

## Supplementary data

The following shows supplementary data referred to in the main body of text.

**Table S1:** Plasma HCO<sub>3</sub><sup>-</sup>, Cl<sup>-</sup>, Na<sup>+</sup>, K<sup>+</sup> and osmolality in exposure to elevated temperatures: 25, 27, 30, 33 and 36°C. The data at 27°C were collected from Gam et al., 2018. A one-way ANOVA was used for comparison between different temperatures at a given time. Different letters indicated significant differences within each column ( $P<0.05$ ). Data are mean  $\pm$  S.E.M (N=6).

	0 h	24 h	48 h	72 h
<b>HCO<sub>3</sub><sup>-</sup> (mM)</b>				
25°C	7.95 $\pm$ 0.3 <sup>a</sup>	8.01 $\pm$ 0.5 <sup>a</sup>	7.97 $\pm$ 0.7 <sup>a</sup>	8.05 $\pm$ 0.3 <sup>a</sup>
27°C	7.64 $\pm$ 0.4 <sup>a</sup>	7.60 $\pm$ 0.4 <sup>a</sup>	7.78 $\pm$ 0.4 <sup>a</sup>	7.62 $\pm$ 0.3 <sup>a</sup>
30°C	7.76 $\pm$ 0.2 <sup>a</sup>	7.75 $\pm$ 0.1 <sup>a</sup>	7.75 $\pm$ 0.2 <sup>a</sup>	7.74 $\pm$ 0.1 <sup>a</sup>
33°C	8.14 $\pm$ 0.3 <sup>a</sup>	8.19 $\pm$ 0.4 <sup>a</sup>	8.12 $\pm$ 0.5 <sup>a</sup>	8.17 $\pm$ 0.5 <sup>a</sup>
36°C	8.06 $\pm$ 0.3 <sup>a</sup>	7.91 $\pm$ 0.5 <sup>a</sup>	8.11 $\pm$ 0.4 <sup>a</sup>	8.00 $\pm$ 0.3 <sup>a</sup>
<b>Cl<sup>-</sup> (mM)</b>				
25°C	104.5 $\pm$ 1.9 <sup>ab</sup>	102.3 $\pm$ 3.2 <sup>ab</sup>	102.2 $\pm$ 2.4 <sup>ab</sup>	103.0 $\pm$ 2.1 <sup>ab</sup>
27°C	109.0 $\pm$ 1.4 <sup>a</sup>	109.2 $\pm$ 1.7 <sup>a</sup>	107.5 $\pm$ 1.1 <sup>a</sup>	108.7 $\pm$ 1.6 <sup>a</sup>
30°C	100.5 $\pm$ 1.3 <sup>b</sup>	100.2 $\pm$ 2.1 <sup>b</sup>	99.7 $\pm$ 2.4 <sup>b</sup>	102.0 $\pm$ 3.4 <sup>b</sup>
33°C	100.2 $\pm$ 3.1 <sup>b</sup>	100.0 $\pm$ 1.4 <sup>b</sup>	99.8 $\pm$ 2.8 <sup>b</sup>	99.3 $\pm$ 1.6 <sup>b</sup>
36°C	99.8 $\pm$ 2.3 <sup>b</sup>	100.2 $\pm$ 1.1 <sup>b</sup>	101.5 $\pm$ 2.5 <sup>b</sup>	100.5 $\pm$ 2.1 <sup>b</sup>
<b>Na<sup>+</sup> (mM)</b>				
25°C	127.8 $\pm$ 1.7 <sup>a</sup>	128.2 $\pm$ 1.5 <sup>a</sup>	127.9 $\pm$ 1.0 <sup>a</sup>	128.2 $\pm$ 1.7 <sup>a</sup>
27°C	132.8 $\pm$ 2.4 <sup>a</sup>	132.5 $\pm$ 0.1 <sup>a</sup>	133.5 $\pm$ 1.2 <sup>a</sup>	132.7 $\pm$ 1.6 <sup>a</sup>
30°C	128.9 $\pm$ 1.0 <sup>a</sup>	129.1 $\pm$ 1.2 <sup>a</sup>	128.7 $\pm$ 2.4 <sup>a</sup>	127.9 $\pm$ 1.8 <sup>a</sup>
33°C	134.1 $\pm$ 3.1 <sup>b</sup>	132.9 $\pm$ 3.6 <sup>a</sup>	131.9 $\pm$ 4.9 <sup>a</sup>	131.5 $\pm$ 2.2 <sup>a</sup>
36°C	131.4 $\pm$ 1.5 <sup>a</sup>	130.2 $\pm$ 1.4 <sup>a</sup>	131.3 $\pm$ 0.9 <sup>a</sup>	130.3 $\pm$ 0.1 <sup>a</sup>
<b>K<sup>+</sup> (mM)</b>				
25°C	3.40 $\pm$ 0.2 <sup>a</sup>	3.42 $\pm$ 0.2 <sup>a</sup>	3.45 $\pm$ 0.1 <sup>a</sup>	3.40 $\pm$ 0.2 <sup>a</sup>
27°C	3.63 $\pm$ 0.1 <sup>a</sup>	3.53 $\pm$ 0.5 <sup>a</sup>	3.65 $\pm$ 0.1 <sup>a</sup>	3.55 $\pm$ 0.2 <sup>a</sup>
30°C	3.52 $\pm$ 0.2 <sup>a</sup>	3.43 $\pm$ 0.2 <sup>a</sup>	3.67 $\pm$ 0.2 <sup>a</sup>	3.50 $\pm$ 0.2 <sup>a</sup>
33°C	3.43 $\pm$ 0.1 <sup>a</sup>	3.48 $\pm$ 0.3 <sup>a</sup>	3.49 $\pm$ 0.4 <sup>a</sup>	3.47 $\pm$ 0.3 <sup>a</sup>
36°C	3.63 $\pm$ 0.2 <sup>a</sup>	3.60 $\pm$ 0.1 <sup>a</sup>	3.65 $\pm$ 0.2 <sup>a</sup>	3.68 $\pm$ 0.3 <sup>a</sup>
<b>Osmolality (mOsm)</b>				
25°C	288.0 $\pm$ 4.9 <sup>ab</sup>	290.5 $\pm$ 3.7 <sup>ab</sup>	289.0 $\pm$ 2.5 <sup>ab</sup>	291.8 $\pm$ 3.4 <sup>ab</sup>
27°C	296.5 $\pm$ 3.2 <sup>a</sup>	299.3 $\pm$ 1.3 <sup>a</sup>	299.4 $\pm$ 2.7 <sup>a</sup>	298.8 $\pm$ 2.3 <sup>a</sup>
30°C	284.5 $\pm$ 4.4 <sup>ab</sup>	286.7 $\pm$ 2.1 <sup>ab</sup>	285.5 $\pm$ 2.7 <sup>ab</sup>	283.3 $\pm$ 2.9 <sup>bc</sup>
33°C	291.5 $\pm$ 3.6 <sup>abc</sup>	289.0 $\pm$ 2.0 <sup>abc</sup>	290.7 $\pm$ 4.4 <sup>abc</sup>	290.5 $\pm$ 3.9 <sup>abc</sup>
36°C	282.3 $\pm$ 1.9 <sup>bc</sup>	283.5 $\pm$ 2.2 <sup>bc</sup>	286.3 $\pm$ 2.0 <sup>bc</sup>	282.5 $\pm$ 1.9 <sup>c</sup>

**Table S2:** Heam concentration, Hct and MCHC in exposure to elevated temperatures: 25, 27, 30, 33 and 36°C. The data at 27°C were collected from Gam et al., 2018. A one-way ANOVA was used for comparison between different temperatures at a given time. Different letters indicated significant differences within each column ( $P<0.05$ ). Data are mean  $\pm$  S.E.M (N=6)

	0 h	24 h	48 h	72 h
<b>Heam (mM)</b>				
<b>25°C</b>	6.33 $\pm$ 0.6 <sup>a</sup>	6.34 $\pm$ 0.3 <sup>a</sup>	6.35 $\pm$ 0.2 <sup>a</sup>	6.30 $\pm$ 0.2 <sup>a</sup>
<b>27°C</b>	5.77 $\pm$ 0.1 <sup>b</sup>	5.72 $\pm$ 0.1 <sup>b</sup>	5.80 $\pm$ 0.1 <sup>b</sup>	5.73 $\pm$ 0.1 <sup>b</sup>
<b>30°C</b>	5.54 $\pm$ 0.2 <sup>bc</sup>	5.45 $\pm$ 0.4 <sup>bc</sup>	5.48 $\pm$ 0.1 <sup>bc</sup>	5.51 $\pm$ 0.1 <sup>bc</sup>
<b>33°C</b>	5.31 $\pm$ 0.1 <sup>bc</sup>	5.35 $\pm$ 0.4 <sup>bc</sup>	5.32 $\pm$ 0.4 <sup>bc</sup>	5.36 $\pm$ 0.6 <sup>bc</sup>
<b>36°C</b>	5.07 $\pm$ 0.2 <sup>c</sup>	5.10 $\pm$ 0.4 <sup>c</sup>	5.12 $\pm$ 0.2 <sup>c</sup>	5.18 $\pm$ 0.2 <sup>c</sup>
<b>Hct (%)</b>				
<b>25°C</b>	31.9 $\pm$ 1.7 <sup>a</sup>	32.0 $\pm$ 0.9 <sup>a</sup>	31.5 $\pm$ 1.0 <sup>a</sup>	31.2 $\pm$ 0.4 <sup>a</sup>
<b>27°C</b>	30.6 $\pm$ 0.2 <sup>a</sup>	30.2 $\pm$ 0.3 <sup>a</sup>	30.9 $\pm$ 0.4 <sup>a</sup>	30.6 $\pm$ 0.1 <sup>a</sup>
<b>30°C</b>	28.7 $\pm$ 0.6 <sup>ab</sup>	28.2 $\pm$ 1.1 <sup>ab</sup>	28.9 $\pm$ 0.7 <sup>ab</sup>	28.3 $\pm$ 1.2 <sup>ab</sup>
<b>33°C</b>	27.0 $\pm$ 0.9 <sup>bc</sup>	26.7 $\pm$ 1.0 <sup>bc</sup>	27.5 $\pm$ 1.5 <sup>bc</sup>	26.9 $\pm$ 1.0 <sup>bc</sup>
<b>36°C</b>	25.5 $\pm$ 1.2 <sup>c</sup>	25.2 $\pm$ 1.0 <sup>c</sup>	25.6 $\pm$ 0.9 <sup>c</sup>	25.3 $\pm$ 1.3 <sup>c</sup>
<b>MCHC (mM)</b>				
<b>25°C</b>	20.1 $\pm$ 2.0 <sup>a</sup>	19.9 $\pm$ 1.2 <sup>a</sup>	20.2 $\pm$ 0.8 <sup>a</sup>	20.2 $\pm$ 0.9 <sup>a</sup>
<b>27°C</b>	18.9 $\pm$ 1.3 <sup>a</sup>	19.0 $\pm$ 1.4 <sup>a</sup>	18.8 $\pm$ 0.3 <sup>a</sup>	18.8 $\pm$ 1.4 <sup>a</sup>
<b>30°C</b>	19.3 $\pm$ 0.5 <sup>a</sup>	19.3 $\pm$ 1.2 <sup>a</sup>	19.1 $\pm$ 0.7 <sup>a</sup>	19.7 $\pm$ 1.2 <sup>a</sup>
<b>33°C</b>	19.8 $\pm$ 0.7 <sup>a</sup>	20.1 $\pm$ 1.5 <sup>a</sup>	19.5 $\pm$ 1.4 <sup>a</sup>	20.0 $\pm$ 0.9 <sup>a</sup>
<b>36°C</b>	20.2 $\pm$ 1.5 <sup>a</sup>	20.5 $\pm$ 1.9 <sup>a</sup>	20.1 $\pm$ 0.8 <sup>a</sup>	20.7 $\pm$ 1.1 <sup>a</sup>

**Table S3:** Plasma ions and osmolality under combined effects of temperature and hypercapnia in the four exposure groups: normocapnia at 25°C, 21 mmHg CO<sub>2</sub> at 25°C, normocapnia at 33°C, 21 mmHg CO<sub>2</sub> at 33°C. The data of normocapnia at 25 and 33°C were collected from series 1. A two-way ANOVA was used for showing significant difference from 0 h within treatment (asterisks) and showing significant difference to controls (normocapnia at 25°C or 33°C) at a sampling time (plus signs). Data are mean ± S.E.M (N=6).

	0 h	24 h	48 h	72 h
<b>Na<sup>+</sup> (mM)</b>				
<b>Normocapnia at 25°C</b>	127.8±1.7	128.2±1.5	127.9±1.0	128.2±1.7
<b>21 mmHg CO<sub>2</sub> at 25°C</b>	128.8±2.2	127.9±1.8	128.2±1.2	122.5±0.8*,+
<b>Normocapnia at 33°C</b>	134.1±3.1	132.9±3.6	131.9±4.9	131.5±2.2
<b>21 mmHg CO<sub>2</sub> at 33°C</b>	132.4±3.4	128.6±1.6	126.9±1.5	123.0±1.2*,+
<b>K<sup>+</sup> (mM)</b>				
<b>Normocapnia at 25°C</b>	3.40±0.2	3.42±0.2	3.45±0.1	3.40±0.2
<b>21 mmHg CO<sub>2</sub> at 25°C</b>	3.47±0.2	3.95±0.1*,+	4.58±0.1*,+	4.15±0.2*,+
<b>Normocapnia at 33°C</b>	3.43±0.1	3.48±0.3	3.48±0.4	3.47±0.3
<b>21 mmHg CO<sub>2</sub> at 33°C</b>	3.52±0.2	4.17±0.2*	4.55±0.2*,+	4.30±0.31*,+
<b>Cl<sup>-</sup> (mM)</b>				
<b>Normocapnia at 25°C</b>	104.5±1.9	102.3±3.2	102.2±2.4	103.0±2.1
<b>21 mmHg CO<sub>2</sub> at 25°C</b>	106.2±2.6	101.5±2.8	96.7±1.8*	94.5±2.0*,+
<b>Normocapnia at 33°C</b>	100.2±3.1	100.0±1.4	99.8±2.8	99.3±1.6
<b>21 mmHg CO<sub>2</sub> at 33°C</b>	100.0±2.2	95.2±2.7	94.7±2.8	89.7±2.0*,+
<b>Osmolality (mOsm)</b>				
<b>Normocapnia at 25°C</b>	288.0±4.9	290.5±3.7	289.0±2.5	291.8±3.4
<b>21 mmHg CO<sub>2</sub> at 25°C</b>	285.5±3.6	279.3±4.1	273.3±5.6*,+	265±5.2*,+
<b>Normocapnia at 33°C</b>	291.5±3.6	289.0±2.0	290.7±4.4	290.5±3.9
<b>21 mmHg CO<sub>2</sub> at 33°C</b>	291.3±3.4	282.0±3.6	280.7±4.1*,+	278.8±2.2*,+

**Table S4:** Haem concentration (Hb), haematocrit (Hct) and mean corpuscular haemoglobin concentration (MCHC) under combined effects of temperature and hypercapnia in the four exposure groups: normocapnia at 25°C, 21 mmHg CO<sub>2</sub> at 25°C, normocapnia at 33°C, 21 mmHg CO<sub>2</sub> at 33°C. The data of normocapnia at 25 and 33°C were collected from series 1. A two-way ANOVA was used for showing significant difference from 0 h within treatment (asterisks) and showing significant difference to controls (normocapnia at 25°C or 33°C) at a sampling time (plus signs). Data are mean ± S.E.M (N=6).

	<b>0 h</b>	<b>24 h</b>	<b>48 h</b>	<b>72 h</b>
<b>Haem (mM)</b>				
<b>Normocapnia at 25°C</b>	6.33±0.6	6.33±0.3	6.35±0.2	6.30±0.2
<b>21 mmHg CO<sub>2</sub> at 25°C</b>	6.23±0.1	5.57±0.5	5.75±0.1	5.93±0.2
<b>Normocapnia at 33°C</b>	5.31±0.1	5.35±0.4	5.32±0.4	5.36±0.2
<b>21 mmHg CO<sub>2</sub> at 33°C</b>	5.41±0.3	4.99±0.3	5.09±0.3	5.03±0.3
<b>Hct (%)</b>				
<b>Normocapnia at 25°C</b>	31.9±1.7	32.0±0.9	31.5±1.0	31.2±0.4
<b>21 mmHg CO<sub>2</sub> at 25°C</b>	31.4±2.0	28.1±2.0	27.1±2.5	28.2±0.5
<b>Normocapnia at 33°C</b>	27.0±0.9	26.7±1.0	27.5±1.5	26.9±1.0
<b>21 mmHg CO<sub>2</sub> at 33°C</b>	29.2±0.5	25.2±2.4 <sup>*,+</sup>	25.5±1.3	26.5±1.4
<b>MCHC (mM)</b>				
<b>Normocapnia at 25°C</b>	20.1±2.0	19.9±1.3	20.2±0.8	20.2±0.9
<b>21 mmHg CO<sub>2</sub> at 25°C</b>	20.2±1.2	20.2±2.2	20.2±2.0	21.1±0.9
<b>Normocapnia at 33°C</b>	19.8±0.7	20.1±1.5	19.5±1.4	20.0±0.9
<b>21 mmHg CO<sub>2</sub> at 33°C</b>	18.5±0.8	20.9±2.6	20.2±1.7	18.9±0.8