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ACID-BASE BALANCE AND GAS EXCHANGE IN BROOK TROUT (*SALVELINUS FONTINALIS*) EXPOSED TO ACIDIC ENVIRONMENTS

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SUMMARY

Brook trout (*Salvelinus fontinalis* Mitchill) with a chronically implanted dorsal aortic cannula were exposed to acidic environments. During exposure, trout developed severe metabolic acidosis as shown by decreases in standard plasma bicarbonate levels as well as negative base excess values. Reduced oxygen consumption seen in acidotic trout resulted from decreased gill oxygen transfer and reduced available blood oxygen capacity.

INTRODUCTION

There have been a number of studies documenting the effects of acidic environments on fish. Fish death at low environmental pH has been attributed to suffocation due to coagulation of mucus on the gills (Ellis, 1937; Westfall, 1945; Plonka & Neff, 1969), decreased blood P_{O_2} (Vaala & Mitchell, 1970), and to a decreased blood oxygen-carrying capacity as blood pH is lowered (Green & Root, 1933). Packer & Dunson (1970, 1972) found that exposure of brook trout (*Salvelinus fontinalis*) to a low pH environment caused a significant loss of body sodium, a decrease in blood pH, and decreased oxygen consumption ($\dot{V}_{O_2}$). Janssen & Randall (1975) reported a decrease in arterial blood pH (pH_a) and a slow gradual increase in ventilation volume ($\dot{V}_G$) in rainbow trout (*Salmo gairdneri*) exposed to water of pH 5·0 (HCl added). Injection of HCl into the dorsal aorta caused an immediate reduction in pH_a and a rapid and marked increase in $\dot{V}_G$. Eddy (1976) also found that injections of HCl into the blood of rainbow trout caused a decrease in pH_a and plasma bicarbonate concentration ($[HCO_3^-]$) accompanied by increases in $\dot{V}_{O_2}$ and $\dot{V}_G$.

In the present study I attempted to determine if the previously described acidosis seen in acid exposed fish was respiratory, metabolic, or a combination of the two. I also investigated the relative importance of inhibition of branchial gas exchange compared to decreased blood oxygen carrying capacity as factors contributing to decreased $\dot{V}_{O_2}$ during acid exposure.

MATERIALS AND METHODS

These experiments were done using brook trout (*Salvelinus fontinalis*) of both sexes. Origin of the trout used, maintenance of trout in the laboratory, cannulation

procedures, the respirometer system, methods for measuring blood pH, P_{CO_2}, and haemoglobin concentrations, as well as the nomogram used to measure base excess (BE) and standard and actual bicarbonate are described by Packer & Sunkin (1978).

To study the effects of acid exposure six strain H (wt 185–380 g), four strain O (wt 285–465 g) and five strain 15 (wt 235–485 g) trout were cannulated, placed in the respirometer, and allowed to recover for at least 24 h. Water temperature was maintained at $18 \pm 0{\cdot}5$ °C. To expose trout to acid, small volumes (0·1–1·0 ml) of concentrated H_2SO_4 were added periodically to the reservoir of the respirometer system over a period of 1–2 h so that the pH of the water fell gradually from 6·90–7·05 to 3·15–3·50. A Corning model 12B pH meter was used to measure water pH. Water pH was maintained between 3·15 and 3·50 for the duration of the experiment. Trout survived 3–8 h after the addition of acid.

Prior to addition of acid, and at regular intervals during acid exposure, the $\dot{V}_{O_2}$ of the trout was measured. The P_{O_2} of water entering and leaving the respirometer was measured using a Radiometer type E5046/D616 oxygen electrode system in conjunction with a Radiometer PHA934 P_{O_2} analyser. Water flow through the respirometer was measured using a Gilmont model 3294-20 flowmeter, and $\dot{V}_{O_2}$ was calculated by the Fick technique. The oxygen electrode was calibrated using air-saturated water and P_{O_2}-zero solution (Radiometer type 10-S4150). All values were corrected to s.t.p. Prior to and during acid exposure periodic blood samples of approximately 200–300 μl were drawn anaerobically from the dorsal aorta cannula and blood pH, P_{O_2}, haematocrit, and [Hb] were determined. P_{O_2} was measured using the Radiometer system. I also did pH-P_{CO_2} titrations using the Astrup technique, plotted lines on the nomogram, and read values for original P_{CO_2}, BE, and actual and standard bicarbonate.

Dorsal aortic blood P_{O_2}, O_2 content, available O_2 capacity, and % O_2 saturation were measured in eight strain H trout exposed to neutral and low pH in an attempt to explain the decrease in $\dot{V}_{O_2}$ observed in acid-exposed trout. Blood O_2 content was measured using the methods of Tazawa (1970). Approximately 300 μl of blood was collected anaerobically from the dorsal aorta of cannulated trout in the respirometer. P_{O_2}, pH, and [Hb] of this sample was measured and the O_2 content of a 20 μl aliquot was determined. Also, an 80 μl aliquot was placed in the tonometer of the Radiometer blood gas instrument. This blood was equilibrated with room air saturated with H_2O for 12 min. Following equilibration, the pH of this blood was measured and the O_2 content of a 20 μl aliquot was determined. The O_2 content of this air-equilibrated sample was considered to be a measure of the available O_2 capacity, and the % O_2 saturation of the original blood sample was calculated by dividing the O_2 content of the original sample by the available O_2 capacity. The term 'available O_2 capacity' is used rather than 'O_2 capacity' since no measurements of sulf-, met-, or carboxyhaemoglobin were made.

RESULTS

Responses of two trout, which are typical of all 15 fish exposed to acid environments, are shown in Fig. 1. Blood pH decreased as did actual [HCO_3^-]. BE values became increasingly negative and standard [HCO_3^-] decreased, indicating development of an increasingly severe metabolic acidosis.

There was no indication of a serious respiratory acidosis during acid exposure. In

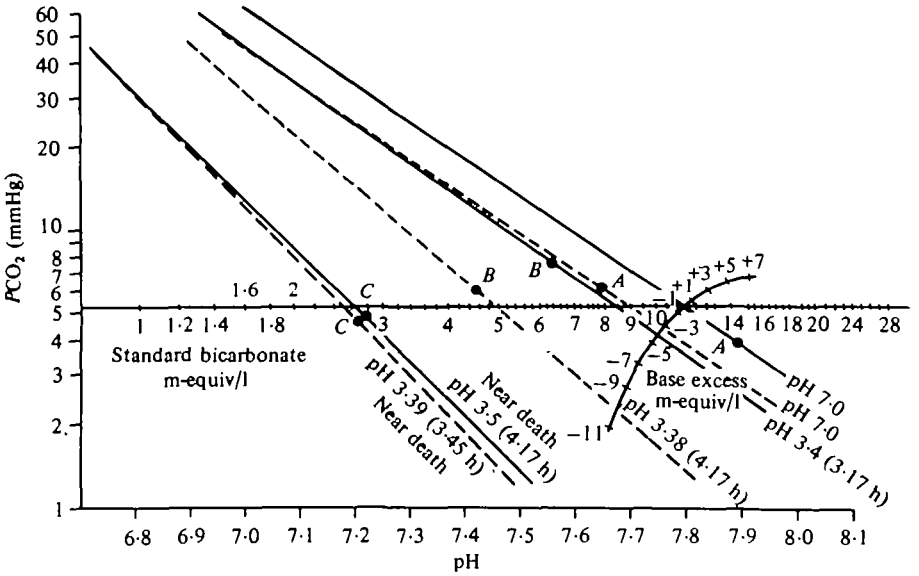

Fig. 1. Changes in blood acid-base status during acid exposure. The response of one trout is shown using three solid pH-P_{CO_2} titration lines, the other by three dashed lines. Closed circles on the titration lines indicate *in vivo* blood pH and P_{CO_2} and are marked *A*, *B*, *C*; denoting the temporal order in which the measurements were made. Environmental pH and time of exposure to low pH are shown near the bottom of pH-P_{CO_2} titration lines.

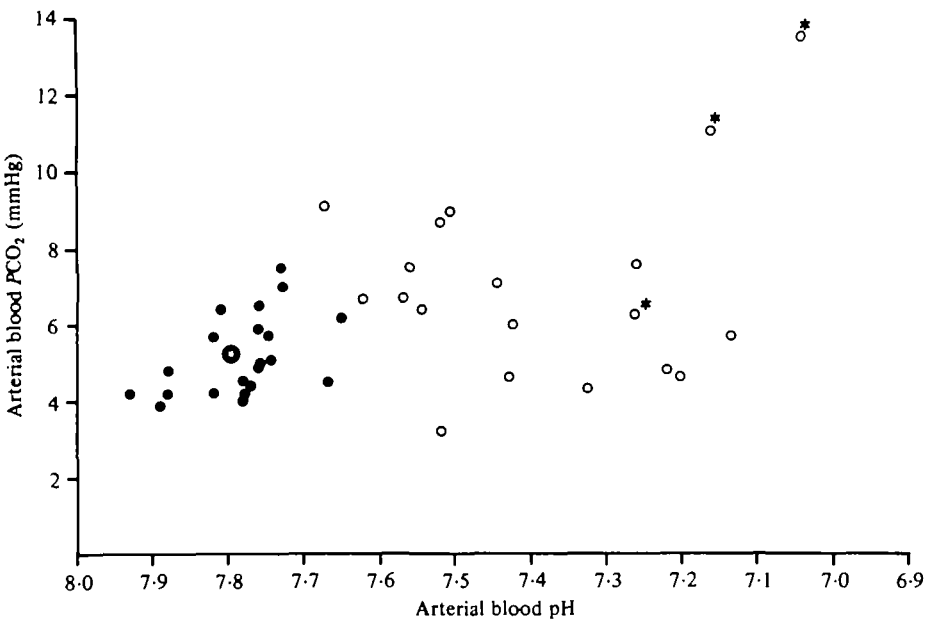

Fig. 2. The relationship between arterial blood pH and P_{CO_2}. Closed circles denote values from fish exposed to a neutral pH. Open circles show values from fish exposed to environments of pH 3·15–3·50 for up to 7 h. Asterisks denote values from fish near death. The large closed circle enclosing a star is plotted at the point of mean arterial blood pH and P_{CO_2} for trout in neutral environments.

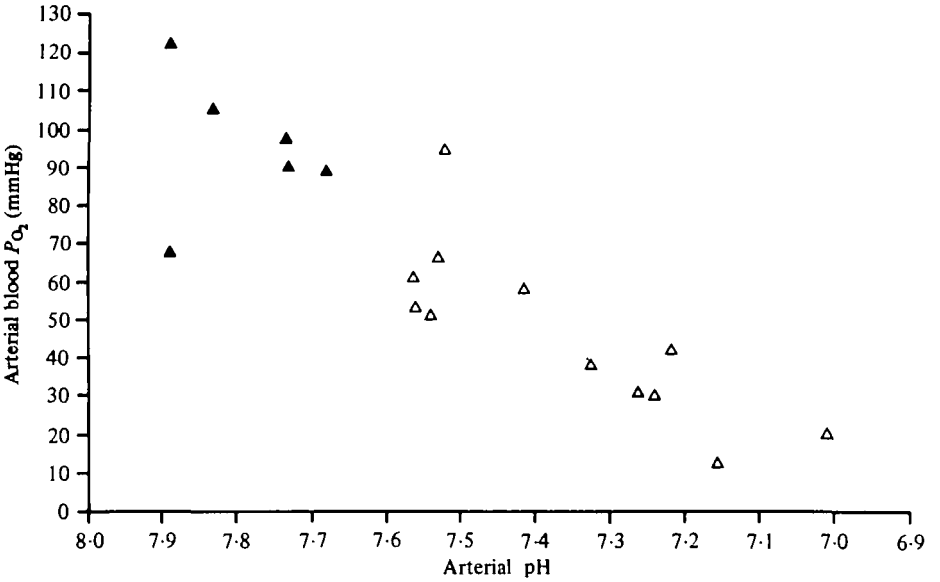

Fig. 3. The relationship between arterial blood pH and P_{O_2}. Closed triangles represent values from fish exposed to a neutral environment. Open triangles show values for fish exposed to environments of pH 3·1–3·4 for up to 8·08 h.

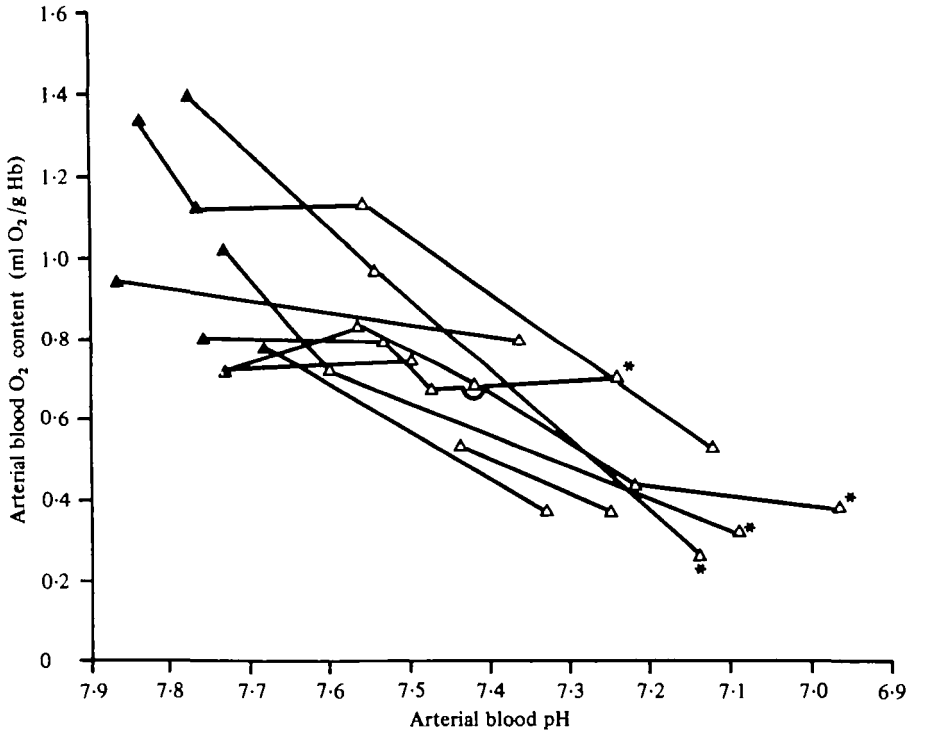

Fig. 4. The relationship between arterial blood pH and oxygen content. Lines connect values obtained from a single fish exposed to a neutral environment (closed triangles) and a low pH environment (open triangles). Asteriaks indicate that the fish were ventilating irregularly when the blood sample was taken.

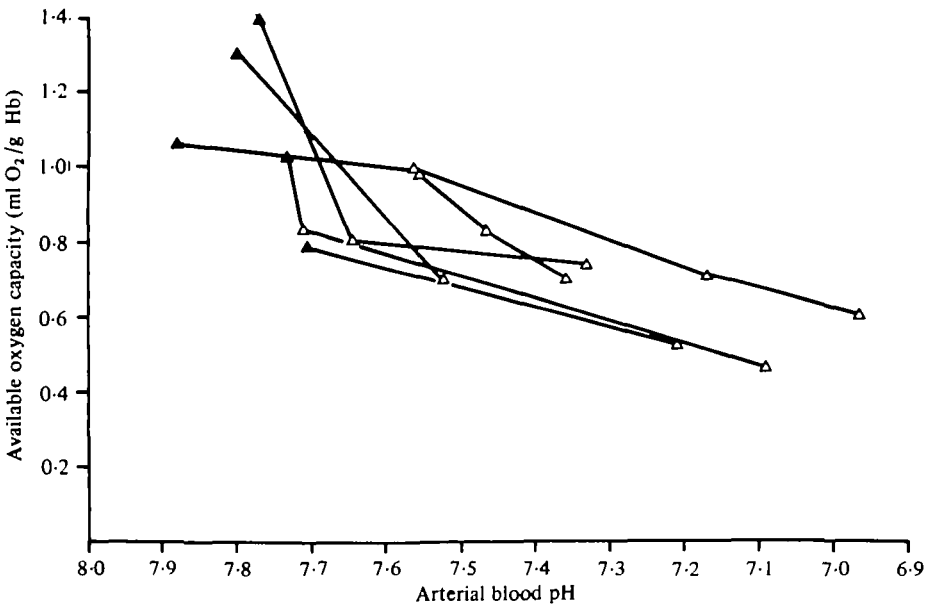

Fig. 5. The relationship between arterial blood pH and available oxygen capacity. Lines connect values obtained from a single fish exposed to a neutral environment (closed triangles) and a low pH environment (open triangles).

some instances arterial P_{CO_2} increased slightly during the initial stage of acid exposure (Fig. 2). $\dot{V}_{O_2}$ also occasionally increased during this time, and the increase in arterial P_{CO_2} may have been caused by an increase in the P_{CO_2} of the respirometer water.

$\dot{V}_{O_2}$ decreased from a mean rate of $10{\cdot}0 \pm 2{\cdot}9$ ml/100 g h^{-1} ($\bar{X} \pm$ S.D., $n = 20$) at neutral pH, as blood pH fell during acid exposure. Blood P_{O_2} also declined as blood pH fell in acid-exposed fish (Fig. 3), indicating an inhibition of O_2 transfer across the gill. The P_{O_2} of inspired water was always considerably higher than that of blood. Water entering the respirometer had a P_{O_2} of 150–157 mmHg and water leaving had a P_{O_2} of 121 mmHg or higher. Judging from the rate of blood flow in the dorsal aortic cannulae, it appeared that blood pressure remained high until blood pH fell below about 7·2 and the trout neared death. Thus, gill blood flow should have remained adequate. At blood pH near or below 7·2, ventilation appeared weak and irregular. A decrease in blood pressure and $\dot{V}_G$ as the animal neared death could help account for the very low P_{O_2} values seen at very low blood pH (Fig. 3). While blood pH was above 7·2 during acid exposure, ventilation appeared rapid and strong with frequent 'coughing'. Therefore, until the blood pH nears the lethal point the decrease in P_{O_2} does not appear to be due to decreases in gill blood flow or $\dot{V}_G$.

Blood oxygen content decreased from control levels ($\bar{X} \pm$ S.D. $= 0{\cdot}96 \pm 0{\cdot}27$ ml O_2/g Hb, $n = 8$) as did available O_2-carrying capacity ($\bar{X} \pm$ S.D. for controls $= 1{\cdot}11 \pm 0{\cdot}24$ ml O_2/g Hb, $n = 5$) as blood pH declined during acid exposure (Figs. 4 and 5). Available O_2 capacity was determined by equilibrating blood samples with room air ($P_{CO_2} < 0{\cdot}3$ mmHg). Therefore the available O_2 capacity values reported are probably higher than *in vivo* values since blood P_{CO_2} of trout in the respirometer

averaged 5·2 mmHg, and O_2 capacity decreases as P_{CO_2} increases (Root effect). No dramatic decrease in % O_2 saturation of Hb was seen consistently during acid exposure.

DISCUSSION

Exposure of trout to a low pH environment results in severe metabolic acidosis, as shown by decreases in blood pH, actual and standard bicarbonate concentration, and negative BE values. Once blood pH fell below about 7·25–7·35, the fish lost equilibrium and died, usually within 15 min. At this point, standard [HCO_3^-] was between 2·5 and 3·5 m-equiv/l, which was about 8 m-equiv/l below the average control value.

The observed metabolic acidosis could be caused by an accumulation of H^+ from the water or accumulation of metabolically produced acid, or both. Trout struggled very infrequently in the respirometer as the water pH was gradually lowered. They usually remained quiescent during the course of acid exposure, with brief struggling at the time of death. Therefore, a large production of lactic acid due to high skeletal muscle activity would not be expected. However, blood oxygen levels indicate that tissue P_{O_2} would be low. This could lead to an increase in anaerobic metabolism and an accumulation of lactic acid. Also, H^+ excretion by the gills may be inhibited by high environmental [H^+].

Increased blood [H^+] and decreased plasma [HCO_3^-] would be expected to impair ionic balance. Packer & Dunson (1970) found that trout in acidic environments lose body Na^+ as a result of inhibition of Na^+ influx and an increase in Na^+ efflux. In a later study (Packer & Dunson, 1972) they reported that the rate of net Na^+ loss was inversely related to survival time. Maetz (1973) demonstrated that Na^+ influx is linked to efflux of NH_4^+ and H^+ and also showed that increased environmental [H^+] inhibited Na^+ influx. In addition to Na^+ imbalance, one would expect that trout suffering metabolic acidosis might also show a Cl^- imbalance. De Renzis & Maetz (1973) demonstrated a Cl^-/HCO_3^- exchange in the gills of goldfish (*Carassius auratus*) in which HCO_3^- serves as a counter-ion for the uptake of Cl^- from the environment. They also found that goldfish suffered acidosis in an environment lacking Na^+ and alkalosis in an environment lacking Cl^-. Presumably, acidosis results from inhibition of H^+ excretion and alkalosis from inhibition of HCO_3^- excretion due to absence of their counterions in the environment. The severe metabolic acidosis found in acid-exposed trout in this study would be expected to cause inhibition of Cl^- uptake due to low plasma [HCO_3^-].

Acid exposure caused decreases in $\dot{V}_{O_2}$, blood P_{O_2} (Fig. 3), blood O_2 content (Fig. 4) and available blood O_2 capacity (Fig. 5). The gills of the fish were exposed to an environment of pH 3·15–3·50. Exposure to low pH causes coagulation of mucus on fish gills (Ellis, 1937; Westfall, 1945; Plonka & Neff, 1969) and this is believed to inhibit O_2 transfer. The low blood P_{O_2} which was found in acid-exposed fish indicates that the depression of O_2 consumption seen during acid exposure is due, at least in part, to a decrease in O_2 transfer across the gill. Blood P_{CO_2} levels did not increase during acid exposure, indicating that CO_2 excretion is unaffected.

The decrease in blood O_2 content can be explained by inhibition of gill oxygen transfer and by the decrease in blood O_2 capacity resulting from Bohr and Root effects caused by a decrease in blood pH.

Table 1

Variable	Equation of regression line	Upper and lower 95% intervals about the confidence slope	Correlation coefficient r
$\dot{V}_{O_2}$	$Y = 86{\cdot}09 + (-11{\cdot}23X)$	$-4{\cdot}96$, $-17{\cdot}51$	$0{\cdot}78$ ($P < 0{\cdot}005$)
P_{O_2}	$Y = 45{\cdot}08 + (-5{\cdot}76X)$	$-3{\cdot}64$, $-7{\cdot}88$	$0{\cdot}87$ ($P < 0{\cdot}001$)
Available blood O_2 capacity	$Y = 20{\cdot}69 + (-2{\cdot}60X)$	$-1{\cdot}43$, $-3{\cdot}77$	$0{\cdot}82$ ($P < 0{\cdot}001$)

Since the decline in blood O_2 content was paralleled by a decrease in available blood O_2 capacity during acid exposure, there was no dramatic decline in % O_2 saturation of Hb. In four of six trout, blood O_2 content decreased more than O_2 capacity and thus % O_2 saturation of haemoglobin declined from nearly 100 to 65–85%. The two trout in which such a decline was not seen had control saturation levels of 60–70%. These low control saturation levels may have been due to high blood P_{CO_2}, or shunting of blood away from respiratory channels in the secondary lamellae. There have been conflicting reports concerning the occurrence of such shunts (Cameron, 1974; Fromm, 1974).

Eddy (1976) found that when he added HCl to the blood of rainbow trout *in vivo*, blood pH fell from 7·83 to 7·55, O_2 content and % O_2 saturation of Hb declined, but in contrast to my findings, blood P_{O_2} and available oxygen capacity were not significantly affected. Since the trout in his study were not exposed to a low environmental pH, one would not expect to see an inhibition of O_2 transfer across the gill leading to low blood P_{O_2}, such as I found. A decline in blood O_2 content (probably due to Bohr and Root effects) concomitant with a constant available O_2 capacity would result in a decline in % O_2 saturation of Hb. Also, the fact that blood pH was often much lower than 7·55 during acid exposure in this study may explain why I found a decrease in available O_2 capacity while Eddy did not.

Acute exposure to low environmental pH caused severe hypoxia and eventual decline in $\dot{V}_{O_2}$. However, $\dot{V}_{O_2}$ transiently increased in some fish during the early stages of acid exposure. Other investigators have reported that acid stress caused increases in $\dot{V}_G$ (Janssen & Randall, 1975; Eddy, 1976) and $\dot{V}_{O_2}$ (Eddy, 1976; Hargis, 1976). Houston, Czerwinski & Woods (1973) found that stress of MS 222 anaesthesia and dorsal aortic cannulation also stimulated $\dot{V}_G$ and $\dot{V}_{O_2}$. Increases in $\dot{V}_G$ in such instances might represent a physiological response to decreased blood pH and resulting decreases in blood oxygen content. Increased $\dot{V}_{O_2}$ could be caused by muscular activity or adrenergic stimulation of tissue respiration.

In this study, at very low blood pH, hypoxia always developed and $\dot{V}_{O_2}$ fell. Reductions in both available blood oxygen capacity and gill oxygen transfer, as evidenced by low blood P_{O_2}, contribute to the decline in $\dot{V}_{O_2}$. The relative contribution of these two factors to reduced $\dot{V}_{O_2}$ during acid exposure was assessed by two-variable linear regression analysis of changes in $\dot{V}_{O_2}$, blood P_{O_2}, and available blood oxygen capacity as blood pH fell during acid exposure (Table 1). To compare the slopes of the regressions, values of $\dot{V}_{O_2}$, blood P_{O_2} and available blood oxygen capacity from acid-exposed trout were expressed as the number of standard deviations each value varied from mean values determined for fish in neutral environments. The slopes of the three regression

lines show that available O_2 capacity decreases at a significantly lower rate than $\dot{V}_{O_2}$ as blood pH falls. Although the slope of regression for P_{O_2} versus pH is less than that of $\dot{V}_{O_2}$ versus pH, the difference is not significant. To the extent that decreases in blood P_{O_2} reflect decreases in gill oxygen transfer, it appears that the decrease in available blood oxygen capacity is a less important factor in restricting $\dot{V}_{O_2}$ in acid-exposed trout than is the decrease in gill oxygen transfer.

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