*J. exp. Biol.* **134**, 463–466 (1988)
*Printed in Great Britain* © *The Company of Biologists Limited 1988*



SHORT COMMUNICATION

# HIGH BLOOD $CO_2$ LEVELS IN RAINBOW TROUT EXPOSED TO HYPERCAPNIA IN BICARBONATE-RICH HARD FRESH WATER – A METHODOLOGICAL VERIFICATION

BY KENTH DIMBERG

*Department of Zoophysiology, University of Uppsala, Box 560, S-75122, Uppsala, Sweden*

*Accepted 11 August 1987*

Heisler (1984, 1986) suggested that fish have a maximum extracellular bicarbonate concentration of about 30 mmol $l^{-1}$, which can be attained and used for the compensation of blood acidosis induced during environmental hypercapnia. In contrast, Börjeson (1977) and Dimberg & Höglund (1987) reported $HCO_3^-$ concentrations, analysed using Astrup's tonometric method (Astrup, 1956), of 40–50 mmol $l^{-1}$ in venous blood drawn by a syringe from captured fish. However, blood pH in rainbow trout is greatly affected by sampling stress induced by venous puncture (Railo, Nikinmaa & Soivio, 1985). This may introduce error into the determination of blood [$HCO_3^-$] when using a tonometric technique based on pH measurements. Consequently, the present study was performed to determine whether the maximal blood $HCO_3^-$ level in hypercapnic fish is limited to 30 mmol $l^{-1}$, and if the higher reported blood bicarbonate values could be the result of sampling and/or analytical errors. Rainbow trout (*Salmo gairdneri*) were exposed to different levels of hypercapnia and acid–base variables were analysed either in venous blood taken with a syringe in the ductus Cuvieri from captured fish or in blood sampled *via* a dorsal aortic catheter from undisturbed fish. In addition, tonometry and gasometry were carried out separately to determine total blood $CO_2$ concentration.

Rainbow trout, weighing 250–350 g, were acclimated to flowing Uppsala tap water (8–10°C, pH 7·88, $P_{CO_2} = 0{\cdot}36$ kPa, $[O_2] = 11$ mg $l^{-1}$, $[Na^+] = 0{\cdot}6$ mmol $l^{-1}$, $[Cl^-] = 0{\cdot}55$ mmol $l^{-1}$, $[Ca^{2+}] = 2{\cdot}60$ mmol $l^{-1}$, $[HCO_3^-] = 4$ mmol $l^{-1}$) for at least 3 months.

Three weeks before the experiments, fish were anaesthetized with MS-222, and the dorsal aorta was cannulated as described by Soivio, Nyholm & Westman (1975). Six fish were then transferred to 100-l test aquaria with water of the same quality as that used during acclimation, except that the pH was 7·65 and the $P_{CO_2}$ was 0·57 kPa.

Key words: rainbow trout, hard fresh water, hypercapnia, high blood $CO_2$ concentration, methodological verification.

48 h before the experiment began the fish were enclosed in individual restrainers. At the start of the experiment the fish were exposed to 1·73 kPa $P_{CO_2}$ by using hydrochloric acid to acidify continuously the flowing aerated water until it reached pH 7·0. Samples were taken at 0, 4 and 48 h. Approximately 0·3 ml of arterial blood was removed and replaced by an equivalent amount of Ringer's solution (Wolf, 1963). Blood acid–base variables were analysed tonometrically according to the method of Astrup (1956).

Uncannulated and unrestrained fish were pre-acclimated for 3 weeks and then exposed to 1·73 kPa $P_{CO_2}$ as in the previous experiment. Venous blood samples were taken from six trout with a syringe *via* the ductus Cuvieri, and then immediately killed. The venous sampling procedure, including capture, lasted about 45 s. Blood acid–base variables were analysed tonometrically.

Five fish were exposed to 3·47 kPa $P_{CO_2}$ to investigate the possible maximum blood $CO_2$ level attained during hypercapnia. Only uncannulated trout were used and venous blood samples were taken as before. Trout were pre-acclimated for 3 weeks in test aquaria and then subjected to steadily increasing hypercapnia for 3 days, reaching a final level of 3·47 kPa $P_{CO_2}$ (water pH = 6·70). This level of hypercapnia was then maintained for an additional 3 days. Total blood $CO_2$ content was analysed both tonometrically and gasometrically.

Blood pH values were measured in samples using a micro-pH electrode unit (Radiometer, G 297/G 2). Blood [$HCO_3^-$] and total [$CO_2$] were calculated according to the Hendersson–Hasselbalch equation, using values of pKa and $CO_2$ solubility from Boutilier, Heming & Iwama (1984). When gasometry was used for the total blood $CO_2$ determination, samples of 50–100 $\mu$l of whole blood were acidified with 0·1 mol $l^{-1}$ HCl in a Warburg flask. The amount of $CO_2$ evolved from the blood was compared with a standard curve using $Na_2CO_3$ as a reference. In addition, samples were also treated with 20 % KOH in the Warburg flask to correct for the $O_2$ content in the blood.

Results were statistically analysed using Wilcoxon rank-sum or signed-rank tests (Colquhoun, 1971); 5 % was taken as the fiducial limit of significance.

The effect of 1·73 kPa external $P_{CO_2}$ on acid–base variables followed the 'classical' response: the induced acidosis returned to normal values as blood $HCO_3^-$ levels increased (Table 1). Both blood sampling procedures revealed that the hypercapnic treatment elevated blood $HCO_3^-$ levels to about 55 mmol $l^{-1}$. In the third experimental series, with 3·47 kPa hypercapnia, venous blood pH was 7·634 ± 0·052, blood $P_{CO_2}$ rose to 4·56 ± 0·47 kPa $P_{CO_2}$, and blood $CO_2$, which approximately equals the $HCO_3^-$ concentration, increased to as high as 66 mmol $l^{-1}$ (means ± S.D., $N = 5$). There was no statistically significant difference between tonometrically and gasometrically obtained blood $CO_2$ values.

From the present results it is evident that blood $CO_2$ concentration is not affected by the 'grab and stab' technique as applied under the conditions used in this study. Moreover, the consistency between the tonometric and the gasometric estimates of blood $CO_2$ concentration excludes any sort of analytical error attributable to the Astrup method (1956), used by Börjeson (1977) and Dimberg & Höglund (1987).

Table 1. *Effect of external hypercapnia (1·73 kPa $P_{CO_2}$) on venous and arterial blood acid–base variables in rainbow trout*

| Time (h) | pH | $P_{CO_2}$ (kPa) | $HCO_3^-$ (mmol $l^{-1}$) | $C_{CO_2}$ (mmol $l^{-1}$) |
|---|---|---|---|---|
| Venous blood taken with a syringe from captured fish ($N = 6$) | | | | |
| 0 | 7·670 ± 0·052 | 1·32 ± 0·2 | 20·6 ± 1·1 | 21·1 ± 1·3 |
| 4 | 7·430 ± 0·064* | 2·69 ± 0·3* | 23·1 ± 5·4 | 24·4 ± 5·5 |
| 48 | 7·805 ± 0·065* | 2·52 ± 0·17* | 55·2 ± 5·2* | 56·4 ± 5·2* |
| Arterial blood taken *via* dorsal aortic cannulae ($N = 6$) | | | | |
| 0 | 7·987 ± 0·080 | 0·6 ± 0·1 | 18·4 ± 1·0 | 18·6 ± 0·98 |
| 4 | 7·663 ± 0·030* | 1·6 ± 0·1* | 24·6 ± 3·3* | 25·4 ± 3·3* |
| 48 | 7·935 ± 0·040 | 1·8 ± 0·03* | 53·8 ± 5·2* | 54·7 ± 5·2* |

Values are means ± S.D.

* Significantly different from value at zero time, Wilcoxon rank-sum or signed-rank tests, $P < 0·05$.

Finally, for comparison, the concentration of $CO_2$ in blood from rainbow trout adapted to Uppsala tap water diluted 1 : 10 with deionized water for several months at pH 7·60 and $P_{CO_2} = 0·13$ kPa was measured and found to be about 10 mmol $l^{-1}$. This is in agreement with the data given by Perry (1982) for control fish adapted to water with a low $P_{CO_2}$ and low bicarbonate concentration. It therefore seems reasonable to conclude that the high blood $CO_2$ concentration demonstrated in this study was real, although it is confusing that the level is at least twice as high as that found in most other investigations. However, as discussed by Heisler (1986), the bicarbonate in the hypercapnic fish has to be increased by the same factor as blood $P_{CO_2}$ to achieve complete pH compensation. Accordingly, if the blood $P_{CO_2}$ is increased from 0·6 to 1·79 kPa, i.e. about three times, a fish with an initial blood $CO_2$ concentration of 19 mmol $l^{-1}$ has to increase the $CO_2$ content to 57 mmol $l^{-1}$. This is indeed the case in the present study with 1·73 kPa hypercapnia. An explanation for the discrepancy between the present high level of blood $CO_2$ with that value in the literature may be the use of the Uppsala water, characterized by high bicarbonate concentration and relatively high $P_{CO_2}$. The importance of the ion composition and buffering capacity of the external medium during hypercapnia has been discussed previously by Heisler & Neuman (1977) and Perry (1982). However, the possible maximal blood $CO_2$ level attained in hypercapnic rainbow trout and to what extent the external bicarbonate concentration interact with the adaptory process must be explored further.

This work was supported by a grant from the Hierta Retzius Foundation. Thanks are given to Docent L. B. Höglund for his comments on the manuscript.

## REFERENCES

ASTRUP, P. (1956). A simple electrometric technique for the determination of carbon dioxide tension in blood and plasma, total content of carbon dioxide in plasma, and bicarbonate in "separated" plasma at a fixed carbon dioxide tension (40 mmHg). *Scand. J. clin. Lab. Invest.* **8**, 33–43.

BÖRJESON, H. (1977). Effects of hypercapnia on the buffer capacity and haematological values in *Salmo salar* (L). *J. Fish Biol.* **11**, 133–142.

BOUTILIER, R. G., HEMING, T. A. & IWAMA, G. K. (1984). Physicochemical parameters for use in fish respiratory physiology. In *Fish Physiology*, vol. XA (ed. W. S. Hoar & D. J. Randall), pp. 403–430. New York, London: Academic Press.

COLQUHOUN, D. (1971). *Lectures on Biostatistics. An Introduction to Statistics with Applications in Biology and Medicine.* Oxford: Oxford University Press.

DIMBERG, K. & HÖGLUND, L. B. (1987). Carbonic anhydrase activity in the blood and the gills of rainbow trout during long-term hypercapnia in hard, bicarbonate-rich freshwater. *J. comp. Physiol.* B (in press).

HEISLER, N. (1984). Acid–base regulation in fishes. In *Fish Physiology*, vol. XA (ed. W. S. Hoar & D. J. Randall), pp. 315–401. New York, London: Academic Press.

HEISLER, N. (1986). Acid–base regulation in fishes. In *Acid–base Regulation in Animals* (ed. N. Heisler), pp. 309–356. Amsterdam: Elsevier.

HEISLER, N. & NEUMANN, P. (1977). Influence of seawater pH upon bicarbonate uptake induced by hypercapnia in an elasmobranch fish (*Scyliorhinus stellaris*). *Pflügers Arch. ges. Physiol.* (Suppl.) **368**, R19.

PERRY, S. F. (1982). The regulation of hypercapnic acidosis in two salmonids, the freshwater trout (*Salmo gairdneri*) and the seawater salmon (*Oncorhynchus kisutch*). *Mar. Behav. Physiol.* **9**, 73–79.

RAILO, E., NIKINMAA, M. & SOIVIO, A. (1985). Effects of sampling on blood parameters in the rainbow trout (*Salmo gairdneri* Richardson). *J. Fish Biol.* **26**, 725–732.

SOIVIO, A., NYHOLM, K. & WESTMAN, K. (1975). A technique for repeated sampling of the blood of individual resting fish. *J. exp. Biol.* **62**, 207–217.

WOLF, K. (1963). Physiological salines for freshwater teleosts. *Progrve Fish. Cult.* **25**, 135–140.