Exceptional CO_2 Tolerance in White Sturgeon (*Acipenser transmontanus*) Is Associated with Protection of Maximum Cardiac Performance during Hypercapnia In Situ

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ABSTRACT

White sturgeon rank among the most CO_2-tolerant fish species examined to date. We investigated whether this exceptional CO_2 tolerance extended to the heart, an organ generally viewed as acidosis intolerant. Maximum cardiac output (Q_{max}) and maximum cardiac power output (PO_{max}) were assessed using a working, perfused, in situ heart preparation. Exposure to a P_{CO_2} of 3 kPa for 20 min had no significant effect on maximum cardiac performance, while exposure to 6-kPa P_{CO_2} reduced heart rate, Q_{max}, PO_{max}, and rate of ventricular force generation (F_O) by 23%, 28%, 26%, and 18%, respectively; however, full recovery was observed in all these parameters upon return to control conditions. These modest impairments during exposure to 6-kPa P_{CO_2} were associated with partially compensated intracellular ventricular acidosis. Maximum adrenergic stimulation (500 nmol L^{-1} adrenaline) during 6-kPa P_{CO_2} protected maximum cardiac performance via increased inotropy (force of contraction) without affecting heart rate. Exposure to higher CO_2 levels associated with morbidity in vivo (i.e., 8-kPa P_{CO_2}) induced arrhythmia and a reduction in stroke volume during power assessment. Clearly, white sturgeon hearts are able to increase cardiac performance during severe hypercapnia that is lethal to other fishes. Future work focusing on atypical aspects of sturgeon cardiac function, including the lack of chronotropic response to adrenergic stimulation during hypercapnia, is warranted.

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Introduction

Aquatic hypercarbia (elevated water carbon dioxide partial pressure; P_wCO_2) is a common environmental challenge in ecosystems such as estuaries and seasonal ponds (Heisler et al. 1982; Ultsch 1996) that has adverse effects on fishes (e.g., Graham et al. 1990; Wood et al. 1990; Wood and LeMoigne 1991; Hayashi et al. 2004). For most fishes, short-term exposure to a P_wCO_2 of more than 2 kPa results in uncompensated acidosis (see Brauner and Baker 2009) that can be lethal, although the exact mechanism(s) of CO_2 toxicity are unknown (Putnam and Roos 1997). In contrast, white sturgeon *Acipenser transmontanus* are remarkably tolerant of elevated CO_2 and the associated blood acidosis, exhibiting morbidity only when exposed to ≥8-kPa P_wCO_2 (D. W. Baker and C. J. Brauner, unpublished observations). An exceptional capacity for intracellular pH (pHi) regulation during hypercarbia is thought to contribute to this tolerance (Baker et al. 2009*a*).

The fish heart is particularly sensitive to hypercapnia and the associated acidosis. The routine heart rate (f_H) of rainbow trout *Oncorhynchus mykiss* decreased by 19% within minutes of exposure to 1.2-kPa P_wCO_2 (Perry et al. 1999). Likewise, both routine cardiac output (Q) and f_H in both Atlantic salmon *Salmo salar* and dogfish *Squalus acanthias* decreased (>20%) rapidly during exposure to more severe hypercarbia (4–5-kPa P_wCO_2 Kent and Peirce 1978; Perry and McKendry 2001). In addition, hypercapnia decreases maximum cardiac performance as assessed in working, perfused fish heart preparations. For example, perfused sea raven (*Hemitripterus americanus*) hearts exposed to P_{CO_2} of just 1.8 kPa significantly decreased maximum cardiac output (Q_{max}) and maximum cardiac power output (PO_{max}) as well as f_H (Farrell et al. 1984). Also, perfused hearts of rainbow trout (Farrell et al. 1986) and ocean pout (*Macrozoarces americanus*; Farrell et al. 1983) exhibited reductions in Q_{max} (29% and 18%, respectively) and PO_{max} (29% and 22%, respectively) during equilibration with P_{CO_2} of only 2 kPa.

Nevertheless, not all fish hearts are so sensitive to hypercapnia. CO_2-tolerant fish species include the armored catfish *Pterygoplichthys pardalis* and the European eel *Anguilla anguilla*, both of which can tolerate direct transfer to CO_2 tensions of ~5 kPa

for days (McKenzie et al. 2003; Brauner et al. 2004). Correspondingly, ventricular strips from the eel recover contractility within 20 min after exposure to sustained severe hypercapnia (10-kPa P_{CO_2}; Gesser et al. 1982), while a similar treatment in rainbow trout results in a 20%–60% reduction in isometric force generation (Gesser et al. 1982). The armored catfish heart also stands out for its high CO_2 tolerance, since a 6.5-kPa P_{CO_2} increase above control levels was required to decrease PO_{max} and Q_{max} by ~50% in an isolated perfused (pericardium intact) heart preparation (Hanson et al. 2009).

Still unclear, however, are the potential roles of both pHi and circulating catecholamines in protecting cardiac performance during elevated CO_2. For example, some CO_2-sensitive hearts show adrenergic cardiac protection (Gesser et al. 1982; Farrell 1985) but not ventricular pHi regulation (Farrell and Milligan 1986). In contrast, armored catfish hearts at a higher P_{CO_2} and saturating levels of exogenous adrenaline exhibited reduced cardiac performance in situ, suggesting less cardiac protection through adrenergic pathways in CO_2-tolerant fishes (Hanson et al. 2009). These reductions were observed despite complete ventricular pHi compensation (Hanson et al. 2009). White sturgeon hearts may also be CO_2 tolerant, as routine cardiac output was unchanged in vivo during short-term hypercarbia (P_{CO_2} of 22.5 mm Hg; Crocker et al. 2000). White sturgeon also regulate heart pHi during hypercapnia in vivo (Baker et al. 2009*a*), but whether the protection of in vivo resting cardiac function during hypercapnia extends to maximum cardiac performance or is dependent on pHi regulation or adrenergic stimulation remains unknown.

The objective of this study was to investigate the effect of hypercapnia on maximum cardiac performance in perfused white sturgeon hearts, a preparation free of potentially confounding effects, such as changes in vagal and endocrine influence not controlled experimentally. Specifically, the aims of this study were (1) to quantify changes in maximum cardiac performance at CO_2 tensions approaching the limit of white sturgeon CO_2 tolerance in vivo ($P_{CO_2} \leq 8$ kPa), (2) to determine whether cardiac recovery occurs after a decrease in maximum cardiac performance associated with short-term exposure to hypercapnia, (3) to determine whether high levels of exogenous adrenaline protect maximum cardiac performance during hypercapnia, and (4) to identify whether the ventricular pHi of perfused hearts is protected during hypercapnia, as has been observed in vivo.

Material and Methods

Experimental Animals

Juvenile hatchery-reared white sturgeon *Acipenser transmontanus* were provided by the Upper Columbia White Sturgeon Recovery Initiative's white sturgeon hatchery in Wardner, British Columbia. They were transported by tanker truck to the University of British Columbia, Vancouver, and maintained in holding tanks supplied with dechlorinated flow-through city water (in mmol L^{-1}: Na^+, 0.06; Cl^-, 0.05; Ca^{2+}, 0.03; Mg^{2+}, 0.007; K^+, 0.004; alkalinity, 3.3 mg as $CaCO_3$ L^{-1}; hardness, 3.55 mg as $CaCO_3$ L^{-1} [Fu et al. 2010]; temperature, 10°–11°C; pH, ~6.7–6.9) under a natural photoperiod at densities no greater than 15 kg m^{-3}. Fish were fed to satiation three times per week with a Moore-Clark trout chow, but food was withheld for 24 h before experimental use.

Surgical Procedures

The in situ heart preparation used in this study has been described in detail for different species, with a variety of minor modifications (Farrell et al. 1983; Farrell and Milligan 1986; Hanson et al. 2006, 2009). In brief, white sturgeon (300–1,300 g; relative ventricular weight 0.096% ± 0.003%) were anesthetized in buffered tricaine methane sulfonate (0.3 g L^{-1} of both MS-222 and $NaHCO_3$), weighed, and transferred to an operating table. A solution of heparinized saline (1 mg kg^{-1}, 150 IU mL^{-1} heparin) was injected into the caudal vessel, and the spinal cord was severed and the brain destroyed, eliminating vagal input to the heart. Within 2–3 min, a shallow lengthwise incision was made along the ventral surface of the abdominal cavity of the fish, from the anal opening to the pectoral girdle. The abdominal wall was then excised to expose the liver, which varied in size, location, and appearance. It was typically flat, thin, and delicate, wrapping around other organs and having connective adhesions with many tissues, especially the gastrointestinal tract. The right hepatic vein (consistently the largest) was used for cannula insertion, and all other major hepatic veins were tied off, including vessels along the gastrointestinal tract. A small incision was made in the right hepatic vein, and a beveled stainless steel input cannula was inserted into the vein (and advanced into the sinus venosus) and secured with silk suture. The heart was immediately (and continuously) perfused with saline (composition below) containing 10 IU mL^{-1} sodium heparin and a tonic level of adrenaline (5–10 nmol L^{-1} adrenaline bitartrate salt; AD). Then the gills were removed, and a stainless steel output cannula was inserted into the bulbus arteriosus (which in sturgeon is distal to the conus arteriosus; Guerrero et al. 2004; Icardo et al. 2004) via the ventral aorta and secured with silk suture. These surgical procedures were completed within 10–15 min. The fish was transected (approximately midabdomen) to allow for easier handling, and the large venous sinus that was severed as a result of transection was sutured to the trunk. After surgery, fish were transferred to a temperature-controlled saline bath (0.7% NaCl), the input cannula was connected to an adjustable, constant-pressure reservoir, and the output cannula was connected to a separate constant-pressure head set at 2.0 kPa to simulate resting in vivo ventral aortic diastolic blood pressure. The input pressure head was in turn connected to a set of isolated, water-jacketed glass reservoirs containing aerated perfusate. All experiments were conducted at 10°C.

Input (P_{in}) and output (P_{out}) pressures were measured through saline-filled side arms (PE50 tubing) connected to disposable pressure transducers (DPT 6100; Smiths Medical, Kirchseon, Germany), and cardiac outflow was measured through the output line with a previously calibrated, in-line

electromagnetic flow probe (SWF-4; Zepeda Instruments, Seattle, WA). The height of the input pressure reservoir was adjusted to set Q at ~17 mL min^{-1} kg^{-1}, a rate derived from in vivo cardiac output estimates for white sturgeon (Crocker et al. 2000) and adjusted for differences in ambient temperature using a Q_{10} value of 2 (Lillywhite et al. 1999). Mean P_{in} during routine cardiac output ranged from 0.04 to 0.16 kPa. Heart rate was independent of filling and output pressures, as has been observed in isolated perfused ventricles of *Acipenser naccarii* (Agnisola et al. 1999). While sturgeon possess coronary circulation (Icardo et al. 2004), the coronary arteries were not perfused in this preparation, which can result in reduced ventricular contractility in some fishes (Farrell 1987; Davie et al. 1992). After surgery, hearts were allowed to recover at routine workloads for 20 min at control (i.e., normocapnic) CO_2 tension (0.5 kPa) before the first maximum-cardiac-performance test.

Perfusate Composition

For all experiments, a freshwater fish perfusate (in mmol L^{-1}: NaCl, 125; KCl, 3.0; $MgSO_4$ $7H_2O$, 1.0; $CaCl_2$ $2H_2O$, 2.5; D-glucose, 5.6; $NaHCO_3$, 11.9; all chemicals from Sigma-Aldrich, Oakville, Ontario) was used. Depending on the experimental protocol (see below), the perfusate was gassed with 0.5- (control), 3.0-, 6.0-, or 8.0-kPa P_{CO_2} prepared gas mixtures (Praxair, Vancouver, British Columbia; certified to be within 0.1 kPa but reported nominally [or by relative in vivo challenge] as 0.5 [control], 3 [moderate], 6 [severe], and 8 [lethal] kPa hereafter) containing 21.2-kPa P_{O_2} balance N_2. When aerated with the control CO_2 mixture (P_{CO_2} = 0.5 kPa), the equilibrated perfusate had a pH of 7.80. As CO_2 tension in the perfusate was increased progressively to 3.0, 6.0, and 8.0 kPa, perfusate pH decreased to 7.25, 6.85, and 6.70, respectively. These perfusate pH values corresponded closely to blood pH values measured in vivo during exposure to similar water CO_2 tensions (Baker et al. 2009*a*). As routine heart rate in sturgeon is under modest β-adrenergic tonus (McKenzie et al. 1995), all perfusates were supplemented with a tonic level of adrenaline (see "Experimental Protocols" for concentrations); preliminary investigation supported the need for adrenaline to ensure routine cardiac function.

Experimental Protocols

Maximum cardiac performance (Q_{max} and PO_{max}) was assessed first under control CO_2 conditions (P_{CO_2} = 0.5 kPa) and then during each treatment condition, as described below for each series of experiments. Because of this sequence, each heart acted as its own control. To assess Q_{max}, P_{in} was raised in a stepwise manner (~0.05-kPa steps) over 3–5 min until Q reached a plateau; this was recorded as Q_{max}. Similarly, with input pressure remaining at its maximum, P_{out} was raised incrementally until cardiac PO_{max} was reached. After the maximum-performance tests, P_{out} and then P_{in} were returned to routine levels, and the heart was allowed to recover for ~5 min before being subjected to the next experimental saline. Preliminary investigation showed that under normocapnic conditions, maximum cardiac performance could be repeatedly assessed at least four times with no loss of performance (with a 15–20-min rest period between each test) and that no change in maximum cardiac performance occurred over a 3-h period, which was 1 h longer than any experimental protocol used in this study. For each hypercapnic condition, hearts were allowed to equilibrate for 10–20 min at routine workloads before their maximum cardiac performance was assessed.

Series 1: Maximum Cardiac Performance during Hypercapnia (3-, 6-, and 8-kPa P_{CO_2})

This series determined the CO_2 tension at which Q_{max} and PO_{max} became impaired in white sturgeon (body mass: 374 ± 14 g; ventricular mass: 366 ± 18 mg). Maximum cardiac performance was assessed under control conditions and then, after a 20-min equilibration period, at one of the following CO_2 tensions: 3 (n = 4), 6 (n = 4), or 8 kPa (n = 4). To reduce fish usage, three hearts were assessed under two CO_2 tensions, first at 3 kPa and then at either 6 (n = 1) or 8 (n = 2) kPa. No differences in performance were seen between hearts exposed to 3-kPa P_{CO_2} before exposure to a higher level of hypercapnia and those exposed directly to a higher level of hypercapnia. Consequently, data from hearts tested at a given CO_2 were pooled for all analyses. Multistep protocols have been used to assess maximum cardiac performance in other CO_2-tolerant fishes (Hanson et al. 2009). All perfusates contained 10 nmol L^{-1} [AD].

Series 2: Recovery of Maximum Cardiac Performance after Severe Hypercapnia (6-kPa P_{CO_2})

This series determined whether the sturgeon heart could recover after exposure to 6-kPa P_{CO_2}. Maximum cardiac performance was assessed in 6 fish (body mass: 382 ± 15 g; ventricular mass: 364 ± 42 mg) after 20-min equilibration periods under (1) control CO_2 tension (P_{CO_2} = 0.5 kPa), (2) hypercapnia (P_{CO_2} = 6 kPa), and then (3) posthypercapnic recovery (P_{CO_2} = 0.5 kPa). All perfusates contained 10 nmol L^{-1} [AD].

Series 3: Maximum Cardiac Performance during Severe Hypercapnia (6-kPa P_{CO_2}) with Maximal Exogenous Stimulation by Adrenaline (500 nmol L^{-1} [AD])

This series determined whether maximum adrenergic stimulation may alleviate declines in Q_{max} and PO_{max} during severe hypercapnia in 8 fish (body mass: 991 ± 82 g; ventricular mass: 943 ± 88 mg). Each heart was exposed to the following sequence of perfusates: (1) control CO_2 tension (P_{CO_2} = 0.5 kPa), with 5 nmol L^{-1} [AD], (2) hypercapnia (P_{CO_2} = 6 kPa), with 5 nmol L^{-1} [AD], and (3) hypercapnia with 500 nmol L^{-1} [AD]. This level of adrenaline was selected to allow for comparison with other studies (e.g., Hanson et al. 2009), and similar levels have been measured in vivo (Burggren and Randall 1978).

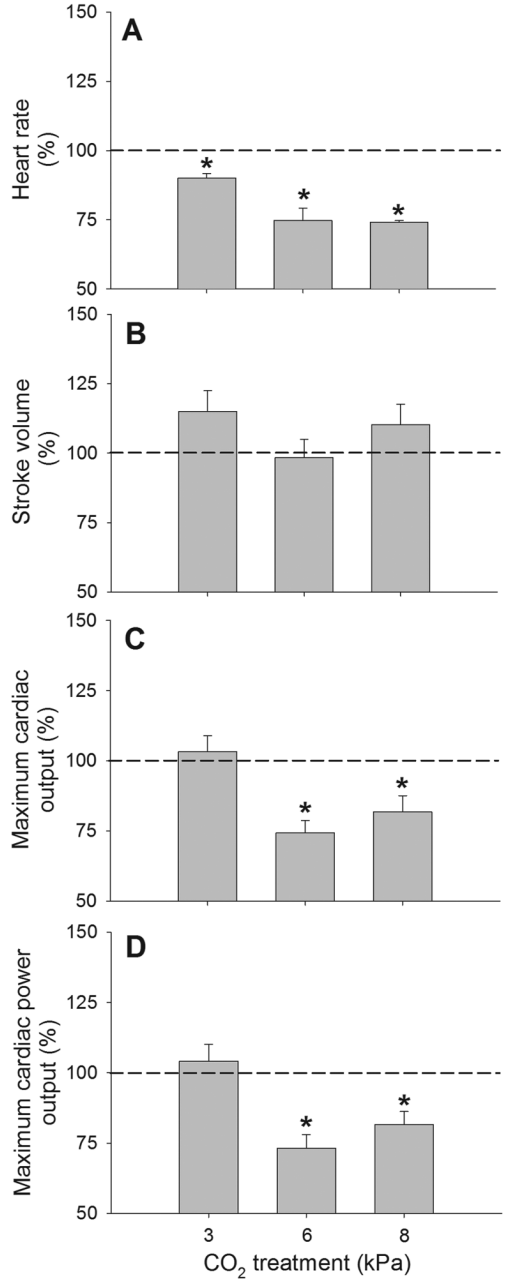

Figure 1. Effect of hypercapnia (P_{CO_2} = 3, 6, or 8 kPa) on heart rate (*A*), stroke volume (*B*), maximum cardiac output (*C*), and maximum cardiac power output (*D*), expressed as a percentage of control values assessed on perfused white sturgeon hearts in situ. Values are means + SEM. An asterisk indicates a statistically significant change from control values within that CO_2 tension. Dotted line represents control values (i.e., 100%) for comparative purposes.

Before the addition of AD (500 nmol L^{-1}), perfusates used in this series of experiments contained 5 nmol L^{-1} [AD] (as opposed to 10 nmol L^{-1}, as in series 1 and 2) to reduce the possibility of prematurely saturating adrenergic receptors, as little is known about the adrenergic sensitivity of sturgeon.

Tissue pHi Determination

The ventricle was rapidly excised and weighed after hearts were exposed to control CO_2 levels (P_{CO_2} = 0.5 kPa, n = 2; CO_2-unexposed hearts, n = 8), hypercapnia (series 1; P_{CO_2} = 6 kPa, n = 4), or hypercapnia with saturating levels of adrenaline (series 3; P_{CO_2} = 6 kPa with the addition of 500 nmol L^{-1} [AD], n = 8) and then flash-frozen in liquid nitrogen for later analysis of pHi. In addition, as a limited number of ventricles (n = 2) were sampled during exposure to control CO_2 tension (P_{CO_2} = 0.5 kPa), pHi was also measured in ventricles from hearts excised from resting normocarbic fish (as listed above; n = 8). Ventricular pHi was measured with the metabolic-inhibitor tissue homogenate method (Pörtner et al. 1990), which has been validated for use in tissues exposed to large changes in CO_2 tension (Baker et al. 2009*b*). In brief, freeze-clamped ventricles were ground under liquid nitrogen and added to a precooled centrifuge tube (2.0 mL) with a precooled scoop. A 1-mL aliquot of a metabolism-inhibiting solution (150 mmol L^{-1} potassium fluoride and 5 mmol L^{-1} nitrilotriacetic acid disodium salt; Sigma Aldrich) was then added, and the mixture was placed on ice. The pH of the resulting supernatant was measured via a thermostatted capillary electrode (BMS 2, Radiometer, London, Ontario) attached to a pH meter (PMS 83, Radiometer).

Calculations and Statistical Analyses

All cardiac measurements were recorded in real time with data acquisition software (Labview, ver. 5.1, National Instruments, Austin, TX). The P_{in}, P_{out}, f_H, Q, and cardiac power output (PO) were recorded simultaneously at a sampling rate of 10 s^{-1}. Rate of cardiac outflow force generation (F_O), a surrogate for ventricular force generation, was calculated from raw data as the average maximum change in P_{out} ($\Delta P_{out}/\Delta t$, in kPa s^{-1}) when the heart was performing at PO_{max}. In series 1, statistically significant differences were determined by paired *t*-tests and data were reported as relative changes, while in series 2 and 3 differences were determined by one-way repeated-measures ANOVA. Comparisons of ventricular pHi were made with one-way ANOVA. Where differences were indicated by ANOVA, a Student-Newman-Keuls post hoc test was used to determine homogenous subsets. For comparisons, α = 0.05 was determined to be appropriate for detecting statistical differences. All values are reported as means ± SEM, unless otherwise indicated.

Results

Series 1: Maximum Cardiac Performance during Hypercapnia (3-, 6-, and 8-kPa P_{CO_2})

Relative to their levels under control conditions (P_{CO_2} = 0.5 kPa), Q_{max} and PO_{max} were unaffected by 3-kPa P_{CO_2} but were significantly reduced by severe hypercapnia (both 6- and 8-kPa P_{CO_2}; Fig. 1*C*, 1D). Hypercapnia significantly slowed f_H during Q_{max} measurements (by 10%, 25%, and 25% at 3-, 6-, and 8-

kPa P_{CO_2}, respectively; Fig. 1*A*) and produced arrhythmia at 8-kPa P_{CO_2} (Fig. 2) but had no significant effect on stroke volume (V_S) during Q_{max} at any CO_2 tension (Fig. 1*B*). In contrast, at 8-kPa P_{CO_2}, V_S during PO_{max} measurement was significantly reduced (53% ± 3%). When assessed at PO_{max}, F_O was reduced at 6- and 8-kPa P_{CO_2}, but not at 3-kPa P_{CO_2} (Table 1).

Series 2: Recovery of Maximum Cardiac Performance after Severe Hypercapnia (6-kPa P_{CO_2})

As in series 1, 6-kPa P_{CO_2} significantly decreased Q_{max}, PO_{max}, and f_H but did not affect V_S (Fig. 2). Control performance was completely restored after a 20-min recovery at 0.5-kPa P_{CO_2} (Fig. 3).

Attempts to similarly recover hearts from 8-kPa P_{CO_2} at 0.5-kPa P_{CO_2} ($n = 4$, data not shown) were abandoned because this level of severe hypercapnia induced arrhythmia (Fig. 2) and, in some hearts, cessation of cardiac rhythm entirely. As a result, two hearts were unable to maintain V_S when P_{out} was increased. The two hearts that continued to work during exposure to 8-kPa P_{CO_2} did not recover maximum cardiac performance upon return to control conditions, suggesting permanent cardiac damage.

Series 3: Maximum Cardiac Performance during Severe Hypercapnia (6-kPa P_{CO_2}) with Maximal Exogenous Stimulation by Adrenaline (500 nmol L^{-1} [AD])

Hearts exposed to 6-kPa P_{CO_2} exhibited significant decreases in Q_{max}, PO_{max}, F_O, and f_H but not V_S (Table 1; Fig. 4), as in series 1 and 2. Addition of 500 nmol L^{-1} [AD] during severe hypercapnia fully restored Q_{max} and PO_{max}, protecting F_O and enhancing V_S but without recovery of f_H (Fig. 4; Table 1). Therefore, maximal adrenergic stimulation protected against the negative inotropy but not the negative chronotropy of severe hypercapnia.

Tissue pHi Determination

Given the negative effects of severe hypercapnia and the protective effect of adrenaline during severe hypercapnia, it was anticipated that intracellular ventricular acidosis would be ameliorated by maximum adrenergic stimulation. Relative to that of control hearts sampled either in situ or in vivo (Table 2), mean ventricular pHi was significantly reduced by exposure to 6-kPa P_{CO_2}. Mean ventricular pHi in the presence of 500 nmol L^{-1} [AD] at 6-kPa P_{CO_2} was significantly higher than that without maximum adrenergic stimulation. Even so, ventricular pHi still remained significantly lower than ventricular pHi measured under control conditions both in situ and in vivo (Table 2).

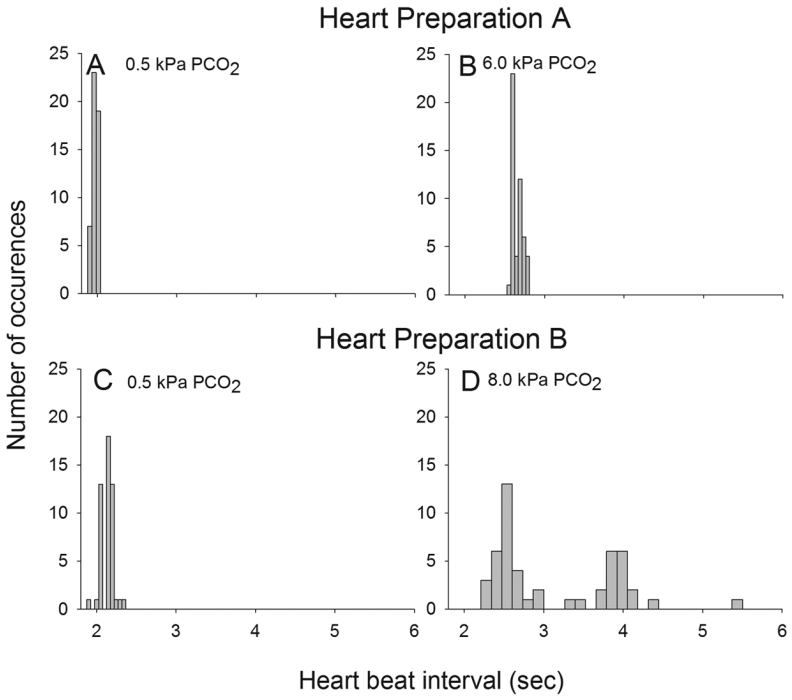

Figure 2. Effect of hypercapnia (P_{CO_2} = 6 or 8 kPa) on heartbeat interval (time in seconds between beats) during cardiac-performance testing. *A*, *B*, Data from an in situ perfused heart sequentially exposed to 0.5-kPa P_{CO_2} (*A*) and 6-kPa P_{CO_2} (*B*). *C*, *D*, Data from an in situ perfused heart sequentially exposed to 0.5-kPa P_{CO_2} (*C*) and 8-kPa P_{CO_2} (*D*). Note the bimodal distribution of long and short heartbeat intervals in *D*.

Table 1: Effect of hypercapnia (3-, 6-, or 8-kPa P_{CO_2}) and maximal adrenergic stimulation (6-kPa P_{CO_2} with 500 nmol L^{-1} [AD]) on the rate of ventricular force generation (F_O) in perfused white sturgeon hearts in situ

		Rate of cardiac output force generation (F_O; kPa s^{-1})		
Hypercapnic P_{CO_2} (kPa)	No. hearts (n)	Normocapnia	Hypercapnia	Hypercapnia + AD
3	4[a]	6.5 ± .3	7.0 ± .2	...
6	7[b]	9.3 ± .6	7.7 ± .4[c]	8.8 ± .6
8	4[a]	6.1 ± .2	5.2 ± .3[c]	...

Note. Values are means ± SEM.
[a]Assessed in hearts from series 1.
[b]Assessed in hearts from series 3.
[c]Statistically significant ($\alpha = 0.05$) difference from normocapnia-exposed hearts within a given P_{CO_2} treatment.

Discussion

The exceptional CO_2 tolerance of white sturgeon exposed to hypercarbia clearly extends to perfused cardiac tissue working at maximal rates of performance. In situ perfused white sturgeon hearts maintained maximum cardiac performance during exposure to a P_{CO_2} of 3 kPa (Fig. 1*A*), a level of hypercapnia known to impair performance of less CO_2-tolerant fish. Exposure to severe hypercapnia (P_{CO_2} = 6 kPa) significantly reduced Q_{max} and PO_{max} (both by ~25%) of working hearts through changes in f_H. These reductions were associated with an intracellular ventricular acidosis and a reduction in F_O yet still represented cardiac impairment that was extremely modest compared to that in other fishes (Farrell et al. 1983, 1986). Furthermore, the decrease in maximum cardiac performance at 6-kPa P_{CO_2} was not permanent (complete recovery was observed in subsequent normocapnia) and could be fully reversed by addition of exogenous adrenaline to provide maximum adrenergic stimulation, illustrating the remarkable CO_2 tolerance of the sturgeon heart. An increase in P_{CO_2} to 8 kPa reduced Q_{max} and PO_{max} to a degree similar to that observed at 6 kPa but also resulted in arrhythmia without recovery in subsequent normocapnia. These data imply that cardiac damage, or at the very least temporary myocardial dysfunction (e.g., Hanson et al. 2006), occurs at 8-kPa co_2, a level of hypercarbia known to induce morbidity in vivo (D. W. Baker and C. J. Brauner, unpublished observations).

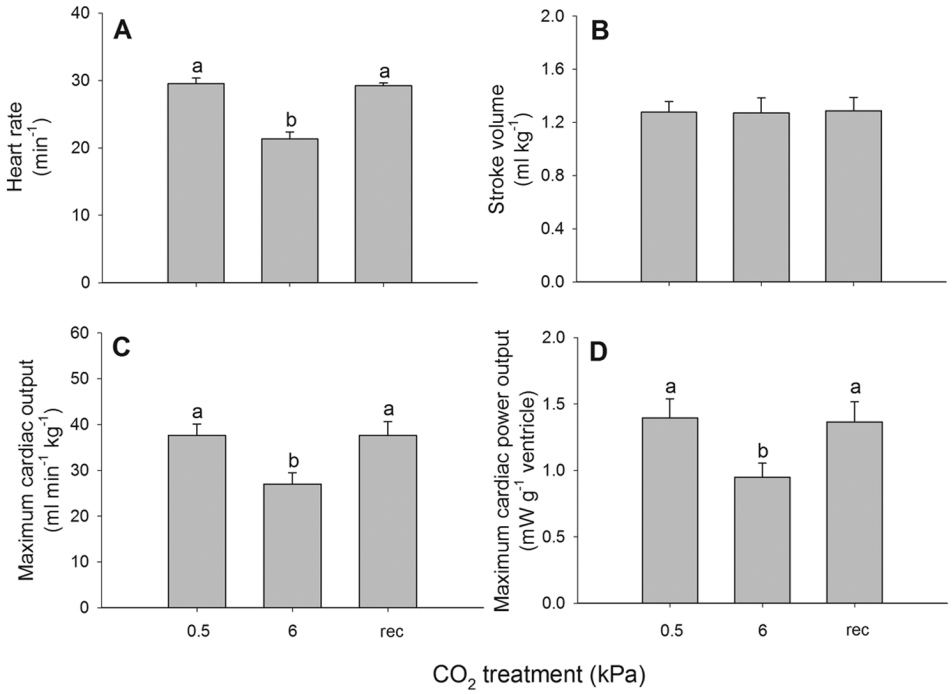

Figure 3. Effect of hypercapnia (P_{CO_2} = 6 kPa) and return to control CO_2 tension (P_{CO_2} = 0.5 kPa; *rec*) on heart rate (*A*), stroke volume (*B*), maximum cardiac output (*C*), and maximum cardiac power output (*D*), assessed in perfused white sturgeon hearts in situ. Values are means + SEM. (n = 6). Letters indicate statistically significant differences between treatment groups.

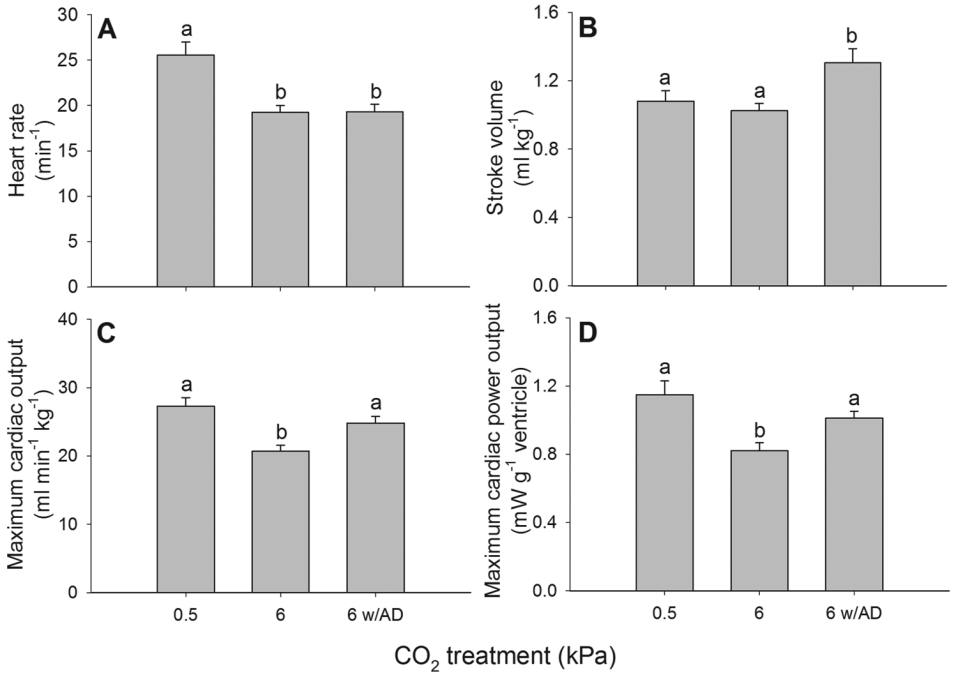

Figure 4. Effect of hypercapnia (P_{CO_2} = 6 kPa) in the absence and presence of adrenaline (500 nmol L^{-1} [AD]; *6 w/AD* indicates the latter) on heart rate (*A*), stroke volume (*B*), maximum cardiac output (*C*), and maximum cardiac power output (*D*), assessed in perfused white sturgeon hearts in situ. Values are means + SEM (n = 8). Letters indicate statistically significant differences between treatment groups.

Maximum Cardiac Performance during Hypercapnia

White sturgeon lack the aerobic scope of pelagic fishes, such as salmonids (Peake 2004), and are often described as benthic cruisers because of their tendency to cover vast distances in search of prey and spawning sites. Maximum cardiac performance of white sturgeon heart has not been previously assessed in situ, but it would be expected to be lower than that of a pelagic predator such as rainbow trout. Relative to those of rainbow trout, white sturgeon hearts exhibited a 50% lower intrinsic f_H (at 10°C; Arthur et al. 1992; Hanson et al. 2006), a 20%–40% lower Q_{max} (Hanson et al. 2006), and a 75% lower PO_{max} (Hanson et al. 2006). The lower power output of white sturgeon hearts (~1.2–1.5 mW g ventricle^{-1}) may reflect their limited athletic prowess.

Hypercapnia-induced reductions in cardiac performance are typically due to both negative chronotropic (frequency of contraction) and inotropic (force of contraction) effects on fish hearts. Hearts of rainbow trout (Farrell et al. 1986), sea raven (Farrell et al. 1983), and ocean pout (Farrell et al. 1983) all exhibited reductions in both f_H (10%–15%) and V_S (5%–10%) during exposure to 1.8–2.0-kPa P_{CO_2}. Perfused hearts of the CO_2-tolerant armored catfish *Pterygoplichthys pardalis* exhibited no change in f_H or V_S at 2.5-kPa P_{CO_2}, but f_H and V_S decreased significantly (by ~30% and ~35%, respectively) at 7.5-kPa P_{CO_2} (Hanson et al. 2009). Like catfish hearts, sturgeon hearts exhibited no decrease in V_S (12% above control; P = .058) or F_O (11% above control; P = .143) at 3-kPa P_{CO_2}, although f_H was significantly lower (8% below control). Furthermore, the V_S of white sturgeon hearts was unchanged at P_{CO_2} = 6 or 8 kPa, even though F_O decreased slightly (~18%), indicating that the decreased Q_{max} and PO_{max} (~25% each) reflected negative chronotropic effects (with f_H decreasing by ~25% at 6-kPa

Table 2: The effect of hypercapnia (6-kPa P_{CO_2}) and maximal adrenergic stimulation (500 nmol L^{-1} [AD]) on white sturgeon ventricular intracellular pH (pHi)

CO_2 tension (kPa)	Fish (n)	[AD] (nmol L^{-1})	Perfusate pH (pHe)	Ventricular pH (pHi)
In vivo	8	In vivo	7.80 ± .01	6.91 ± .013^A
.5 (control)	2	10	7.80	6.95 ± .050^A
6	4	10	6.85	6.77 ± .015^B
6	8	500	6.85	6.83 ± .011^C

Note. In vivo values were obtained from ventricles excised from white sturgeons under resting conditions. Control group represents ventricles sampled during 0.5-kPa P_{CO_2}. Values are means ± SEM. Letters indicate statistically significant differences between treatment groups. pHe = extracellular pH.

P_{CO_2}). Thus, the remarkable CO_2 tolerance of white sturgeon hearts appears to be associated more with a protection of inotropy than with protection of chronotropy.

In vertebrate hearts, high CO_2 can induce chronotropic effects by, for example, alterations in the activity of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels in pacemaker cells, which set the intrinsic rate of the heart (Bers 2001). HCN channel activity can be reduced during acidosis by a concurrent reduction in cyclic adenosine monophosphate (cAMP), resulting in a subsequent bradycardia. However, whether similar mechanisms are responsible for the hypercapnic bradycardia displayed in white sturgeon awaits further experimentation.

Previous work with CO_2-sensitive species has shown that myocardial acidosis exerts a negative inotropic effect through H^+/Ca^{2+} competition for binding sites on troponin (Williamson et al. 1976; Gesser and Jørgensen 1982). White sturgeon have demonstrated an exceptional capacity for pHi regulation during short-term hypercapnia in vivo (Baker et al. 2009*a*), and this could explain preservation of inotropy. Here, the magnitude of the acidosis measured in white sturgeon ventricles exposed to 6-kPa P_{CO_2} (~0.18 pH units; Table 2) was less than half that predicted from its intrinsic buffer capacity (~0.4 pH units; Baker et al. 2009*a*). Thus, we suggest that some of the protection of cardiac performance in situ may be the result of the exceptional pH regulatory response of white sturgeon. While *P. pardalis* better maintained ventricular pHi in situ (Hanson et al. 2009), inotropy during severe hypercapnia was still better protected in white sturgeon hearts, suggesting greater insensitivity to intracellular acidosis in the white sturgeon. Inotropic effects due to acidosis in cardiac muscle tissue have generally been attributed to changes in Ca^{2+} affinity and transport (Williamson et al. 1976; Gesser and Jørgensen 1982; Shiels et al. 2010), and therefore further research using ventricular strip preparations to examine, for example, Ca^{2+} affinity is warranted.

Protective Effects of Adrenergic Stimulation on Cardiac Performance during Hypercapnia

White sturgeon hearts are under some degree of adrenergic tone during normocapnic conditions in vivo (Crocker et al. 2000), but relatively little is known about their adrenergic sensitivity. Even so, differences in control cardiac performance between series 2 and 3 (a 20% lower Q_{max} and PO_{max} in the latter; Figs. 3*C*, 3*D*, 4*C*, 4*D*) are unlikely to be related to tonic levels of perfusate adrenaline used (10 and 5 nmol L^{-1} [AD], respectively), as the relative effects of 6-kPa P_{CO_2} were almost identical. Instead, these differences between control values for Q_{max} and PO_{max} may be related to size (2.5-fold larger hearts in series 3), as larger hearts may lack coronary perfusion and thus potential limitations in oxygen diffusion in a thicker compact myocardium (Lillywhite et al. 1999). Further studies are needed to describe dose-response effects of adrenaline on cardiac function in sturgeon.

Hypercapnia (3-kPa P_{CO_2}) induces a persistent (96-h) elevation of plasma adrenaline in white sturgeon (~5 times resting levels; Crocker and Cech 1998), suggesting an important role for this hormone in ameliorating the negative effects of hypercapnia on cardiorespiratory function. Increased AD can, for example, stimulate transsarcolemmal Ca^{2+} influx and sarcoplasmic reticulum Ca^{2+} uptake, thus increasing cardiac contractility and accelerating relaxation (Bers 2001). Adrenergic protection of cardiac performance during hypercapnia is commonly observed in fish hearts; high [AD] has been demonstrated to increase inotropy in ventricular strips of both rainbow trout and eel during hypercapnia. Likewise, increasing [AD] (from 5 to 500 nmol L^{-1}) during 6-kPa P_{CO_2} in our study restored Q_{max} and PO_{max} to control levels by increasing f_O (Table 1) and V_S (Fig. 4*B*). Although addition of high concentrations of adrenaline completely protected cardiac performance during hypercapnia, heart rate remained depressed (Fig. 4*A*). Thus, although Q_{max} and PO_{max} were maintained at control levels during 6-kPa P_{CO_2}, heart function was qualitatively very different from that during control conditions.

A lack of effect of adrenaline on f_H was unexpected, as AD is known to stimulate HCN channel activity in pacemaker cells, and increase f_H in vertebrate hearts. In addition, in most fishes examined, adrenergic protection is attributable to increases in both f_H and contractile force. Only rainbow trout hearts working routinely exhibited no increase in f_H in the presence of high adrenaline levels (Farrell 1985), albeit at a much lower CO_2 tension. We speculate that this absence of an effect on f_H may be a direct effect of perfusate pH (6.85 at P_{CO_2} = 6 kPa) on pacemaker cells rather than of pHi effects, because pHi increased in response to elevated AD. This possibility might also explain why hearts exposed to 8-kPa P_{CO_2} (perfusate pH = 6.7) exhibited arrhythmia. Thus, while cardiac inotropy may be CO_2 tolerant, negative chronotropic effects may be unavoidable, because no blood pH compensation occurs during exposure to severe hypercarbia (>6–8 kPa). Unfortunately, little is known about the effects of severe blood acidosis (blood pH decreases of >0.7) on intrinsic heart rate, as few other vertebrates can tolerate this condition. Cardiac failure as a mechanism of CO_2 toxicity in white sturgeon remains a possibility, particularly considering the absence of sustained, severe intracellular acidosis.

Conclusions

To place our study in a broader perspective, various authors (Heisler 1986; Ultsch 1996; Brauner and Baker 2009) have suggested that aquatic hypercarbia has been underestimated as a selective pressure associated with a number of important vertebrate adaptations. Among fishes, white sturgeon display an exceptional tolerance to hypercapnia, and this tolerance extends to cardiac performance. Severe hypercapnia (6-kPa P_{CO_2}) only modestly reduced Q_{max} and PO_{max}, and both were restored with adrenergic stimulation or upon return to control CO_2 tensions. Furthermore, this loss of performance was observed in an in situ heart preparation, devoid of other possible mediating responses, such as alterations in vagal tone or vascular resistance.

The combination of an emergent coordinated response (Baker et al. 2009*a*) and inherent CO_2 tolerance of cardiac tissue strongly support the hypothesis that CO_2 tolerance is an adaptive response to a selective pressure (i.e., aquatic hypercarbia). As white sturgeon are phylogenetically positioned between elasmobranchs and teleosts, these findings can provide important insight into the evolution of CO_2 tolerance in fish hearts. Research identifying mechanisms associated with protection of cardiac function during hypercapnia remains an exciting future direction.

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