

Predation of freshwater fish in environments with elevated carbon dioxide

Stephen R. Midway^{A,C,D}, Caleb T. Hasler^A, Tyler Wagner^B and Cory D. Suski^A

^ADepartment of Natural Resources and Environmental Science, University of Illinois at Urbana–Champaign, Urbana, IL 61801, USA.

^BU.S. Geological Survey, Pennsylvania Cooperative Fish & Wildlife Research Unit, Pennsylvania State University, University Park, PA 16802, USA.

^CPresent address: Department of Oceanography and Coastal Sciences, Louisiana State University, Baton Rouge, LA 70820, USA.

^DCorresponding author. Email: smidway@lsu.edu

Abstract. Carbon dioxide (CO₂) in fresh-water environments is poorly understood, yet in marine environments CO₂ can affect fish behaviour, including predator–prey relationships. To examine changes in predator success in elevated CO₂, 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) CO₂ concentration and the second factor CO₂ concentration during 20-min predation experiments. Both factors had three treatment levels, including ambient partial pressure of CO₂ (*p*CO₂; 0–1000 µatm), low *p*CO₂ (4000–5000 µatm) and high *p*CO₂ (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 CO₂ 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 CO₂, but we also used CO₂ treatments that were substantially higher than those in past experiments. Our work demonstrated a pronounced resiliency of freshwater predators to elevated CO₂ exposure, and a starting point for future work in this area.

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

Received 28 April 2016, accepted 27 November 2016, published online 15 February 2017

Introduction

The effects of carbon dioxide (CO₂) 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 CO₂ in the atmosphere and the commensurate increase of CO₂ concentrations in aquatic environments (often measured as the partial pressure of CO₂ (*p*CO₂; Solomon *et al.* 2007). Marine systems have been particularly responsive to concerns over elevated *p*CO₂, given the nature of ocean acidification; elevations in atmospheric CO₂ translate into an increase in the *p*CO₂ 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 CO₂ 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 *p*CO₂ 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 *p*CO₂ (Hasler *et al.* 2016a).

In North American fresh waters, *p*CO₂ is demonstrating to be dynamic and variable. Cole *et al.* (1994) found that the majority of sampled US lakes are supersaturated with *p*CO₂, whereas Butman and Raymond (2011) showed that many streams across the US are supersaturated with *p*CO₂. Furthermore, Baumann *et al.* (2015) reported CO₂ dynamics in brackish systems and found additional evidence for high variability in *p*CO₂; e.g. daily summer values ranged from 500 to 4000 µatm. Concentrations of *p*CO₂ 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 CO₂ is the high variability of *p*CO₂ in fresh waters. This high variability is largely absent from CO₂ 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 CO₂ (Noatch and Suski 2012) represent another potential source of significant (but localised) CO₂ 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 pCO₂ 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) CO₂ environments, and found that predation rates increased with elevated pCO₂, 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) pCO₂ 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 pCO₂; predators showed reduced capture success and prey showed longer reaction distances. Thus, exposure to elevated pCO₂ 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 CO₂ exposure would affect predator–prey dynamics for freshwater fishes, as well as the interaction of CO₂ 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 pCO₂. *Micropterus salmoides* was used as a predator species and *Pimephales promelas* was used as a prey species. Understanding the influence of CO₂ on predator–prey dynamics in fresh water will help understand how species and communities exposed to increasing concentrations of CO₂ can expect to respond. We predicted that predation rates, successful consumption, time to consumption, and failed strikes would be affected by increasing pCO₂. Particularly, we expected that time to consumption and failed strikes would increase with pCO₂, 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 pCO₂ (<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 pCO₂ during holding (4-day acclimation) and pCO₂ 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 pCO₂ and exposed to higher pCO₂ only during the experimental phase. Each factor had the following three levels with a target concentration: ambient pCO₂ (0–1000 µatm), low pCO₂ (4000–5000 µatm) and high pCO₂ (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 pCO₂ tolerance. Previous work has shown that extended holding at pCO₂ > 10 000 µatm can have strong negative effects on fish, such that they lose equilibrium (Kates et al. 2012). Experimental pCO₂ values were maintained using the common method of bubbling CO₂ gas into the water through an airstone, using water pH to maintain a target pCO₂ 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 CO₂ probe was used to monitor pCO₂ (GMT221, 0–20%, Vaisala, Vantaa, Finland; Johnson et al. 2010). Temperature and CO₂ measurements for all trials (n = 96) are reported in Table 1. The pCO₂ monitoring was accurate to ± (1.5% of range + 2% of reading) (Vaisala), and probe values were also compared with water with known concentrations of pCO₂ 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 ($p\text{CO}_2$) for each experimental trial
 $p\text{CO}_2$ values are accurate to $\pm 2\%$ 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 $p\text{CO}_2$ 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 $p\text{CO}_2$. The first model was selected to answer the question of whether CO₂ 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 $p\text{CO}_2$, experimental $p\text{CO}_2$, and their interaction, to predict the probability of whether or not *P. promelas* was consumed.

The second question we sought to answer was whether CO₂ 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 $p\text{CO}_2$, experimental $p\text{CO}_2$, 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

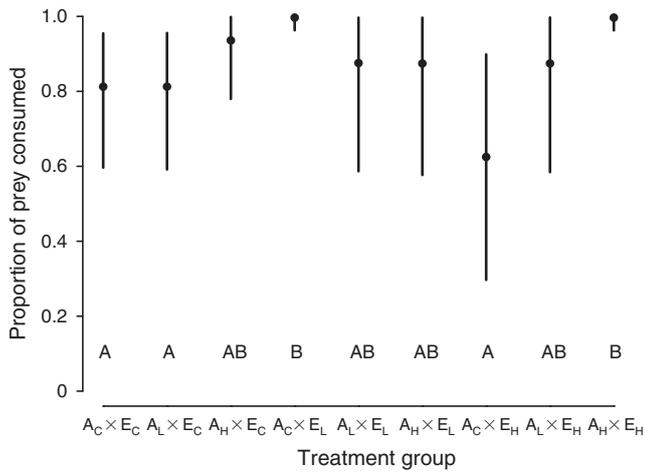


Fig. 1. Estimates of interaction coefficients for an interactions-only logistic regression model. The x-axis lists the interaction combinations, with letters A and E indicating *Acclimation* (4-day holding) and *Experimentation* (20-min trial) treatments respectively, and subscripts C, L, and H indicating CO₂ 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.

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 \times 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 \times experimental low pCO_2 and acclimation high \times 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).

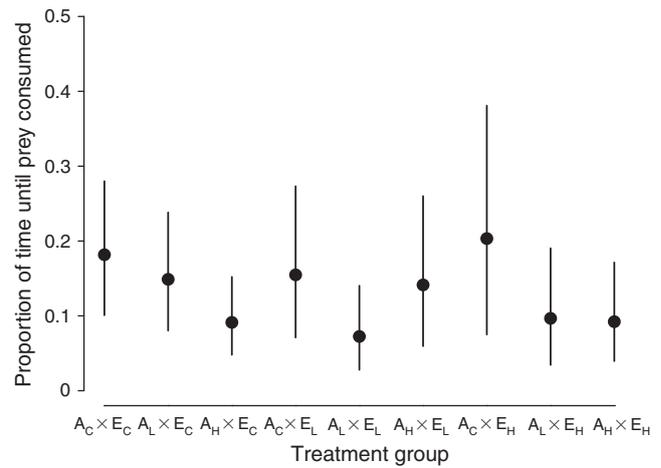


Fig. 2. Estimates of interaction coefficients for an interactions-only beta regression model. The x-axis lists the interaction combinations, with letters A and E indicating *Acclimation* and *Experimentation* treatments respectively, and subscripts C, L and H indicating CO₂ treatment levels control, low and high respectively. No significant differences among groups were found at the 95% credible level.

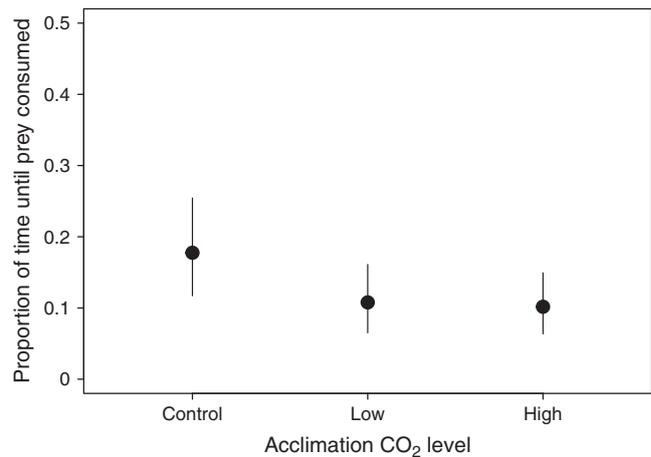


Fig. 3. Coefficient estimates from a one-way beta regression using acclimation CO₂ concentration to predict time until prey consumed. Time until consumption decreased with an increasing CO₂ concentration, although all factor levels overlapped at the 95% credible level.

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.

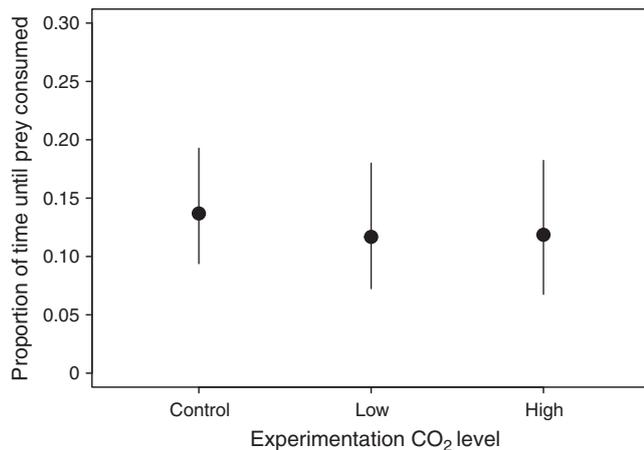


Fig. 4. Coefficient estimates from a one-way beta regression using experimental CO₂ concentration to predict time until prey consumed. Time until consumption decreased with an increasing CO₂ concentration, although all factor levels overlapped at the 95% credible level.

Discussion

Comparison to marine studies

Acclimation to elevated $p\text{CO}_2$ 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 $p\text{CO}_2$ (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 $p\text{CO}_2$ (441–1064 μatm , which is ~ 1.1 – 2.5 times ambient $p\text{CO}_2$; 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 $p\text{CO}_2$ (880 μatm , or $2.0\times$ ambient $p\text{CO}_2$) 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 $p\text{CO}_2$ (600–950 μatm , or 1.5 – $2.4\times$ ambient $p\text{CO}_2$) for 4–7 days. In addition to studies on *P. fuscus*, effects of CO₂ 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 $p\text{CO}_2$ (Green and Jutfelt 2014; Dixson *et al.* 2015; Pistevoš *et al.* 2015). Of particular note is that $p\text{CO}_2$ 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 $p\text{CO}_2$ 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 $p\text{CO}_2$ negatively affects prey detection of predators, leading to reductions in feeding rates (Cripps *et al.* 2011). More specifically, as a result of CO₂-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 $p\text{CO}_2$ 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 $p\text{CO}_2$, 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 $p\text{CO}_2$.

Similarly to feeding after extended exposure to elevated $p\text{CO}_2$, feeding behaviours of *M. salmoides* were not affected by short-term holding at elevated $p\text{CO}_2$. Fish acclimated to ambient $p\text{CO}_2$ and then placed in either low or high $p\text{CO}_2$ 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 $p\text{CO}_2$ is important to understand because freshwater $p\text{CO}_2$ 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, $p\text{CO}_2$ can rise in freshwater lakes within a day (100-fold change in some days; Maberly 1996). In addition, zones of elevated $p\text{CO}_2$ 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 $p\text{CO}_2$ independent of insidious stressors such as climate change. Should short-term increases in $p\text{CO}_2$ be high enough (e.g. $>45\,000\ \mu\text{atm}$), fish may lose equilibrium, because CO₂ 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 $p\text{CO}_2$ ($\sim 50\,000\ \mu\text{atm}$; Ishimatsu *et al.* 2004). However, in natural environments, where a gradient in CO₂ concentrations exist, fish have the capacity to sense elevated $p\text{CO}_2$ (Perry and Gilmour 2002) and will avoid lethal concentrations (Kates *et al.* 2012). Despite fish having the ability to avoid elevated $p\text{CO}_2$, short-term physiological consequences are possible, because physiological changes in response to CO₂ 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 $p\text{CO}_2$ 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 CO₂ do not affect consumption rates or time to consumption.

There are several potential mechanisms to explain why exposure to elevated *p*CO₂ did not alter the feeding behaviours of *M. salmoides*. First, and perhaps foremost, the range of *p*CO₂ values that freshwater fish have been exposed to over evolutionary history is likely to be greater than that of marine fishes. Specifically, *p*CO₂ 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 *p*CO₂ (~400 µatm with very little variability in most marine habitats) and are expected to reach 1000 µatm by 2100 (Solomon *et al.* 2007); however, CO₂ ‘hotspots’ where *p*CO₂ 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, *p*CO₂ levels in coral reefs, where several studies have observed changes to predator–prey dynamics, are typically stable and close to the mean ocean *p*CO₂ (Munday *et al.* 2014). For this reason, freshwater fish may be adapted to a wider range of *p*CO₂ values, as well as higher absolute *p*CO₂, and thus may have the ability to maintain typical feeding behaviours following holding at an elevated *p*CO₂. For example, coho salmon (*Oncorhynchus kisutch*), a species that can transition between marine and freshwater habitats, has also shown to be resistant to increased *p*CO₂, because they have similar somatic growth rates when exposed to a range of *p*CO₂ 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 *p*CO₂. 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 CO₂-exposed fish are returned to ambient conditions. This pathway and feeding in freshwater fish exposed to elevated *p*CO₂ have not been investigated and should be considered for future research, specifically in the context of rising aquatic *p*CO₂, 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 *p*CO₂ during their evolutionary history and, potentially, because they have a more robust physiological response to high concentrations of CO₂.

Prey item and other considerations

Although the focus of the current study was on the response of a predatory freshwater fish to CO₂ exposure, our data also allowed us to make inferences related to the effect of CO₂ on the prey fish in our study. The topic of how prey fishes respond following CO₂ 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 *p*CO₂ (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 *p*CO₂, 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 CO₂, it would be expected that *M. salmoides* acclimated to ambient conditions (i.e. no change in *p*CO₂) would have had higher consumption rates in the two treatments where *p*CO₂ was elevated, which was not observed. Future studies should monitor behaviour and activity of prey fish to gain a clearer picture of how elevated *p*CO₂ 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 CO₂ plays in the ecology of freshwater systems. Similar to the marine environment, freshwater CO₂ 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 *p*CO₂ 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 CO₂ in fresh water, *p*CO₂ 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 CO₂ barriers (Noatch and Suski 2012). Together, in many freshwater systems, biota will likely be exposed to higher and variable CO₂ concentrations. Data from the current study would suggest that there is minimal effect of elevated *p*CO₂ on the feeding behaviour of *M. salmoides*.

Acknowledgements

This work was supported by the United States Geological Survey, through funds provided by the United States Environmental Protection Agency’s Great Lakes Restoration Initiative. We thank Cody Sullivan for technical support and Jen Jeffrey for helpful comments. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

References

- Allan, B. J. M., Domenici, P., McCormick, M. I., Watson, S.-A., and Munday, P. L. (2013). Elevated CO₂ affects predator–prey interactions through altered performance. *PLoS One* **8**(3), e58520–e58527. doi:10.1371/JOURNAL.PONE.0058520
- Baumann, H., Wallace, R. B., Tagliapietra, T., and Gobler, C. J. (2015). Large natural pH, CO₂ and O₂ fluctuations in a temperate tidal salt marsh on diel, seasonal, and interannual time scales. *Estuaries and Coasts* **38**, 220–231. doi:10.1007/S12237-014-9800-Y
- Bignami, S., Sponaugle, S., and Cowen, R. K. (2013). Response to ocean acidification in larvae of a large tropical marine fish, *Rachycentron canadum*. *Global Change Biology* **19**, 996–1006. doi:10.1111/GCB.12133
- Butman, D., and Raymond, P. A. (2011). Significant efflux of carbon dioxide from streams and rivers in the United States. *Nature Geoscience* **4**(10), 1–4.

- Caldeira, K., and Wickett, M. E. (2003). Oceanography: anthropogenic carbon and ocean pH. *Nature* **425**, 365. doi:10.1038/425365A
- Chivers, D. P., McCormick, M. I., Nilsson, G. E., Munday, P. L., Watson, S.-A., Meekan, M. G., Mitchell, M. D., Corkill, K. C., and Ferrari, M. C. O. (2014). Impaired learning of predators and lower prey survival under elevated CO₂: a consequence of neurotransmitter interference. *Global Change Biology* **20**, 515–522. doi:10.1111/GCB.12291
- Claireaux, G., and Lefrançois, C. (2007). Linking environmental variability and fish performance: integration through the concept of scope for activity. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **362**, 2031–2041. doi:10.1098/RSTB.2007.2099
- Clements, J. C., and Hunt, H. L. (2015). Marine animal behaviour in a high CO₂ ocean. *Marine Ecology Progress Series* **536**, 259–279. doi:10.3354/MEPS11426
- Clements, K. D., and Raubenheimer, D. (2006). Feeding and nutrition. In 'The Physiology of Fishes'. (Eds D. H. Evans, and J. B. Claiborne.) pp. 47–82. (CRC Press: Boca Raton, FL.)
- Cole, J. J., Caraco, N. F., Kling, G. W., and Kratz, T. K. (1994). Carbon-dioxide supersaturation in the surface waters of lakes. *Science* **265**, 1568–1570. doi:10.1126/SCIENCE.265.5178.1568
- Cripps, I. L., Munday, P. L., and McCormick, M. I. (2011). Ocean acidification affects prey detection by a predatory reef fish. *PLoS One* **6**, e22736–e22737. doi:10.1371/JOURNAL.PONE.0022736
- Dixon, D. L., Munday, P. L., and Jones, G. P. (2010). Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. *Ecology Letters* **13**, 68–75. doi:10.1111/J.1461-0248.2009.01400.X
- Dixon, D. L., Jennings, A. R., Atema, J., and Munday, P. L. (2015). Odor tracking in sharks is reduced under future ocean acidification conditions. *Global Change Biology* **21**, 1454–1462. doi:10.1111/GCB.12678
- Fabry, V. J., Seibel, B. A., Feely, R. A., and Orr, J. C. (2008). Impacts of ocean acidification on marine fauna and ecosystem processes. *ICES Journal of Marine Science* **65**, 414–432. doi:10.1093/ICESJMS/FSN048
- Ferrari, S., and Cribari-Neto, F. (2004). Beta regression for modelling rates and proportions. *Journal of Applied Statistics* **31**, 799–815. doi:10.1080/0266476042000214501
- Ferrari, M. C. O., McCormick, M. I., Munday, P. L., Meekan, M. G., Dixon, D. L., Lonnstedt, Ö., and Chivers, D. P. (2011). Putting prey and predator into the CO₂ equation: qualitative and quantitative effects of ocean acidification on predator–prey interactions. *Ecology Letters* **14**, 1143–1148. doi:10.1111/J.1461-0248.2011.01683.X
- Ferrari, M. C. O., McCormick, M. I., Munday, P. L., Meekan, M. G., Dixon, D. L., Lonnstedt, Ö., and Chivers, D. P. (2012). Effects of ocean acidification on visual risk assessment in coral reef fishes. *Functional Ecology* **26**, 553–558. doi:10.1111/J.1365-2435.2011.01951.X
- Ferrari, M. C. O., Munday, P. L., Rummer, J. L., McCormick, M. I., Corkill, K., Watson, S.-A., Allan, B. J. M., Meekan, M. G., and Chivers, D. P. (2015). Interactive effects of ocean acidification and rising sea temperatures alter predation rate and predator selectivity in reef fish communities. *Global Change Biology* **21**, 1848–1855. doi:10.1111/GCB.12818
- Goldstein, R. M. (1993). Size selection of prey by young largemouth bass. *Proceedings of the Southeastern Association of Fish and Wildlife Agencies* **47**, 596–604.
- Green, L., and Jutfelt, F. (2014). Elevated carbon dioxide alters the plasma composition and behaviour of a shark. *Biology Letters* **10**, 20140538. doi:10.1098/RSTB.2014.0538
- Hambright, K. D. (1991). Experimental-analysis of prey selection by large-mouth bass: role of predator mouth width and prey body depth. *Transactions of the American Fisheries Society* **120**, 500–508. doi:10.1577/1548-8659(1991)120<0500:EAOPSB>2.3.CO;2
- Hamilton, T. J., Holcombe, A., and Tresguerres, M. (2013). CO₂-induced ocean acidification increases anxiety in rockfish via alteration of GABAA receptor functioning. *Proceedings. Biological Sciences* **281**, 20132509. doi:10.1098/RSPB.2013.2509
- Hasler, C. T., Butman, D., Jeffrey, J. D., and Suski, C. D. (2016a). Freshwater biota and rising pCO₂? *Ecology Letters* **19**, 98–108. doi:10.1111/ELE.12549
- Hasler, C. T., Midway, S. R., Jeffrey, J. D., Tix, J., Sullivan, C., and Suski, C. D. (2016b). Exposure to elevated pCO₂ alters diel movement patterns of largemouth bass over short time scales. *Freshwater Biology* **61**, 1590–1600. doi:10.1111/FWB.12805
- Heuer, R. M., and Grosell, M. (2014). Physiological impacts of elevated carbon dioxide and ocean acidification on fish. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **307**, R1061–R1084. doi:10.1152/AJPREGU.00064.2014
- Ishimatsu, A., Kikkawa, T., Hayashi, M., Lee, K. S., and Kita, J. (2004). Effects of CO₂ on marine fish: larvae and adults. *Journal of Oceanography* **60**, 731–741. doi:10.1007/S10872-004-5765-Y
- Iwama, G. K., McGeer, J. C., and Pawluk, M. P. (1989). The effects of five fish anaesthetics on acid–base balance, hematocrit, blood gases, cortisol, and adrenaline in rainbow trout. *Canadian Journal of Zoology* **67**, 2065–2073. doi:10.1139/Z89-294
- Johnson, M. S., Billett, M. F., Dinsmore, K. J., Wallin, M., Dyson, K. E., and Jassal, R. S. (2010). Direct and continuous measurement of dissolved carbon dioxide in freshwater aquatic systems: method and applications. *Ecohydrology* **3**, 68–78.
- Jutfelt, F., and Hedgärde, M. (2013). Atlantic cod actively avoid CO₂ and predator odour, even after long-term CO₂ exposure. *Frontiers in Zoology* **10**, 81. doi:10.1186/1742-9994-10-81
- Jutfelt, F., and Hedgärde, M. (2015). Juvenile Atlantic cod behavior appears robust to near-future CO₂ levels. *Frontiers in Zoology* **12**, 11. doi:10.1186/S12983-015-0104-2
- Kates, D., Dennis, C., Noatch, M. R., Suski, C. D., and MacLachy, D. L. (2012). Responses of native and invasive fishes to carbon dioxide: potential for a nonphysical barrier to fish dispersal. *Canadian Journal of Fisheries and Aquatic Sciences* **69**, 1748–1759. doi:10.1139/F2012-102
- Kitchell, J. F., Eby, L. A., He, X., Schindler, D. E., and Wright, R. A. (1994). Predator–prey dynamics in an ecosystem context. *Journal of Fish Biology* **45**, 209–226. doi:10.1111/J.1095-8649.1994.TB01094.X
- Leduc, A. O. H. C., Ferrari, M. C. O., Kelly, J. M., and Brown, G. E. (2004). Learning to recognize novel predators under weakly acidic conditions: the effects of reduced pH on acquired predator recognition by juvenile rainbow trout. *Chemoecology* **14**, 107–112. doi:10.1007/S00049-003-0268-7
- Lima, S. L. (1998). Nonlethal effects in the ecology of predator–prey interactions. *Bioscience* **48**, 25–34. doi:10.2307/1313225
- Maberly, S. C. (1996). Diel, episodic and seasonal changes in pH and concentrations of inorganic carbon in a productive lake. *Freshwater Biology* **35**, 579–598. doi:10.1111/J.1365-2427.1996.TB01770.X
- Marking, L. L., and Meyer, F. P. (1985). Are better anesthetics needed in fisheries? *Fisheries* **10**(6), 2–5. doi:10.1577/1548-8446(1985)010<0002:ABANIF>2.0.CO;2
- McNeil, B. I., and Sasse, T. P. (2016). Future ocean hypercapnia driven by anthropogenic amplification of the natural CO₂ cycle. *Nature* **529**, 383–386. doi:10.1038/NATURE16156
- Munday, P. L., Jones, G. P., Pratchett, M. S., and Williams, A. J. (2008). Climate change and the future for coral reef fishes. *Fish and Fisheries* **9**, 261–285. doi:10.1111/J.1467-2979.2008.00281.X
- Munday, P. L., Dixon, D. L., McCormick, M. I., Meekan, M., Ferrari, M. C. O., and Chivers, D. P. (2010). Replenishment of fish populations is threatened by ocean acidification. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 12930–12934. doi:10.1073/PNAS.1004519107
- Munday, P. L., Cheal, A. J., Dixon, D. L., Rummer, J. L., and Fabricius, K. E. (2014). Behavioural impairment in reef fishes caused by ocean acidification at CO₂ seeps. *Nature Climate Change* **4**, 487–492. doi:10.1038/NCLIMATE2195

- Näslund, J., Lindström, E., Lai, F., and Jutfelt, F. (2015). Behavioural responses to simulated bird attacks in marine three-spined sticklebacks after exposure to high CO₂ levels. *Marine and Freshwater Research* **66**, 877–885. doi:10.1071/MF14144
- Nilsson, G. E., Dixon, D. L., Domenici, P., McCormick, M. I., Sørensen, C., Watson, S.-A., and Munday, P. L. (2012). Near-future carbon dioxide levels alter fish behaviour by interfering with neurotransmitter function. *Nature Climate Change* **2**, 201–204. doi:10.1038/NCLIMATE1352
- Noatch, M. R., and Suski, C. D. (2012). Non-physical barriers to deter fish movements. *Environmental Reviews* **20**, 71–82. doi:10.1139/A2012-001
- Ou, M., Hamilton, T. J., Eom, J., Lyall, E. M., Gallup, J., Jiang, A., Lee, J., Close, D. A., Yun, S.-S., and Brauner, C. J. (2015). Responses of pink salmon to CO₂-induced aquatic acidification. *Nature Climate Change* **5**, 950–955. doi:10.1038/NCLIMATE2694
- Perry, S. F., and Gilmour, K. M. (2002). Sensing and transfer of respiratory gases at the fish gill. *The Journal of Experimental Zoology* **293**, 249–263. doi:10.1002/JEZ.10129
- Phillips, J. C., McKinley, G. A., Bennington, V., Bootsma, H. A., Pilcher, D. J., Sterner, R. W., and Urban, N. R. (2015). The potential for CO₂-induced acidification in freshwater: a great lakes case study. *Oceanography* **28**, 136–145. doi:10.5670/OCEANOGRAPHY.2015.37
- Pilcher, D. J., McKinley, G. A., Bootsma, H. A., and Bennington, V. (2015). Physical and biogeochemical mechanisms of internal carbon cycling in Lake Michigan. *Journal of Geophysical Research: Oceans* **120**, 2112–2128.
- Pistevos, J. C., Nagelkerken, I., Rossi, T., Olmos, M., and Connell, S. D. (2015). Ocean acidification and global warming impair shark hunting behaviour and growth. *Scientific Reports* **5**, 16293. doi:10.1038/SREP16293
- Rahel, F. J., and Nutzman, J. W. (1994). Foraging in a lethal environment: fish predation in hypoxic waters of a stratified lake. *Ecology* **75**, 1246. doi:10.2307/1937450
- Regan, M. D., Turko, A. J., Heras, J., Andersen, M. K., Lefevre, S., Wang, T., Bayley, M., Brauner, C. J., Huong, d.T.T., Phuong, N. T., and Nilsson, G. E. (2016). Ambient CO₂, fish behaviour and altered GABAergic neurotransmission: exploring the mechanism of CO₂-altered behaviour by taking a hypercapnia dweller down to low CO₂ levels. *The Journal of Experimental Biology* **219**, 109–118. doi:10.1242/JEB.131375
- Solomon, S., Qin, D., and Manning, M. (2007). 'Climate Change 2007: the Physical Science Basis: Working Group I Contribution to the Fourth Assessment Report of the IPCC (Vol. 4).' (Cambridge University Press: New York, NY, USA.)
- Sundin, J., and Jutfelt, F. (2016). 9–28 d of exposure to elevated pCO₂ reduces avoidance of predator odour but had no effect on behavioural lateralization or swimming activity in a temperate wrasse (*Ctenolabrus Rupestrus*). *ICES Journal of Marine Science* **73**, 620–632. doi:10.1093/ICESJMS/FSV101