7.5 ± 0.2	172 ± 14	7.4 ± 0.4	20.5 ± 0.7	28.8 ± 2.7	10,160 ± 1752

*Colour disc test kit, Hach Company, Loveland, CO.

†pH meter, WTW pH 3310 meter with a SenTix 41 probe, Xylem, Rye Brook, NY.

‡Alkalinity titration kit, Model AL-AP, Hach Company, Loveland, CO.

§Dissolved oxygen meter, YSI 6600, YSI Instruments, Yellow Springs, OH.

¶CO₂ titration kit, Model CA-23, Hach Company, Loveland, CO.

**Infrared CO₂ transmitter, GMT221 infrared CO₂ probe, Vaanta.

10,160 ± 1752 µatm (mean ± SD). The 'control' tank that received no additional CO₂ had a pCO₂ level of <100 µatm (the lowest detectable limit of the probe). The elevated level of pCO₂ chosen was based on previous work showing exposure of largemouth bass to pCO₂ levels in excess of 10 000 µatm can reduce ventilation rates, cause loss of equilibrium and induce physiological changes (Kates *et al.*, 2012), suggesting this may be the maximum pCO₂ level to which fish can be exposed to without inducing negative consequences. Furthermore, the elevated level of pCO₂ represents realistically high values that might be expected downstream of a release point of a CO₂ barrier, and would be considered rare in the context of current global freshwater lakes and rivers (Cole *et al.*, 1994; Butman & Raymond, 2011), but not necessarily for future freshwater conditions, which are unknown (Hasler *et al.*, 2016). Fish were held at elevated pCO₂ levels for 5 days (no loss of equilibrium or changes in fish behaviour were observed in the holding tank), which is a period of time that has been found to induce alterations in fish behaviour in marine environments, though at levels lower than those used in this study (e.g. <1000 µatm, Ferrari *et al.*, 2011; Munday *et al.*, 2010; Heuer & Grosell, 2014). Following CO₂ exposure, fish were released on 8 June 2015 into an earthen-bottom pond containing natural vegetation, equipped with a wireless telemetry array (see below). On 22 September 2015, the pond was drained to quantify fish survival. This study was conducted in accordance with University of Illinois Institutional Animal Care and Use Committee protocol #14168.

Study site

Largemouth bass movements were monitored in a rectangular earthen-bottom pond (c. 53 m × 18 m × 2 m; 954 m² or 0.095 ha; mean pCO₂, 800 µatm (±200 [SD]) at

the Aquatic Research Facility at the University of Illinois, Urbana-Champaign, USA (40°4' 25.5066", -88°13' 13.1598"). Prior to the start of the study, the pond was drained and most submergent and emergent macrophytes were removed as part of an annual servicing protocol. A wireless telemetry array consisting of six hydrophones mounted to steel posts was then installed (WHS 3000 series, Lotek Wireless Inc., Newmarket, ON). The pond was re-filled with municipal water, and tagged fish were added to the pond after chlorine off-gassed (c. 2 weeks for chlorine to be <0.1 mg L⁻¹; chlorine test kit, Pocket Colourimeter II, Hach Company, Loveland, CO). Fathead minnow (*Pimephales promelas*: Cyprinidae) and bluegill (*Lepomis macrochirus*: Centrarchidae) were also released into the pond.

Positional data and trajectory calculation

After 35 days, on 13 July 2015, the hydrophones/data loggers were removed from the pond and files were processed using U-MAP software (Version 1.3.1, Lotek Wireless Inc., Newmarket, ON) to obtain positional data. Data for trajectory analysis and home range calculation were manually assessed, and four positions per day for each fish over a period of 15 days (9 June 2015–23 June 2015; note, the first day of monitoring was omitted from analysis due to low accuracy in position estimates) were selected (a total of 60 positions per fish) and included single points nearest to the hours of 00:00:00 (representing 12:00 am midnight), 06:00:00, 12:00:00 and 18:00:00. Thus, the four time periods monitored were as follows: time period 1, 00:00:00–06:00:00; time period 2, 06:00:00–12:00:00; time period 3, 12:00:00–18:00:00 and time period 4, 18:00:00–24:00:00. The reasons for focusing on a small subset of position estimates for each fish were to prevent potential erroneous positions from hindering the comparison between the two treatment groups, and to

reduce the likelihood of spatial and temporal autocorrelation from biasing the statistical models (Kie *et al.*, 2010). A low position dilution of precision (PDOP) was also a requirement for positions to be included in the data set (i.e. only position estimates close to 1 were selected). PDOP is a measurement of the accuracy of an obtained position, and an ideal PDOP is <1, an excellent PDOP is <2 and a good PDOP is <5 (see Baktoft *et al.*, 2015, for further information related to PDOP and the use of a similar hydrophone array). Furthermore, the 15 days observation period for movement behaviours and home range calculation was chosen to represent a length of time that would allow for the potential to observe effects following the 5 days CO₂ exposure period and potential recovery from the exposure (Hamilton, Holcombe & Tresguerres, 2013). Data beyond the 15 days period was only used to assess whether performance of the hydrophone array differed between CO₂-exposed fish and unexposed fish. For this *post hoc* analysis, the day when less than 10 position estimates (~1% of possible transmissions) was determined for each fish.

Selected positional data (criteria described above) were analysed using the program R (R Development Core Team, 2010). The R package *adehabitatLT* (Calenge, 2006) was used to calculate the minimum distance travelled (MDT) for each subsequent position per fish (Calenge, Dray & Royer-Carenzi, 2009). Ninety-five per cent kernel densities were calculated using the R package *adehabitatHR* (Calenge, 2006) and were used to measure home range size.

Statistical analysis

Linear mixed effects models were used to define the effects of different factors on the distance travelled for individual fish ($N = 19$). For the entire 15 days data set, individual fish (i.e. evaluation unit; Hurlbert, 2009) nested in period and separately nested in day, were treated as random effects (i.e. a within-subjects design), resulting in random slopes and random intercepts. Main effects, including treatment (factor), day (continuous) and time period (factor), as well as the interactions of time period \times day, treatment \times time period and treatment \times day were treated as fixed effects. To quantify the potential for recovery following exposure to elevated levels of $p\text{CO}_2$ (should any fixed factor be significant), the data set was parsed into three 5 days period (given visual observations in distance travelled and to allow for an appropriate sample size). In these models, day and fish ID, nested in time period, were treated as random effects, while treatment, time period and

treatment \times time period were included as fixed effects. All models were fit using 'lmer' from the R package *lme4* (Bates, 2010), and coefficients were estimated using restricted maximum likelihood. To define the importance of fixed effects, we used the *sim* function ('arm' package in R) to generate $N = 1000$ posterior simulations of each fixed effect. To determine significance of the effects, we evaluated whether the resulting posterior distribution of effect estimates overlapped 0 at the 95% level (i.e. distributions of fixed effects whose 95% credible intervals did not overlap 0 were said to be significant). To complete multiple comparisons between levels of significant factors, changes in means and 95% credible intervals of simulated changes in model intercepts were compared. Note that for log-linear models, changes in model intercepts represent percent changes in the transformed response variable. Means are presented $\pm 95\%$ credible interval.

A similar model as described above was also used to quantify the effect of the treatment on space-use-related metrics. Specifically, a linear mixed effects model with individuals included as a random effect was used to compare the home range size of the two treatment groups across three temporal subsets (i.e. days 1–5, 6–10 and 11–15). Effect significance was determined as described above. A Cox proportional hazard analysis was used to compare the two groups of fish and the dates when positional estimates (PDOP < 2) were fewer than 10 positions per day. The Cox hazard analysis was completed using the 'survival' package in R (Therneau, 2015) and included the entire 35-day data set.

Results

Exposure of largemouth bass to elevated $p\text{CO}_2$ resulted in significant changes to some, but not all, of the response variables assessed. Significant interaction effects of treatment and time period on MDT were found for time period 2 (06:00–12:00 hours) and time period 3 (12:00–18:00 hours) (Table 2). The significant interaction effect for CO₂ \times time period 2 indicates that MDT for CO₂-treated fish during time period 2 compared to time period 1 is different relative to largemouth bass not exposed to elevated CO₂ (Fig. 1). The change in the regression intercept is greater for largemouth bass not exposed to elevated CO₂ than for CO₂-treated fish (Fig. 2a), as unexposed fish demonstrated a 37% (–73%, –4%) decrease in movement during period 2, compared to a 7% increase for CO₂-treated fish (–28%, 44%) (Fig. 2a). Likewise, MDT in unexposed largemouth bass for time period 3 compared to time period 1 was 36%

Table 2 Statistical outputs of linear mixed effects models using random slopes and intercepts. Mean intercept and change in intercept values and 95% credible intervals were calculated using posterior simulations of each fixed effect. The intercept value represents the baseline values (e.g. non-exposed fish, Day 1 or Period 1). Values for factors represent the percent change in the model intercept associated with the factor. Values in bold represent significant factors.

Response	Model	Parameter	Mean	95% Credible interval
Log (MDT)	Linear mixed effects model	Intercept	1.86	1.28, 2.41
		CO ₂	-0.47	-1.19, 0.29
		Day	-0.03	-0.01, 0.08
		Period 2	-0.37	-0.73, -0.04
		Period 3	-0.36	-0.77, 0.00
		Period 4	-0.23	-0.58, 0.10
		CO ₂ × Day	0.02	-0.04, 0.07
		CO₂ × Period 2	0.46	0.12, 0.77
		CO₂ × Period 3	0.36	0.03, 0.70
		CO ₂ × Period 4	0.22	-0.08, 0.53
		Day × Period 2	0.01	-0.02, 0.05
		Day × Period 3	0.02	-0.01, 0.06
		Day × Period 4	0.00	-0.03, 0.03
		Log(MDT) (Day 1–5)	Linear mixed effects model	Intercept
CO ₂	-0.56			-1.13, -0.02
Period 2	-0.35			-0.73, 0.01
Period 3	-0.33			-0.76, 0.06
Period 4	-0.23			-0.59, 0.11
CO₂ × Period 2	0.51			0.01, 0.99
CO ₂ × Period 3	0.48			-0.09, 1.01
Log(MDT) (Day 5–10)	Linear mixed effects model	Intercept	2.08	1.60, 2.56
		CO ₂	-0.28	-0.98, 0.42
		Period 2	-0.28	-0.67, 0.12
		Period 3	-0.23	-0.63, 0.16
		Period 4	-0.22	-0.55, 0.11
		CO₂ × Period 2	0.64	0.07, 1.19
		CO ₂ × Period 3	0.47	-0.09, 1.05
Log(MDT) (Day 10–15)	Linear mixed effects model	Intercept	2.29	1.93, 2.65
		CO ₂	-0.10	-0.59, 0.38
		Period 2	-0.14	-0.53, 0.24
		Period 3	-0.03	-0.40, 0.35
		Period 4	-0.25	-0.61, 0.10
		CO ₂ × Period 2	0.18	-0.34, 0.67
		CO ₂ × Period 3	0.13	-0.39, 0.66
Home range	Linear mixed effects model	Intercept	431.38	283.23, 580.84
		CO ₂	-38.82	-215.51, 127.31
		Days 6–10	116.43	-4.10, 237.03
		Days 11–15	158.99	33.75, 284.27

(-77%, 0%) lower, while CO₂-treated largemouth bass had no change (-36%, 36%) (Fig. 2b). Taken together, these two significant interaction effects indicate that largemouth bass not exposed to elevated pCO₂ showed a decrease in MDT between 06:00 and 18:00 hours, whereas fish exposed to elevated pCO₂ do not have a discernible change in MDT (Fig. 1). No significant

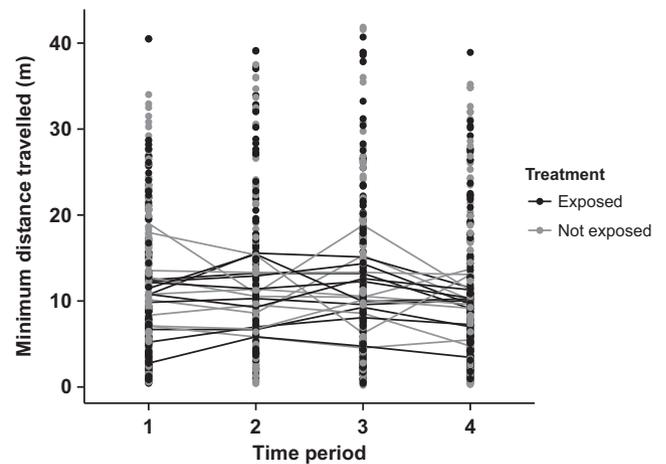


Fig. 1 Minimum distance travelled (m) for each tagged largemouth bass for each of the four distinct time periods (1, 00:00–06:00 hours; 2, 06:00–12:00 hours; 3, 12:00–18:00 hours; 4, 18:00–24:00 hours) during the 15-day monitoring period. Local polynomial regression fitting smooth lines represent the general trend observed for each fish. Exposed refers to fish treated with elevated levels of CO₂ (~10 000 µatm) for 5 days. Not exposed refers to fish held at ambient levels of CO₂ for 5 days. Grey dots and lines represent fish not exposed to elevated pCO₂ and black dots and lines represent fish exposed to elevated pCO₂.

change in MDT per day was found between fish exposed to elevated pCO₂ and unexposed fish (Table 2; Fig. 3).

During days 1–5 and days 5–10, significant interaction effects were found for the CO₂ × time period 2 (Table 2). For days 1–5, the change in MDT for time period 2 compared to time period 1 was greater in unexposed fish (a negative change in intercept of -0.35 (-0.73, 0.01) when compared to CO₂-exposed fish [change in intercept = 0.16 (-0.19, 0.52)] (Fig. 4a). Conversely, during days 5–10, the change in MDT during time period 2 compared to time period 1 was greater for largemouth bass exposed to elevated pCO₂ [change in intercept = 0.36 (-0.02, 0.74)] compared to fish not exposed to elevated pCO₂ [change in intercept = -0.28 (-0.67, 0.12)] (Fig. 4b). No significant effects were found for the 11–15 days group (Table 2), suggesting that the MDT for unexposed and exposed largemouth bass were no longer different from one another.

The home range size of largemouth bass was unaffected by pCO₂ exposure, but were larger after 11 days (Table 2; Fig. 5). However, the ability of the acoustic array to calculate positional estimates degraded 4 days faster (95% confidence limit: 1.43, 13.42) for fish exposed to elevated pCO₂ compared to unexposed fish (Cox proportional hazard analysis, likelihood ratio test = 7.25, d.f. = 1, P = 0.0071), suggesting that after the 15 days

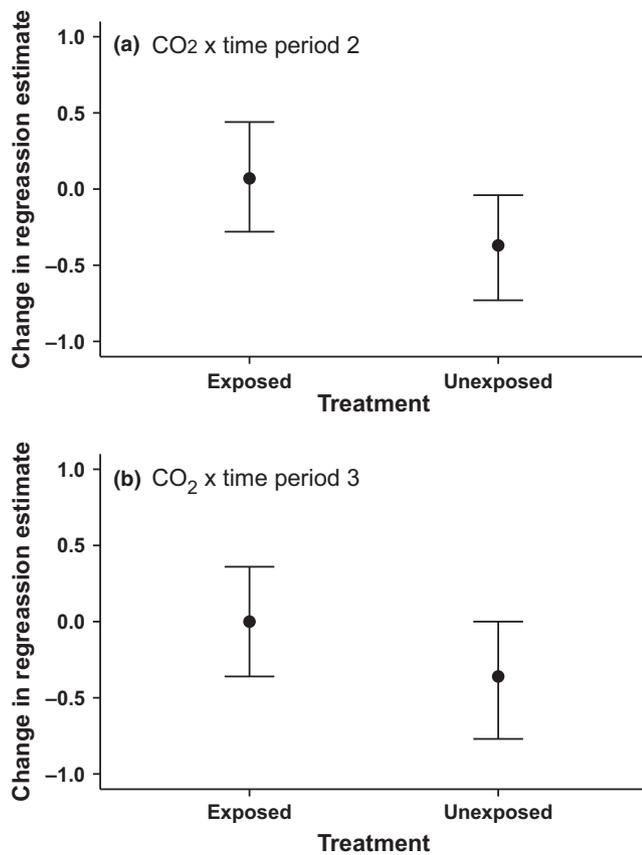


Fig. 2 Change in the intercept estimate during (a) 06:00–12:00 hours (time period 2) and (b) 12:00–18:00 hours (time period 3). Dots represent the mean change in the estimate and whiskers represent the 95% credible intervals. Both mean and credible intervals were calculated from estimates obtained using posterior simulations of each fixed effect.

monitoring period, largemouth bass exposed to elevated $p\text{CO}_2$ were using different parts of the pond. Note that all fish were found alive when the pond was drained in late September, 91 days after the completion of the telemetry monitoring period, thus no dead fish were included in the above data analyses.

Discussion

Exposure of largemouth bass to elevated $p\text{CO}_2$ for 5 days had a significant impact on the MDT following release. More specifically, fish not exposed to elevated $p\text{CO}_2$ showed 37% and 36% decrease in MDT between 06:00–12:00:00 and 12:00:00–18:00 hours, respectively, whereas fish exposed to elevated $p\text{CO}_2$ did not have a discernible change in MDT. Direct measures of field activity (e.g. swimming speed and distance travelled) have shown that largemouth bass typically increase activity during daylight periods, likely due to foraging

and warmer temperatures relative to the nocturnal period (Demers *et al.*, 1996; Hanson *et al.*, 2007, 2008; Binder *et al.*, 2012). However, foraging requires ambush and pursuit behaviour (Howick & O'Brien, 1983), which utilises burst swimming (i.e. increased swimming speeds) and anaerobic metabolism, and therefore periods of resting during the day are required for metabolic recovery (Sherwood *et al.*, 2002). The reduction in MDT during the daylight observed in largemouth bass not exposed to elevated $p\text{CO}_2$ in this study may be representative of resting behaviour in largemouth bass after foraging, which was also found in sparsely collected data using radio telemetry where it was suggested that largemouth bass exhibit short periods of increased distance travelled followed by longer periods of sedentary movements during the day (Winter, 1977), or due to cruising more at night (Howick & O'Brien, 1983). Furthermore, our study pond was small (<0.1 ha), meaning foraging fish would not have to move far to locate prey items and could have returned to their previous position to rest. Regardless of what constitute typical diurnal movements of wild largemouth bass, exposure to elevated $p\text{CO}_2$ resulted in an absence of a diel pattern in MDT despite the use of relatively few positions each day for each fish.

The relatively higher MDT during the daytime exhibited by largemouth bass exposed to elevated levels of $p\text{CO}_2$ may have resulted from physiological and behavioural changes that have been shown to occur in other fishes exposed to elevated $p\text{CO}_2$. Specifically, several marine-focused studies have found that acidosis in the blood caused by elevated $p\text{CO}_2$ results in increased extracellular Cl^- levels (Heuer & Grosell, 2014), and thus, increased neuronal depolarisation due to altered function of the neurotransmitter GABA and its receptors (Hamilton *et al.*, 2013). The GABA pathway plays an important function in the precise control of circadian rhythms and changes in GABA_A levels can destabilise typical diel patterns (Freeman *et al.*, 2013), which has also been noted for marine organisms exposed to ocean acidification (Kaniewska *et al.*, 2015). Therefore, it is plausible that the fish in this study may have experienced a disruption in their GABA pathway function from exposure to high levels of $p\text{CO}_2$ and this may explain the lack of typical diel activity patterns observed in the MDT of fish exposed to elevated $p\text{CO}_2$ when compared to fish not exposed to elevated $p\text{CO}_2$.

Differences between the diel movement patterns of largemouth bass exposed and not exposed to elevated $p\text{CO}_2$ were only found for days 1–5 and days 5–10 post-release, and not for the 11–15 days grouping. The lack

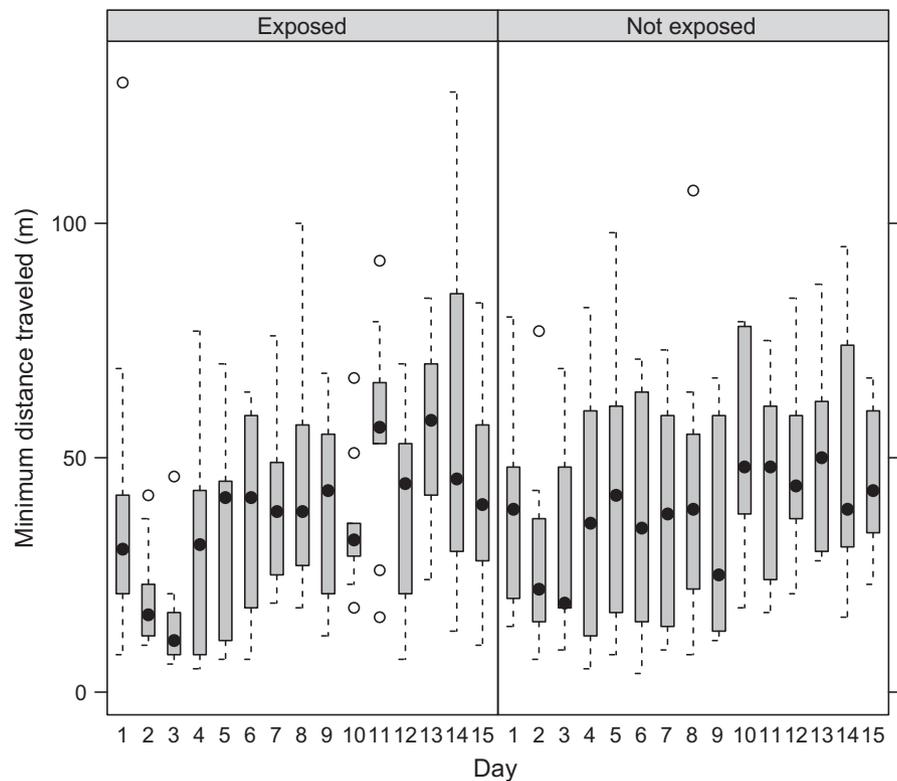


Fig. 3 Minimum distance travelled (m) each day for fish exposed (left) and not exposed (right) to elevated $p\text{CO}_2$. Black dots represent medians, grey areas represent the interquartile ranges (IQR), dashed lines are whiskers and represent $1.5 \times \text{IQR}$ and black circles represent outliers. No statistical difference was observed.

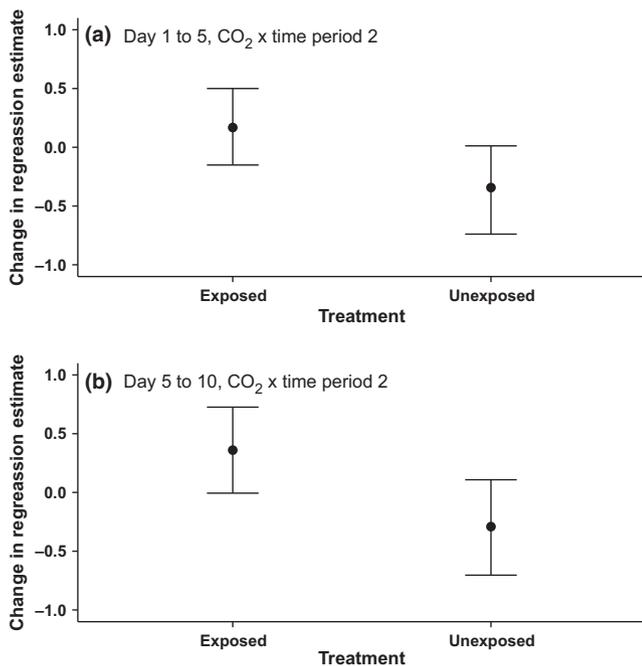


Fig. 4 Change in the intercept estimate for fish exposed and not exposed to elevated $p\text{CO}_2$. (a) Days 1–5 for 06:00–12:00 hours (time period two) and (b) days 5–10 for 12:00–18:00 hours (time period three). Dots represent the mean change in the estimate and whiskers represent the 95% credible intervals. Both mean and credible intervals were calculated from estimates obtained using posterior simulations of each fixed effect.

of statistical difference between the exposed and unexposed fish indicate that the abnormal within-day movement pattern observed during the first 10 days (i.e. 1–5 days, 5–10 days groups) were no longer detectable, and fish were moving similar distances regardless of treatment, indicating that animals likely recover from elevated $p\text{CO}_2$ exposure. Recovery from exposure to elevated $p\text{CO}_2$ levels has been shown in previous studies, as Hamilton *et al.* (2013) found that anxiety-related behaviours of juvenile Californian rockfish (*Sebastes diploproa*) affected by CO_2 exposure returned to normal after returning to ambient seawater for 12 days. Interestingly, in our study, the observed difference between within-day movement patterns of exposed and unexposed fish were no longer detectable on 12 days after release (day 11 in model due to the initial release day being omitted from the data set). A potential explanation for the recovery is the changes in the GABA pathway induced by extended holding at elevated levels of $p\text{CO}_2$ returned to the typical inhibitory function and normal neuronal activity and diel behaviours commenced (Nilsson *et al.*, 2012; Hamilton *et al.*, 2013). Future studies should more closely examine diel patterns in activity and potentially manipulate the GABA pathway using gabazine (an antagonist of the GABA pathway) (Nilsson *et al.*, 2012) to gain a more complete understanding of how elevated

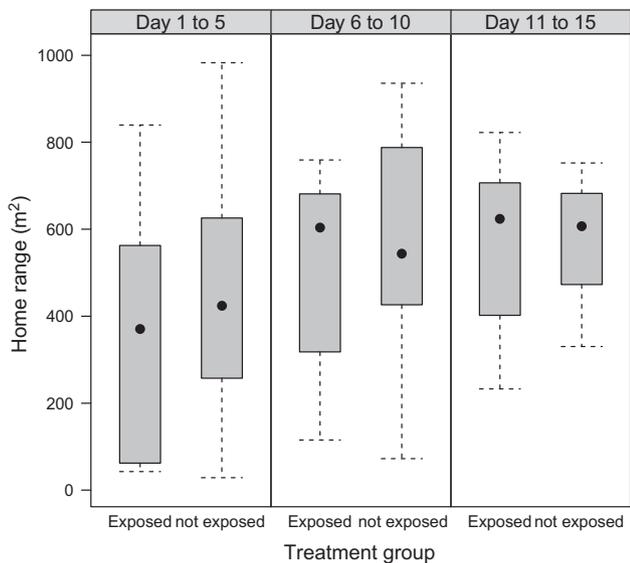


Fig. 5 Home range size (m^2) calculated using 95% kernel density estimations of fish relocations during days 1–5 (left), days 6–10 (centre) and days 11–15 (right) for fish exposed and not exposed to elevated $p\text{CO}_2$. Black dots represent medians, grey areas represent the interquartile ranges (IQR) and dashed lines are whiskers and represent $1.5 \times \text{IQR}$.

$p\text{CO}_2$ affects diel behaviours in fish and potential preventive solutions.

Space use assessed using both home range size and the degradation of accurate position estimates indicated a limited influence of elevated $p\text{CO}_2$ on largemouth bass habitat use. Specifically, home range size did not change regardless of exposure to elevated $p\text{CO}_2$ or not, likely due to the pond being smaller than the typical size of largemouth bass home range (0.3–1.4 ha; Winter, 1977). However, space use may have been altered by exposure to elevated $p\text{CO}_2$, as the *post hoc* analysis of space use using positional estimates after the 15 days monitoring period revealed that fish exposed to elevated $p\text{CO}_2$ could not be positioned by the system 4 days earlier than unexposed fish (at least 1.3 days earlier based on the lower confidence limit). Because the accuracy of position estimates is strongly influenced by the position of the fish with respect to the position of the hydrophone array (Baktoft *et al.*, 2015), the disproportionate reduction in position estimates between treatment groups indicates that fish exposed to elevated $p\text{CO}_2$ were likely spending the majority of time during the latter part of the study (i.e. after day 15) beyond the foot print of the array, likely in near shore areas where spatial coverage was reduced due to shallow water and vegetation. This measured difference in habitat use may be related to a variety of factors including exclusion by non-exposed fish (e.g. Hasegawa *et al.*, 2004), or a compensatory response to account for

the physiological changes noted above (e.g. recovery from acidosis; Heuer & Grosell, 2014). Interestingly, there may be a carryover effect (i.e. conditions in one period effect an outcome in an subsequent period; e.g. Harrison *et al.*, 2011) of exposure to elevated $p\text{CO}_2$ that alters habitat use by largemouth bass, which has also been found in wild largemouth bass injected with the stress hormone, cortisol. O'Connor *et al.* (2010) found that largemouth bass injected with cortisol several months prior were unable to cope with anoxic conditions and died at a faster rate than fish not artificially stressed; presumably because stressed fish were using more anoxic habitats than non-stressed fish. In the case of this study, the exposure to elevated $p\text{CO}_2$ (a stressor known to increase cortisol in fish; Kates *et al.*, 2012) may have caused fish several weeks after exposure to use poorer quality, peripheral habitat in the pond. However, because we were unable to assess the other movement metrics during this time period, it is difficult to fully assess the potential that elevated $p\text{CO}_2$ has significant carryover effects on habitat selection.

Broadly, our results indicate that short-term exposure (5 days) to elevated $p\text{CO}_2$ at a level of approximately $10\,000\ \mu\text{atm}$ has limited impact on movement behaviours of largemouth bass, and changes that do occur in diel movement patterns, appear to be corrected by 12 days following exposure. To our knowledge, no other telemetry studies have characterised daily movement and space use in free-ranging freshwater or marine fishes exposed to elevated levels of $p\text{CO}_2$, therefore making comparisons to other studies is difficult. Fish demonstrating irregular movement behaviours have been found for some marine species observed using underwater observation of externally tagged fish, and fish moved further and had larger home ranges due to abnormal searching and lack of homing ability (e.g. Devine *et al.*, 2012). The loss of a typical diel movement pattern that was observed in this study may have consequences for energetics, specifically if foraging success is in some way impacted. However, we did not observe any changes to daily movement, so it is unclear if fish exposed to higher $p\text{CO}_2$ expended more total energy each day. A potential reason for not finding changes in daily movement may be due to freshwater fish being adapted to a wider range of $p\text{CO}_2$ levels in comparison to marine fishes (suggested in Hasler *et al.*, 2016). For example, in a global assessment of direct measurements of $p\text{CO}_2$ in freshwater lakes, $p\text{CO}_2$ ranged from $100\ \mu\text{atm}$ to over $4000\ \mu\text{atm}$ (Cole *et al.*, 1994), and wide diel and episodic fluctuations have been found to increase $p\text{CO}_2$ over 15-fold (Maberly, 1996). The findings of this study may apply to future scenarios of freshwater $p\text{CO}_2$, as

some systems, specifically the Laurentian Great Lakes, may exhibit higher pCO₂ as a result of higher atmospheric pCO₂ (Phillips *et al.*, 2015). Furthermore, hatchery-reared fish are often exposed to high pCO₂ to assist with safe handling and because of crowding (Carmichael *et al.*, 1984). In general, further studies should be completed to understand how freshwater fish behaviour changes as pCO₂ rises, and are warranted given the statistical limitations imposed by the experimental design used in this study (e.g., one treatment tank, one pond) (Cornwall & Hurd, 2016). Studies specifically targeted at understanding behaviours related to reproduction, predator–prey dynamics and studies on sensitive species should be prioritised. Given the findings from marine studies on fish behaviour and rising pCO₂, it will be vital for the health of freshwater ecosystems to fully understand the influence of pCO₂ on freshwater fishes and their ecology.

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