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RESEARCH ARTICLE

Valve movement of three species of North American freshwater mussels exposed to elevated carbon dioxide

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Abstract Freshwater mussels are at-risk taxa and may be exposed to high levels of carbon dioxide (CO_2) because of the potential use of CO_2 to control the movement of invasive aquatic fish species. One potential behavioral response to a change in the partial pressure of CO_2 (pCO_2) may be altered valve movement. In this study, three species of mussels were fitted with modified sensors and exposed to two regimes of pCO_2 to define thresholds of impaired valve movement. The first experiment demonstrated that *Pyganodon grandis* were much more tolerant to rising pCO_2 relative to *Lampsilis siliquoidea* (acute closure at ~200,000 μatm in comparison to ~80,000 μatm). The second experiment consisted of monitoring mussels for 6 days and exposing them to elevated pCO_2 (~70,000 μatm) over a 2-day period. During exposure to high pCO_2, *Lampsilis cardium* were open for nearly the entire high pCO_2 period. Conversely, *P. grandis* were closed for most of the period following exposure to high pCO_2. For *L. siliquoidea*, the number of closures decreased nearly 40-fold during high pCO_2. The valve movement responses observed suggest species differences, and exposure to elevated pCO_2 requires a reactive response.

Keywords Unionidae · Acidification · Conservation physiology

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Introduction

Mussels provide many vital ecosystem services and are integral components of freshwater food webs (Vaughn et al. 2008). Globally, freshwater mussel species are among the most at-risk taxa on the planet (Ricciardi and Rasmussen 1999) with 71% of freshwater mussels either extinct or under threat (Williams et al. 1993). Habitat degradation, invasive species, overexploitation, and climate change are among the main reasons for declines of mussel populations (e.g., Strayer and Dudgeon 2010; Strayer et al. 2004; Williams et al. 1993); therefore, monitoring the responses of mussels to environmental stressors is important for predicting population outcomes and to inform conservation practices.

Carbon dioxide (CO_2) is a naturally occurring component of freshwater ecosystems, but one that also has the potential to be a stressor that can negatively impact freshwater organisms (Hasler et al. 2016). Many rivers (i.e., unionid mussel habitat) are currently supersaturated with CO_2 (Butman and Raymond 2011), can reach partial pressures upwards of 35,000 μatm, and often have wide variation in CO_2 across daily and seasonal time scales (Crawford et al. 2016). To date, it remains unclear how the partial pressure of CO_2 (pCO_2) will change in freshwater in the future (Hasler et al. 2016). However, as a result of climate change, pCO_2 in the Great Lakes may increase concomitantly with the atmosphere (Phillips et al. 2015), which would expose freshwater biota to relatively marginal increases in pCO_2. Perhaps, the most impending action that could change pCO_2 in freshwater is the proposal to use zones of elevated pCO_2 (e.g., 50–100 times current levels) to deter the movement of invasive fishes (Noatch and Suski 2012; Donaldson et al. 2016; Cupp et al. 2017). More importantly, elevated pCO_2 similar to what might be used to deter fish movement in freshwater has been shown to induce ionic, molecular, and metabolic responses for mussels, inducing

several physiological costs (Hannan et al. 2016a, 2016b, 2016c; Jeffrey et al. 2016). Taken together, the increased likelihood of mussels experiencing elevated pCO_2 in the future makes it imperative that we quantify signs of disturbance to understand the impacts of potential environmental stressors on these threatened taxa.

Valve movement, the opening and closing of the two valves of a mussel, is a rhythmic behavior that may be one of the several behaviors or physiological processes altered by exposure to elevated pCO_2. Valve movement is completed through contraction of the adductor muscles, and this cyclical opening and closing of the valves has been shown to be essential for feeding (Robson et al. 2009; Robson et al. 2010), respiration (Schick et al. 1986), and metabolism (Schick et al. 1988). In addition, valve closure is an important anti-predator behavior, but extended valve closure can induce energetic costs by limiting oxygen uptake, reducing feeding rates, and impairing acid-base regulation (Robson et al. 2007; Robson et al. 2010). In past studies, mussels have been found to alter valve movements (i.e., open or closed more frequently) in response to challenges such as exposure to conspecific cues present in water (e.g., masticated conspecifics; Robson et al. 2007), various substances, including serotonin applied to the cerebral ganglia (Salánki 1963), toxic environments (e.g., maganese and uranium, Markich et al. 2000; noxious dinoflagellates, Nagai et al. 2006), and elevated water temperatures (Dowd and Somero 2013). At present, the pCO_2 levels that induce changes to valve movement, and how mussels alter valve movement in response to exposure to elevated pCO_2, are not known; however, some evidence suggest that high pCO_2 can be used as an anesthetic in molluscs (Salánki 1963; Cooper 2011). Defining both the levels of ambient pCO_2 that result in closure, as well as the patterns of closure, may be critical for defining thresholds for mussel valve movement. Understanding how mussel behavior responds to high pCO_2 may define the behavioral and physiological consequences of exposure, as well as how mussel communities may change in environments that undergo a rise in pCO_2. Species differences with respect to valve movement and tolerance to rising pCO_2 may also be possible given the range of pCO_2 different species may have experience in the wild over both recent and evolutionary time.

Thus, the objectives of the present study were to (1) define an upper level of pCO_2 that mussels would abruptly respond to by observing when changes in valve movement occurred during an exposure to incrementally rising pCO_2 and (2) quantify valve movement of three freshwater mussel species in response to a 48-h exposure to elevated pCO_2, as well as during a 48-h period before and after the exposure.

Materials and methods

Mussel collection and husbandry

Fatmucket (*Lampsilis siliquoidea* [Barnes, 1823], hereinafter "FM") were reared from the glochidial stage and delivered overnight from an aquaculture research facility at Missouri State University, Springfield, MO, USA, to the Aquatic Research Facility, Urbana, IL, in June 2015. Giant floater (*Pyganodon grandis* [Say, 1829], hereinafter "GF") and plain pocketbook (*Lampsilis cardium* [Rafinesque, 1820], hereinafter "PB") were collected by benthic grab from a barrow pit near Champaign, IL, USA, and the Mississippi River near Cordova, IL, USA, respectively, between July and August 2015, and transported to the Aquatic Research Facility at the University of Illinois, Urbana-Champaign, IL, USA, in coolers. Following transport, all three species were placed into separate 128.7 L recirculating tank systems supplied with 64.4 L of fresh water that was drawn from a 0.04 ha naturalized, earthen-bottom pond. The recirculating systems consisted of three plastic holding containers (one of which was only used to treat water and house the recirculating pump), a UV sterilizer (TMC Vecton 8 W, 11 L min^{-1} flow rate, Pentair, Apopka, FL, USA), an aquarium heater/chiller (Teco 500, TECO-US, Aquarium Specialty, Columbia, SC, USA), and a low-pressure air blower (SL24H, Sweetwater/Pentair, Apopka, FL, USA). Each holding tank also had 5 cm of sand (Old Castle all-purpose sand, Atlanta GA) as mussel habitat. Mussels were provided a commercial shellfish diet (a mix of algae species ranging in particle sizes; ration = 1 L; Instant Algae, Reed Mariculture Inc., Campbell, CA, USA) every other day. Within holding containers, each sensor-equipped mussel was placed in a small, plastic, open container (15.6 cm × 15.6 cm × 6.35 cm, Ziploc, S. C. Johnson & Son, Racine, WI) to prevent movement towards other mussels and tangling of sensors across individuals, but mussels were still exposed to the recirculating water. Experiments commenced 1–3 months after mussels were brought to the facility, and food was withheld during the experiments. Length of time spent in laboratory conditions does not seem to alter species-specific physiological responses to elevated pCO_2 (Hannan et al. 2016a, 2016b).

Valve gape measurements

In the experiments described below, the valve gape of each mussel (i.e., the distance between two valves of the shell) was monitored every 10 s (Robson et al. 2009) using linear Hall effect circuits modified from Wilson et al. (2005). The sensor (transducer) component of the circuit (1.5 mm × 4 mm × 3 mm; 0.1 g; A1324, Allegro Microsystems, Inc., Worcester, MA, USA) was sealed using a plastic sealant and affixed near the siphon (posterior end) of the right valve of each mussel, while

a neodymium iron boron nickel-plated magnet (4 mm diameter; 3 mm height; 0.3 g; M1219-3; Comos Group of Companies, Clifton, NJ, USA) was attached on the other valve directly opposite to the sensor. Both the sensors and magnets were affixed using a cyanoacrylate adhesive. Sensors were connected to a microcontroller board (Arduino Uno; Somerville, MA, USA), which was connected to a computer for data logging and power supply purposes. The sensor, when near a magnetic field, produces a voltage change that decreases non-linearly as the distance between the sensor and a magnet increases. Thus, closure of the valves can be inferred from monitoring the voltage output of the sensor for each mussel. Because voltage output varies based on relative placement of the sensor and magnet, output voltages cannot be grouped across individuals.

Acute incremental exposure

Using FM ($N = 6$) and GF ($N = 5$) mussels, an experiment was carried out to define the CO_2 level when abrupt valve closure occurred and to observe the duration of time that the mussels remained closed (PB mussels were not used due to sensor malfunction). Carbon dioxide was added to the central basin of the recirculating system using the common technique of bubbling compressed CO_2 gas (commercial grade, 99.9% purity) through an air stone (Summerfelt and Sharrer 2004; Clingerman et al. 2007). Bubbling lasted for a 5-min period at a rate of 0.95 m^3 s^{-1} (17,000 L), followed by a 5-min wait period. After this 5-min wait period, additional CO_2 was added, followed by another wait period. This wait period was necessary to allow the pCO_2 level to stabilize and be accurately determined. The process of incrementally adding CO_2 continued until all the mussels in the experiment were closed and pCO_2 increased approximately 14,000 μatm every 5 min. Upon cessation of CO_2 addition, and the subsequent decline in pCO_2 (pCO_2 decreased approximately 5000 μatm every 5 min), the pCO_2 at which mussels opened and the duration of the closure were recorded. The CO_2 level was measured using a modified infrared probe (GMT221, 0–20%, Vaisala, Vantaa, Finland) (Johnson et al. 2009), and both the time of each mussel's closure and re-opening, as well as the duration of the closure, were recorded.

Monitoring of valve movement across differing pCO_2

An impact assessment approach was used to determine if elevated pCO_2 changed valve movement. The monitoring period to define valve movement before, during, and after an exposure to elevated pCO_2 lasted a total of 144 h. For the first 48 h, beginning at noon on the first day of the experiment, animals were held at ambient CO_2 (Table 1). Exposure to elevated CO_2 began at noon on the third day and ended at noon on the fifth day of the experiment. Mussels ($N = 7$ FM, 6 PB, 4 GF) were subsequently monitored for 48 h after the exposure to CO_2 (from noon on day 5 to noon on day 7 of the experiment).

Elevated pCO_2 was achieved by bubbling into the central basin of the recirculating system through an air stone until the target pCO_2 was reached (Table 1). The target pCO_2 used, ~70,000 μatm, in the current study was chosen based on levels known to induce sub-lethal physiological disturbances in freshwater mussels (Hannan et al. 2016c) as well as a level that would not result in an immediate valve movement response. Target pCO_2 was maintained using a pH controller (PINPOINT®, American Marine Inc., Ridgefield, CT, USA) that would automatically add CO_2 to the basin should the pH rise above the target level during the exposure period (Reynaud et al. 2003). Dissolved CO_2 and total alkalinity (TA) levels were measured using digital titration kits (Model CA-23 (dissolved CO_2) and Model AL-AP (TA), Hach Company, Loveland, CO, USA) before, during, and after exposure to CO_2, and pCO_2 was monitored using a modified infrared probe. Other water quality parameters monitored included pH (WTW pH 3310 meter, Cole Palmer, Vernon Hills, IL, USA), dissolved oxygen, and temperature (YSI 550A, Yellow Springs Instruments, Irvine, CA, USA) (Table 1). All measurements were taken every 6 h over the course of the experiment ($n = 8$ for each period).

Data analyses

Sensor data were assessed to determine when each mussel was closed by comparing voltage output values during the experiment to outputs of the mussel during the sensor attachment process when mussels were out of water and closed, and by visually inspecting the data (Fig. 1). Three response variables were measured for each mussel during the 6-day CO_2 exposure experiment; however, only total time spent closed and number of closures were analyzed due to average duration of closure per individual correlating positively with total time spent closed. To compare these two response variables across the different exposure periods (levels: before, during, and after CO_2 exposure) and across species (levels: FM, PB, and GF), generalized linear mixed models were conducted using the 'glmmPQL' function in R (R Development Core Team 2010), which uses a penalized quasi-likelihood to estimate model parameters (Breslow and Clayton 1993; Bolker et al. 2009). Fixed factors in each model included exposure period and species, as well as the interaction between exposure period and species. Mussel ID was included in each model as a random factor to account for repeated measures across the same individual within a treatment. A quasipoisson error distribution was fitted to the count data (i.e., number of closures), while the model for total time spent closed was fitted using quasi error distributions (quasipoisson and quasi error distributions model over-dispersion because the dispersion parameter is not fixed) (Bolker et al. 2009). Visual inspection of

Table 1 Means and standard deviations ($n = 8$, for each period) of water quality parameters measured during the CO_2 exposure experiments

CO_2 exposure period	pCO_2 (μatm)	Temperature (°C)	Dissolved oxygen (mg L^{-1})	Total alkalinity (mEq L^{-1})	pH	Dissolved CO_2 (mg L^{-1})
Fatmucket (July 02–08, 2015)						
Before	<100 (DL)	21.4 ± 0.2	8.6 ± 0.2	4300 ± 107	8.7 ± 0.1	5.7 ± 0.9
During	72,551 ± 17,189	21.4 ± 0.3	7.8 ± 0.3	4885 ± 345	6.6 ± 0.1	136 ± 31
After	<100 (DL)	21.2 ± 0.3	8.5 ± 0.3	5432 ± 85	8.8 ± 0.2	7.7 ± 0.9
Pocketbook and giant floater (October 20–26, 2015)						
Before	<100 (DL)	21.3 ± 0.3	8.2 ± 0.1	4420 ± 98	8.5 ± 0.1	9.8 ± 0.8
During	69,430 ± 14,342	21.4 ± 0.2	7.6 ± 0.2	4640 ± 290	6.6 ± 0.2	124 ± 40
After	<100 (DL)	21.5 ± 0.3	8.2 ± 0.4	5080 ± 112	8.6 ± 0.1	10.2 ± 0.7

DL detectable limit

model residuals was used to assess normality and heteroscedasity, and models were graphically validated following Zuur et al. (2009). Significance of fixed factors, including interactions, was assessed at the 95% significance level. The 'multcomp' package (Torsten et al. 2008) was used to complete Tukey's post hoc analyses to define pairwise differences between factor levels. Pairwise comparisons were only considered significant at the 99% significant level.

For the acute incremental exposure experiment, a similar statistical test as described above was used to compare the

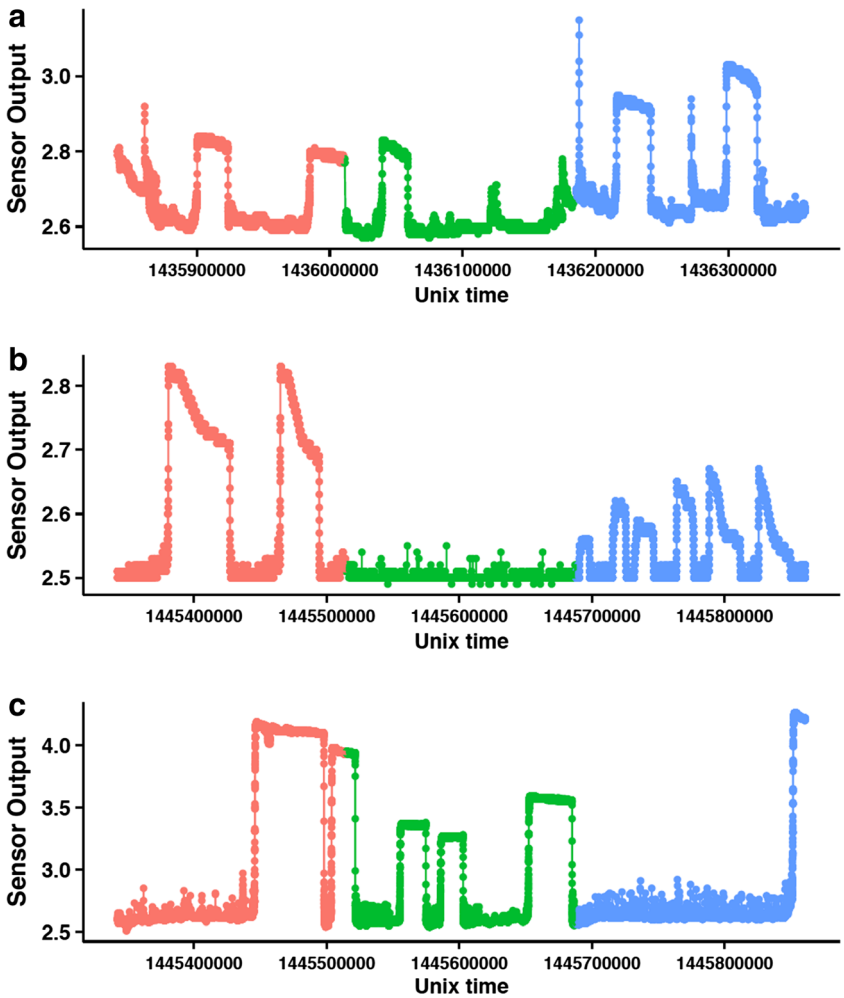

Fig. 1 Examples of the raw sensor output (no unit) before (*red*), during (*green*), and after (*blue*) the CO_2 exposure for a fatmucket, *Lampsilis siliquoidea* (**a**), pocketbook, *Lampsilis cardium* (**b**), and giant floater, *Pyganodon grandis* (**c**). Time is expressed as Unix time, which is the number of seconds elapsed since January 1, 1970. High sensor output values represent values typical of closed mussels and low sensor output values represent values typical of mussels that are open (i.e., magnet is not close to sensor)

pCO_2 at which mussels of the two species (FM and GF) closed and subsequently re-opened. For this model, species and valve action (levels = closed and re-open) were included as fixed effects, mussel ID was included as a random factor, and a quasipoisson error distribution was used. Duration of the closure during the acute experiment was compared across species using a Cox proportional hazard survival analysis using the 'survival' package in R (Therneau 2015) because the response was a time to an event (i.e., valve closure).

Results

The pCO_2 at which mussels closed following incremental increases in pCO_2 and subsequently re-opened following cessation of CO_2 additions differed across species (Table 2). GF mussels closed at pCO_2 more than twice as high as FM mussels (170,263 ± 56,141 μatm vs 81,925 ± 44,160 μatm, respectively) and re-opened at a significantly lower pCO_2 compared to FM mussels (21,210 ± 26,070 μatm vs 88,411 ± 31,661 μatm, respectively; Fig. 2). Within species, FM mussels closed and then re-opened at a similar pCO_2 level, while GF mussels re-opened at a pCO_2 over two-thirds lower than the pCO_2 value at the time of their closure (Fig. 2). The duration of the closure between the times when the mussels initially closed and then re-opened was significantly longer for GF mussels compared to FM mussels (333 ± 329 min vs 109 ± 15 min, respectively; Cox proportional hazard, $z = -1.79$, $df = 1$, $P = 0.07$).

Table 2 Generalized linear mixed effects models (PQL estimation) with coefficient estimates and *P* values. Terms in italics represent significant parameters

Response	Model	Parameter	Value	S.E.	*df*	*t*	*P*
No. of closures	Quasipoisson	*Intercept*	*3.39*	*0.28*	*36*	*12.27*	*<0.001*
		Period—during	*−1.92*	*0.51*	*36*	*−3.75*	*<0.001*
		Period—after	−0.29	0.28	36	−1.04	0.307
		Species—giant floater	*−2.76*	*0.94*	*36*	*−2.94*	*0.006*
		Species—pocketbook	*−2.51*	*0.69*	*36*	*−3.62*	*<0.001*
		During × giant floater	2.21	1.32	36	1.68	0.102
		After × giant floater	−0.52	1.68	36	−0.31	0.759
		During × pocketbook	*2.18*	*1.03*	*36*	*2.12*	*0.041*
		After × pocketbook	0.59	0.92	36	0.64	0.526
Duration of closures	Quasi	Intercept	2339.86	3233.80	36	0.72	0.474
		Period—during	−883.29	4573.28	36	−0.19	0.848
		Period—after	−819.57	4573.28	36	−0.18	0.859
		Species—giant floater	*33,755.64*	*5362.65*	*36*	*6.29*	*<0.001*
		Species—pocketbook	*21,467.64*	*4760.02*	*36*	*4.51*	*<0.001*
		During × giant floater	1120.54	7583.93	36	0.15	0.883
		After × giant floater	*−17,778.43*	*7583.93*	*36*	*−2.34*	*0.025*
		During × pocketbook	*−22,920.88*	*6731.69*	*36*	*−3.40*	*0.002*
		After × pocketbook	−10,954.60	6731.69	36	−1.63	0.112
Total time closed	Quasi	*Intercept*	*22,781.00*	*8206.80*	*36*	*2.78*	*0.009*
		Period—during	−17,533.86	11,435.29	36	−1.54	0.133
		Period—after	8067.57	11,445.41	36	0.70	0.485
		Species—giant floater	*49,724.12*	*13,456.47*	*36*	*3.70*	*<0.001*
		Species—pocketbook	*42,337.45*	*11,914.86*	*36*	*3.55*	*0.001*
		During × giant floater	*48,133.86*	*18,963.29*	*36*	*2.54*	*0.016*
		After × giant floater	*−53,110.07*	*18,963.29*	*36*	*−2.80*	*0.008*
		During × pocketbook	*−47,599.48*	*16,832.29*	*36*	*−2.83*	*0.008*
		After × pocketbook	−26,599.24	16,847.18	36	−1.58	0.123
Acute test, pCO_2 at time closure	Quasipoisson	*Intercept*	*81,925.17*	*16,691.25*	*13*	*4.91*	*<0.001*
		Species—giant floater	*88,338.03*	*24,757.12*	*13*	*3.57*	*0.003*
		Valve action—open	6486.00	23,605.00	13	0.27	0.788
		Giant floater × open	*−155,538.60*	*35,011.85*	*13*	*−4.44*	*<0.001*

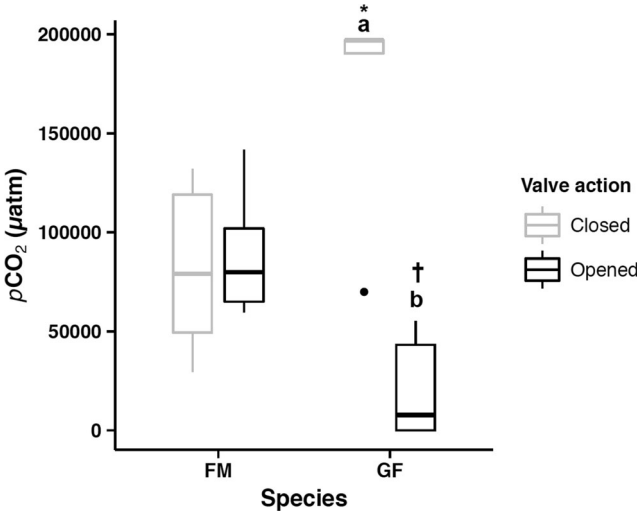

Fig. 2 Carbon dioxide (CO_2) partial pressures (µatm) that fatmucket (FM, $N = 6$) and giant floater (GF, $N = 5$) mussels "closed at" or "opened at" during the acute exposure to incremental rising of CO_2 levels. Valve closure occurred during the step-wise increase in pCO_2 that occurred every 5 min until all mussels were closed. Valve opening occurred after CO_2 addition had ended and the system began to return to starting conditions. Box plots that do not share a *letter* represent within-species differences determined using a generalized linear mixed model. An *asterisk* or *dagger* represents across-species differences within valve actions. *Horizontal bars* in the box plot represent the median response value, and the 75 and 25% quartiles. Whiskers represent ±1.5 times the interquartile range, and outliers are indicated as *dots*

Before, during, and after the 2-day exposure to elevated pCO_2, differences in valve movement, time spent closed and number of closures, were observed both within and across the three species of mussels examined (Table 2). During the elevated pCO_2 exposure, PB mussels were closed for nearly 0% of the time, which was significantly different than the periods before and after exposure (Fig. 3a). Conversely, the duration that GF mussels were closed was not affected until after the period of CO_2 exposure when closure duration decreased to approximately a third of the time spent closed during the CO_2 exposure, which was significant relative to during the CO_2 exposure period, but not compared to the pre-exposure period (Fig. 3a). In addition, CO_2 exposure did not result in a significant change in the duration that FM mussels remained closed (Fig. 3a), but the number of closures decreased over 40-fold during the CO_2 exposure period compared to before the CO_2 exposure period (Fig. 3b). Among the three species of mussels, FM mussels were closed for a third of the duration that PB and GF mussels were closed before the pCO_2 exposure period (Fig. 3a); however, FM had on average 10 times more closures than PB and GF during the same period (Fig. 3b). Finally, PB and GF mussels did not vary the number of closures across treatments (Fig. 3b).

Discussion

All three freshwater mussel species responded to a 48-h exposure to elevated pCO_2 by changing valve movement, likely responding to elevated pCO_2 as a stressor. When mussels are exposed to elevated pCO_2, they experience changes such as an increase in heat shock protein 70 production (Jeffrey et al. 2016), increased Na^{2+} and glucose (Hannan et al. 2016b), and decreased Mg^{2+} (Hannan et al. 2016c) in hemolymph, likely from reduced internal or external pH, which can act as a stressor and cause a departure from homeostasis. Interestingly, however, the different species of mussels appeared to have utilized different strategies to deal with the stress of high pCO_2. More specifically, during the exposure to elevated pCO_2, PB and FM mussels decreased either the time they spent closed or the number of closures, respectively, resulting in longer periods of time spent with their shell open. It is possible that spending more time open during exposure to high pCO_2 may assist with mediating acid/base disturbances, which have been shown to occur in several species of mussels exposed to high levels of CO_2 (Hannan et al. 2016a, 2016b, 2016c). Furthermore, should mussels remain closed during exposure to high pCO_2, mussels may be forced to rely on anaerobic metabolism and incur additional energetic costs (Byrne et al. 1990); thus, remaining open may be a behavioral adaptation to prevent the accumulation of harmful end products of anaerobic respiration. In addition to potential respiratory acidosis, the tendency for PB and FM mussels to remain open during pCO_2 exposure suggests that the increase in pCO_2 caused the posterior adductor muscle to relax, which could indicate anesthesia (Salánki 1963). PB and FM also showed similar valve movement after the elevated pCO_2 exposure period ended. In fact, based on the individual measurements of sensor output, valve movement observed before the elevated pCO_2 exposure period returned almost immediately once the stressor was removed. Similarly, when Dowd and Somero (2013) exposed *Mytilus* congeners to elevated temperature, a pattern that was prevalent during recovery periods was a tendency for valve movement to quickly return to control levels, even after subsequent bouts of exposure to high temperatures. Return to normal valve movement activity after exposure to a stressor is likely important for maintaining homeostasis (Romero et al. 2009) and may have ecological consequences, as prolonged periods of being open can lead to increased predation (Norton-Griffiths 1967; Robson et al. 2010).

Unlike FM and PB mussels, GF mussels utilize a different strategy during exposure to elevated pCO_2. During elevated pCO_2 exposure, GF mussels remained closed for a longer duration compared to FM and PB mussels. Furthermore, during the acute experiment, GF mussels did not close until CO_2 levels reached nearly 200,000 µatm suggesting that they may be more tolerant to acute exposure to elevated pCO_2 compared to FM,

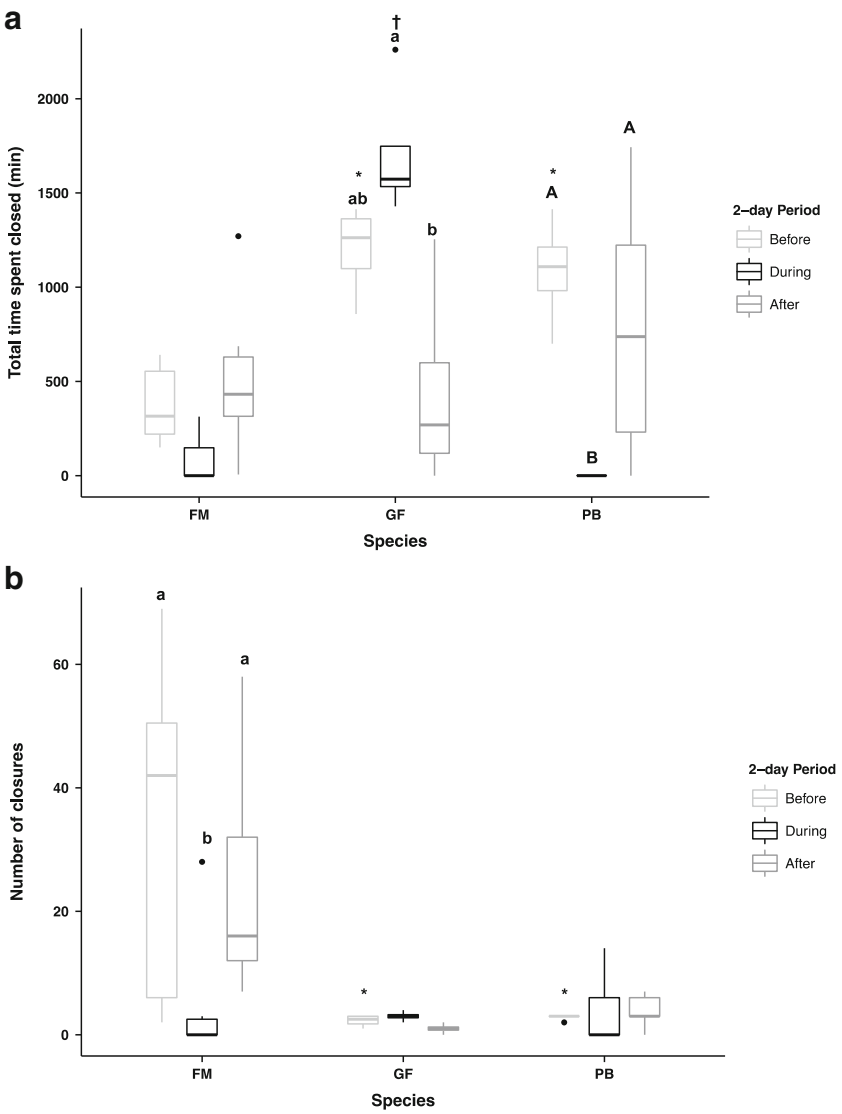

Fig. 3 Total time spent closed (min; **a**) and number of closures (**b**) before, during, and after the 48 h of elevated CO_2 exposure for fatmucket (FM, $N = 7$), giant floater (GF, $N = 4$), and pocketbook (PB, $N = 6$). Box plots that do not share a *letter* represent within-species differences and box plots that do not share an *asterisk* or *dagger* represent across-species differences within periods of exposure determined using a generalized linear mixed model. *Horizontal bars* in the box plot represent the median response value, and the 75 and 25% quartiles. Whiskers represent ±1.5 times the interquartile range, and outliers are indicated as *dots*

which closed at approximately 80,000 μatm. This greater tolerance to an acute elevation in pCO_2 somewhat contradicts the finding that GF remained closed longer during elevated pCO_2 exposure when compared to the other species. However, it may suggest that GF responds to elevated pCO_2 based on exposure time following a threshold value of pCO_2 less than 200,000 μatm, rather than absolute pCO_2. Being closed longer during exposure, elevated pCO_2 was likely an attempt to limit exposure to the external environment, thus providing protection against the potential for respiratory acidosis induced by high pCO_2 (see above). However, in intertidal mussels, there is a clear trade-off, as remaining closed for an extended duration also increases anaerobic respiration (Zandee et al. 1985), reduces food intake (Robson et al. 2010), and causes a rise in metabolic wastes due to impaired excretion (Nagai et al. 2006). A higher tolerance to elevated pCO_2 may have been an attempt to limit these trade-offs, though because we used freshwater mussels, it may be that these trade-offs are more or less severe. Furthermore, if GF mussels in this study experienced these trade-offs while being closed for extended durations, it is likely that the significant increased time spent open by GF mussels during the recovery period following the elevated pCO_2 exposure allowed mussels to release harmful by-products and to increase feeding (McCorkle et al. 1979; Famme 1980; Schick et al. 1986). Similar responses in valve movement have been observed in freshwater mussels exposed to emersion, where mussels decrease their time spent closed following emersion (Byrne et al. 1990).

Differences in how species responded to changes in pCO_2 were not unexpected, as species differences in response to stressors are known, including elevated pCO_2 and acidification (Widdicombe and Spicer 2008; Kroeker et al. 2010). Specifically for unionid mussels in response to elevated pCO_2, Hannan et al. (2016b) found that FM and threeridge (*Amblema plicata*; [Say 1817]) mussels demonstrated species-specific difference in hemolymph Ca^{2+} and Cl^{-}

following exposure to pCO_2 of 55,000 μatm for 28 days and suggested that shell thickness and access to internal bicarbonate play a role in physiological responses to high pCO_2. PB and FM are closely related phylogenetically (i.e., both *Lampsilis* genus; Campbell et al. 2005), tend to have thick shells, and showed similar responses to elevated pCO_2 (i.e., closed less often or less frequently), while GF mussels have thinner shells (Pennak 1989) and may have a higher tolerance to elevated pCO_2. Taken together, observed species differences in valve movement may have been due to shell thickness and access to internal bicarbonate, which has been suggested by physiological-based studies on the mussels monitored in this study (Hannan et al. 2016a, 2016b). It is also possible that species differences with respect to preferred habitat may be an important factor in why the species differed in their responses to elevated pCO_2; however, because FM were hatchery-reared, it is difficult to link species differences in all three mussels monitored. For the wild mussels used in this study (PB and GF), PB are found in small creeks to large rivers, and GF are found in sluggish waters, lakes, and ponds (Cummings and Mayer 1992). It is possible that these systems would differ widely with respect to pCO_2 with GF habitat potentially having higher pCO_2 for longer periods of the year (Cole et al. 1994; Butman and Raymond 2011; Hasler et al. 2016), and this might suggest that GF would be more tolerant to elevated pCO_2 and why their response differed from PB.

Valve movement is a behavior that is critical to maintain homeostasis in mussels. Modifications in valve movement as a result of external challenges can be an effective measure of demonstrating thresholds for disturbance, as well as potential outcomes. Overall, valve movement was shown to be a useful measurement for understanding the behavioral response of mussels to elevated pCO_2. Mussel species reacted to elevated pCO_2 by altering valve movement, presumably in response to acid-base disturbances as a result of reduced internal or external pH. Furthermore, the three species of mussels demonstrated differences in tolerance to elevated pCO_2 as well as the duration of valve closures and number of closures. The consequences of these species-specific differences in valve movement are not well understood; however, as specific strategies of valve movement during exposure to elevated pCO_2 maximize survival, community-level changes could arise if environments become elevated in pCO_2. Specifically, should CO_2 barriers be used to control the movement of invasive fishes, it is likely that one response by mussels will be to alter valve movement. Future work should aim to understand how physiological stress experienced during exposure to elevated pCO_2 may be modulated by valve movement and to monitor both physiological and behavioral responses to elevated pCO_2 in individuals. Other intrinsic factors such as sex and size of individuals may also yield differences in how individuals respond to elevated pCO_2.

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