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RESEARCH ARTICLE

Acid–base regulation in the air-breathing swamp eel (*Monopterus albus*) at different temperatures

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ABSTRACT

Vertebrates reduce arterial blood pH (pHa) when body temperature increases. In water breathers, this response occurs primarily by reducing plasma HCO_3^- levels with small changes in the partial pressure of CO_2 (P_{CO_2}). In contrast, air breathers mediate the decrease in pHa by increasing arterial P_{CO_2} (Pa_{CO_2}) at constant plasma HCO_3^- by reducing lung ventilation relative to metabolic CO_2 production. Much less is known about bimodal breathers, which utilize both water and air. Here, we characterized the influence of temperature on arterial acid–base balance and intracellular pH (pH_i) in the bimodal-breathing swamp eel, *Monopterus albus*. This teleost uses the buccopharyngeal cavity for gas exchange and has very reduced gills. When exposed to ecologically relevant temperatures (20, 25, 30 and 35°C) for 24 and 48 h, pHa decreased by −0.025 pH units (U) °C^{-1} in association with an increase in Pa_{CO_2}, but without changes in plasma [HCO_3^-]. pH_i was also reduced with increased temperature. The slope of pH_i of liver and muscle was −0.014 and −0.019 U °C^{-1}, while the heart muscle showed a smaller reduction (−0.008 U °C^{-1}). When exposed to hypercapnia (7 or 14 mmHg) at either 25 or 35°C, *M. albus* elevated plasma [HCO_3^-] and therefore seemed to defend the new pHa set-point, demonstrating an adjusted control of acid–base balance with temperature. Overall, the effects of temperature on acid–base balance in *M. albus* resemble those in air-breathing amniotes, and we discuss the possibility that this pattern of acid–base balance results from a progressive transition in CO_2 excretion from water to air as temperature rises.

KEY WORDS: P_{CO_2}, Bimodal breathing, Blood gases, Intracellular pH

INTRODUCTION

All animals, with a few notable exceptions, decrease the pH of their bodily fluids as temperature increases (e.g. Truchot, 1987; Ultsch and Jackson, 1996; Stinner and Hartzler, 2000; Burton, 2002; Wang and Jackson, 2016). In aquatic ectothermic vertebrates, the reduction in blood pH is primarily associated with a reduction in the plasma HCO_3^- concentration at constant partial pressure of CO_2 (P_{CO_2}), whilst air-breathing vertebrates reduce blood pH by increasing P_{CO_2} through a reduction in ventilation relative to metabolic CO_2 production (Randall and Cameron, 1973; Larry, 1979; Austin et al., 1927; Smatresk and Cameron, 1982; Cameron and Kormanik, 1982; Boutilier et al., 1987; Amin-Naves et al., 2004). This marked difference in strategy probably stems from water breathers being obliged to maintain high ventilation rates because of the low solubility of oxygen in water, whilst air breathers are endowed with the luxury of modulating acid status through ventilatory adjustments (Rahn, 1966). As an obvious benefit, the respiratory modulation of acid–base status obviates the need for transepithelial ion exchange and the associated osmotic disturbances that ensue (Austin et al., 1927; Burton, 2002).

While differences between water and air breathers remain a cherished topic among comparative physiologists, relatively little is known about the influence of temperature on acid–base status in bimodal breathers (e.g. air-breathing fishes that utilize both air and water for gas exchange). The transition from water to air breathing is associated with an elevation of blood CO_2 levels due to the reduced ventilation in air relative to water (Rahn, 1966). Consequently, many air-breathing fish have much higher arterial P_{CO_2} (Pa_{CO_2}) levels than water-breathing fish (e.g. Cameron and Wood, 1978; Shartau and Brauner, 2014). This has implications for the influence of temperature on acid–base status because an increase in temperature is expected to increase the reliance on air breathing as metabolism rises ('the Q_{10} effect'). Therefore, an increase in temperature is likely to induce a passive rise in Pa_{CO_2} in bimodal breathers as the partitioning of gas exchange shifts from water to air. Indeed, two studies on the facultative air-breathing freshwater gar (*Lepisosteus oseus* and *Lepisosteus oculatus*) have demonstrated a clear and reversible elevation of Pa_{CO_2} with an increase in temperature (Rahn et al., 1971; Smatresk and Cameron, 1982). Further, when forced to employ air breathing through exposure to hypoxic water, the facultative air breather *Pangasianodon hypophthalmus* also exhibits an increase in Pa_{CO_2} with an increase in temperature (Damsgaard et al., 2018).

The purpose of the present study was to extend our understanding of the influence of temperature on acid–base regulation in an air-breathing teleost, *Monopterus albus* (Asian swamp eel, Zuiew 1793). *Monopterus albus* is an obligate air-breathing teleost that thrives in muddy ponds, swamps and other stagnant freshwater bodies in tropical Southeast Asia. The metabolic rate of *M. albus* conforms to a normal Q_{10} of around 2 (Lefevre et al., 2016). The gills of *M. albus* are greatly reduced and have a low capacity for gas exchange. Instead, oxygen uptake occurs primarily over the highly vascularized epithelium of the buccopharyngeal cavity and oesophagus, and is facilitated by a very high blood–oxygen affinity (Shih, 1940; Rainboth, 1996; Iversen et al., 2013; Damsgaard et al., 2014). Current climate models have suggested that temperature in the Mekong area might increase from 27°C to as much as 33°C within the coming century (MRC, 2009). As an air breather with gills that possess a modest capacity for gas exchange, *M. albus* has a rather high Pa_{CO_2} (10–20 mmHg; Damsgaard et al.,

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2014) and has been reported to tolerate a broad thermal range (8–40°C; Shafland et al., 2009; Lefevre et al., 2016). However, the effects of temperature on intracellular pH (pH_i) changes are not known.

Our study had three specific aims. Firstly, we wished to establish how arterial acid–base balance and plasma ion concentrations were affected by temperatures within the range of 20 to 35°C. These measurements were performed on chronically cannulated fish exposed to a progressive rise in temperature. In addition, although most proteins and their biological functions reside within the cells, little is known about the influence of temperature on intracellular acid–base status. A second aim of our study was therefore to measure pH_i in separate groups of fish kept at four temperatures (20, 25, 30 and 35°C). Whilst these previous two objectives describe the influence of temperature, our third aim was to establish whether the reduction in arterial pH (pHa) with temperature is indeed regulated. To address this question, we exposed *M. albus* to two levels of hypercapnia (7 and 14 mmHg in water and air) at both 25 and 35°C to investigate whether they defend pHa at a given temperature by metabolic compensation in response to the induced mild respiratory acidosis.

MATERIALS AND METHODS

Experimental animals

Asian swamp eels (*Monopterus albus*) of both sexes and a mean body mass of 342±9 g were purchased from a commercial farm in the Mekong Delta in southern Vietnam and kept at the University of Can Tho for 3–6 weeks before the experiments were performed. During this period, the eels were kept in aerated 300 l tanks maintained at 27°C and provided with stiff plastic tape as shelter. Animal experiments were conducted in accordance with European Union guidelines.

Surgery and catheterization of the dorsal aorta

The swamp eels were anaesthetized by submergence in a benzocaine solution (225 mg l^{-1}) for 20–30 min until they stopped spontaneous movement, and were then transferred to an operating table in a supine position. A 2–4 cm incision in the abdominal cavity provided access to the coeliac artery for cannulation with polyethylene tubing (PE50) containing heparinized saline (50 IU per 1 ml saline). The incision was closed with stitches and the catheter was secured to the skin. The surgery lasted <20 min and eels were allowed to recover for 24 h individually in small tanks with aerated water at room temperature (27°C).

Experimental design

Series 1: effects of temperature on arterial acid–base status

We measured arterial acid–base status and plasma ion concentration in six eels as temperature was increased progressively from 20 to 25, 30 and 35°C. Each temperature was maintained for 48 h whereupon fish were sampled, and the temperature was gradually increased to the next target value over the subsequent 2–3 h. Sampling consisted of drawing 1 ml of arterial blood from the dorsal aorta at 24 and 48 h for each temperature level, which was analysed immediately for pHa, Pa_{CO_2}, total CO_2 concentration of the plasma, haemoglobin concentration ([Hb]) and haematocrit (Hct), while plasma samples were frozen for subsequent measurement of plasma ion concentration and osmolality.

Series 2: effects of temperature on pH_i

We measured pH_i of liver, heart and muscle at each of the four temperatures (20, 25, 30 and 35°C). For each temperature, we performed measurements on six individuals that had been maintained at the relevant temperature for 24 h. None of these fish were instrumented with catheters and they had therefore not been anaesthetized prior to sampling. The eels were killed quickly by a sharp blow to the head, allowing rapid tissue removal (2–4 min) through an incision. All tissue samples were wrapped in aluminium foil before immersion in liquid N_2, after which they were held at −80°C until measurements were made.

Series 3: effects of temperature on the metabolic compensation of arterial pH

After recovering from surgery, eels were maintained at either 25 or 35°C in aerated normoxic water. A 1 ml arterial blood sample (control) was drawn, and the eels were then exposed to either

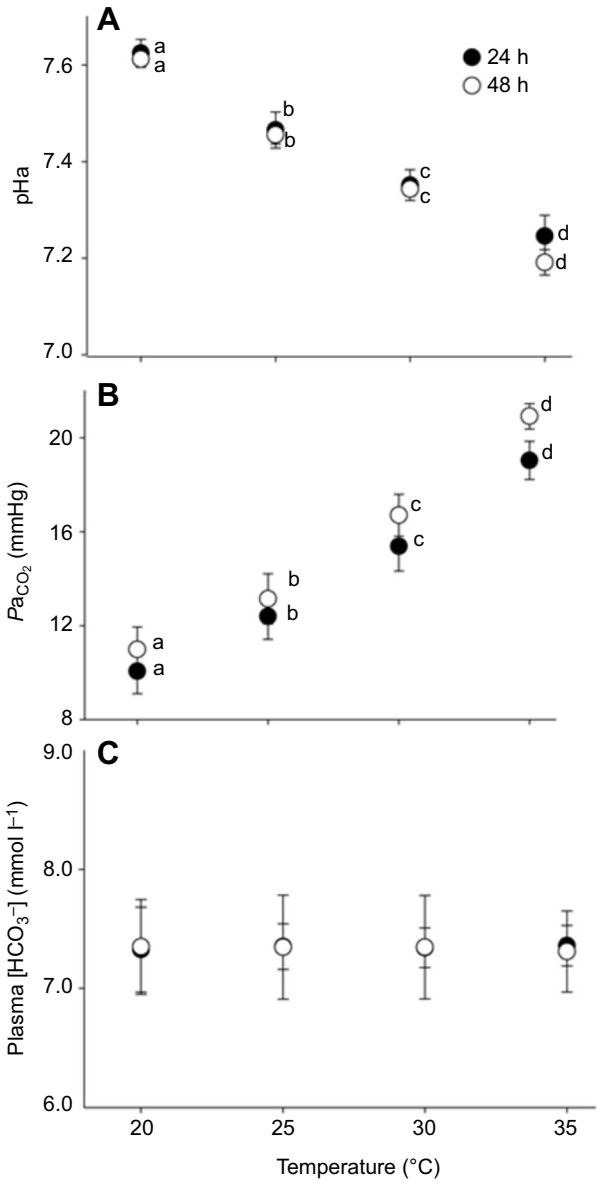

Fig. 1. Effects of elevated temperature on arterial blood acid–base status. Arterial pH (pHa; A), arterial partial pressure of carbon dioxide (Pa_{CO_2}; B) and plasma [HCO_3^-] (C) in cannulated *Monopterus albus* held at different temperatures for 24 and 48 h. Within each panel, letters that differ indicate statistically significant differences (P<0.05). A one-way ANOVA was used for comparison between different temperatures at a given time. Values are means±s.e.m. (N=6).

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Table 1. Effects of elevated temperature on arterial plasma ion concentration

	[Na^+] (mmol l^{-1})		[K^+] (mmol l^{-1})		[Cl^-] (mmol l^{-1})		Osmolality (mOsm)	
Temperature (°C)	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
20	123.5±1.3^a	123.3±1.2^a	2.8±0.2^a	2.9±0.2^a	111.1±3.1^a	112.0±2.2^a	287.3±3.1^a	290.8±3.8^a
25	123.1±1.8^a	122.3±1.8^a	2.9±0.2^a	2.8±0.1^a	111.2±4.0^a	110.8±2.5^a	290.0±2.5^a	287.2±2.5^a
30	124.4±1.6^a	122.7±2.2^a	2.9±0.2^a	2.8±0.2^a	111.8±2.0^a	112.8±2.1^a	287.2±3.5^a	288.0±5.2^a
35	123.3±1.7^a	122.4±1.3^a	3.3±0.1^a	3.5±0.1^b	112.8±3.5^a	112.0±4.3^a	287.0±2.3^a	288.1±3^a

Arterial plasma Na^+, K^+ and Cl^- concentrations and osmolality of cannulated *Monopterus albus* following 24 and 48 h at 20, 25, 30 and 35°C. Letters that differ within a column indicate statistically significant differences (P<0.05). A one-way ANOVA was used for comparison between different temperatures at a given time. Values are means±s.e.m. (N=6).

normocapnia or hypercapnia (in both water and air simultaneously) of 7 or 14 mmHg CO_2 for 72 h (six fish in each group). During these exposures, a 1 ml arterial blood sample was taken at 24, 48 and 72 h. At both temperatures, we included an untreated control group that was maintained normocapnic in both air and water for the duration of the experiment. All blood samples were then analysed for arterial acid–base status and plasma ion concentration.

Analytical methods

pHa and Pa_{CO_2} were measured with CG3+ cartridges on an iSTAT hand-held blood gas analyser where values were temperature compensated to the fish temperature, using the equation from the iSTAT manual (i-STAT Corporation, Princeton, NJ, USA) (Harter et al., 2014; Damsgaard et al., 2015). The total CO_2 concentration in the plasma was measured according to Cameron (1971), allowing for bicarbonate concentration in the plasma ([HCO_3^-]) to be calculated by subtraction of dissolved CO_2 ($Pa_{CO_2}\times\alpha_{CO_2}$), using an appropriate carbon dioxide solubility (α_{CO_2}) (Boutilier et al., 1985). [Hb] was measured spectrophotometrically at 540 nm after conversion to cyanmethaemoglobin using Drabkin's reagent, whilst Hct was determined as the fraction of packed red cells following centrifugation at 14,500 ***g*** (radius 9 cm) for 3 min. Plasma chloride concentration ([Cl^-]) was determined by titration using a Sherwood chloride analyser (model 926S MK II, Sherwood Scientific Ltd, Cambridge, UK), while sodium and potassium concentrations were measured by flame photometry (model 420, Sherwood). Total osmolality was measured on a Fiske Model 210 Micro Osmometer (Advanced Instruments™ Fiske™ 210 Micro-Sample Osmometer, Norwood, MA, USA).

To measure pH_i, heart, liver and muscle samples were ground to a fine powder under liquid N_2 (Pörtner et al., 1990; Brauner et al., 2004; Baker et al., 2009a,b). Approximately 0.1 g of the powder was placed in a 1.5 ml Eppendorf tube containing 0.8 ml of metabolic inhibitor cocktail [150 mmol l^{-1} potassium fluoride and 6 mmol l^{-1} nitrilotriacetic sodium (Na_2NTA)]. The solution was immediately vortexed for 30 s and centrifuged at 3000 ***g*** for 45 s. Then the pH of the supernatant (0.2 ml) was measured with a Mettler Toledo pH electrode. In all cases, the pH electrode was calibrated and

Table 2. Effects of elevated temperature on arterial blood haemoglobin concentration and haematocrit

	[Hb] (mmol l^{-1})		Hct (%)	
Temperature (°C)	24 h	48 h	24 h	48 h
20	8.1±0.1^a	8.4±0.1^a	51.9±0.2^a	52.5±0.3^a
25	8.3±0.1^a	8.3±0.1^a	52.2±0.3^a	52.7±0.3^a
30	8.4±0.1^a	8.3±0.1^a	53.7±0.3^a	53.9±0.3^a
35	8.8±0.1^a	8.8±0.1^a	54.4±0.2^a	55.4±0.2^a

Arterial blood haemoglobin concentration ([Hb]) and haematocrit (Hct) in cannulated *M. albus* following 24 h and 48 h at 20, 25, 30 and 35°C. A two-way ANOVA was used for comparison between different temperatures in a given time; there were no statistically significant differences. Values are means ±s.e.m. (N=6).

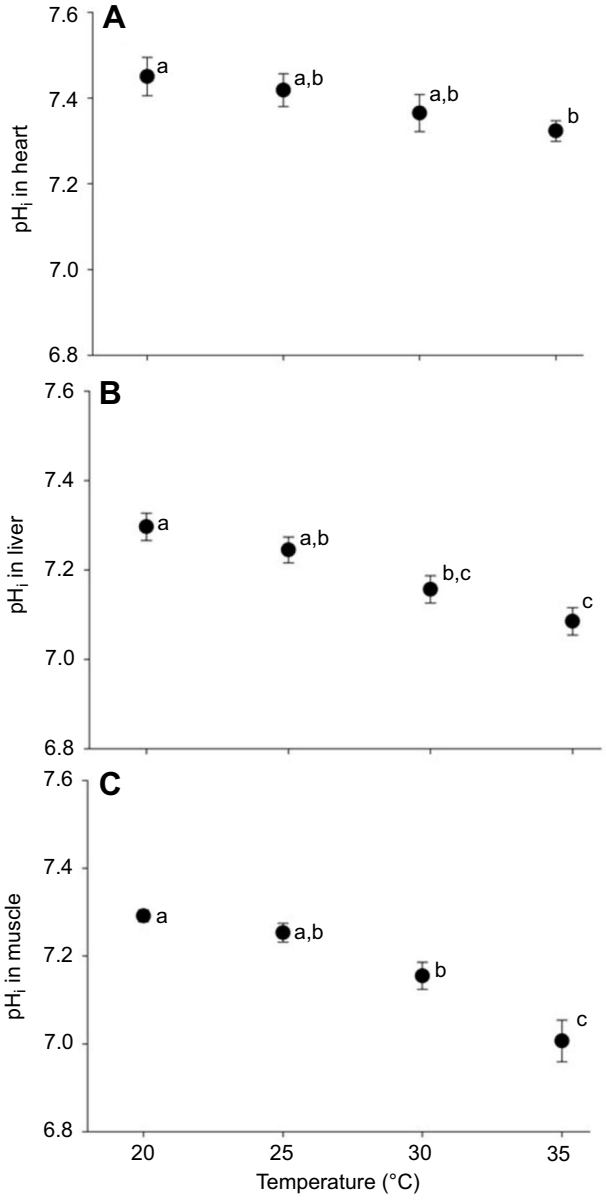

Fig. 2. Effects of elevated temperature on intracellular pH. Intracellular pH (pH_i) in heart (A), liver (B) and muscle (C) of *M. albus* held at 20, 25, 30 or 35°C for 24 h. Letters that differ within a panel indicate statistically significant differences (P<0.05). A one-way ANOVA was used for comparison between different temperatures at a given time. Values are means±s.e.m. (N=5).

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maintained at the same temperature as the fish, and the supernatant was measured at this temperature.

Statistical analysis

A two-way ANOVA for repeated measures (a multivariate analysis of covariance) was used to test for an overall effect of temperature and hypercapnia on blood parameters followed by Duncan's *post hoc* test to identify difference amongst means. To evaluate the isolated effects of temperature, a one-way ANOVA was used. Data are presented as means±s.e.m. A probability (P) value of less than 0.05 was considered significant. All statistical analyses were performed using PASW Statistic 18.

RESULTS

Series 1: effects of temperature on arterial acid–base status

Arterial acid–base status in response to the progressive rise in temperature from 20 to 35°C is shown in Fig. 1; plasma ion concentrations and osmolality are shown in Table 1. The rise in temperature caused a virtually linear reduction in pHa with a slope of 0.025±0.001 and 0.028±0.001 U °C^{-1} after 24 and 48 h at each temperature, respectively. These slopes did not differ significantly. The Pa_{CO_2} rose progressively from 10.1±1.0 mmHg at 20°C to 19.1±1.0 mmHg at 35°C (P<0.05). Plasma [HCO_3^-] and ion concentrations were unaffected by temperature (Table 1). [Hb] and Hct were not affected by temperature (Table 2).

Series 2: effects of temperature on pH_i

Increased temperature also led to a reduction in pH_i (Fig. 2). In cardiac tissue, pH_i decreased from 7.45±0.04 (20°C) to 7.36±0.04 (30°C) (P>0.05), with a stronger and significant fall to 7.32±0.02 at 35°C (P<0.05). Over the same temperature range, liver tissue pH_i decreased rapidly from 7.29±0.03 (20°C) to 7.08±0.03 (35°C) (P<0.05). The skeletal muscle showed the lowest pH_i values, falling significantly from 7.29±0.02 to 7.00±0.04 (P<0.05) over the same temperature range.

Series 3: effects of temperature on the metabolic compensation of arterial pH

The regulation of arterial acid–base status at the two levels of hypercapnia (7 and 14 mmHg in water and air) at 25 and 35°C is shown in Fig. 3 in comparison with the normocapnic controls. At 25°C, both levels of hypercapnia (7 and 14 mmHg) led to an elevation of Pa_{CO_2}, but pHa remained unaffected at 72 h because of an elevation of HCO_3^- in the plasma. The effects of hypercapnia were less pronounced at 35°C, where Pa_{CO_2} was higher and pHa lower than at 25°C. Nevertheless, in response to 14 mmHg CO_2, there was still a significant rise in plasma [HCO_3^-] whilst pHa remained unaffected. Thus, while exposure to increased P_{CO_2} had little effect on pHa (P=0.944), increased temperature caused a significant reduction in pHa (P=0.00001). Further, there was a significant effect of the combination of temperature and P_{CO_2} (P=0.012). Plasma ion concentrations and osmolality remained unaffected by hypercapnia at either temperature (Table 3). Additionally, [Hb] and Hct did not change during hypercapnia (Table 4).

DISCUSSION

Monopterus albus exhibited an archetypical reduction in plasma pH and a reduction in pH_i of three major tissue types with an increase in temperature. The magnitude of the pHa reduction was larger than reported for most vertebrates, but the change in pH over the change in temperature ($\Delta pH/\Delta T$) in *M. albus* is not exceptional (Malan et al., 1976; Heisler et al., 1976; Moalli et al., 1981; Cameron and Kormanik, 1982; Walsh and Moon, 1982; Boutilier et al., 1987; Amin-Naves et al., 2004; Fobian et al., 2014). The responses to temperature were relatively fast and appeared to be complete within 24 h as there were no changes from 24 to 48 h after the change in temperature, but we cannot exclude short-term effects (hours) upon temperature changes.

The pattern of acid–base regulation in *M. albus* differed markedly from the typical water-breathing teleost pattern because the

Table 3. Combined effects of temperature and hypercapnia on arterial plasma ion concentration

	0 h	24 h	48 h	72 h
Na^+ (mmol l^{-1})				
25°C normocapnia	139.8±1.6	140.2±1.5	140.7±2.4	139.3±1.3
25°C 7 mmHg CO_2	138.7±1.2	138.5±1	138.2±1.1	138.2±1.0
25°C 14 mmHg CO_2	139.6±1.7	138.8±1.8	138.5±1.4	137.9±1.4
35°C normocapnia	139.5±0.9	139.6±1	139.4±1.0	139.3±1.5
35°C 7 mmHg CO_2	140.7±1.4	139.6±2.1	138.4±1.2	137.0±0.9
35°C 14 mmHg CO_2	138.9±1.4	137.1±1.4	136.8±1.2	136.2±1.0
K^+ (mmol l^{-1})				
25°C normocapnia	2.9±0.4	2.9±0.2	3.0±0.4	3.0±0.4
25°C 7 mmHg CO_2	3.0±0.2	3.1±0.1	3.4±0.3	3.6±0.3
25°C 14 mmHg CO_2	3.4±0.4	3.3±0.3	3.4±0.2	3.4±0.2
35°C normocapnia	3.2±0.4	3.1±0.2	3.2±0.4	3.5±0.3
35°C 7 mmHg CO_2	3.2±0.3	3.5±0.6	3.4±0.2	3.3±0.6
35°C 14 mmHg CO_2	2.5±0.6	3.4±0.3	3.4±0.3	3.5±0.4
Cl^- (mmol l^{-1})				
25°C normocapnia	112.4±3.8	111.6±1.9	112.4±2.0	112.4±2.3
25°C 7 mmHg CO_2	112.0±3.6	111.2±1.2	112.1±1.6	111.9±2.4
25°C 14 mmHg CO_2	111.9±0.8	110.2±1.5	109.9±0.4	109.7±0.9
35°C normocapnia	112.0±1.4	112.2±2.2	112.3±2.3	111.8±2.0
35°C 7 mmHg CO_2	112.0±2.4	111.4±1.2	110.9±2.0	110.8±1.2
35°C 14 mmHg CO_2	112.2±2.2	110.6±1.4	109.7±0.7	109.7±1.1
Osmolality (mOsm)				
25°C normocapnia	288.7±3.8	288.3±3.6	288.5±2.1	288.3±2.0
25°C 7 mmHg CO_2	288.5±3.3	288.1±3.3	288.3±3.1	288.1±1.5
25°C 14 mmHg CO_2	288.3±2.8	286.3±2.9	285.5±2.1	286.1±3.4
35°C normocapnia	289.6±1.0	289.5±1.2	289.3±1.0	289.5±1.3
35°C 7 mmHg CO_2	290.6±2.9	289.3±4.5	288.5±2.9	288.0±2.3
35°C 14 mmHg CO_2	288.6±2.9	286.3±2.7	286.1±2.2	286.1±3.3

Arterial plasma Na^+, K^+ and Cl^- concentration and osmolality of cannulated *M. albus* after exposure to normocapnia or a P_{CO_2} of 7 or 14 mmHg CO_2 at 25 or 35°C. Two-way ANOVA was used for comparison between different temperatures at a given time and data are means±s.e.m. (N=6).

Table 4. Combined effects of temperature and hypercapnia on arterial blood [Hb] and Hct

	0 h	24 h	48 h	72 h
[Hb] (mmol l^{-1})				
25°C normocapnia	8.5±0.8	8.3±0.4	8.5±0.7	8.2±0.5
25°C 7 mmHg CO_2	8.3±0.3	8.4±0.3	8.3±0.3	8.3±0.4
25°C 14 mmHg CO_2	8.4±0.2	8.3±0.2	8.4±0.2	8.3±0.2
35°C normocapnia	8.5±0.3	8.6±0.4	8.5±0.5	8.6±0.2
35°C 7 mmHg CO_2	8.4±0.3	8.6±0.4	8.6±0.3	8.7±0.2
35°C 14 mmHg CO_2	8.5±0.3	8.6±0.2	8.7±0.3	8.7±0.2
Hct (%)				
25°C normocapnia	49.6±1.5	49.5±1.3	49.4±0.7	49.5±1.6
25°C 7 mmHg CO_2	50.3±0.5	49.9±1.0	49.8±1.0	49.1±2
25°C 14 mmHg CO_2	50.5±0.4	50.0±0.4	49.6±0.5	49.2±0.4
35°C normocapnia	51.7±1.6	51.1±1	51.8±1	52.1±1.9
35°C 7 mmHg CO_2	52±1.4	51.0±1.3	51.4±1.3	51.4±1.8
35°C 14 mmHg CO_2	50.5±0.5	51±0.5	51. 5±0.7	51.8±0.7

Arterial blood [Hb] and Hct of cannulated *M. albus* after exposure to normocapnia, or a P_{CO_2} of 7 or 14 mmHg at 25 or 35°C. Two-way ANOVA was used for comparison between different temperatures at a given time and data are means±s.e.m. (N=6).

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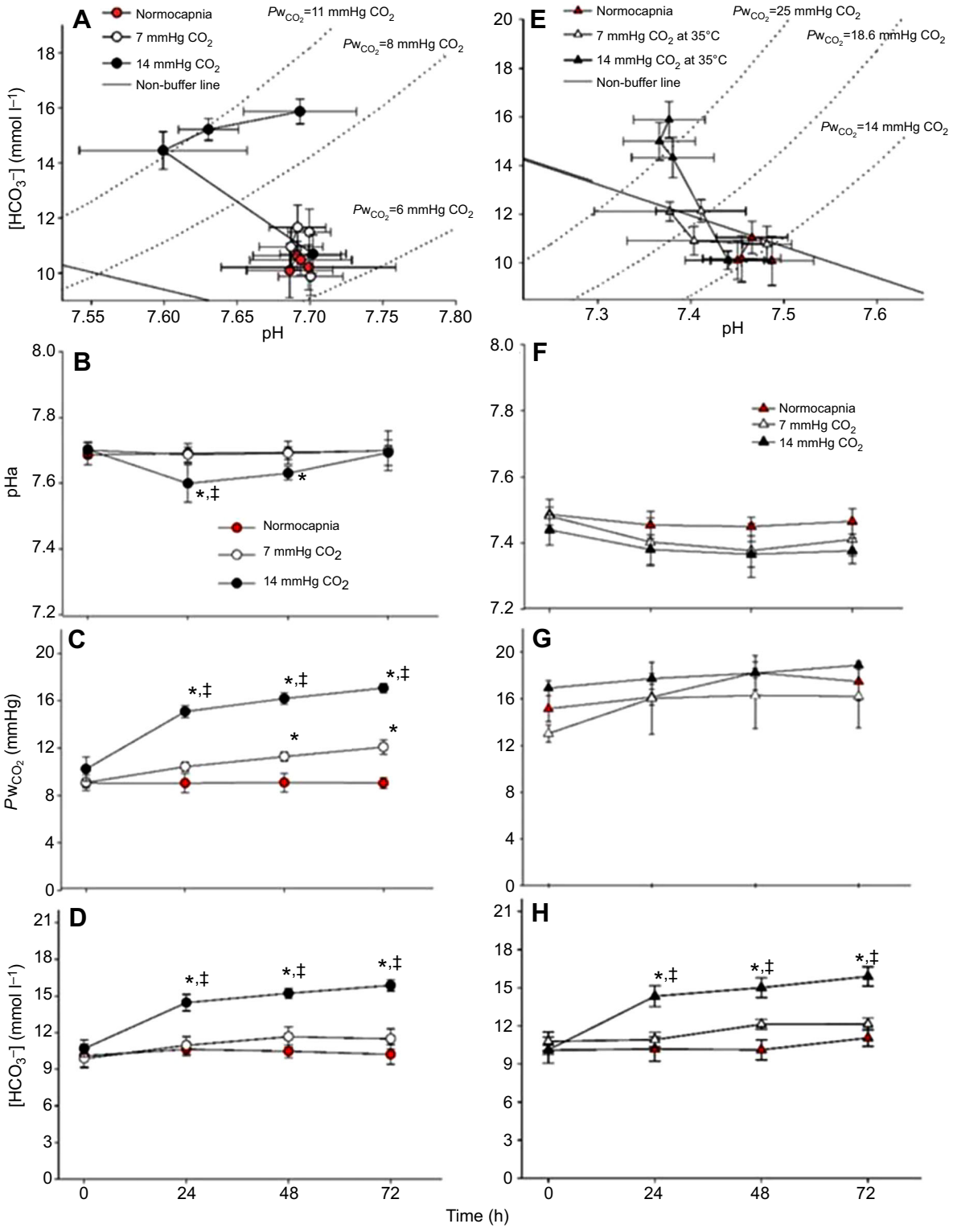

Fig. 3. Effects of temperature on the metabolic compensation of pHa. (A,E) Davenport diagram with CO_2 isopleths at Pa_{CO_2} upon exposure to 7 mmHg CO_2 and 14 mmHg CO_2 at 25°C (A) and 35°C (E). Pw_{CO_2}, partial pressure of CO_2 in water. pHa (B,F), partial pressure of CO_2 (P_{CO_2}; C,G) and [HCO_3^-] (D,H) after exposure to normocapnia, or a P_{CO_2} of 7 or 14 mmHg CO_2 at 25°C (A–C) or 35°C (D–F). Asterisks indicate statistically significant differences from day 0 at a given P_{CO_2} and double daggers indicate a statistically significant difference from normocapnia at the respective sampling time. A two-way ANOVA was used for comparison between different temperatures at a given time. Values are means±s.e.m. (N=6).

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Table 5. Comparison of extracellular and intracellular pH changes with temperature (ΔpH/Δ*T*) in fish, amphibians and reptiles for comparison with values obtained in this study

		ΔpH/Δ*T*				
Species	Temperature (°C)	Blood	White muscle	Heart	Red muscle	Liver
Frog (*Rana catesbeiana*)[1]	3.5–30	−0.0204	−0.0152			
Turtle (*Pseudemys scripta*)[2]	9–32	−0.021	−0.014			−0.023
Dogfish (*Scyloirhinus stellaris*)[3]	10–23	−0.0148	−0.0178	−0.0098	−0.0334	
American eel (*Aguilia rostrata*)[4]	5–20	−0.0076	−0.009	−0.0205	−0.0033	−0.0177
Channa catfish (*Ictalurus punctatus*)[5]	15–31	−0.0132	−0.015	−0.012	−0.018	
Anuran amphibians (*Xenopus laevis*)[6]	10–30	−0.017		−0.007	−0.017	
Anuran amphibians (*Bufo marinus*)[7]	10–30	−0.015		10.026	−0.023	
Swamp eel (*Monopterus albus*)[8]	**20–35**	**−0.025**	**−0.019**	**−0.0084**		**−0.014**

[1,2]Malan et al. (1976); [3]Heisler et al. (1976); [4]Walsh and Moon (1982); [5]Cameron and Kormanik (1982); [6,7]Boutilier et al. (1987); and [8]present study (bold).

reduction in pH was mediated entirely by an elevation of Pa_{CO_2} and plasma [HCO_3^-] remained unaffected. The influence of temperature on acid–base balance in *M. albus* therefore resembles the effect on air-breathing vertebrates where a fall in pHa with increased temperature is achieved through a reduction in ventilation relative to CO_2 production, which alleviates the need for transepithelial ion exchange. Consistent with this pattern, plasma ion concentrations remained unaffected by temperature in *M. albus*. This is the first clear report of this pattern in a teleost.

The pronounced rise in Pa_{CO_2} of *M. albus* with elevated temperature is larger than that reported for other teleosts, but the overall response appears to reflect a general trend amongst the few air-breathing fishes that have been studied to date. In the lobe-finned South American lungfish (*Lepidosiren paradoxa*), Pa_{CO_2} increases with temperature as a result of increased reliance on pulmonary gas exchange as metabolic CO_2 production rises (Amin-Naves et al., 2004). Similarly, in the non-teleost ray-finned air-breathing garfish (*Lepisosteus osseus*), Pa_{CO_2} is elevated during the higher summer temperatures compared with the colder winter months (Rahn et al., 1971). Within the teleost taxa, the facultative air-breathing striped catfish *Pangasianodon hypophthalmus* also elevates Pa_{CO_2} with temperature when forced to air breathe in hypoxic water (Damsgaard et al., 2018). However, in this case, the elevation in Pa_{CO_2} with elevated temperature is associated with a significant elevation of plasma [HCO_3^-], and Damsgaard et al. (2018) argued that the transfer of gas exchange from water to air is attended by a passive increase in Pa_{CO_2}, and the pH drop is fine-tuned by elevations in HCO_3^- through branchial ion exchange.

In *M. albus*, the acid–base changes in response to temperature resemble the classic pattern of tetrapods and lungfishes, where central chemoreception for CO_2 (and probably pH) in the cerebrospinal fluid plays a major role in reducing pulmonary ventilation relative to metabolic CO_2 production to elevate Pa_{CO_2} as temperature rises (e.g. Hitzig and Jackson, 1978; Jackson, 1989; Branco et al., 1993; Amin-Naves et al., 2004). However, as a teleost fish, it seems unlikely that *M. albus* is in possession of central CO_2 chemoreception (Milsom, 2010). Nevertheless, *M. albus* exhibits a vigorous ventilatory response to hypercapnia (M. Thomsen, M.B. and T.W., unpublished observation), and the putative role of peripheral chemoreceptors should be considered in future studies on the influence of temperature on the ventilatory regulation of acid–base balance in air-breathing fishes.

A simple explanation for the rise in Pa_{CO_2} and the associated reduction in pH in *M. albus* is that it is likely that the rise in metabolic CO_2 production with increased temperature imposes a transition from aquatic to aerial CO_2 excretion. In this scenario, the rise in Pa_{CO_2} can be seen as a simple passive consequence of the increased reliance on air breathing with increased temperature (Lefevre et al., 2016) and the associated retention of CO_2 (Rahn, 1966). Similar responses are characteristic of amphibians where gas exchange is partitioned between the lungs and skin (Wang et al., 1998a,b; Boutilier and Heisler, 1987), as well as in the lungless salamanders where the reduction in pHa with increased temperature is due to an elevation of Pa_{CO_2} because cutaneous conductance for CO_2 changes very little with temperature (Moalli et al., 1981).

Following this argument, it was pertinent to investigate whether the temperature-induced change in pHa constitutes a new regulated set-point. To address this question, we exposed *M. albus* to mild levels of hypercapnia at 20 and 30°C. The results clearly showed that *M. albus* responds to the induced respiratory acidosis with an increase in plasma [HCO_3^-] and thus regulates pHa to a temperature-specific value. There remains, therefore, little doubt that temperature changes the pHa set point in a tightly regulated manner in this species. Further, this experiment also demonstrates that this species possesses the necessary capacity for compensation of the extracellular respiratory acidosis. This is a common trait amongst water-breathing fishes, but has been found to be lacking in some air-breathing fishes (Shartau and Brauner, 2014), though not in others (Damsgaard et al., 2015; Gam et al., 2017).

The observation that the reduction in pHa is indeed regulated, however, provides little novel insight into the regulated variable. The magnitude of ΔpHa/Δ*T* in *M. albus* is consistent with the notion of maintaining constant protein (mainly imidazole) ionization (e.g. Reeves, 1972), but this obviously does not provide conclusive evidence for protein ionization being the regulated variable. Nevertheless, it is striking that plasma [HCO_3^-] remained constant across temperature, which, in combination with the lack of change in other extracellular ion concentrations, is consistent with the idea of constancy of protein ionization (Stewart, 1978). The biological significance of the reduction in pH with increased temperature is obviously of particular importance for the intracellular compartments where most protein function occurs (Malan et al., 1976; Reeves, 1977). Our measurements of pH_i from muscle, liver and heart indicate that the increase in temperature affected intracellular acid–base status, but the $\Delta pH_i/\Delta T$ values were lower than those for blood pH, with the heart being the least affected. These data contrast with other studies where the ΔpH/Δ*T* of the heart was greater than that of other organs (muscle, liver and brain) (Table 5). However, Cameron and Kormanik (1982) reported $\Delta pH_i/\Delta T$ to be lower in heart tissue than in blood in *Ictalurus punctatus*. Heisler et al. (1976) also reported differences amongst organs in dogfish and carp (Heisler, 1980) and the lower $\Delta pH_i/\Delta T$ in the cardiac tissue of *Monopterus* be may be associated with its very high levels of myoglobin in the ventricle (Damsgaard et al., 2014).

In conclusion, we demonstrate that *M. albus* reduces both pHa and pH_i as temperature increases. The reduction in pH clearly seems to

reflect a change in the regulated set-point. In contrast to water-breathing fishes, but in accordance with an emerging pattern amongst air-breathing fishes, the lowering of pH is accomplished by a rise in Pa_{CO_2} that may be a passive consequence of increased reliance on the air-breathing organ for CO_2 excretion, as increased temperature stimulates metabolic CO_2 production. It remains to be investigated, however, whether CO_2/pH-sensitive chemoreceptors also play a role in this response; clearly, this is an area for further research.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: P.V.T., C.J.B., M.B., T.W.; Methodology: P.V.T., N.T.P., C.J.B., D.T.T.H., A.T.W., G.T.K., J.C., M.B., T.W.; Writing - original draft: P.V.T., T.W.; Writing - review & editing: N.T.P., C.J.B., D.T.T.H., A.T.W., G.T.K., J.C., M.B., T.W.; Supervision: N.T.P., D.T.T.H., M.B., T.W.; Funding acquisition: M.B., T.W.

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