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Intrinsic mechanical properties of the perfused armoured catfish heart with special reference to the effects of hypercapnic acidosis on maximum cardiac performance

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SUMMARY

The armoured catfish, *Pterygoplichthys pardalis*, is known to be extremely tolerant of environmental hypercarbia (elevated water CO_2 tensions), which occurs in their natural environment. In addition, previous studies have demonstrated that during exposure to hypercarbia, *P. pardalis* does not exhibit extracellular pH compensation and thus the heart and other organs must continue to function despite a severe extracellular acidosis. We used an *in situ* perfused heart preparation to determine the effects of an extracellular hypercapnic (elevated CO_2 in the animal) acidosis (1–7.5% CO_2) on heart function, specifically cardiac output, power output, heart rate and stroke volume. The present study is the first to comprehensively examine cardiac function in an acidosis-tolerant teleost. When compared with control conditions, maximum cardiac performance was unaffected at levels of CO_2 as high as 5%, far exceeding the hypercapnic tolerance of other teleosts. Moreover, *P. pardalis* exhibited only a moderate decrease (~35%) in cardiac performance when exposed to 7.5% CO_2, and full cardiac performance was restored in six out of seven hearts upon return to control conditions. Myocardial intracellular pH (pH_i) was protected *in situ*, as has been found *in vivo*, and this protection extended to the highest level of CO_2 (7.5%) investigated. Thus, maintained heart function during a hypercapnic acidosis in *P. pardalis* is probably associated with preferential pH_i regulation of the heart, but ultimately is not sufficient to prevent loss of cardiac function. Our findings suggest the need for further study to elucidate the mechanisms behind this remarkable cardiac hypercapnic tolerance.

Key words: hypercapnia, heart, carbon dioxide, intracellular pH, *Pterygoplichthys pardalis*, acid–base physiology.

INTRODUCTION

The waters of the Amazon are prone to episodes of both hypoxia (low dissolved oxygen) (Val et al., 1995) and hypercarbia (high dissolved carbon dioxide). These events are the result of high biomass and water stratification, and occur most frequently at night; CO_2 tensions as high as 8 kPa have been measured under dense vegetative mats (Heisler, 1982; Ultsch, 1996), suggesting that severe hypercarbia may be a relatively common occurrence in tropical environments. CO_2 levels of this magnitude will induce a severe extracellular acidosis in fishes, which no fish studied to date is able to compensate for. Consequently, organs which are bathed in, and rely on, venous blood, such as the heart, must continue to function in spite of the potentially debilitating effects of an extracellular acidosis if the animal is to survive. As a result of considerable study on the inotropic (force of contraction) and chronotropic (frequency of contraction) effects of hypercapnic acidosis in both isolated cardiac muscle strips (Poupa et al., 1978; Gesser and Jorgensen, 1982; Gesser et al., 1982; Kalinin and Gesser, 2002) and working perfused heart preparations (Farrell et al., 1986; Farrell and Milligan, 1986; Farrell et al., 1988; Hanson et al., 2006), it is believed that the origin of the negative effects on both myocardial contractility and pacemaker cells is in fact intracellular acidification (Satoh and Hashimoto, 1983).

Despite periodic hypercarbic challenges, a great number of teleost species thrive in the Amazon. One such species, the armoured catfish, *Pterygoplichthys pardalis* (formerly known as *Liposarcus pardalis*), is a facultative air breather and possesses a great capacity to tolerate aquatic hypercarbia (Brauner et al., 2004). This tolerance is not related to extracellular pH (pH_e) compensatory capacity as, during exposure to hypercarbia, *P. pardalis* does not compensate for the extracellular acidosis, and thus pH_e can decrease from pH 7.9 to below pH 7.0 for hours or days without apparent adverse effects (Brauner et al., 2004). In most fishes studied to date, decreases in pH_e are qualitatively matched by decreases in intracellular pH (pH_i) in tissues such as the heart, white muscle and liver (Milligan and Farrell, 1986; Milligan and Wood, 1986; Wood and LeMoigne, 1991; Wood et al., 1990; McKenzie et al., 2002). Intriguingly, *P. pardalis* has been shown to regulate heart, liver and white muscle intracellular pH at normocarbic levels during the severe extracellular acidosis induced by hypercarbia (Brauner et al., 2004). This unusual pattern of pH_i protection during severe pH_e depression, which has only been observed in *P. pardalis*, *Acipenser transmontanus* (Brauner and Baker, 2009) and *Synbranchus marmoratus* (Heisler, 1982), may be the basis for CO_2 tolerance in fishes (Brauner and Baker, 2009) and we hypothesize here, protection of heart function in *P. pardalis*. The objective of this study was to determine the effects of a severe extracellular acidosis on maximum cardiac performance of *P. pardalis*, using an *in situ* perfused heart preparation subjected to different levels of hypercapnia. In order to appropriately examine these effects, characterization of baseline

cardiac parameters and cardiac function was necessary, as these variables have not been previously reported in this species. Of special interest was the effect of hypercapnia on heart pH_i in this *in situ* preparation, where hearts were forced to work maximally, representing a very different condition to that examined previously *in vivo* (Brauner et al., 2004). This study provides insight into the physiological basis for CO_2 tolerance in fish that has recently been considered to be associated with the evolution of air-breathing (Brauner and Baker, 2009).

MATERIALS AND METHODS

Experimental animals

Armoured catfish (*Pterygoplichthys pardalis* Castelnau 1855) of both sexes (mass, 473±39 g; relative ventricular mass, 0.028±0.002%) were obtained from a local commercial fish supplier and held in aerated well water at Instituto Nacional de Pesquisas da Amazônia (INPA), Manaus, Brazil prior to and during experimentation. Fish were maintained under a natural photoperiod in aerated outdoor tanks containing well water at 28°C.

Surgical procedures

Fish were anaesthetized in an oxygenated solution of buffered tricaine methane sulfonate (MS-222, 0.15 g l^{-1}, with 0.30 g l^{-1} $NaHCO_3$), weighed and placed on an operating table where their gills were continuously irrigated with chilled, oxygenated anaesthetic (0.05 g l^{-1} MS-222 buffered with 0.1 g l^{-1} $NaHCO_3$). Fish were then injected with 1 ml kg^{-1} of heparinized saline (150 i.u. ml^{-1}) into the caudal vessels. An *in situ* perfused heart preparation was prepared as previously described (Farrell et al., 1986) but modified (Farrell et al., 1989); however, further modifications were necessary because of the anatomical differences between rainbow trout and armoured catfish. A shallow lengthwise incision was made from the anal opening to an area just posterior to the pectoral girdle to expose the viscera and allow a stainless steel input cannula to be introduced into the sinus venosus *via* a hepatic vein. In armoured catfish there are two major hepatic veins, one from each lobe of the liver. These two vessels are highly visible as they exit the liver and merge to form a single vessel. This single vessel is only visible for a very short distance before it becomes enveloped by the layer of connective tissue that encloses the entrance to the pericardium. Owing to these anatomical limitations, the input cannula was always inserted into the left hepatic vein at a point just upstream (posterior) from the junction. The input cannula was advanced along the vessel to the point at which the hepatic vein joined the sinus venosus. In the ideal preparation, the tip of the input cannula was introduced fully into the sinus venosus; however, the anatomy of the junction between the two structures was such that this was not always possible. Consequently, even with careful placement, partial occlusion of the tip of the cannula by the walls of the hepatic vessel was often unavoidable, thus high input pressures (0.11–0.25 kPa) were needed to supply adequate perfusate. The use of high perfusion pressures is not ideal; nevertheless, it provides far superior results than the only alternative, a preparation in which the integrity of the pericardium is not maintained. In addition, previous studies have used even higher perfusion pressure (Stuart et al., 1983) with no apparent ill effects.

Following insertion of the input cannula, the heart was immediately perfused with saline (composition below) containing 500 nmol l^{-1} adrenaline (adrenaline bitartrate salt; AD) and 10 i.u. sodium heparin per millilitre. A stainless steel output cannula was then secured into the ventral aorta at a point confluent with the bulbus arteriosus. This was facilitated by removal of the lower jaw and gills. The total time to prepare the perfused heart preparation was 15–20 min. All experimental procedures complied with the policies of INPA, the University Animal Care Committee of the University of British Columbia, and the Canadian Council on Animal Care.

Following surgery, the fish was transferred to a physiological saline bath (7‰ NaCl). The input cannula was immediately connected to an adjustable, constant-pressure reservoir, and the output cannula was connected to a separate constant pressure head set at 3.0 kPa. The height of the input pressure reservoir was adjusted to set routine cardiac output ($\dot{Q}$) at approximately 35 ml min^{-1} kg^{-1}. Input (P_{in}) and output (P_{out}) pressure were measured through saline-filled side arms (PE50 tubing) connected to disposable pressure transducers (DPT 6100, Smiths Medical, Kirchseeon, Germany). Cardiac outflow was continuously measured through the output line with an in-line electromagnetic flow probe (SWF-4, Zepeda Instruments, Seattle, WA, USA) that had been previously calibrated with known flow rates of perfusate. Hearts were allowed to equilibrate for 5-10 min under control conditions (see below) before the experiment commenced. Previous examination of *P. pardalis* ventricles revealed no evidence of a coronary circulation.

Perfusate composition

For the control perfusate, freshwater fish saline (125.0 mmol l^{-1} NaCl, 3.0 mmol l^{-1} KCl, 1.0 mmol l^{-1} $MgSO_4 \cdot 7H_2O$, 2.5 mmol l^{-1} $CaCl_2 \cdot 2H_2O$, 5.6 mmol l^{-1} D-glucose, 11.9 mmol l^{-1} $NaHCO_3$; all chemicals from Sigma-Aldrich, Oakville, ON, Canada) was aerated with 1.0% CO_2 (balance air), supplied by a Wösthoff gas mixing pump (Bochum, Germany), to achieve a pH of 7.8 and an oxygen level of 20 kPa. Previous experiments in our laboratory (Hanson et al., 2006) have shown no significant difference in maximum cardiac performance between hyperoxic hearts (95.5% O_2, 0.5% CO_2) and hearts perfused with air-saturated saline, which was the control level of oxygen for all experiments. For the hypercapnic test conditions the perfusate was aerated with varying levels of CO_2 (2.5, 5.0 and 7.5% CO_2, balance air), however, the ionic composition of the perfusate remained the same. Regardless of the test conditions, the perfusate contained 500 nmol l^{-1} AD. Preliminary studies suggested that this high level of adrenergic stimulation was necessary for the heart to maintain consistent performance. Furthermore, previous studies on cardiac strips (trout and eel) demonstrated that adrenaline increases the force of contraction during hypercapnia without altering the relative changes in force seen under different exposure conditions [anoxia, hypercapnia, recovery (Gesser et al., 1982)], thus minimizing any concerns that potentially excess adrenergic stimulation affected the results of the present study. Additionally, 500 nmol l^{-1} AD was shown not to affect pH_i during extracellular acidosis in perfused rainbow trout hearts, although contractility was restored in failing hearts (Farrell and Milligan, 1986).

Experimental protocols

Maximum cardiac performance was assessed in each heart preparation under every test condition. By initially measuring both maximum cardiac output ($\dot{Q}_{max}$) and maximum cardiac power output (PO_{max}) under control conditions, each heart acted as its own control. To determine $\dot{Q}_{max}$, P_{in} was gradually increased in increments of approximately 0.05 kPa until cardiac output reached a plateau (usually around 0.4 kPa). To assess PO_{max}, P_{in} was left at its maximum and P_{out} was increased in a stepwise fashion in ~0.1 kPa increments until PO reached a plateau. After PO_{max} was determined, P_{out} and P_{in} were returned to resting levels and the heart was allowed

to recover (~5 min) before being exposed to the next perfusate. To mimic natural conditions all experiments were performed at 27.0±0.2°C.

Series 1: characterization of cardiac performance

The purpose of this series was to characterize the Starling response (the change in cardiac performance *versus* preload) and the pressure development of armoured catfish hearts. Following 5–10 min acclimation period to control conditions, the Starling response was determined by increasing P_{in} in 0.05 kPa increments, with the heart being allowed to equilibrate for approximately 1 min at each step. P_{in} was increased until such time as $\dot{Q}$ reached a maximum. Immediately following this procedure maximum pressure generation was determined by increasing P_{out} in a similar stepwise fashion (with P_{in} held constant at its maximum) until PO reached a maximum. Hearts were then returned to resting levels of P_{in} and P_{out} so that their routine, post-test performance could be compared with their initial performance to assess preparation viability and ensure that the heart had not been damaged by the test.

Series 2: hypercapnia

The purpose of Series 2 was to quantify the effect of hypercapnia and associated acidosis on mechanical characteristics of the perfused heart in maximally stimulated hearts (i.e. presence of 500 nmol l^{-1} AD). Recovery from severe hypercapnia was assessed by re-testing hearts with control perfusate following the hypercapnic exposures. After assessing $\dot{Q}_{max}$ as described above (i.e. control, 1% CO_2, resulting in a pH of 7.83), hearts were then sequentially subjected to: (1) 2.5% CO_2 (resulting in a pH of 7.56), (2) 5.0% CO_2 (resulting in a pH of 7.26), (3) 7.5% CO_2 (resulting in a pH of 7.10), (4) control with a pH of 7.83 and (5) 7.5% CO_2 (resulting in a pH of 7.10). Hearts were re-exposed to 7.5% CO_2 during this final step so that intracellular pH (pH_i) could be measured under these conditions. Hearts were exposed to each perfusate for a total of 15 min during which time $\dot{Q}_{max}$, PO_{max}, heart rate and stroke volume were measured; this time period also ensured continued viability of the photosensitive AD. Following the sixth and final 15 min exposure the heart was rapidly excised, the ventricle was dissected out, weighed and frozen in liquid nitrogen for later measurement of pH_i as described below.

Series 3: hypercapnic preconditioning

The purpose of this series was to determine if the maximum cardiac performance observed during the highest level of hypercapnia (7.5% CO_2 resulting in a pH of 7.10) in Series 2 was affected by the previous exposure to an intermediate level of hypercapnia. Individual hearts were subjected to the following protocol: (1) control (1% CO_2) resulting in a pH of 7.83, (2) 7.5% CO_2 resulting in a pH of 7.10, (3) control with a pH of 7.83. As in the previous experiments, maximum cardiac performance was assessed under each test condition. Following the final (15 min) step hearts were excised, ventricle removed, weighed and frozen in liquid nitrogen for later measurement of pH_i (termed recovery pH_i) as described below.

Intracellular pH determination

Intracellular pH was determined on freeze clamped ventricles using the tissue homogenate technique (Pörtner et al., 1990). In brief, the method involved homogenization of ventricular tissue using a liquid nitrogen cooled mortar and pestle. Pulverized tissue was then transferred using a pre-cooled metal scoop to a pre-cooled 1.5 ml centrifuge tube. An 800 µl aliquot of an isotonic metabolic inhibitor solution (150 mmol l^{-1} KCl and 5 mmol l^{-1} nitrilotriacetic acid disodium salt) was then added to the tissue, and pH of the resultant mixture was measured using a thermostated capillary pH electrode (Radiometer, BMS 2, London, Ont., Canada). Although this technique has been validated for both water breathing (blood ~0.5% CO_2) and air breathing (blood ~3–4% CO_2) animals, further validation (Baker et al., 2009) was provided by comparing measurements of pH_i of red blood cells separated from blood exposed to high CO_2 in tonometers (between 0.5% and 10% CO_2) using the freeze–thaw technique (Zeidler and Kim, 1977) and the metabolic inhibitor homogenate method (Pörtner et al., 1990). The high correlation between results (R^2=0.95) indicated that the latter can be used to measure pH_i at very high CO_2 tensions despite the potential for CO_2 loss during tissue processing. For comparison purposes pH_i was also measured on hearts taken immediately from euthanized, uncannulated, resting control fish (N=6), hereafter referred to as control pH_i.

Calculations and statistical analysis

All experimental data were collected using data acquisition software (Labview version 5.1, National Instruments, Austin, TX, USA), which allowed for real-time measurements of f_H, P_{in}, P_{out}, $\dot{Q}$ and PO. Statistical differences within test groups were determined by one-way repeated measures analysis of variance (ANOVA). When warranted, the Holm–Sidak procedure was used for *post-hoc* multiple comparisons. Comparisons of pH_i between test groups were made using a Students' *t*-test. Sigma Stat (3.0, SPSS, San Rafael, CA, USA) was used for all statistical analysis. For statistical comparisons, α=0.05 was used for determining statistical differences.

Owing to the anatomical limitations discussed above, control preloads ranged from 0.11 kPa to 0.25 kPa. Consequently, results in Fig. 1 are presented as change from routine P_{in}, where routine P_{in} is defined as the preload necessary to achieve a $\dot{Q}$ of 35 ml min^{-1} kg^{-1}. Preloads were normalized by fitting (r^2>0.98) the raw data for each individual fish (N=6) to a first order sigmoidal equation of the form $\dot{Q}=a/\{1+e^{-[(P\text{in}-\text{routine } P\text{in})/b]}\}$ where a and b are coefficients derived from the fit for each individual fish. These equations were used to calculate $\dot{Q}$ for individual fish at specific, relative preload values. Results are presented as the mean for each preload value ± s.e.m.

RESULTS

Series 1: characterization of cardiac performance

Heart rate (107±4 beats min^{-1}) increased 0–3 beats min^{-1} during the first 0.05 kPa increase in P_{in} (P>0.05) but no further changes in f_H occurred subsequent to that (data not reported). The maximum response to preload was usually produced around 0.3 kPa to 0.4 kPa above routine P_{in} and resulted in an average $\dot{Q}_{max}$ of 62.8±4.1 ml min^{-1} kg^{-1} (Fig. 1). Stroke volume (V_S) during $\dot{Q}_{max}$ averaged 0.55±0.05 ml kg^{-1}. Maximum cardiac power output (PO_{max}) was reached between 3.7 kPa and 3.9 kPa and averaged 10.31±0.53 mW g^{-1} ventricle (Fig. 2).

Series 2: hypercapnia

When compared with cardiac performance under control conditions, there was no significant decrease in either $\dot{Q}_{max}$ (67.6± 3.8 ml min^{-1} kg^{-1}) or PO_{max} (10.86±0.06 mW g^{-1} ventricle) under levels of hypercapnia as high as 5% CO_2 (Fig. 3). Conversely, exposure to 7.5% CO_2 resulted in a ~35% decrease in both PO_{max} and $\dot{Q}_{max}$. The decrease in $\dot{Q}_{max}$ was associated with a 20% decrease in f_H (P<0.05) and a 15% decrease in V_S, although the decrease in V_S was not statistically significant (Fig. 3). Similarly, a 15–20%

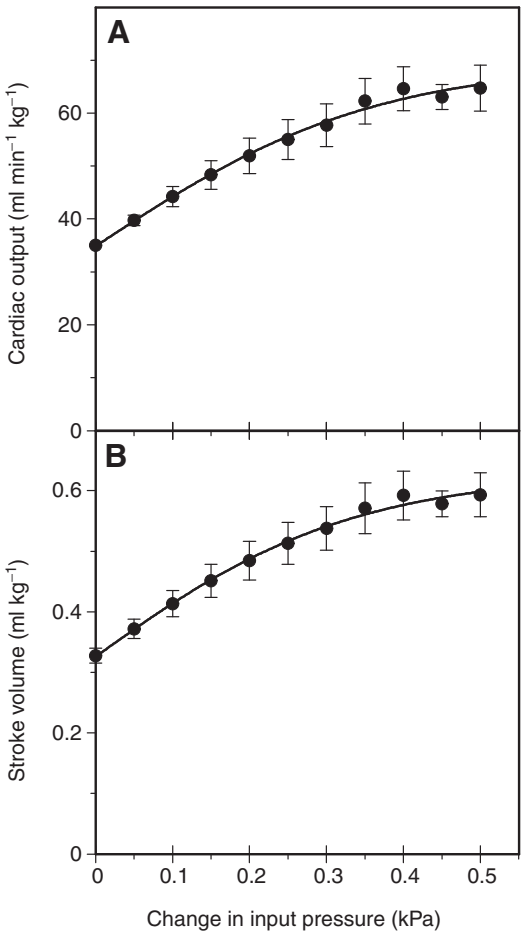

Fig. 1. The effect of changes in input pressure on cardiac output (A) and cardiac stroke volume (B) in *in situ* perfused *Pterygoplichthys pardalis* hearts under control, normocapnic conditions at 27.0±0.2°C (*N*=6). Results are presented as change from routine preload pressure and were normalized to a first order sigmoidal equation (r^2>0.98) as discussed in the Results. Data are presented as the means for each preload value ± s.e.m.

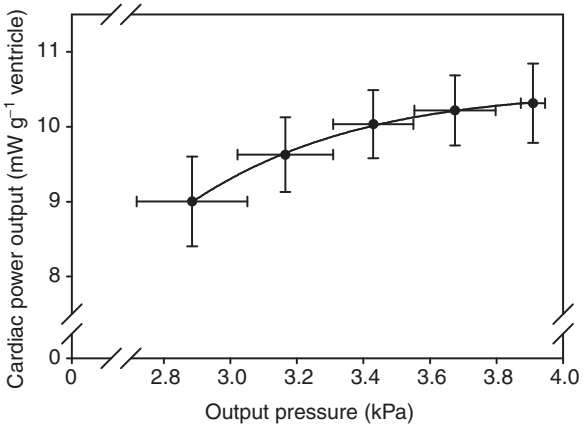

Fig. 2. The effect of changes in after-load on cardiac power output in *in situ* perfused *Pterygoplichthys pardalis* hearts under control, normocapnic conditions at 27.0±0.2°C (*N*=6). Data are presented as means ± s.e.m.

decrease in both f_H and V_S were observed at PO_{max} although as a result of high inter-individual variation these changes were not statistically significant (data not shown).

The effects of hypercapnia on heart function were usually not permanent as performance was restored following recovery to control conditions in all but one preparation (Fig. 3). Note that although results are only shown for five fish an additional two fish were tested under this protocol and also showed a complete recovery upon return to control conditions. Unfortunately, recordings from these two fish were lost due to equipment malfunction.

Series 3: hypercapnic preconditioning

Hearts exposed directly to 7.5% CO_2 following control perfusate showed a trend toward an additional 17–20% decrease in $\dot{Q}_{max}$ and PO_{max} when compared with the cardiac performance of hearts exposed to 7.5% CO_2 following a stepwise increase (Series 2). However, this difference between the two exposure methods was not statistically significant (possibly because of the small sample size, *N*=3). Nevertheless, these preliminary results suggest the potential for hypercapnic preconditioning, such that a gradual (stepwise) exposure to extreme hypercapnia reduces the impact of the final hypercapnic exposure. However, confirmation of this will need further study. Similarly to Series 2, the reduction in cardiac performance seen under hypercapnia appeared to result from decreases in both f_H and V_S.

Intracellular pH (pH_i)

Intracellular pH of hearts exposed to 7.5% CO_2 (Series 2) was found to be significantly (P<0.05) higher than that of non-perfused control hearts (7.02±0.05, *N*=6 *versus* 6.92±0.04, *N*=6). However, there was no significant difference in pH_i between hearts exposed to 7.5% CO_2 compared with those sampled under normocapnic conditions (1.0% CO_2) during hypercapnic recovery (7.06±0.01, *N*=3, Series 3). In addition, regression analysis showed that myocardial pH_i under hypercapnia was negatively correlated with cardiac performance ($\dot{Q}_{max}$) under hypercapnia (r^2=0.64; P<0.01) such that the ventricle with the lowest pH_i (i.e. closest to control values) showed the least decline in performance.

DISCUSSION

This study demonstrates the great capacity of the heart of *P. pardalis* to both tolerate and perform maximally under a severe hypercapnic acidosis. Maximum cardiac performance was not significantly different from that seen under normocapnic conditions at levels of hypercapnia as high as 5% CO_2 (5.1 kPa). Moreover, *P. pardalis* exhibited only moderate decreases (~35%) in cardiac performance when exposed to 7.5% CO_2. Equally as remarkable, full cardiac performance was restored upon return to normocapnic conditions in six out of seven preparations.

The present study is the first to comprehensively examine cardiac function in an acidosis-tolerant teleost. Previous studies looking at cardiac function in intact hearts during hypercapnic acidosis have all been conducted on species considered to be intolerant of acidosis (or sensitive to pH perturbation), i.e. rainbow trout (Farrell and Milligan, 1986; Farrell et al., 1986; Farrell et al., 1988), ocean pout (Turner and Dredzic, 1980; Farrell et al., 1983) and sea raven (Turner and Dredzic, 1980; Farrell et al., 1983). When compared with *P. pardalis* (present study) these species show significant decreases in cardiac performance (14–58%) during exposure to a far less severe hypercapnia (1–2% CO_2; Table 1). The degree of acidosis associated with a particular CO_2 tension in previous studies is not as great as in the present study (i.e. for equivalent CO_2 tensions hearts in the present study are exposed to a lower pH), but this is largely due to the effect of temperature on pH (0.016 pH°C^{-1}). When ocean pout

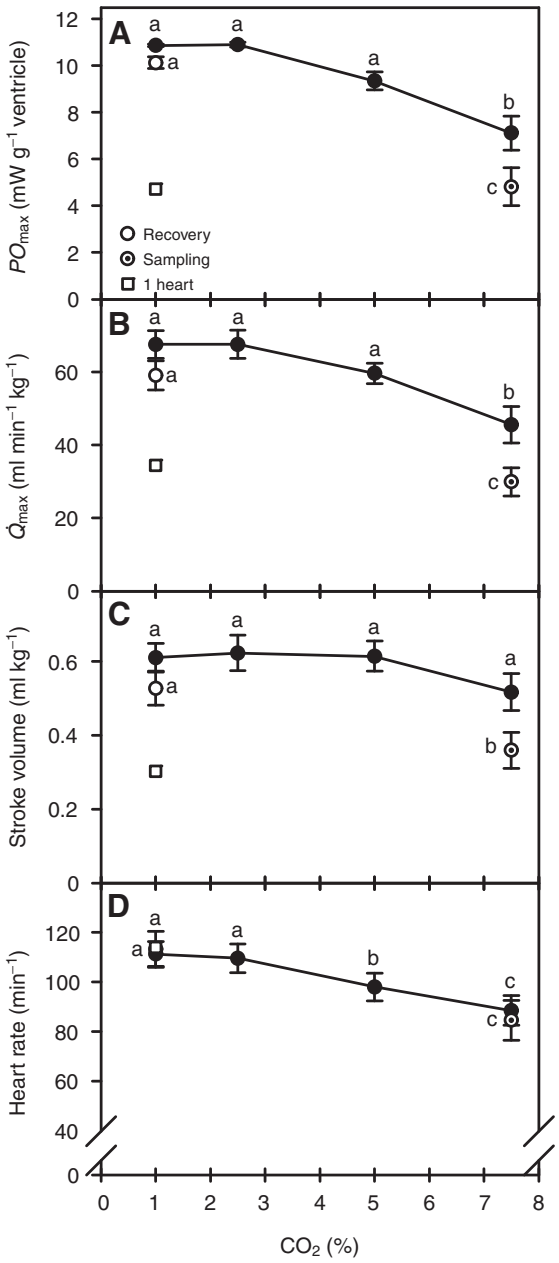

Fig. 3. The effect of hypercapnia on (A) maximum cardiac power generation, PO_{max}, (B) maximum cardiac output, $\dot{Q}_{max}$, (C) cardiac stroke volume and (D) heart rate of *in situ* perfused *Pterygoplichthys pardalis* hearts at 27.0±0.2°C (*N*=5). Hearts were sequentially exposed to CO_2 tensions of 1.0% (control), 2.5%, 5.0% and 7.5% before being returned to 1.0% CO_2 to assess recovery (open circles). Hearts were then re-exposed to 7.5% CO_2 to permit sampling for intracellular pH determinations (marked circles). One heart failed to recover from exposure to extreme hypercapnia and thus has been presented separately (squares).

hearts at 10°C were perfused with saline containing 2% CO_2, the pH of the perfusate was 7.4 and they exhibited a 20% decline in cardiac performance (Farrell et al., 1983). Conversely, performance of *P. pardalis* hearts at 27°C perfused with an equivalent CO_2 tension but at a lower pH (pH 7.2) did not differ from normocapnic values (present study). Thus, cardiac function is clearly maintained in *P. pardalis* at CO_2 tensions that greatly reduce cardiac function in seemingly acidosis-sensitive fishes.

Eels and sturgeons are known to tolerate severely hypercarbic water (10% and 3% CO_2, respectively) for short durations (several hours) with no decrease in cardiac output *in vivo* (Crocker et al., 2000; McKenzie et al., 2002). However, equivalent *in situ* studies of heart function have not been conducted for direct comparison with the present work. In addition, it is difficult to extrapolate the results of previous *in vitro* findings on isolated cardiac muscles to the present work. Nevertheless, if one assumes that isometric cardiac force generation is analogous to maximum power generation then *P. pardalis* would be slightly less acidosis tolerant than the turtle (*Trachemys scripta*, formerly *Pseudemys scripta*). *In vitro* turtle myocardium preparations showed no significant decrease in contractile force after 15–30 min at a similar level of respiratory acidosis (Poupa et al., 1978; Gesser and Jorgensen, 1982) as that which resulted in a ~35% decrease in performance in the present study. Thus, although it appears that *P. pardalis* is remarkably acidosis tolerant for a teleost, and falls within the range of the few other acidosis-tolerant vertebrates, caution should be used when comparing air and water breathers since the former have higher *in vivo* resting blood CO_2 tensions and lower blood pH.

In the absence of published values for *in vivo* cardiovascular performance of *P. pardalis*, we compare our values with those reported for other teleost species and consider the effect of temperature on cardiac output ($\dot{Q}$ increases with temperature). Given the rather sedentary nature of *P. pardalis*, maximum cardiac performance under routine (normocapnic) conditions ($\dot{Q}_{max}$=62.8±4.1 ml min^{-1} kg^{-1}; PO_{max}=10.5±0.3 mW g^{-1} ventricle) appears impressive compared with rainbow trout at 18°C, where $\dot{Q}_{max}$ ranges from 54–78 ml min^{-1} kg^{-1} and PO_{max} ranges from 5.9–9.3 mW g^{-1} ventricle (Keen and Farrell, 1994; Farrell et al., 1996; Hanson and Farrell, 2007). Although maximum cardiac power output of *P. pardalis* under normocapnic conditions was comparable to that seen in rainbow trout, the maximum pressure generation was not (~4 kPa *versus* 6–8 kPa). This suggests that *P. pardalis* probably possesses a much lower arterial blood pressure. Maximum V_S of *P. pardalis* (0.55±0.05 ml kg^{-1}) is also lower than that of other teleosts where V_S ranges from 0.8–1.1 ml kg^{-1} (Farrell et al., 1986; Mendonça et al., 2007), as is its relative ventricular mass (0.03% *versus* ~0.07% in salmonids). If V_S is expressed per gram of ventricle (2.09±0.10 ml g^{-1} ventricle), it is similar to that found in another benthic species, winter flounder (2.3 ml g^{-1} ventricle) and greater than that of more active species [rainbow trout ~1.2 ml g^{-1} ventricle (Farrell et al., 1986); Atlantic salmon, 1.4 ml g^{-1} ventricle, Atlantic cod, 1.7 ml g^{-1} ventricle (Mendonça et al., 2007)]. In addition to the present results, a previous study (Mendonça et al., 2007) suggests that winter flounder also has a comparatively low arterial blood pressure. These sorts of comparisons lend some support to the possibility that higher relative ventricular mass in active fishes is more important for pressure generation than for control of stroke volume (Gamperl and Farrell, 2004). Our heart rates (~110 min^{-1}) are higher than those recorded previously (76 min^{-1}) in resting individuals of this species (MacCormack et al., 2003a). We attribute the majority of this discrepancy to the removal of vagal cholinergic tone in the present experiment. Previous studies have found cholinergic tone to be exceptionally high in air breathing fishes (Sundin et al., 1999; McKenzie et al., 2007) and thus the removal of cholinergic control can have dramatic effects on heart rate. For example, administration of a cholinergic antagonist caused heart rate to nearly triple in the facultative air breathing jeju (*Hoplerythrinus unitaeniatus*) (McKenzie et al., 2007). An additional explanation is that some of the increase in heart rate may be due to adrenergic stimulation. Tonic catecholamine concentrations are

Table 1. The effect of hypercapnia on *in situ* and *in vitro* cardiac performance in fish

Species	Temp (°C)	ΔCO_2	Perfusate pH†	Percent change from control: PO_{max} (mW g^{-1} ventricle)	$\dot{Q}_{max}$ (ml min^{-1} kg^{-1})	f_H (beats min^{-1})	V_S ml kg^{-1}	Reference
Pterygoplichthys pardalis	27	+1.5%	7.56	0	0	–2	0	Present study (*in situ*)
		+4.0%	7.23	–14	–12	–12	0	
		+6.0%	7.10	–35	–33	–21	–15	
Oncorhynchus mykiss	10	+1.3%	7.4	–12	–8	–11	0	Farrell et al., 1986 (*in situ*)
Hemitripterus americanus	10	+1.5%	7.4	–14	–12	–8	–2	Farrell et al., 1983 (*in situ*)
Macrozoarces americanus	10	+1.5%	7.4	–20	–18	–10	–10	Farrell et al., 1983 (*in situ*)
*Hemitripterus americanus**	10	+1.0%	6.8–7.0	–50	–	Paced	–	Turner and Driedzic, 1980 (*in vitro*)
*Macrozoarces americanus**	10	+1.0%	6.8–7.0	–58	–	Paced	–	Turner and Driedzic, 1980 (*in vitro*)

*Values reached after 15 minutes of exposure to hypercapnia, no adrenaline in perfusate.
†Initial pH ranged from 7.8–7.9 between studies, initial CO_2 was between 0%–1%.
f_H, heart rate; PO_{max}, maximum cardiac power output; $\dot{Q}_{max}$, maximum cardiac output; V_S, stroke volume.

currently unknown for *P. pardalis* and thus preliminary experiments were conducted to determine the appropriate level of adrenergic stimulation. These experiments revealed that high levels of adrenaline (500 nmol l^{-1}) were necessary to ensure a stable preparation.

When perfused hearts were exposed to the most severe hypercapnia (7.6 kPa; 7.5% CO_2) both $\dot{Q}_{max}$ and PO_{max} decreased by ~35%. The reduction in both measures of cardiac performance appears to be mainly due to hypercapnic bradycardia (23 beats min^{-1} at $\dot{Q}_{max}$ and 19 beats min^{-1} at PO_{max}), although in the case of PO_{max} the ~20% reduction in f_H (19 beats min^{-1}) was not statistically significant. A similar degree of hypercapnic bradycardia has been well documented in other species (Farrell et al., 1983; Farrell et al., 1986; McKendry and Perry, 2001). The current study used CO_2 aeration to induce an extracellular acidosis, however, the acidosis did not manifest intracellularly. This suggests that preferential regulation of myocardial pH_i is occurring in this *in situ* preparation, as CO_2 tensions within the myocardium are expected to equilibrate with the perfusate quite rapidly (Gesser and Jorgensen, 1982). Previous studies on the CO_2-sensitive rainbow trout demonstrated that during a hypercapnic acidosis both *in vivo* and *in situ* myocardial pH_i fell within 3 h (Farrell and Milligan, 1986; Wood and LeMoigne, 1991).

Interestingly, in *P. pardalis* myocardial pH_i is regulated both *in situ* (present study) and *in vivo* (Brauner et al., 2004). Robust tissue pH regulation has been identified in only a few other fishes, for example, the white sturgeon, *Acipenser transmontanus* (Brauner and Baker, 2009), and the facultative air breather, the marbled swamp eel, *Synbranchus marmoratus* (Heisler, 1982). In both of these species, intrinsic buffering was not great enough to account for pH_i protection, and therefore, active cellular pH regulation was hypothesized to be driving pH_i compensation. Trans-membrane cellular pH_i regulation capacity could explain the incongruity between the findings in rainbow trout and the armoured catfish. Elucidation of these mechanisms will have to await further study.

This study is the first to comprehensively examine cardiac function in a CO_2-tolerant teleost. Our results reveal that the heart of *P. pardalis* possesses a remarkable ability to both tolerate and perform maximally in the face of a severe hypercapnic acidosis; a situation that freshwater tropical fish may experience in their natural environment (Ultsch, 1996), and a condition that also occurs when a facultative air-breathing fish breathes air during exposure to hypoxia (Heisler, 1982). Our results also imply a role for pH_i protection in maintaining heart function at high CO_2 tensions, consistent with our hypothesis that CO_2 tolerance in fishes is associated with preferential pH_i regulation; although this regulation is not sufficient to ultimately prevent loss of cardiac function. Recent studies have suggested that *P. pardalis* is better able to regulate myocardial intracellular calcium (MacCormack et al., 2003b) than more acidosis-sensitive species. Intracellular calcium levels and myofilament calcium sensitivity play a vital role in determining myocardial contractility. In addition, the positive inotropic effects of intracellular calcium are thought to counteract the negative inotropic effects of hypercapnic acidosis. Consequently, intracellular calcium handling has been implicated in the restoration of force and contractility during hypercapnic acidosis, however, little is currently known about the mechanistic basis for this. If improved intracellular Ca^{2+} handling is involved in alleviating the effects of hypercapnia, future exploration of this topic may reveal the mechanisms responsible for the remarkable hypercapnic tolerance of *P. pardalis*.

List of abbreviations

AD	adrenaline
f_H	heart rate
pH_e	extracellular pH
pH_i	intracellular pH
P_{in}	input pressure
P_{out}	output pressure
PO	cardiac power output
PO_{max}	maximum cardiac power output
$\dot{Q}$	cardiac output
$\dot{Q}_{max}$	maximum cardiac output
V_S	stroke volume

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