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RESEARCH ARTICLE

Renal acid excretion contributes to acid–base regulation during hypercapnia in air-exposed swamp eel (*Monopterus albus*)

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ABSTRACT

The swamp eel (*Monopterus albus*) uses its buccal cavity to air breathe, while the gills are strongly reduced. It burrows into mud during the dry season, is highly tolerant of air exposure, and experiences severe hypoxia both in its natural habitat and in aquaculture. To study the ability of *M. albus* to compensate for respiratory acidosis, we implanted catheters to sample both arterial blood and urine during hypercapnia (4% CO_2) in either water or air, or during whole-animal air exposure. These hypercapnic challenges caused an immediate reduction in arterial pH, followed by progressive compensation through a marked elevation of plasma HCO_3^- over the course of 72 h. There was no appreciable rise in urinary acid excretion in fish exposed to hypercapnia in water, although urine pH was reduced and ammonia excretion did increase. In the air-exposed fish, however, hypercapnia was attended by a large elevation of ammonia in the urine and a large rise in titratable acid excretion. The time course of the increased renal acid excretion overlapped with the time period required to elevate plasma HCO_3^-, and we estimate that the renal compensation contributed significantly to whole-body acid–base compensation.

KEY WORDS: Respiratory acidosis, Arterial pH, Metabolic compensation, Kidney, Urine, Renal function, Air-breathing fish

INTRODUCTION

Vertebrates continuously regulate acid–base balance typically in both the extracellular and intracellular fluid compartments (Truchot, 1987). In fishes, the gills are generally considered to play the major role in re-establishing normal acid–base balance upon disturbances brought about by external hypercapnia (Cameron, 1971; Jensen et al., 1993; Evans et al., 2005; Perry and Gilmour, 2006; Brauner and Baker, 2009). However, a number of studies, particularly on trout, have shown that renal acid secretion may contribute significantly to restoration of arterial pH (pHa) by elevation of plasma HCO_3^- concentration ($[HCO_3^-]$) (e.g. Wood and Caldwell, 1978; Perry et al., 1987; Wood et al., 1999). This renal compensation in freshwater fishes is particularly pronounced during respiratory versus metabolic acid–base disturbances, and it seems that exposure to hypercapnia elicits a similar renal response in both fish and mammals (e.g. Wood et al., 1999; Perry et al., 2003; Wright et al., 2014). Nevertheless, compared with the focus on branchial ion exchange, the role of the kidneys has received much less attention in fish (see Wood et al., 1999; Perry and Gilmour, 2006). This is also the case for air-breathing fishes. Thus, while there certainly was a transition from branchial to renal compensation for respiratory acid–base disturbances as vertebrates underwent the transition from an aquatic to a terrestrial lifestyle (Wood et al., 2016), it remains to be clarified whether extant air-breathing fishes rely more on renal regulation of acid–base balance than their water-breathing relatives. In *Synbranchus marmoratus*, the kidneys do not appear to be involved in acid–base balance when arterial P_{CO_2} increases in response to air breathing (Heisler, 1982). However, in the African lungfish (*Protopterus annectans*), the kidneys contribute significantly, but less than the gills, during metabolic acid–base disturbances (Gilmour et al., 2007). Furthermore, in a direct comparison of the physiological responses to hypercapnia in two erythrinid species from the Amazon, Cameron and Wood (1978) reported greater renal compensation in the air-breathing species (*Hoplerythrinus unitaeniatus*) than in the water-breathing species (*Hoplias malabaricus*). However, only one specimen of each species was studied, and because the renal involvement in acid–base regulation during hypercapnia has not been studied in any other air-breathing teleost, we decided to investigate this problem in the air-breathing swamp eel, *Monopterus albus*.

Monopterus albus is native to tropical South East Asia (Rosen and Greenwood, 1976) where it thrives in muddy ponds and swamps (Tay et al., 2003; Ip et al., 2004). Being considered a delicacy, *M. albus* is also a popular fish in aquaculture, where it is farmed in stagnant water bodies with very high CO_2 levels (reaching 30–35 mmHg, P.V.T., unpublished observation). Unlike most other air-breathing fishes, *M. albus* does not possess a separate air-breathing organ, and aerial gas exchange occurs over the highly vascularized epithelium of the buccopharyngeal lining and the anterior oesophagus (Wu and Kung, 1940; Liem, 1967; Iversen et al., 2013; Lefevre et al., 2016b). These air-breathing surfaces and the strongly reduced gills are supplied with blood from the ventral aorta through a uniquely evolved circulatory structure (Hyrtl, 1858; Volz, 1906; Liem, 1961; Munshi et al., 1990; Iversen et al., 2013). Despite its low capacity for aquatic gas exchange, *M. albus* tolerates prolonged submersion in normoxic water without changes in arterial blood gases, demonstrating that the combination of skin, buccopharyngeal surface and gills can support resting levels of CO_2 excretion and oxygen uptake (Wu and Liu, 1940; Liem, 1967; Iversen et al., 2013). In the wild, *M. albus* often resides within deep (up to 1.5 m) burrows in the mud, where it may remain for more than 4 months during the dry season (Volz, 1906; Shih, 1940; Rainboth, 1996). It remains a matter of debate whether *M. albus* aestivates during the dry season, but it seems to maintain air access and to burrow down to the water table. Vietnamese farmers report that the fish reproduce in these summer burrows.

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List of abbreviations

Hb	haemoglobin concentration in the blood (mmol l^{-1})
Hct	haematocrit (the fraction of red blood cells in the blood)
Pa_{CO_2}	partial pressure of CO_2 in arterial blood (mmHg)
pHa	arterial pH
pHe	extracellular pH
TA	net titratable acidity of the urine
TAN	total concentration of NH_4^+ and NH_3
UFR	urine flow rate (ml h^{-1} kg^{-1})
β_{NB}	non-bicarbonate buffering capacity

Given its rather unusual natural history with regular extended air exposures, *M. albus* can be considered an amphibious teleost, and we hypothesized that the kidney plays a greater than usual role in the compensatory response to a respiratory acidosis. To investigate this possibility, we measured acid–base variables of arterial blood and urine, as well as plasma ion levels, when *M. albus* was exposed to hypercapnia. We exposed the fish to hypercapnia either while they were kept in water or when they were air exposed, where the latter exposure was designed to curtail the possibility of branchial regulation.

MATERIALS AND METHODS

Experimental animals and catheterization of the dorsal aorta

Asian swamp eels, *Monopterus albus* (Zuiew 1793), order Synbranchiformes, were purchased from a commercial farm in the Mekong Delta (southern Vietnam) and kept in 500 l tanks with freshwater at 27–29°C for several months at the College of Aquaculture and Fisheries (Can Tho University, Vietnam). The fish had a body mass of 350±10 g and body length of 35±5 cm when the studies were performed. Two-thirds of the water was changed on a daily basis to maintain acceptable environmental conditions [oxygen >90% air saturation, pH 7.7–7.8 and total ammonia nitrogen (TAN) <0.05 mol l^{-1}] as measured by a YSI 556 handheld instrument (YSI, Yellow Springs, OH, USA) and methods described below. The fish were fed twice daily with live food. All experiments were performed in accordance with national guidelines for the protection of animal welfare in Vietnam.

All studies were performed on chronically cannulated fish. To insert the catheters, the swamp eels were anaesthetized in benzocaine solution (225 mg l^{-1}) for 20 min until cessation of movement and placed in a supine position on an operating table. A surgical incision (4–5 cm) into the abdominal cavity allowed for occlusive cannulation of the posterior end of the coeliac artery using polyethylene tubing (PE50) containing heparinized saline. The incision was closed with continuous sutures (nylon 3/0) and the catheter secured to the skin. An additional catheter was inserted into the urethra to collect urine, secured with cyanoacrylate glue in the urethra and sutures to the outside skin. Eels from all treatments were allowed to recover for 24 h in well aerated and normocapnic freshwater at 27°C to ensure a common baseline prior to CO_2 exposure. We have shown in previous studies that arterial acid–base variables remain stable after this surgery (Thinh et al., 2018), but we cannot rule out that reduced gastric blood flow could have compromised acid–base regulation in hypercapnia.

Effects of hypercapnia on arterial acid–base status

We measured arterial blood gases, plasma ions and urine composition under four different treatments: (i) normocapnic water; (ii) air exposure; (iii) combination of hypercapnia in water and air (4% CO_2, approximately 30 mmHg or 40,000 µatm); and (iv) hypercapnia (4% CO_2) of air-exposed eels. Six eels were studied in each protocol and all exposures were continued for 72 h. Water P_{CO_2} was controlled using an Oxyguard Pacific system, and the hypercapnic gases were generated by a Wösthoff gas-mixing pump (Bochum, Germany). Temperature was maintained at 27°C throughout. Water pH was 7.7–7.8 in normocapnic water and decreased to 6.6–6.8 in hypercapnia. Oxygen in the water was more than 80% saturation and ammonia levels were very low (around 0.2–0.3 mmol l^{-1}).

Determination of non-bicarbonate buffering capacity (β_{NB}) of blood *in vitro*

The non-bicarbonate buffering capacity (β_{NB}) was determined *in vitro* on blood samples obtained from six fish. Large blood samples (approximately 10 ml) were drawn from an arterial catheter and divided into two portions. One was centrifuged slowly at 38.2 ***g*** (600 rpm, radius 9.5 cm) for 3 min at 27°C to remove plasma, which could then be added to the other sample. This procedure gave two samples with either low or high haematocrit (Hct) (around 30% and 55%, respectively; normal values are between 40% and 50%). Each subsample was placed in a rotating Eschweiler tonometer and equilibrated with 7, 15 or 30 mmHg CO_2 for at least 30 min, with gas mixtures generated using Wösthoff gas-mixing pumps (Bochum, Germany). At each P_{CO_2}, we measured total CO_2 concentration of the plasma, blood pH and Hct. For each blood sample, β_{NB} was calculated from the linear relationship between plasma [HCO_3^-] and pH, and we evaluated the influence of Hct as the linear relationship between βN_B and Hct, as previously shown in fish (e.g. Wood et al., 1982).

Analytical methods

During all exposures, a 1 ml blood sample was carefully drawn through the catheter at 0, 3, 6, 24, 48 and 72 h, without disturbing the fish. Some of the blood (around 0.5 ml) was re-infused after analysis and approximately 1 ml of saline was given after each blood sampling to flush the catheter. We did not add any protein or plasma expanders. Partial pressure of CO_2 in the arteries (Pa_{CO_2}) and extracellular pH (pHe) were measured using an iStat with G3+cartridges (Abbot Point of Care Inc., Princeton, NJ, USA) and temperature compensated using the provided equation. Similar values for pHe were also measured using an InLab Microelectrode (SevenCompact pH meter, Mettler Toledo Ltd, Greifensee, Switzerland). Total plasma CO_2 concentration was measured according to Cameron (1971) and used to calculate plasma [HCO_3^-]. Haemoglobin (Hb) concentration was measured spectrophotometrically at 540 nm after conversion to cyano-methaemoglobin using Drabkin's solution (Zijlstra et al., 1983), and Hct was determined as the proportional volume of packed red blood cells after centrifugation at 14,500 ***g*** for 3 min.

Urine was collected by passive urine flow into an Eppendorf tube placed at the surface level. The collected volume was measured at 8–12 h intervals to enable calculation of the mean urine flow rate (UFR) over that period. Urine pH was measured with the same Mettler Toledo pH electrode and urinary CO_2 concentration was determined with the same technique (Cameron, 1971) as described above. Titratable acidity (TA) was measured by titrating the urine samples with 0.2 mol l^{-1} NaOH to reach the measured *in vivo* pHa in the corresponding blood sample, while the vials were shaken regularly on a vortex for a couple of minutes. TAN, the total concentration of NH_4^+ and NH_3 in urine, was measured according to Scheiner (1976) in an assay of total ammonia nitrogen. Chloride concentrations in plasma and urine were measured using a chloride titrator (model 926S MK II, Sherwood Scientific, Cambridge, UK),

Journal of Experimental Biology (2019) 222, jeb198259. doi:10.1242/jeb.198259

while Na^+ and K^+ concentrations were determined using flame photometry (model 420, Sherwood Scientific). Total osmolality was measured on a micro-osmometer (Advanced Instruments™ Fiske™ 210 Micro-Sample Osmometer, Norwood, MA, USA).

Statistics

A two-way ANOVA (the Holm–Šídák multiple comparison method, pair-wise comparison) was used to identify differences between treatments and sampling times for all parameters related to hypercapnic exposure (acid–base variables, ion in plasma and urine). Data were tested for normality using the Shapiro–Wilk test and log transformed when necessary. Statistical analyses were performed with PASW statistics (SPSS) considering a *P*-value smaller than 0.05 as significant.

RESULTS

Effects of hypercapnia on arterial acid–base status

The temporal changes in arterial acid–base variables during the various exposures are shown in Fig. 1, and as a Davenport diagram in Fig. 2. Pa_{CO_2} increased from around 6–8 mmHg to almost 30 mmHg within the first 6 h of exposure to hypercapnia and remained stable at this elevated level for the following 72 h (Fig. 1B). In both hypercapnic treatments, the rise in Pa_{CO_2} caused an immediate acidification, where pHe fell by approximately 0.3 units (Fig. 2), and with an initial decrease that fell below the prediction from the *in vitro* non-bicarbonate buffer line (Fig. 2). After 6 h, the respiratory acidosis was progressively compensated by an elevation of plasma [HCO_3^-], and pHa was almost completely restored by 72 h of hypercapnia (Figs 1 and 2). There was an unexpected tendency for the air-exposed fish to exhibit a more complete pH compensation than the fish exposed to hypercapnia in both water and air. Acid–base status remained unchanged in the six eels measured in normocapnic water over 72 h and, similarly, there was no effect on acid–base status in the six eels that were air exposed in normocapnic air for 72 h.

The attendant changes in plasma ion concentrations and osmolality are depicted in Fig. 3. Both plasma Na^+ and Cl^- fell during hypercapnia (Fig. 3A,B) in combination with a reduction in plasma osmolality (Fig. 3D) that, surprisingly, was most pronounced in fish exposed to hypercapnia in water and air. Plasma potassium increased slightly during the exposures, but remained below 4 mmol l^{-1} in all treatments (Fig. 3B). Both Hb and Hct decreased with time similarly in all groups, a likely consequence of the repeated blood sampling (Table 1).

Effects of hypercapnia on urine composition and renal acid excretion

In control fish (normocapnia in water), UFR was stable at approximately 1.5 ml kg^{-1} h^{-1}, while hypercapnia, air exposure and the combination of hypercapnia and air exposure elicited an approximately 5-fold (significant) reduction in UFR (Fig. 4A). Within the first 24 h of hypercapnia in the air-exposed fish, urinary sodium decreased significantly, but had recovered at the termination of the experiment (Table 2). Urine chloride increased rapidly in both hypercapnia groups, reaching a maximum at 24 h, after which it fell again over the following 48 h to a value slightly (but significantly) above controls at termination (Table 2). Urine potassium concentration did not change, except for a significant rise in the eels exposed to hypercapnia in air (Table 2). In control and air-exposed fish, urine pH remained constant, but it was strongly reduced within the first 12 h of hypercapnia in both air and water (Fig. 4B). During the next 72 h in hypercapnia, urine pH returned to control

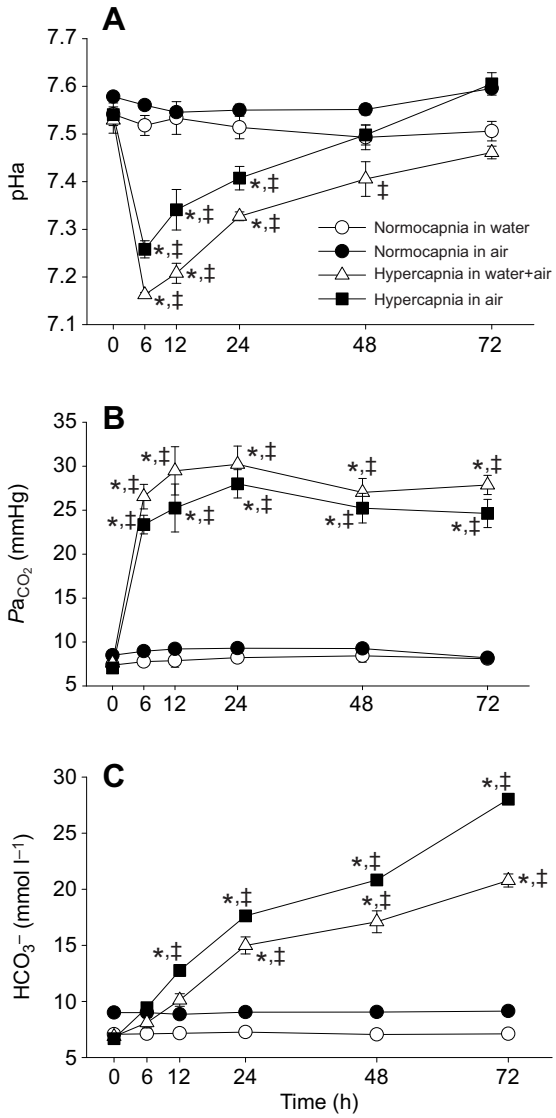

Fig. 1. Influence of environmental hypercapnia in water and air on arterial acid–base status in *Monopterus albus*. Arterial pH (A), arterial partial pressure of CO_2 (Pa_{CO_2}) (B) and plasma [HCO_3^-] (C). One group of fish was measured in normocapnic water for 72 h and another group was measured when air exposed in normocapnia. A third group was exposed to 4% CO_2 in water (hypercapnia, 30 mmHg; water+air), while the fourth group was exposed to hypercapnia during air exposure. Statistical differences within a given treatment are marked with an asterisk, and statistical differences from normocapnic water exposure are shown by a double-dagger. All data are presented as means±s.e.m. (*N*=6 in each group).

levels. Hypercapnia was associated with a 3- to 4-fold elevation of total CO_2 concentration in the urine (Fig. 4D), a large increase in urine TAN (Fig. 4C) and a large increase in titratable acid (Fig. 4E). Although UFR was reduced in hypercapnia, the calculated excretion rates of TAN and titratable acid were significantly elevated in both hypercapnic treatments, and the absolute rise in the rate of renal acid excretion was particularly pronounced in fish exposed to hypercapnia during air exposure (Fig. 5).

Non-bicarbonate buffering capacity (β_{NB}) of blood

Based on our *in vitro* measurements, β_{NB} in slykes ($\Delta[HCO_3^-]$ ΔpH^{-1}) at 27°C is described as a function of Hct (in %) as follows:

$$\beta_{NB} = 9.79 + (0.2017 \times \text{Hct}). \quad (1)$$

Journal of Experimental Biology (2019) 222, jeb198259. doi:10.1242/jeb.198259

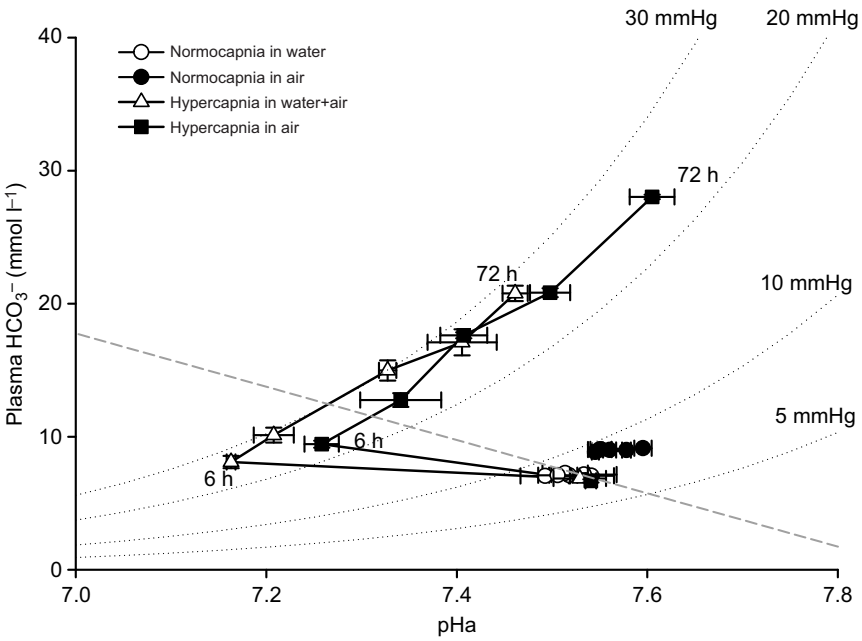

Fig. 2. Davenport diagram depicting arterial acid–base status in *M. albus* exposed to hypercapnia in water and air. Same data as presented in Fig. 1. The blood buffer line (dashed grey line) is derived from our *in vitro* measurements and calculated for a haematocrit (Hct) of 50% to resemble *in vivo* values (see Table 1). All data are presented as means±s.e.m. (*N*=6 in each group).

The dissociation constant for CO_2 hydration (pK') was calculated as:

$$pK' = pHe - \log([HCO_3^-]_{plasma}/(\alpha CO_2 \times Pa_{CO_2})), \quad (2)$$

and the pH dependence of pK' could be described as pK' =−0.3049×pH+8.4496, using a temperature-compensated αCO_2 from Boutilier et al. (1985).

DISCUSSION

Monopterus albus achieved almost complete restoration of pHa by elevating plasma [HCO_3^-] during exposure to hypercapnia levels that resemble those in aquaculture and natural settings (Willmer, 1934; Wakeman and Ultsch, 1975; Ultsch, 1987; Damsgaard et al., 2015). When exposed to hypercapnia in water, the pHa compensation occurred without noticeable changes in urine H^+ excretion and the elevation of plasma HCO_3^- was therefore probably mediated by branchial ion exchange as in most water-breathing fish. Consistent

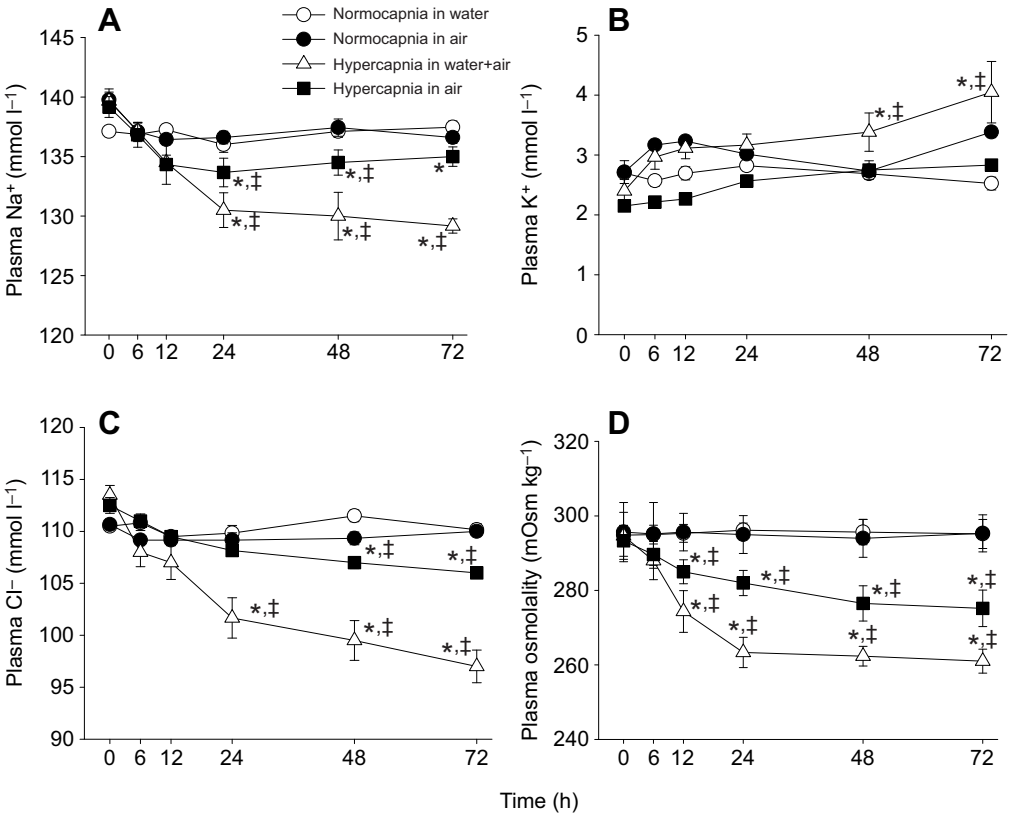

Fig. 3. The influence of environmental hypercapnia in water and air on plasma ion concentrations and plasma osmolality in *M. albus*. Plasma Na^+ concentration (A), plasma K^+ concentration (B), plasma Cl^- concentration (C) and plasma osmolality (D). Statistical differences within a given treatment are marked with an asterisk, and statistical differences from normocapnic water exposure are shown by a double-dagger. All data are presented as means±s.e.m. (*N*=6 in each group).

Journal of Experimental Biology (2019) 222, jeb198259. doi:10.1242/jeb.198259

Table 1. Blood haemoglobin (Hb) concentration and haematocrit (Hct) in *Monopterus albus* following exposure to normocapnia or hypercapnia in air or water

	0 h	6 h	12 h	24 h	48 h	72 h
Hb concentration (mmol l^{-1})						
Normocapnic water	12.8±0.7	12.7±0.6	12.3±0.6	11.3±0.7	10.6±0.7	9.4±0.8
Air exposure	10.0±0.4	11.2±0.8	12.4±1.0	9.2±0.4	10.3±0.4	8.7±0.3
Hypercapnic water	10.6±0.7	10.2±0.3	9.7±1.0	10.1±0.3	8.2±0.5	9.2±0.5
Hypercapnic air exposure	12.0±0.5	11.4±0.6	11.0±0.6	10.5±0.4	9.9±0.5	9.4±0.5
Hct (%)						
Normocapnic water	53.7±2.9	52.0±2.3	49.7±2.7	46.8±3.0	45.2±2.2	37.7±1.7
Air exposure	51.8±0.7	50.1±1.3	47.6±1.4	44.6±1.8	44.4±0.7	37.8±2.2
Hypercapnic water	49.3±1.6	48.2±1.4	46.7±1.5	43.8±2.0	41.5±2.3	39.8±2.3
Hypercapnic air exposure	51.1±3.8	49.3±3.1	47.8±1.9	44.0±3.7	36.3±1.6	38.2±4.8

Monopterus albus were exposed to combinations of normocapnia or hypercapnia in air and water over a period of 72 h. Data are means±s.e.m. (*N*=6 in each treatment).

with the high reliance on aerial gas exchange (Lefevre et al., 2016b), there were no effects of air exposure on arterial blood gases, but UFR was greatly reduced, which probably serves to alleviate dehydration. Accordingly, during air exposure, where branchial ion exchange is curtailed by a lack of water contact, aerial hypercapnia places *M. albus* in an interesting conflict where increased demands for renal acid secretion must be accomplished in the face of the lowered UFR. Nevertheless, there was a remarkable rise in renal acid excretion with

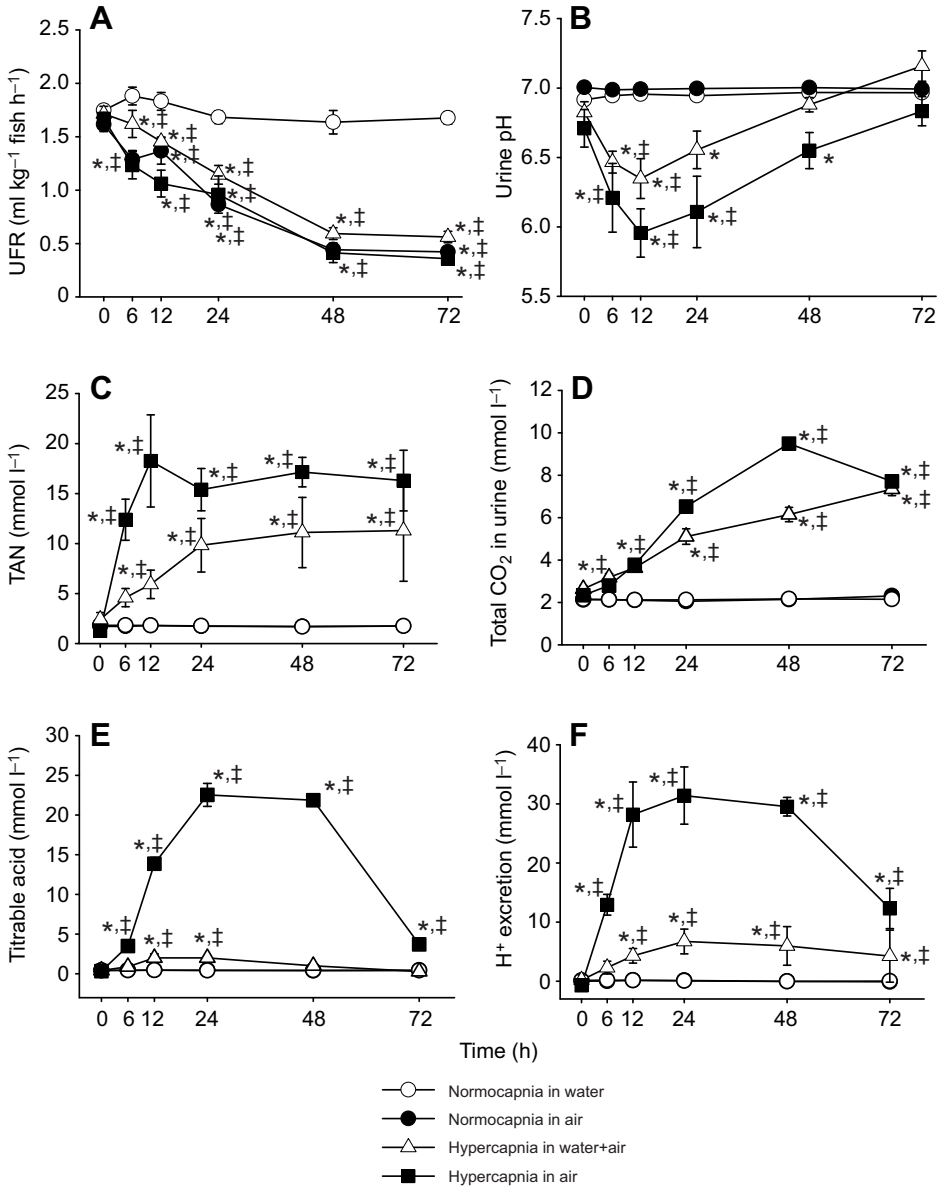

Fig. 4. The influence of environmental hypercapnia in water and air on urine. Urine formation rate (UFR; A), urine pH (B), total ammonia nitrogen (TAN; C), total CO_2 concentration in the urine (D), titratable acid (E) and the calculated rate of urinary proton excretion (F). Statistical differences within a given treatment are marked with an asterisk, and statistical differences from normocapnic water exposure are shown by a double-dagger. All data are presented as means±s.e.m. (*N*=6 in each group).

Journal of Experimental Biology (2019) 222, jeb198259. doi:10.1242/jeb.198259

Table 2. Ion concentrations in the urine of *Monopterus albus* following exposure to normocapnia or hypercapnia in air and water

	0 h	6 h	24 h	48 h	72 h
Urine [Na^+] (mmol l^{-1})					
Normocapnic water	13.7±0.2	14.0±0.1	14.1±0.1	13.5±0.4	13.2±0.3
Air exposure	13.2±0.2	13.5±0.3	13.2±0.2	13.5±0.3	12.9±0.2
Hypercapnic water	15.0±1.5	15.5±2.7	17.1±2.8*,‡	11.5±0.7	13.0±0.6
Hypercapnic air exposure	14.2±0.3	9.6±0.5*,‡	7.0±0.2*,‡	8.3±0.3*,‡	10.8±0.3*,‡
Urine [K^+] (mmol l^{-1})					
Normocapnic water	3.6±0.1	3.4±0.3	3.8±0.2	3.4±0.2	3.4±0.3
Air exposure	3.2±0.1	3.5±0.3	3.7±0.3	3.8±0.2	3.5±0.2
Hypercapnic water	3.3±0.3	2.7±0.2	3.0±0.3	2.9±0.4	3.1±0.3
Hypercapnic air exposure	4.6±0.2	3.2±0.2	4.8±0.3‡	3.9±0.1	5.1±0.2‡
Urine [Cl^-] (mmol l^{-1})					
Normoxia in water	4.2±0.2	4.0±0.1	3.6±0.1	3.9±0.1	3.7±0.3
Air exposure	4.2±0.2	4.1±0.2	4.1±0.5	4.0±0.2	3.9±0.2
Hypercapnic water	4.0±0.6	7.3±0.8*,‡	13.5±1*,‡	12.0±2.3*,‡	9.6±1.4*,‡
Hypercapnic air exposure	4.5±0.4	8.3±0.4*,‡	11.2±0.3*,‡	7.7±0.5*,‡	5.8±0.3

Monopterus albus were exposed to combinations of normocapnia or hypercapnia in air and water over a period of 72 h. Data are means±s.e.m. (*N*=6 in each group).

*Statistical differences within a given treatment.

‡Statistical differences to normocapnic water exposure.

depressed urine pH and a large rise in total titratable acidity and TAN (NH_4^+ and NH_3).

Urine composition and renal function in water and during air exposure

The low ion concentrations and the mild acidity of urine are typical of freshwater fish, and the low concentration of renal ammonia excretion is also consistent with other species, indicating a modest renal acid output in normocapnic fish. The UFR of approximately 1.5 ml kg^{-1} h^{-1} in *M. albus* in normocapnic water is 2- to 3-fold lower than in common water-breathing teleosts (Cameron, 1980; Wood et al., 1999; Wright et al., 2014; Lawrence et al., 2015). This low UFR indicates a low water influx in *M. albus*, which probably stems from the very strongly reduced surface area of the gills, but a low water permeability of the skin may also contribute. The UFR measured in other air-breathing fish, *H. malabaricus*, *H. unitaeniatus* and *P. dolloi*, is also higher than that in *M. albus* (Cameron and Wood, 1978; Patel et al., 2009), but none of these have gills as reduced as those in *M. albus*.

When exposed to hypercapnia in water, UFR decreased 3-fold in a progressive fashion over 48 h. This reduction occurred in the face of a small fall in plasma ions and osmolality, and the reduced UFR,

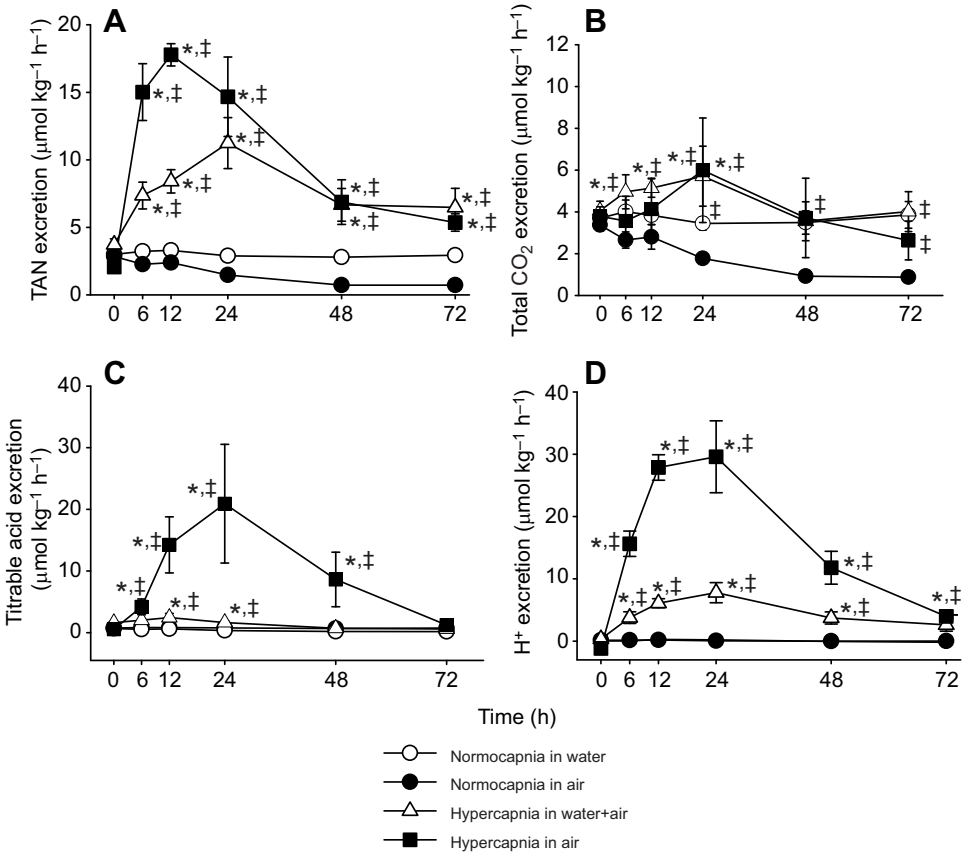

Fig. 5. The influence of environmental hypercapnia in water and air on renal excretion rates. Rate of renal excretion of NH_4^+ and NH_3 (TAN) (A), total CO_2 (B) and titratable acid (C) and the calculated rate of renal H^+ excretion (D). Statistical differences within a given treatment are marked with an asterisk, and statistical differences from normocapnic water exposure are shown by a double-dagger. All data are presented as means±s.e.m. (*N*=6 in each group).

therefore, does not appear to result from dehydration. If blood sampling caused hypovolaemia, which might have been indicated by the reduced Hct, it would be expected that the control fish should also decrease UFR in response to repeated blood sampling, which was not the case. *Cyprinus carpio* also exhibits a marked reduction in UFR during water acidification (Wright et al., 2014), whereas UFR increases markedly when *Carassius auratus* is exposed to acidic water (Lawrence et al., 2015). In trout, the effects of hypercapnia on UFR are less dramatic, with modest changes during respiratory and metabolic acidosis (Wood et al., 1999) or a slight reduction when water pH is lowered (McDonald and Wood, 1981).

Effects of air exposure

Monopterus albus obtains most of its oxygen from air breathing (Wu and Liu, 1943; Liem, 1967; Lefevre et al., 2016a,b), a feature shared with the closely related *Monopterus cuchia* (Lomholt and Johansen, 1974). Whilst the partitioning of CO_2 excretion remains to be determined, the maintenance of normal blood gases during air exposure demonstrates that the normal rates of oxygen uptake and CO_2 excretion can be satisfied by aerial respiration alone (see Bayley et al., 2019).

Acid–base regulation in water

When in water, *M. albus* displayed a pronounced capacity for pHa compensation by a progressive rise in plasma [HCO_3^-] during hypercapnia. It is noteworthy that the initial rise in plasma HCO_3^- was smaller than predicted from the blood buffer line, which may indicate fast regulation of the intracellular space with a net uptake of HCO_3^- from the extracellular space. The pronounced compensation of pHe in *M. albus* resembles the typical piscine acid–base compensatory response to hypercapnia (Jensen et al., 1993; Brauner and Baker, 2009), but differs markedly from that of the closely related South American Synbranchid *S. marmoratus* (Heisler, 1982). When *S. marmoratus* made the transition from water breathing in normoxic water to air breathing in hypoxic water, the 5-fold elevation in Pa_{CO_2} (from approximately 5 to 26 mmHg) elicited a pH fall of approximately 0.6 units that persisted for 18 h without changes in plasma [HCO_3^-] (Heisler, 1982). Although *S. marmoratus* and *M. albus* have a strikingly similar external appearance, the gills of *S. marmoratus* are well developed, which clearly demonstrates that regression of the gills in *M. albus* has occurred without hindering effective ion regulation to elevate plasma [HCO_3^-]. A similar lack of pHe compensation in hypercapnia has been reported in other air-breathing fishes, such as *Liposarcus pardalis* and *Arapaima gigas* (Brauner et al., 2004; Gonzalez et al., 2010), whereas *Hypostomus* sp. and *Pangasianodon hypophthalmus* exhibit effective compensation (Wood et al., 1979; Damsgaard et al., 2015). *Amia calva* exhibits a more variable response (McKenzie and Randall, 1990; Brauner and Baker, 2009). It is possible that some of the species differences serve to avoid the loss of ions in ion-poor water (Heisler, 1978), but it is certainly clear that air-breathing fishes exhibit large variations in their pH regulation, ranging from full compensation to very little regulation of the extracellular fluid compartment.

Teleosts with well-developed gills typically elevate plasma [HCO_3^-] through a net excretion of Cl^- over the gills, and numerous studies show reciprocal and equimolar changes in plasma HCO_3^- and Cl^- concentration in response to hypercapnia (e.g. Brauner and Baker, 2009). In a number of other species, including the air-breathing siluriform catfish *P. hypophthalmus*, branchial Na^+/H^+ exchange may also contribute significantly (e.g. Evans et al., 2005; Goss et al., 1992; Perry and Gilmour, 2006; Hvas et al., 2016; Damsgaard et al., 2018). In *M. albus*, plasma [Cl^-] decreased approximately 16 mmol l^{-1} at 72 h of hypercapnia, whilst [HCO_3^-] rose approximately 10 mmol l^{-1} (when the rise in [HCO_3^-] due to buffering was subtracted). There was also an unexplained reduction in plasma [Na^+] of 10 mmol l^{-1} and an associated reduction in osmolality, which may indicate a net uptake of water in aquatic hypercapnia.

The kidney appeared to play only a minor role in the elevation of plasma [HCO_3^-] when *M. albus* was submerged. However, hypercapnia did lead to an elevation in renal excretion of ammonia, but the attendant rise in the total CO_2 concentration meant that total titratable acid of the urine and hence total H^+ excretion rate was only marginally elevated. Nevertheless, the observation that total CO_2 concentration in the urine remained well below the plasma levels during the compensated state certainly demonstrates a capacity to reabsorb tubular HCO_3^- following glomerular filtration. If mediated by Cl^-/HCO_3^- exchange, as in the proximal tubule in the mammalian kidney (e.g. Romero and Boron, 1999), this reabsorption might explain the urinary rise in [Cl^-] during hypercapnia.

Regulation of acid–base status during hypercapnia in air-exposed *M. albus*

During air exposure, the bottom of the tanks was still covered by a film of water and the fish skin remained moist. This water may have provided some opportunity for the exchange of acid–base relevant ions despite its low volume. Nevertheless, it is both striking and surprising that *M. albus* was fully capable of restoring pHa through a marked elevation of plasma [HCO_3^-]. In contrast to the hypercapnic exposure in water, there appeared to be a significant renal contribution to the recovery of pHa whilst fish were kept in air. Thus, hypercapnia caused a large rise in renal ammonia excretion, probably reflecting a marked capacity for renal ammoniagenesis (e.g. Wood et al., 1999; Lawrence et al., 2015), and there was an associated large rise in titratable acid excretion. The time course of the elevated renal acid excretion overlapped with the time period required to elevate plasma HCO_3^- and attain pH compensation. Thus, although the kidneys did excrete similar levels of HCO_3^- to those measured during water exposure, the net acid excretion was sizeable. Under the assumption that the extracellular space constitutes 20% of the body (determined in the closely related *S. marmoratus*; Heisler, 1982), we estimate the renal generation of HCO_3^- from ammoniagenesis and ammonia excretion may account for around 30% of the extracellular HCO_3^- accumulation during hypercapnia in this group. It is reasonable to assume that the pH of the intracellular compartments was fully restored, so the renal contribution to whole-animal acid–base regulation in hypercapnia would be less. The remaining non-renal transfer of acid–base relevant ions probably occurred over the gills in the thin film of water that prevailed during air exposure, but it is also possible that cutaneous exchange contributed.

Perspectives

Monopterus albus is an extraordinary fish with numerous cardiorespiratory adaptations to air breathing. Most of its gas exchange takes place over the vascularized lining of the buccopharyngeal cavity, but the strongly reduced gills nevertheless appear capable of pronounced acid–base regulation. Nevertheless, when fish are air exposed, a situation that may resemble the conditions prevailing under their summer retreat into mud burrows in the dry season, the kidneys appear to provide a substantial contribution to acid–base balance. It is interesting that the renal

acid–base balance was not recruited when the eels where exposed to hypercapnia in water, which may indicate that priority was given to branchial ion exchange and that there are separate regulatory mechanisms underlying the branchial and renal functions.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: P.T., D.H., C.D., M.B., T.W.; Methodology: P.T., L.G., M.B.; Formal analysis: P.T., C.D., T.W.; Investigation: D.H., T.W.; Data curation: P.T.; Writing - original draft: P.T., M.B., T.W.; Writing - review & editing: P.T., D.H., L.G., C.D., N.P., M.B., T.W.; Supervision: D.H., N.P., M.B., T.W.; Project administration: L.G., N.P., M.B., T.W.; Funding acquisition: D.H., N.P., M.B., T.W.

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