Predation of freshwater fish in environments with elevated carbon dioxide

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Abstract. Carbon dioxide (CO_2) in fresh-water environments is poorly understood, yet in marine environments CO_2 can affect fish behaviour, including predator–prey relationships. To examine changes in predator success in elevated CO_2, we experimented with predatory *Micropterus salmoides* and *Pimephales promelas* prey. We used a two-factor fully crossed experimental design; one factor was 4-day (acclimation) CO_2 concentration and the second factor CO_2 concentration during 20-min predation experiments. Both factors had three treatment levels, including ambient partial pressure of CO_2 (pCO_2; 0–1000 μatm), low pCO_2 (4000–5000 μatm) and high pCO_2 (8000–10 000 μatm). *Micropterus salmoides* was exposed to both factors, whereas *P. promelas* was not exposed to the acclimation factor. In total, 83 of the 96 *P. promelas* were consumed ($n = 96$ trials) and we saw no discernible effect of CO_2 on predator success or time to predation. Failed strikes and time between failed strikes were too infrequent to model. Compared with marine systems, our findings are unique in that we not only saw no changes in prey capture success with increasing CO_2, but we also used CO_2 treatments that were substantially higher than those in past experiments. Our work demonstrated a pronounced resiliency of freshwater predators to elevated CO_2 exposure, and a starting point for future work in this area.

Additional keywords: climate change, predator–prey dynamics, *Micropterus salmoides*, *Pimephales promelas*.

Introduction

The effects of carbon dioxide (CO_2) on aquatic systems has recently received a great deal of interest (Fabry *et al.* 2008; Munday *et al.* 2008). Many of the questions on this topic, and much of the work, is motivated by increasing concentrations of CO_2 in the atmosphere and the commensurate increase of CO_2 concentrations in aquatic environments (often measured as the partial pressure of CO_2 (pCO_2; Solomon *et al.* 2007). Marine systems have been particularly responsive to concerns over elevated pCO_2, given the nature of ocean acidification; elevations in atmospheric CO_2 translate into an increase in the pCO_2 in the oceans, which, in turn, causes acidification that can negatively affect a range of biological processes, including fish behaviour (Caldeira and Wickett 2003). Fewer studies have been undertaken to understand and quantify how the concentration of fresh-water CO_2 may change in the future (but see (Butman and Raymond 2011; Hasler *et al.* 2016a). However, currently evidence is mounting to support the idea that pCO_2 in North American fresh waters may be higher and more variable than conventionally assumed (Maberly 1996; Phillips *et al.*

2015), and freshwater biota may be at risk from increasing pCO_2 (Hasler *et al.* 2016a).

In North American fresh waters, pCO_2 is demonstrating to be dynamic and variable. Cole *et al.* (1994) found that the majority of sampled US lakes are supersaturated with pCO_2, whereas Butman and Raymond (2011) showed that many streams across the US are supersaturated with pCO_2. Furthermore, Baumann *et al.* (2015) reported CO_2 dynamics in brackish systems and found additional evidence for high variability in pCO_2; e.g. daily summer values ranged from 500 to 4000 μatm. Concentrations of pCO_2 can be particularly high and variable in low-order streams in watersheds, with high terrestrial primary productivity and precipitation rates (Butman and Raymond 2011), and in watersheds underlain with geology that does not allow for pH buffering (Cole *et al.* 1994). In addition to interest in the overall magnitude of CO_2 is the high variability of pCO_2 in fresh waters. This high variability is largely absent from CO_2 investigations in the marine realm, at least with respect to coral-reef environments that have been investigated (Munday *et al.* 2014). In addition to natural sources, proposed deployments of fish

barriers that use CO_2 (Noatch and Suski 2012) represent another potential source of significant (but localised) CO_2 input to fresh waters.

Predator–prey dynamics can be among the most important interactions in which fish participate. Successful predation is not only needed for survival, but effective predation can lead to variation in growth, maturation and other life-history traits that shape individual selection, population growth and evolution (Lima 1998). The same can be said for avoiding predation; those individuals best equipped to avoid predation will have higher fitness within their population. Because predator–prey dynamics are critically important for both species involved, but also for the structuring of the food web and ecosystem, minor changes in this dynamic can have substantial effects (Kitchell *et al.* 1994). Water-quality parameters are known to affect predator success and prey avoidance (e.g. turbidity; Rahel and Nutzman 1994); however, very little work has looked at how increases in pCO_2 may alter this interspecies dynamic, with studies thus far having been limited to marine environments. Ferrari *et al.* (2011) examined predation with *Pseudochromis fuscus* preying on *Pomacentrus* spp. in control (440 µatm) and elevated (700 µatm) CO_2 environments, and found that predation rates increased with elevated pCO_2, but that there was no change in prey selectivity on smaller prey (and a reversal of prey species at larger sizes). Allan *et al.* (2013) also experimented with reef-fish predation under control (440 µatm) and elevated (880 µatm) pCO_2 conditions. In a cross-factored design, *Pseudochromis fuscus* and prey *Pomacentrus amboinensis* were exposed to either treatment, with results suggesting negative effects for both species in elevated pCO_2; predators showed reduced capture success and prey showed longer reaction distances. Thus, exposure to elevated pCO_2 can induce changes to the predator–prey dynamics of marine fishes, which, in turn, can translate into population-level changes (Munday *et al.* 2010).

In the current study, we sought to expand on previous work in the marine environment by quantifying how CO_2 exposure would affect predator–prey dynamics for freshwater fishes, as well as the interaction of CO_2 concentrations, along with the duration of exposure. More specifically, the objective of the present study was to quantify predator–prey outcomes in fishes in an experimental setting, following exposure to various levels of pCO_2. *Micropterus salmoides* was used as a predator species and *Pimephales promelas* was used as a prey species. Understanding the influence of CO_2 on predator–prey dynamics in fresh water will help understand how species and communities exposed to increasing concentrations of CO_2 can expect to respond. We predicted that predation rates, successful consumption, time to consumption, and failed strikes would be affected by increasing pCO_2. Particularly, we expected that time to consumption and failed strikes would increase with pCO_2, and overall predation success (capture of prey after a fixed amount of time) would decrease.

Materials and methods

Species and acclimation

We used *Micropterus salmoides* as a predator species and *Pimephales promelas* as a prey species. Both of these fish species are common in North American fresh waters and have a well

documented predator–prey relationship (Hambright 1991). All fish were captive-reared and acquired from Keystone Hatcheries in Richmond, Illinois, USA, in early November 2015. Fish were transported to and held at the Aquatic Research Facility at the University of Illinois Urbana–Champaign. On arrival to the facility, conspecifics were held in three 1100-L aquaria (no more than 40 *M. salmoides* individuals per aquaria) with ambient pCO_2 (<1000 µatm) and a constant temperature of 24°C (range 23.8–24.7°C). *Pimephales promelas* was initially introduced to the *M. salmoides*-holding tank to confirm a predation response (which was unknown because of hatchery diet), and, almost instantly, *M. salmoides* aggressively pursued prey. After this confirmation of predation response, *M. salmoides* were left for 1 week without food, to ensure that all predators were sufficiently hungry during the experiments. Ammonia and nitrate concentrations were monitored (LaMotte Co., Ammonia Nitrogen kit number 3351-02, Chestertown, MD, USA) and daily water changes took place. To prevent hypoxia, air was bubbled in and dissolved oxygen monitored (YSI, 550A Yellow Springs 82 Instruments, Irvine, CA, USA).

Experimental setup

The experimental design included a 3×3 factorial design, in which the two factors were pCO_2 during holding (4-day acclimation) and pCO_2 during the experiment (20 min). These factors were selected to compare the effects of longer-term, pre-predation conditions with those at the time of predation. *Micropterus salmoides* individuals were exposed to both factors, whereas *P. promelas* individuals were all acclimated to ambient pCO_2 and exposed to higher pCO_2 only during the experimental phase. Each factor had the following three levels with a target concentration: ambient pCO_2 (0–1000 µatm), low pCO_2 (4000–5000 µatm) and high pCO_2 (8000–10 000 µatm). These levels were chosen such that the control represented normal conditions, 4000–5000 µatm represented higher than normal conditions (but values that are not considered extreme in fresh water) and 8000–10 000 µatm was selected as an upper limit of pCO_2 tolerance. Previous work has shown that extended holding at $pCO_2 > 10 000$ µatm can have strong negative effects on fish, such that they lose equilibrium (Kates *et al.* 2012). Experimental pCO_2 values were maintained using the common method of bubbling CO_2 gas into the water through an airstone, using water pH to maintain a target pCO_2 level (Pinpoint pH controller, American Marine Inc., Ridgefield, CT, USA; Kates *et al.* 2012; Hasler *et al.* 2016b). This was performed in a header tank, from which treated water was then mixed into the experimental tanks. A modified infrared CO_2 probe was used to monitor pCO_2 (GMT221, 0–20%, Vaisala, Vantaa, Finland; Johnson *et al.* 2010). Temperature and CO_2 measurements for all trials ($n = 96$) are reported in Table 1. The pCO_2 monitoring was accurate to $\pm$ (1.5% of range + 2% of reading) (Vaisala), and probe values were also compared with water with known concentrations of pCO_2 multiple times throughout the study period.

After the 20-min acclimation phase for the predator, one prey fish was introduced to the aquaria holding nothing but the predator fish. Aquaria contained no shelter and low light (aquaria were in a lighted room, but behind black curtains on all sides to minimise visual distraction), suggesting that visual

Table 1.   Measured temperature and partial pressure of carbon dioxide (pCO$_2$) for each experimental trial
pCO$_2$ values are accurate to ±2% of reading

Trial	Temperature (°C)	Acclimation CO$_2$	Experimental CO$_2$	Acclimation CO$_2$ (µatm)	Experimental CO$_2$ (µatm)
1	24	Control	Control	<100	<100
2	24	Control	Control	<100	<100
3	24.2	Control	Low	<100	4100
4	24.2	Control	Low	<100	4100
5	24.7	Control	High	<100	8800
6	24.7	Control	High	<100	8600
7	23.8	Low	Control	4600	<100
8	24	Low	Control	4600	<100
9	23.4	Low	Low	4400	4400
10	23.4	Low	Low	4400	4400
11	24.1	Low	High	4000	8200
12	24.2	Low	High	4000	8200
13	24	High	Control	8500	<100
14	24	High	Control	8500	<100
15	24.1	High	Low	8400	4800
16	24.1	High	Low	8400	4800
17	24	High	High	8800	8800
18	24	High	High	8800	8800
19	24.1	Control	Control	<100	<100
20	24.1	Control	Control	<100	<100
21	24.3	Low	Control	4400	<100
22	24.3	Low	Control	4400	<100
23	24	High	Control	8300	<100
24	24	High	Control	8300	<100

cues were the primary means of predation. All trials were recorded from an overhead camera. We watched videos of all trials, and determined whether the prey was consumed (yes or no), the time to consumption (s), the number of total strikes, the number of failed strikes, and the time between multiple strikes (s). Water temperature was constant (varying less than 1°C throughout the experiment) and was not included in the analysis, and pCO$_2$ concentrations were treated as factors because their measurements did not vary meaningfully within factor level. Although prey size can be viewed as a factor and some studies seek to quantify this, we selected sufficiently large predators and small prey, so that fish sizes were not considered an important influence on predation (Goldstein 1993). For example, mean predator size was 180 mm ($n = 24$; s.d. $= 14$) and prey size was 51 mm ($n = 24$; s.d. $= 5$). Such a large size differential and strong support in the literature suggests that size and gape limitation were not a factor in our experiments.

Data analysis

We focussed on two statistical models to describe predation at varying levels of pCO$_2$. The first model was selected to answer the question of whether CO$_2$ treatments affected successful consumption of *P. promelas*. The model used to address this question was a binomial generalised linear model (i.e. logistic regression) that used the categorical predictors of acclimation pCO$_2$, experimental pCO$_2$, and their interaction, to predict the probability of whether or not *P. promelas* was consumed.

The second question we sought to answer was whether CO$_2$ treatments affected the time it took a *M. salmoides* individual

to successfully consume a *P. promelas* individual. The model used to address this question was a beta regression, which is commonly used to model rates and proportions (Ferrari and Cribari-Neto 2004). In our case, time to consume was bounded at 0 and 1200 s, and the individual trial times were divided by the total time (1200), resulting in the proportion used as the response variable. Again, we used the categorical predictors of acclimation pCO$_2$, experimental pCO$_2$, and their interaction, to model the time it took for *P. promelas* to be consumed.

All models were implemented in the BUGS language and used Bayesian estimation for model fitting. *Post hoc* comparisons among groups were conducted by determining whether the 95% credible interval (CI) of the differences in posterior distributions among groups overlapped with zero. For all models we ran three concurrent Markov chains (comprising 12 000 iterations), beginning each chain with randomly generated values. The first 8000 iterations of each chain were discarded as burn-in, thinned by removing every other iteration, and the remaining 6000 values were assessed for convergence using the Brooks–Gelman–Rubin statistic ($\hat{R}$) with values <1.1 indicating convergence (all our reported values were <1.01). Analyses were run through JAGS in the rjags package (M. C. Plummer, see http://www.sourceforge.net/projects/mcmc-jags/, accessed 4 January 2017) run from within R (R Foundation for Statistical Computing, Vienna, Austria, see https://www.R-project.org/).

Results

Overall, 83 of the 96 *P. promelas* individuals were consumed during the 96 trials. For both the logistic- and beta-regression
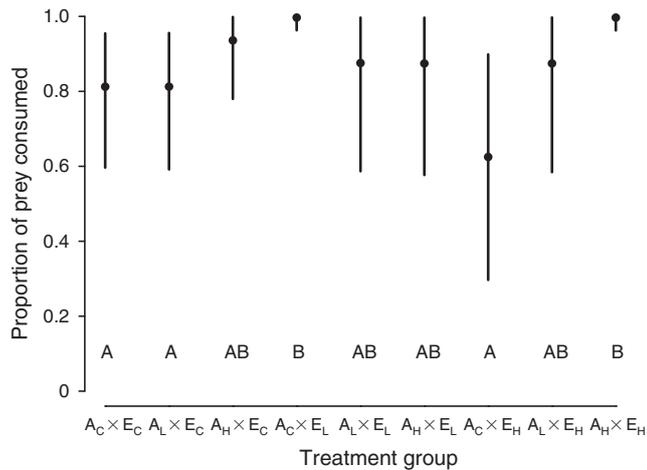
Fig. 1. Estimates of interaction coefficients for an interactions-only logistic-regression model. The *x*-axis lists the interaction combinations, with letters A and E indicating *Acclimation* (4-day holding) and *Experimentation* (20-min trial) treatments respectively, and subscripts C, L, and H indicating CO_2 treatment levels control, low and high respectively. Within the plotting surface, letters A and B are groupings for multiple comparisons; groups sharing the same letter are not significantly different at the 95% credible level.
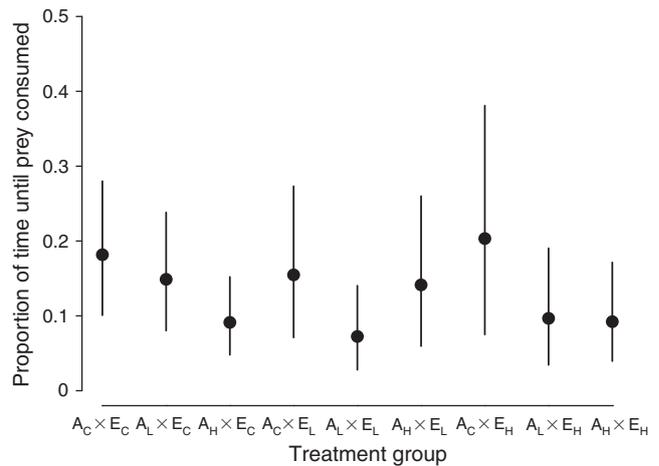

Fig. 2. Estimates of interaction coefficients for an interactions-only beta regression model. The *x*-axis lists the interaction combinations, with letters A and E indicating *Acclimation* and *Experimentation* treatments respectively, and subscripts C, L and H indicating CO_2 treatment levels control, low and high respectively. No significant differences among groups were found at the 95% credible level.

models, we first ran an interaction-only model to test for an interaction between the main effects of acclimation pCO_2 and experimental pCO_2, the presence of which would exclude the need to examine main effects. The interaction-only logistic regression (which modelled predation success) showed significant differences between some groups (i.e. 95% credible intervals of differences between group means did not overlap with zero; Fig. 1), which eliminated the need to run a main-effects model. The treatment combinations showed significant differences, with the acclimation control × experimental high pCO_2 having the lowest proportion of prey-consumption success (mean = 0.64 and 95% credible interval = 0.29–0.90), and the acclimation control × experimental low pCO_2 and acclimation high × experimental high pCO_2 having very high estimated prey-consumption success (both with a mean = 1.0 and 95% credible interval = 0.95–1.00).

The interaction-only beta-regression model (which modelled the time to prey consumption) found no significant differences across treatments (Fig. 2), and, subsequently, main effects were examined. We used separate one-way main-effect models for each factor, because means parameterisations were needed to generate full posterior estimates for all effects required for multiple comparisons (i.e. effects parameterisations require setting one factor level to 0, which eliminates the posterior distribution needed for multiple comparisons). Additionally, means parameterisation of two-way models results in model non-identifiability. The single-factor beta-regression model for acclimation pCO_2 showed a general decline in the proportion of time until prey consumption with an increasing pCO_2, although group means did not differ significantly (Fig. 3). The single-factor beta-regression model for experimental pCO_2 estimated similar proportions of time until prey consumption for all treatments (Fig. 4).
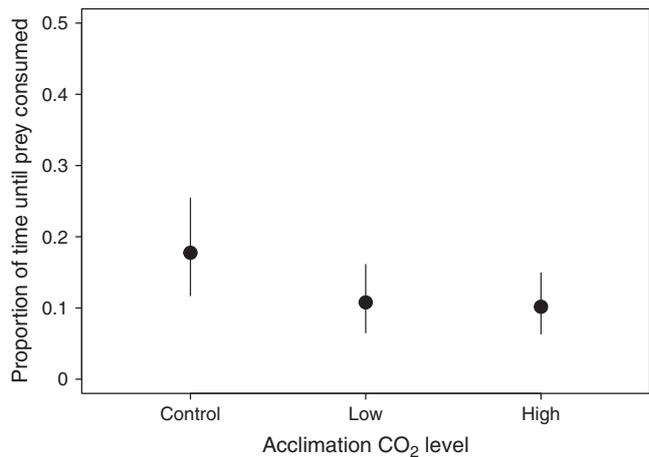
Fig. 3. Coefficient estimates from a one-way beta regression using acclimation CO_2 concentration to predict time until prey consumed. Time until consumption decreased with an increasing CO_2 concentration, although all factor levels overlapped at the 95% credible level.

Owing to the very few trials with failed strikes (only 4 of 96), we did not model failed strikes or time between failed strikes, nor were these few observations of failed strikes occurring within one treatment type. Finally, a logistic regression model was fitted to test for an effect of experimental day on predation success, so as to determine whether predators were more or less likely to successfully consume as the experiment progressed (i.e. examining the potential effect of hunger). Experimental day showed no significant effect, indicating that holding time did not influence a predator's probability of consuming prey.
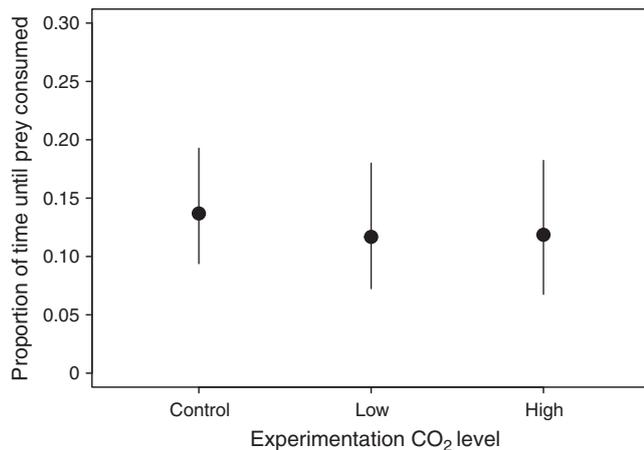
Fig. 4. Coefficient estimates from a one-way beta regression using experimental CO$_2$ concentration to predict time until prey consumed. Time until consumption decreased with an increasing CO$_2$ concentration, although all factor levels overlapped at the 95% credible level.

Discussion

Comparison to marine studies

Acclimation to elevated pCO$_2$ for 4 days did not result in alterations to feeding behaviours for *M. salmoides*, defined as prey consumption rate and time to prey consumption. In fact, *M. salmoides* acclimated to both low (4000–5000 μatm) and high pCO$_2$ (8000–10 000 μatm) consumed 86% of prey items. These findings contradicted recent experiments with marine fishes that found that predator–prey dynamics were compromised when fish were exposed to elevated pCO$_2$ (441–1064 μatm, which is ∼1.1–2.5 times ambient pCO$_2$; reviewed by Clements and Hunt 2015). Specifically, Allan *et al.* (2013) found that *P. fuscus* had a lower capture success when both *P. fuscus* and its prey items were exposed to elevated pCO$_2$ (880 μatm, or 2.0× ambient pCO$_2$) for 4 days. In another study, Cripps *et al.* (2011) found that olfactory response of *P. fuscus* to the smell of injured prey was degraded, fish took more than four times longer to respond to introduced prey, and fish had almost one-third fewer feeding strikes when exposed to elevated pCO$_2$ (600–950 μatm, or 1.5–2.4× ambient pCO$_2$) for 4–7 days. In addition to studies on *P. fuscus*, effects of CO$_2$ on the predator mechanics of other obligate marine fish predators have also been observed, including several shark species, which avoided prey odours after exposure to elevated pCO$_2$ (Green and Jutfelt 2014; Dixson *et al.* 2015; Pistevos *et al.* 2015). Of particular note is that pCO$_2$ in fresh waters tends to be in much higher concentrations, which is why our values are substantially greater than those presented in the (marine) literature.

Possible mechanisms

Mechanistically, the successful ingestion of a prey item requires the coordination of a number sensory modalities, including chemical and visual detection of a prey item, followed by pursuit, capture and ingestion (Clements and Raubenheimer 2006; Allan *et al.* 2013). Precisely how exposure to elevated pCO$_2$ might alter pursuit, capture and ingestion of prey is unknown,

but the influence of environmental variability on fish performance is of interest to researchers (Claireaux and Lefrançois 2007). Currently, it is believed that exposure to elevated pCO$_2$ negatively affects prey detection of predators, leading to reductions in feeding rates (Cripps *et al.* 2011). More specifically, as a result of CO$_2$-induced acidosis in the blood of fish, there is an increase in extracellular Cl$^-$ concentrations (Heuer and Grosell 2014), which causes increased neuronal depolarisation and altered function of the GABA$_A$ neurotransmitter receptor (Nilsson *et al.* 2012). This alteration in the receptor is thought to be responsible for a wide array of behavioural and physiological changes in fishes and could be responsible for altered prey detection (Nilsson *et al.* 2012; Hamilton *et al.* 2013; Chivers *et al.* 2014; Clements and Hunt 2015; Ou *et al.* 2015). Changes in pCO$_2$ can alter the GABA$_A$ neural transmitter in obligate freshwater fishes (Regan *et al.* 2016); however, without a defined link between prey capture and exposure to elevated pCO$_2$, it is difficult to assess whether *M. salmoides* in the present study experienced a compromised GABA$_A$ pathway. Despite the lack of defined physiological links, it is clear that the ability of *M. salmoides* to capture and consume prey was not altered despite exposure to elevated pCO$_2$.

Similarly to feeding after extended exposure to elevated pCO$_2$, feeding behaviours of *M. salmoides* were not affected by short-term holding at elevated pCO$_2$. Fish acclimated to ambient pCO$_2$ and then placed in either low or high pCO$_2$ tanks 20 min before the feeding trial did not show a significant change in either prey consumption rates or in the time to consume prey. Behaviour after short-term exposure to elevated pCO$_2$ is important to understand because freshwater pCO$_2$ can vary over both diel and seasonal time scales (Maberly 1996). For example, as a result of factors that include rain events, season and aquatic respiration, pCO$_2$ can rise in freshwater lakes within a day (100-fold change in some days; Maberly 1996). In addition, zones of elevated pCO$_2$ that are ∼50-fold above the ambient concentration have been proposed for use as a non-physical barrier to influence the movement of fishes (Kates *et al.* 2012; Noatch and Suski 2012). Thus, wild, free-swimming fish have potential to experience sudden increases in pCO$_2$ independent of insidious stressors such as climate change. Should short-term increases in pCO$_2$ be high enough (e.g. >45 000 μatm), fish may lose equilibrium, because CO$_2$ is a known anaesthetic for fish (Marking and Meyer 1985). Also, mortality may be possible because cardiac failure occurs when marine fishes have been exposed to elevated pCO$_2$ (∼50 000 μatm; Ishimatsu *et al.* 2004). However, in natural environments, where a gradient in CO$_2$ concentrations exist, fish have the capacity to sense elevated pCO$_2$ (Perry and Gilmour 2002) and will avoid lethal concentrations (Kates *et al.* 2012). Despite fish having the ability to avoid elevated pCO$_2$, short-term physiological consequences are possible, because physiological changes in response to CO$_2$ exposure are rather quick (e.g. changes occur within minutes (Iwama *et al.* 1989; Ishimatsu *et al.* 2004). In addition, the rate at which the GABA transmitter pathway is altered and causes behavioural changes can occur within 4 days (Ishimatsu *et al.* 2004; Nilsson *et al.* 2012), but physiological effects are possible within hours (Chivers *et al.* 2014). So, it is conceivable that short-term exposure to elevated pCO$_2$ had the potential to influence the feeding behaviours of *M. salmoides*;

however, clearly, data from the current study showed that short-duration exposures to high concentrations of CO_2 do not affect consumption rates or time to consumption.

There are several potential mechanisms to explain why exposure to elevated pCO_2 did not alter the feeding behaviours of *M. salmoides*. First, and perhaps foremost, the range of pCO_2 values that freshwater fish have been exposed to over evolutionary history is likely to be greater than that of marine fishes. Specifically, pCO_2 in freshwater lakes can range from ∼100 to 4100 µatm and can reach as high as 20 000 µatm in some African lakes (Cole *et al.* 1994). In comparison, marine environments are typically at equilibrium with atmospheric pCO_2 (∼400 µatm with very little variability in most marine habitats) and are expected to reach 1000 µatm by 2100 (Solomon *et al.* 2007); however, CO_2 'hotspots' where pCO_2 can be 10-fold above the current level exist, and can be found in the Southern Ocean, Pacific Ocean and North Atlantic Ocean (McNeil and Sasse 2016). Similarly, pCO_2 levels in coral reefs, where several studies have observed changes to predator–prey dynamics, are typically stable and close to the mean ocean pCO_2 (Munday *et al.* 2014). For this reason, freshwater fish may be adapted to a wider range of pCO_2 values, as well as higher absolute pCO_2, and thus may have the ability to maintain typical feeding behaviours following holding at an elevated pCO_2. For example, cobia (*Rachycentron canadum*), a species that can transition between marine and freshwater habitats, has also shown to be resistant to increased pCO_2, because they have similar somatic growth rates when exposed to a range of pCO_2 values (800 and 2100 µatm; Bignami *et al.* 2013). Second, it is possible that the feeding behaviours of freshwater fishes are not tightly linked to the potential physiological changes that fish undergo when exposed to elevated pCO_2. As described above, in marine fishes, changes to the GABA neural-transmitter pathway are thought to be the mechanism by which behavioural changes occur in marine fish, and can be reversed if CO_2-exposed fish are returned to ambient conditions. This pathway and feeding in freshwater fish exposed to elevated pCO_2 have not been investigated and should be considered for future research, specifically in the context of rising aquatic pCO_2, as not all marine fish species have had similar behavioural changes (Jutfelt and Hedgärde 2013, 2015; Näslund *et al.* 2015; Sundin and Jutfelt 2016). Overall, no significant changes in feeding behaviours of *M. salmoides* were observed and this may be due to being exposed to elevated pCO_2 during their evolutionary history and, potentially, because they have a more robust physiological response to high concentrations of CO_2.

Prey item and other considerations

Although the focus of the current study was on the response of a predatory freshwater fish to CO_2 exposure, our data also allowed us to make inferences related to the effect of CO_2 on the prey fish in our study. The topic of how prey fishes respond following CO_2 exposure has been quantified previously using marine fishes, and many studies have demonstrated that prey have reduced escape distances (Allan *et al.* 2013), longer reaction distances (Allan *et al.* 2013), impaired predator detection (Dixson *et al.* 2010; Sundin and Jutfelt 2016), altered visual-risk assessment (Ferrari *et al.* 2012) and increased predation when

exposed to elevated pCO_2 (Ferrari *et al.* 2011). In fact, smaller freshwater fish have been found to have lower ventilation rates (an indication of sedation; Kates *et al.* 2012), along with impaired predator learning (Leduc *et al.* 2004) when exposed to elevated pCO_2, which would presumably make them prone to consumption. In the present study, prey fish were not monitored for activity, but, presumably, if prey fish used in the present study were experiencing behavioural changes from the short-term exposure to elevated CO_2, it would be expected that *M. salmoides* acclimated to ambient conditions (i.e. no change in pCO_2) would have had higher consumption rates in the two treatments where pCO_2 was elevated, which was not observed. Future studies should monitor behaviour and activity of prey fish to gain a clearer picture of how elevated pCO_2 potentially may change predator–prey dynamics of freshwater fishes, which could have both synergistic and antagonistic effects (Ferrari *et al.* 2015).

Further to understanding the predator–prey dynamics of freshwater fishes, it is important to understand the role that CO_2 plays in the ecology of freshwater systems. Similar to the marine environment, freshwater CO_2 is expected to increase in some systems in the future, including the Laurentian Great Lakes (Phillips *et al.* 2015; Pilcher *et al.* 2015), but future changes in pCO_2 will vary widely on the basis of several environmental factors, including terrestrial primary productivity, substrate and biological respiration (Hasler *et al.* 2016a). In addition to changing concentrations of CO_2 in fresh water, pCO_2 may also become more variable in the future as a result of changing precipitation patterns (Butman and Raymond 2011), and could be altered because of management activities such as the installation of CO_2 barriers (Noatch and Suski 2012). Together, in many freshwater systems, biota will likely be exposed to higher and variable CO_2 concentrations. Data from the current study would suggest that there is minimal effect of elevated pCO_2 on the feeding behaviour of *M. salmoides*.

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