Journal of Fish Biology (1998) **52,** 621–637

Carbon dioxide anaesthesia in rainbow trout: effects of hypercapnic level and stress on induction and recovery from anaesthetic treatment

N. J. BERNIER*‡ AND D. J. RANDALL†

**University of Ottawa, Department of Biology, 30 Marie Curie, Ottawa, Ont. K1N 6N5 Canada and †University of British Columbia, Department of Zoology, 6270 University Blvd, Vancouver, BC V6T 2A9 Canada*

(*Received 10 June 1997, Accepted 29 October 1997*)

The physiological and anaesthetic effects of three different levels of air-saturated and buffered CO_2 anaesthesia, P_wCO_2 = 37, 78, or 125 mmHg, were examined in cannulated rainbow trout *Oncorhynchus mykiss*. Complete anaesthesia (no opercular movements) was not achieved by these hypercapnic levels after 20 min of CO_2 exposure. Although increasing P_wCO_2 reduced the induction times to the early stages of anaesthesia, it also resulted in increasing hyperventilatory, hypoxaemic, and acid-base disturbances. After a 10-min recovery period, while the respiratory acidosis component of the acid-base disturbance was corrected, there was a significant metabolic acidosis. Recovery time was longest in the high P_wCO_2 treatment where 33% of the fish died. Two additional groups (P_wCO_2 = 37 and 78 mmHg) were exposed to an acute stress prior to the anaesthetic treatment. Stress reduced the hypoventilatory effects of the low P_wCO_2 treatment, increased the recruitment of anaerobic metabolism, and prolonged recovery time. Although the increase in plasma catecholamines elicited by the stress was small relative to the response obtained with the anaesthetic, stress prior to CO_2 anaesthesia impaired the efficiency of the treatment. Overall, our results suggest that P_wCO_2 levels above 37 mmHg and/or stress prior to the anaesthesia impair the efficiency of air-saturated and buffered CO_2 anaesthesia by exacerbating the hypoxaemic effects of the hypercapnic treatment.

Key words: anaesthesia; hypercapnia; acid-base balance; stress; salmonid.

INTRODUCTION

The necessity of transporting and handling live fish within various fish enhancement programmes, commercial fisheries, and the fish-farming industry has led to the development of techniques to anaesthetize fish without impairing their health or commercial value. Although a wide variety of chemical anaesthetics are available (Bell, 1964, 1987; Iwama & Ackerman, 1994), economic, safety, and especially regulatory considerations have rendered the use of chemical anaesthetics on food fish inappropriate (Carpenter, 1994; Iwama & Ackerman, 1994; Prince *et al.*, 1995). Potentially, a suitable nonchemical alternative, first described by Fish (1943), is carbon dioxide gas (CO_2). Unlike chemical anaesthetics, CO_2 leaves no toxic residues in the fish, has no secondary effects on the handler or the environment, when used in a ventilated area, and is economical (Post, 1979; Bell, 1964, 1987; Loch, 1991; Iwama & Ackerman, 1994).

Specific problems associated with the use of CO_2 anaesthesia on salmonids reduce its efficiency compared to other anaesthetics (Gilderhus & Marking,

‡Author to whom correspondence should be addressed. Tel.: (613) 562-5800 ext: 6010; fax: (613) 562-5486; email: nbernier@science.uottawa.ca

0022–1112/98/030621+17 $25.00/0/jb970633

1987). For example, CO_2 anaesthesia leads to an acute and transient period of hyperactivity by the fish (Mechelen, 1985; Bell, 1987; Loch, 1991). The induction and recovery times of CO_2 anaesthesia may also be relatively long (Bell, 1987; Gilderhus & Marking, 1987). Deep anaesthesia is usually not reached with CO_2 (Gilderhus & Marking, 1987), and indicators of stress, such as blood adrenaline and cortisol concentrations, increase faster and reach higher levels with unbuffered CO_2 anaesthesia than with other anaesthetics (Iwama *et al.*, 1989).

Although similar problems have been observed with the use of CO_2 anaesthesia on common carp *Cyprinus carpio* L., studies using carp which are implanted chronically with a cannula have allowed detailed relationships to be developed between the characteristics of the anaesthetic water, changes in blood gas, blood pH, and progression in the depth of anaesthesia (Mitsuda *et al.*, 1982; Yoshikawa *et al.*, 1988*a*, 1991). The comprehensive nature of these experiments has shown the importance of an appropriate balance between the partial pressure of CO_2 (P_wCO_2) and the partial pressure of oxygen (P_wO_2) in the anaesthetic water in order to achieve specific induction and recovery rates (Yoshikawa *et al.*, 1988*a*, *b*, 1991). The characteristics of the anaesthetic water may also allow a reduction in the oxygen consumption of *C. carpio* anaesthetized with CO_2 while still maintaining the oxygen carrying capacity of the blood (Itazawa & Takeda, 1982), conditions which may reduce the stress of the anaesthetic treatment. Overall, these studies have shown that the minimum P_wCO_2 required for induction of CO_2 anaesthesia in *C. carpio* is beteen 100 and 125 mmHg, and the preferred range for short term anaesthesia (30 min) is between 175 and 250 mmHg (Mitsuda *et al.*, 1988; Yoshikawa *et al.*, 1988*a*, *b*, 1991). In contrast, the CO_2 content of the anaesthetic water (C_wCO_2) recommended and utilized for anaesthetizing salmonids is in the range of 200 to 500 mg l^{-1} (equivalent to a P_wCO_2 range of approximately 18–44 mmHg at pH 6·9 and 12° C; Mechelen, 1985; Bell, 1987; Gilderhus & Marking, 1987; Loch, 1991). Although there are important differences in the blood respiratory and cardiovascular variables between *C. carpio* and salmonids (Perry & McDonald, 1993), it remains to be seen whether these differences warrant such different approaches to the use of CO_2 as an anaesthetic.

In the only study where cannulated salmonids were exposed to anaesthetizing levels of CO_2, C_wCO_2, P_wCO_2, or P_wO_2 were not recorded (Iwama *et al.*, 1989). Hence, in order to improve the methodology for CO_2 anaesthesia with salmonids and to alleviate some of the problems outlined above, detailed relationships between the anaesthetic conditions and the behavioural and physiological responses to CO_2 anaesthesia must be explored further. Moreover, although Bell (1987) suggests that the hyperactivity exhibited by fish anaesthetized with CO_2 might be reduced if the latter are not agitated prior to the treatment, this area has yet to be investigated. The aim of this study was therefore twofold: (a) to investigate the relative merits of using anaesthetizing levels of CO_2 which are two and three times the presently recommended levels; and (b) to compare the relative efficiency of CO_2 anaesthesia on calm or stressed fish. To achieve these goals, a comprehensive approach was used which included a description of the effects of these various hypercapnic treatments on the induction and recovery times to anaesthesia, their effects on ventilation frequency, and on the blood

chemistry of rainbow trout *Oncorhynchus mykiss* (Walbaum) fitted chronically with cannulae. At the outset, it was hypothesized that under buffered and air-saturated conditions, increasing the level of CO_2 in the anaesthetic water above the recommended level and an avoidance of stressors prior to anaesthesia will improve the efficiency of CO_2 as an anaesthetic.

MATERIALS AND METHODS

This study comprised five experimental groups of rainbow trout, each of which was exposed to one of three different levels of anaesthetizing hypercapnic water: low (P_wCO_2 = 36·5 mmHg), medium (P_wCO_2 = 77·9 mmHg), or high (P_wCO_2 = 124·8 mmHg). In the low and the medium groups, fish were either resting or stressed prior to the anaesthetic treatment. Hence, the five experimental groups consisted of: low (n = 8), low + stress (n = 8), medium (n = 6), medium + stress (n = 6), and high (n = 6).

EXPERIMENTAL ANIMALS

Rainbow trout of either sex were obtained from West Creek Trout Farm (Aldergrove, B. C., Canada), and kept in 800-l outdoor fibreglass tanks supplied with flow-through dechlorinated tap water. The fish were acclimated to these conditions for a month prior to the experiments. They were fed with a commercial trout food and kept on a maintenance ration. The experiments were carried out between 7 June and 20 August at a mean water temperature of 12·1 ± 0·4° C. The trout for these experiments had mean body weights of 551·1 ± 12·6 g.

SURGICAL PROCEDURE

Trout were anaesthetized in a buffered ($NaHCO_3$) MS-222 solution at a concentration of 1:10 000, and transferred to an operating table where they were force-ventilated with a buffered, cooled, and aerated MS-222 solution of 1:16 000. The dorsal aorta was cannulated chronically with polyethylene 50 tubing (Clay Adams) using the technique of Soivio *et al.* (1975), and the fish allowed to recover in separate flow-through black perspex boxes. The cannulae were filled and flushed with heparinized (50 IU ml^{-1} sodium heparin) Cortland saline (Wolf, 1963). The trout were undisturbed for 48 h prior to the CO_2 anaesthesia experiments.

EXPERIMENTAL PROTOCOL AND BLOOD SAMPLING

All experiments were carried out on fish kept in 9·2-l black perspex boxes supplied with an aerated normocapnic water source, or hypercapnic water of known CO_2 content, O_2 partial pressure, and pH. Hypercapnia was produced by bubbling CO_2 gas continuously through ceramic air stones (Point Four Systems) in a 100-l reservoir. Water flow to the reservoir, and from the reservoir to the fish box, were kept constant at 2 l min^{-1} for all the treatments, and gas flow through the reservoir was adjusted to achieve the desired PCO_2.

The sampling regime used throughout each hypercapnic trial was as follows: (1) removal of a blood sample to assess resting control values; (2) change from normocapnic water to hypercapnic water source by switching the inflow line to the fish box; (3) removal of blood samples after 10 and 20 min of hypercapnic anaesthesia; (4) return to normocapnic water; and (5) removal of a blood sample after 10 min of recovery. During change from normocapnic to hypercapnic conditions PCO_2 increased exponentially, and 50% of the transformation from one state to the other was achieved within 2·4 min, 90% in 5·4 min, and 97·5% in 10 min. The return to normocapnic conditions after 20 min of hypercapnia was also achieved at a similar rate. In order to prevent O_2 from being forced out of solution by the CO_2, the water in the 100-l reservoir was aerated continuously. During the hypercapnic treatments, the water PO_2 in the fish box decreased by a maximum of 10%. Water pH was kept constant between treatments by pumping a concentrated solution of $NaHCO_3$ to the 100-l reservoir at a rate that matched the flow

TABLE I. Water carbon dioxide content (C_wCO_2), partial pressure of carbon dioxide (P_wCO_2), partial pressure of oxygen (P_wO_2) and pH in a fish holding box after 20 min of hypercapnia in relation to five different anaesthetic treatments

Treatment	n	C_wCO_2 (mM)	P_wCO_2 (mmHg)	P_wO_2 (mmHg)	pH
Low	8	10·12 ± 0·51	36·48 ± 2·74	137·1 ± 0·6	6·88 ± 0·02
Low + stress	8	11·31 ± 0·60	40·50 ± 3·76	135·9 ± 0·5	6·91 ± 0·03
Medium	6	21·88 ± 0·52	77·88 ± 1·77	136·5 ± 0·6	6·92 ± 0·02
Medium + stress	6	22·51 ± 0·90	84·30 ± 4·65	139·9 ± 0·7	6·90 ± 0·01
High	4	34·26 ± 0·79	124·76 ± 8·74	135·2 ± 1·0	6·92 ± 0·03

Values are means ± 1 S.E.

of CO_2 gas. The CO_2 content, partial pressure of CO_2, partial pressure of O_2, and pH of the anaesthetizing water after 20 min of hypercapnic exposure are given in Table I for the five experimental groups.

In all five experimental groups, a total of four blood samples were taken from each fish: at time 0 (control), 10 min (initial CO_2), 20 min (final CO_2), and 30 min (recovery). Each blood sample (800 µl) was replaced by an equivalent volume of Cortland saline, collected in 1·5 ml micro centrifuge tubes and analysed immediately. From the initial sample, aliquots of whole blood were taken for measurement of arterial blood partial pressure of O_2 (P_aO_2) and arterial blood O_2 content (C_aO_2). Whole blood was also collected in heparinized capillary tubes. These tubes were sealed, centrifuged, and used to determine CO_2 content from the plasma fraction (C_aCO_2). The remaining blood was spun down and plasma removed for measurement of plasma pH (pH_a), and later measurement of plasma [lactate] and plasma [catecholamine]. The plasma aliquot for determination of lactate was deproteinized with ice-cold 0·6 N perchloric acid (PCA), spun down, and the supernatant frozen in liquid nitrogen and stored at −80° C for later analysis.

In the low CO_2 + stress and the medium CO_2 + stress treatments, the fish were stressed prior to the hypercapnic anaesthesia by lowering the water level in the holding box by half, thereby partially air-exposing the fish, and keeping the water static for a period of 10 min. This protocol was chosen in an attempt to mimic the stress imposed on fish by the crowding and netting procedures used in fish culture facilities prior to the anaesthesia.

ASSESSMENT OF THE STAGES OF ANAESTHESIA AND VENTILATION FREQUENCY

Each trial was recorded on videotape using a JVC video-camera mounted above the clear plexiglass cover of the fish holding box. The video-tapes were used to assess how much time had elapsed between the beginning of the hypercapnic treatment and stages I and II of anaesthesia, as well as II and III of recovery (Table II). In this study, the definition used for the different stages of anaesthesia and recovery was taken from Iwama *et al.* (1989). However, stages III of anaesthesia and I of recovery were not determined, since cessation of opercular movement never occurred during the hypercapnic treatments, an essential criterion which defines the above stages (Iwama *et al.*, 1989).

The video-tapes also were used to assess ventilation frequency (V_f). Using a stop watch, V_f was assessed visually for 50 s of every minute from the beginning of the hypercapnic treatment until 20 min into the recovery period.

ANALYTICAL TECHNIQUES AND CALCULATIONS

Measurement of P_wO_2, P_aO_2, and C_aO_2 were made using thermostatted Radiometer PO_2 electrodes (E5046; Radiometer, Copenhagen, Denmark) with Radiometer PHM71 acid-base analysers. C_aO_2 was measured using the method described by Tucker (1967). C_wCO_2 and C_aCO_2 were measured with a Carle gas chromatograph (model III) following the

TABLE II. Behavioural characteristics used to define the stages of anaesthesia and recovery of rainbow trout exposed to anaesthetic levels of hypercapnia

	Behavioural characteristics
Stages of anaesthesia	
I	Loss of equilibrium
II	Loss of gross body movements but with regular opercular movements
Stages of recovery	
II	Regular opercular movements and gross body movements beginning
III	Equilibrium regained and pre-anaesthetic appearance

methods of Boutilier *et al.* (1985). P_wCO_2 and P_aCO_2 were calculated using a rearrangement of the Henderson–Hasselbalch equation with values of plasma and water pK and CO_2 solubility coefficients from Boutilier *et al.* (1984). Whole blood pH was measured using a thermostatted Radiometer G297/G2 glass capillary electrode with a PHM71 acid-base analyser. Whole-blood [lactate] was measured spectrophotometrically using the NAD^+-linked enzymatic procedure no. 826-UV of Sigma Chemical (St Louis, MO, U.S.A.). Plasma adrenaline (AD) and noradrenaline (NA) concentrations were determined on alumina-extracted plasma samples using high pressure liquid chromatography (HPLC) based on Woodward (1982).

STATISTICAL ANALYSIS

All data are presented as mean ± one standard error. The statistical significance of observed effects of treatment exposure within a group were tested by one-way repeated measures ANOVA. To compare pretreatment means with means at subsequent sampling times Dunnett's post-test was used. Where appropriate, the statistical significance of observed differences between the means from all treatments at a particular sampling time was tested by one-way ANOVA. To isolate which group(s) differed from the others, a Student–Newman–Keuls test was used. The significance level for all statistical tests was $P<0{\cdot}05$.

RESULTS

INDUCTION AND RECOVERY TIMES

The induction times for loss of equilibrium (anaesthesia stage I) and loss of gross body movements (anaesthesia stage II) were shorter in the medium CO_2 treatment than in the low CO_2 treatment (Fig. 1). A greater increase in the CO_2 content of the anaesthetic water did not reduce further the induction times of anaesthesia. While stress also shortened the induction times to anaesthesia stages I and II in the low CO_2 treatment, it had no effect on the induction times in the medium CO_2 treatment. Irrespective of whether the trout were quiescent or stressed prior to the anaesthetic treatment, introduction to the CO_2 anaesthetic water was accompanied by a brief and violent struggle.

In comparison to the induction times, the recovery times for gross body movement (recovery stage II) and equilibrium (recovery stage III) in the five anaesthetic treatments had a high variability and were not significantly different from each other (Fig. 1). However, when the effects of stress were excluded, the high CO_2 treatment prolonged recovery stage III significantly compared with the

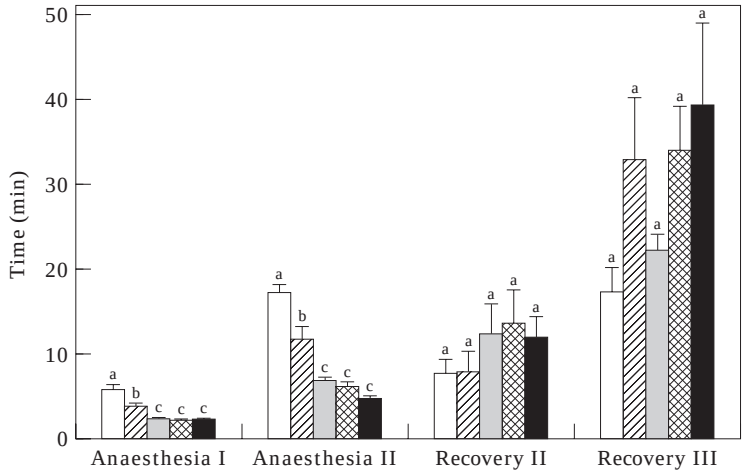

FIG. 1. Time required by rainbow trout to reach anaesthesia stage I, anaesthesia stage II, recovery stage II, and recovery stage III (see Table II for description of stages), after exposure to five different anaesthetic treatments: low, $P_wCO_2 = 36{\cdot}5 \pm 2{\cdot}7$ mmHg ($n = 8$) (□); low + stress, $P_wCO_2 = 40{\cdot}5 \pm 3{\cdot}8$ mmHg ($n = 8$) (▨); medium, $P_wCO_2 = 77{\cdot}9 \pm 1{\cdot}8$ mmHg ($n = 6$) (▢); medium + stress, $P_wCO_2 = 84{\cdot}3 \pm 4{\cdot}7$ mmHg ($n = 6$) (▩), and high, $P_wCO_2 = 124{\cdot}8 \pm 8{\cdot}7$ mmHg ($n = 4$) (■). The letters are used to denote statistical differences, and treatments which do not share a common letter for a given anaesthetic stage are significantly different from each other ($P < 0{\cdot}05$). Values are means + 1 S.E.

low CO_2 treatment. Also, two of the six fish in the high CO_2 treatment never recovered from the anaesthesia and data from these two fish are not included in our analysis throughout the paper.

VENTILATION FREQUENCY

The hypercapnia in the low, medium and high CO_2 treatments caused a significant increase in V_f after only 3–4 min of exposure [Fig. 2(a), (c) and (e)]. This hyperventilation was very brief in the low CO_2 treatment and was followed by a sustained hypoventilation period throughout the rest of the hypercapnia. In the medium CO_2 treatment the hyperventilation lasted a few minutes and then subsided to a frequency not significantly different from the time 0 value for the remaining hypercapnic period. In contrast, the high CO_2 treatment was characterized by a sustained hyperventilation throughout the hypercapnia. In the two hypercapnic treatments preceded by a stress [Fig. 2(b) and (d)], V_f was significantly higher at time 0 than in the three other treatments. Unlike the low, medium, and high CO_2 treatments, the hypercapnia did not cause a further rise in V_f in the two stress groups. Instead, although V_f decreased after the onset of hypercapnia, the values in both stress grups remained high in comparison to the low and medium treatments over the same time period.

Recovery from CO_2 anaesthesia was characterized by a rapid and short period of hypoventilation in the low, medium, medium + stress, and high CO_2 treatments. This transient response to a rapid fall in P_wCO_2 was followed by a gradual rise in V_f back towards control levels in the low CO_2 treatment, and above the latter in the medium and high CO_2 treatments.

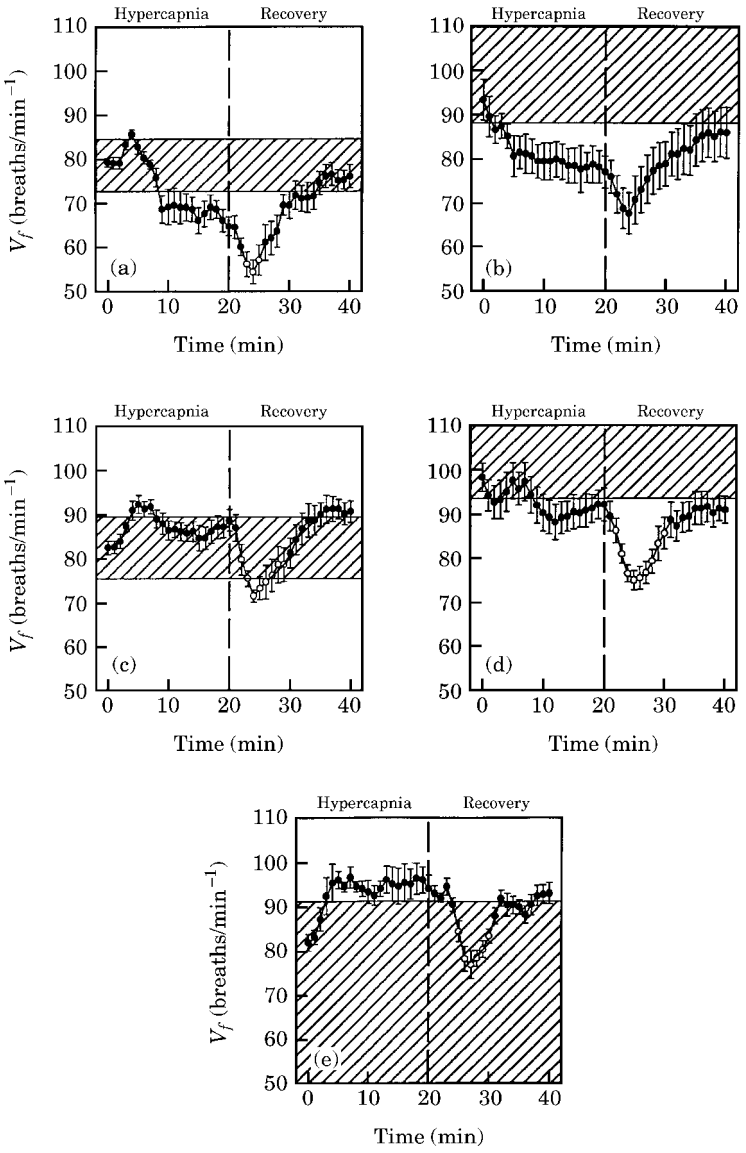

FIG. 2. Ventilation frequency (V_f) of rainbow trout in relation to exposure duration to five different anaesthetic treatments: (a) low, (b) low+stress, (c) medium, (d) medium+stress, and (e) high. Animals were exposed to the hypercapnic conditions for the first 20 min, the following 20 min is a recovery period. Values above or below the hatched area for a given treatment are significantly different from the control time 0 value. Open symbols are significantly different from the time 20 value for a given treatment ($P<0{\cdot}05$). Values are means ± 1 S.E. For further details on the anaesthetic treatments and the *n* values, see the legend of Fig. 1.

BLOOD CHEMISTRY

The overall changes in acid-base balance observed during the normocapnia/hypercapnia transfers are shown in the Davenport diagrams of Fig. 3. In response to the increase in P_wCO_2 of the anaesthetic treatment there is a rapid decrease in pH_a and an increase in arterial $[HCO_3^-]$ and P_aCO_2. In the following

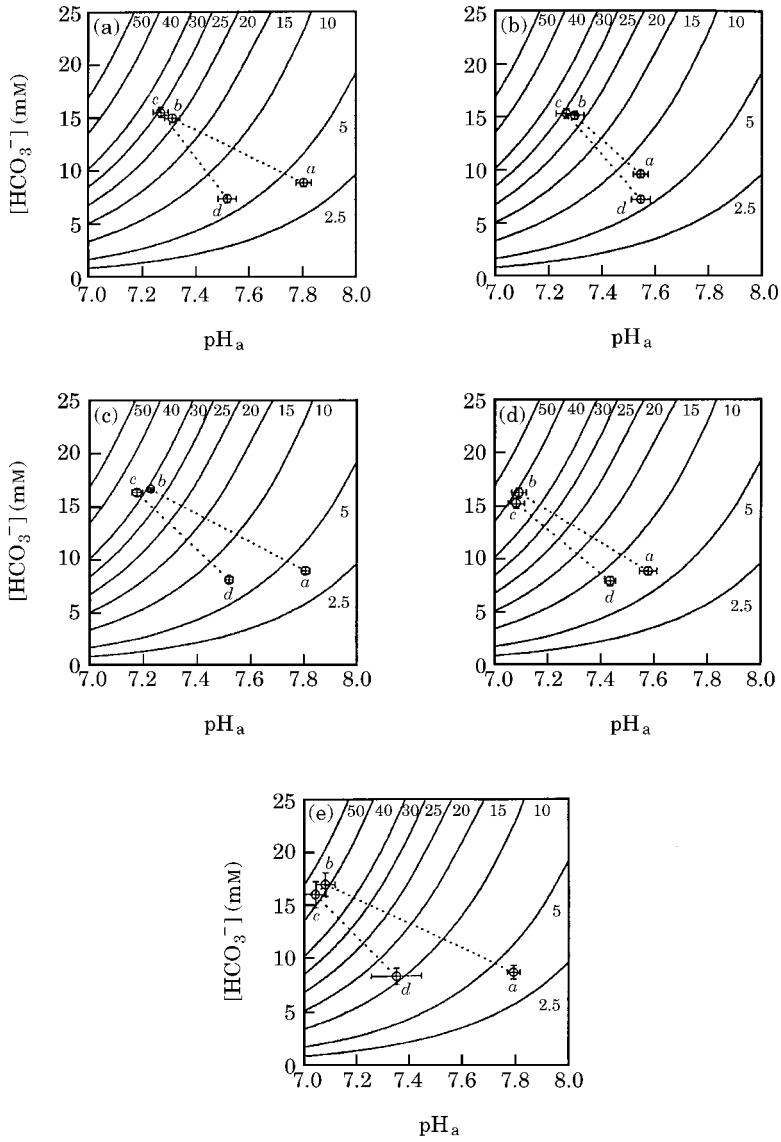

FIG. 3. Plasma bicarbonate concentration ($[HCO_3^-]$) as a function of arterial pH (pH_a) of rainbow trout exposed to five different anaesthetic treatments: (a) low, (b) low+stress, (c) medium, (d) medium+stress, and (e) high. Point *a* are control values, point *b* and *c* are values taken after 10 and 20 min of hypercapnia, respectively, and point *d* is after 10 min of recovery. The isobars are for the arterial partial pressure of CO_2 (P_aCO_2) at 12·1° C. Values are means ± 1 S.E. For further details on the anaesthetic treatments and the *n* values, see the legend of Fig. 1.

10 min of hypercapnia, point *b* to point *c*, there is relatively little change in the acid-base status of the fish. During recovery, point *c* to point *d*, the decrease in P_wCO_2 to control conditions is matched by a similar decrease in arterial $[HCO_3^-]$ but only a partial recovery of P_aCO_2 and pH_a. In the two stress groups [Fig. 3(b) and (d)], while the time 0 control values (point *a*) have a lower pH_a in

comparison to their respective non-stress counterparts, the changes in acid-base balance throughout the hypercapnic treatment follow the same general pattern as described above.

The hypercapnic treatments also resulted in a sustained increase in C_aCO_2 (Table III). After 10 min of anaesthesia the increases in C_aCO_2 were proportional to the relative differences in P_wCO_2 between the five treatments. In recovery, the two low CO_2 treatments had lower C_aCO_2 values than their respective time 0 values, and in the high CO_2 treatment C_aCO_2 was higher than in the four other treatments. Stress, on its own, did not have an effect on C_aCO_2. On the other hand, relative to the other treatments, stress resulted in higher P_aCO_2 values in both stress treatments at time 0, and in the medium CO_2 + stress treatment after 10 min of hypercapnia (Table III). The increase in P_aCO_2 with hypercapnia was proportional to the relative differences in P_wCO_2 between the five treatments. However, the differences in P_aCO_2 between the treatments were more pronounced than the differences in C_aCO_2. The increase in P_aCO_2 associated with hypercapnia was sustained through the recovery period in the low, medium, and high CO_2 treatments. Plasma bicarbonate concentrations ($[HCO_3^-]$) increased in all five treatments throughout the period of anaesthesia (Fig. 3). In recovery $[HCO_3^-]$ returned to control values in the two medium and the high treatments, and in parallel to the C_aCO_2 values, $[HCO_3^-]$ decreased below the control values in the two low CO_2 treatments. All five hypercapnic treatments were characterized also by a marked acidosis which was most pronounced in the medium CO_2 + stress and the high CO_2 treatments during the hypercapnia (Fig. 3).

The hypercapnic treatments were accompanied by a gradual increase in plasma lactate that was sustained even into the 10-min recovery period (Table III). The low CO_2 + stress and medium CO_2 + stress treatments consistently had higher plasma lactate concentratons than their non-stressed counterparts, and the differences were significant between the two medium CO_2 treatments.

Although the two stress groups had significantly lower P_aO_2 values than the other experimental groups at time 0, these differences were not maintained after the first 10 min of hypercapnic anaesthesia (Table III). Decreases in P_aO_2 were observed, however, in the medium CO_2 + stress and the high CO_2 treatments after 20 min of hypercapnia. In recovery, P_aO_2 values were low in all the groups except for the low CO_2 treatment. Unlike the control P_aO_2 values, the C_aO_2 of the two stress treatments were similar to the other treatments at time 0 (Table III). However, C_aO_2 decreased in all groups throughout the hypercapnia and only recovered in the two low CO_2 treatments. The decrease in C_aO_2 was greater in the two medium CO_2 and the high CO_2 treatments than in the two low CO_2 treatments. C_aO_2 was especially low in the high CO_2 treatment 20 min into the hypercapnic exposure.

Finally, plasma catecholamines increased to very high circulating concentrations throughout the hypercapnic exposure in all five treatments (Fig. 4). The concentrations of NA [Fig. 4(a)] and AD [Fig. 4(b)] decreased markedly upon recovery, but in only a few treatments did plasma NA return to control levels. The two stress treatments had significantly higher concentrations of both catecholamines than the three other treatments at time 0, but these differences were not maintained during the hypercapnic exposure or after recovery.

TABLE III. Arterial CO_2 content (C_aCO_2), arterial pressure of CO_2 (P_aCO_2), plasma lactate, arterial partial pressure of O_2 (P_aO_2), and arterial O_2 content (C_aO_2) of rainbow trout at rest (control), after 10 min of hypercapnia (initial CO_2), 20 min of hypercapnia (final CO_2), and 10 min of recovery, in relation to five different anaesthetic treatments (see Table I for physical properties of treatments)

Stage	Treatment	*n*	C_aCO_2 (mM)	P_aCO_2 (mmHg)	Plasma lactate (mM)	P_aO_2 (mmHg)	C_aO_2 (vol%)
Control	Low	8	9·07 ± 0·37	3·69 ± 0·17^{a}	2·44 ± 0·62^{a}	119·9 ± 4·0^{a}	13·4 ± 1·0
	Low + stress	8	10·06 ± 0·36	7·89 ± 0·61^{b}	4·56 ± 0·87^{ab}	46·8 ± 3·8^{b}	12·7 ± 1·2
	Medium	6	9·16 ± 0·34	3·91 ± 0·19^{a}	1·16 ± 0·30^{a}	122·0 ± 3·2^{a}	11·3 ± 0·6
	Medium + stress	6	9·21 ± 0·41	6·90 ± 0·52^{b}	6·41 ± 1·25^{b}	43·5 ± 1·8^{b}	9·2 ± 1·1
	High	4	9·39 ± 0·41	4·18 ± 0·14^{a}	1·68 ± 0·66^{a}	120·0 ± 5·9^{a}	12·5 ± 2·2
Initial CO_2	Low	8	16·29 ± 0·38*^{a}	20·84 ± 1·27*^{a}	4·87 ± 0·67*^{a}	121·6 ± 5·3	8·3 ± 0·8*^{ab}
	Low + stress	8	16·51 ± 0·33*^{a}	20·98 ± 1·69*^{a}	7·51 ± 1·40*^{a}	114·5 ± 4·1	10·5 ± 1·1*^{a}
	Medium	6	18·31 ± 0·16*^{b}	29·47 ± 0·53*^{b}	6·60 ± 0·50*^{a}	117·0 ± 1·9	6·0 ± 0·9*^{b}
	Medium + stress	6	18·48 ± 0·46*^{b}	39·95 ± 1·64*^{c}	14·09 ± 1·73*^{b}	117·5 ± 2·9	5·8 ± 0·7*^{b}
	High	4	21·90 ± 1·09*^{c}	49·23 ± 5·25*^{d}	6·13 ± 0·59*^{a}	108·7 ± 8·7	4·9 ± 1·3*^{b}
Final CO_2	Low	8	17·05 ± 0·47*^{a}	23·79 ± 1·13*^{a}	7·51 ± 0·75*^{a}	115·4 ± 4·0^{a}	8·6 ± 0·7*^{a}
	Low + stress	8	16·84 ± 0·42*^{a}	22·82 ± 1·86*^{a}	12·54 ± 1·89*^{a}	115·1 ± 3·6^{a}	9·9 ± 0·7*^{a}
	Medium	6	18·17 ± 0·28*^{a}	32·63 ± 0·95*^{b}	12·80 ± 0·98*^{a}	111·8 ± 2·9^{a}	4·7 ± 0·5*^{b}
	Medium + stress	6	17·33 ± 0·50*^{a}	38·26 ± 1·94*^{b}	20·44 ± 1·73*^{b}	92·3 ± 6·7†^{b}	4·1 ± 0·6*^{b}
	High	4	20·94 ± 0·82*^{b}	50·67 ± 4·82*^{c}	13·11 ± 0·67*^{a}	40·0 ± 5·9*^{c}	1·1 ± 0·4*^{c}
Recovery	Low	8	7·76 ± 0·40*^{a}	6·33 ± 0·54*^{a}	14·41 ± 1·24*^{a}	109·3 ± 4·6^{a}	11·7 ± 1·5^{a}
	Low + stress	8	7·55 ± 0·32*^{a}	5·93 ± 0·53^{a}	19·81 ± 2·29*^{ab}	103·3 ± 3·1†^{a}	13·7 ± 0·7^{a}
	Medium	6	8·51 ± 0·28^{a}	7·12 ± 0·16*^{a}	18·01 ± 1·57*^{ab}	99·0 ± 5·8*^{a}	7·9 ± 0·9*^{b}
	Medium + stress	6	8·37 ± 0·44^{a}	8·53 ± 0·17^{a}	26·73 ± 3·13*^{b}	105·5 ± 3·5†^{a}	6·7 ± 0·7*^{b}
	High	4	11·65 ± 1·41^{b}	16·68 ± 5·77*^{b}	18·21 ± 1·84*^{ab}	80·3 ± 7·8*^{b}	5·8 ± 0·6*^{b}

*Significant difference from control in given treatment; †significant difference from the initial CO_2 value in given treatment. Treatments which do not share a common letter for a given stage of the experimental protocol are significantly different from each other ($P<0·05$). Values are means ± 1 S.E.

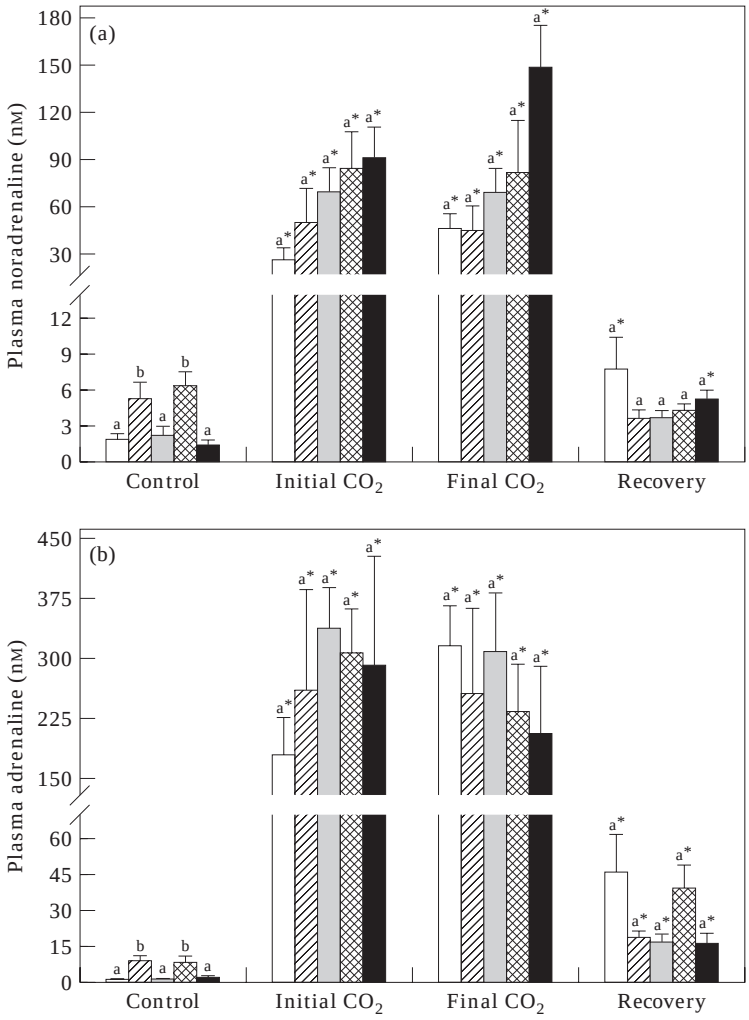

FIG. 4. (a) Plasma noradrenaline and (b) adrenaline concentrations of rainbow trout at rest (control), after 10 min of hypercapnia (initial CO_2), 20 min of hypercapnia (final CO_2), and 10 min of recovery, in relation to five different anaesthetic treatments: low (□), low + stress (▨), medium (▣), medium + stress (▩), and high (■). *Significant difference from control in given treatment. Letters denote statistical differences: treatments which do not share a common letter for a given stage of the experimental protocol are significantly different from each other ($P<0{\cdot}05$). Values are means + 1 S.E. For further details on the anaesthetic treatments and the *n* values, see the legend of Fig. 1.

DISCUSSION

Although the range of P_wCO_2 (37–125 mmHg) utilized to anaesthetize rainbow trout in this study was equivalent to or greater than the level of hypercapnia (18–44 mmHg) recommended for CO_2 anaesthesia in salmonids (Bell, 1964, 1987), complete anaesthesia (no opercular movement) was not achieved in any of the groups within the 20-min treatment. Therefore, at the outset, it may appear that increasing the level of hypercapnia, or keeping the fish in a quiescent state prior to anaesthesia, may not necessarily improve the anaesthetic properties of

CO_2 anaesthesia. Our results are in sharp contrast to the results obtained by Iwama *et al.* (1989) where complete anaesthesia of cannulated rainbow trout was achieved in 4–7 min. In that study, although the P_wCO_2 was not reported, the mean P_aCO_2 throughout the anaesthetic period was approximately 29 mmHg, a value which is similar to the P_aCO_2 in the medium hypercapnic level of this study. While the level of hypercapnia may have been comparable between the two studies, other conditions of the anaesthetic water were not. In this study, as recommended by Bell (1987) for hatchery practices, the P_wO_2 was maintained close to saturation level (normoxic hypercapnia) and the pH was kept at 6·9 by adding $NaHCO_3$. Iwama *et al.* (1989) used a 50:50 mixture of O_2 and CO_2 (hyperoxic hypercapnia) and did not neutralize the pH of the anaesthetic water. The importance of these differences to the overall effectiveness of CO_2 anaesthesia, and to the relative merits of either increasing the level of hypercapnia or keeping the fish in a quiescent state prior to the anaesthetic treatment, will be the focus of the following discussion.

HYPERCAPNIC LEVEL AND CO_2 ANAESTHESIA

The induction times to stages I and II of CO_2 anaesthesia on rainbow trout in this study were reduced significantly by increasing the P_wCO_2 of the anaesthetic water from 37 to 78 mmHg. Increasing P_wCO_2 from 78 to 125 mmHg, did not result in any further reduction in induction time. A reduction in the induction times of stages I and II of CO_2 anaesthesia with increasing hypercapnic level (P_wCO_2 from approximately 18 to 44 mmHg) has been observed also in both coho *Oncorhynchus kisutch* (Walbaum) and chinook *O. tshawytscha* (Walbaum) salmon (Mechelen, 1985). In carp, several studies with P_wCO_2 ranging from 50 to 250 mmHg have shown that the time required for anaesthesia is shortened proportionally by raising the hypercapnic level (Yoshikawa *et al.*, 1988*a*,*b*, 1991). Complete anaesthesia, the cessation of opercular movement, could be achieved in carp within 28 min at a P_wCO_2 of 250 mmHg (Yoshikawa *et al.*, 1991). As with the study of Iwama *et al.* (1989), the studies on *C. carpio* (Itazawa & Takeda, 1982; Yoshikawa *et al.*, 1988*a*, 1991) were done with hyperoxic hypercapnia without buffering the anaesthetic water.

Since the availability of O_2 is the primary stimulus for ventilatory drive in fish (Perry & Wood, 1989; Randall, 1990), the differences in oxygenation of the anaesthetic water between the above studies and ours may explain at least partially why the trout did not reach anaesthesia stage III. A close observation of the C_aO_2 (Table III) and V_f (Fig. 2) data reveals an inverse relationship during the anaesthetic treatment. Although the anaesthetic water was buffered in this study, the fish still experienced a pronounced blood acidosis during the anaesthesia that was proportional to the hypercapnic level. Blood acidosis leads to Root effect reductions in blood-oxygen capacity, even at high PO_2 levels (e.g. see Table III; Randall, 1990). In contrast, the hyperoxic and hypercapnic conditions (P_wO_2 = 379 mmHg, P_wCO_2 = 157 mmHg) used on *C. carpio*, despite a lack of anaesthetic water buffering, resulted in an increase in O_2 content during the anaesthetic treatment (Itazawa & Takeda, 1982). This increase in O_2 content during the anaesthetic treatment may account for the cessation of breathing observed in carp and trout exposed to hyperoxic hypercapnia.

Although CO_2 anaesthesia in *C. carpio* is done under hyperoxic conditions, V_f still increases rapidly upon exposure to the mixed gas, reaches a maximum within a few minutes, and then gradually decreases (Itazawa & Takeda, 1982; Yoshikawa *et al.*, 1988*a*, *b*, 1991). So, while hypoxaemia may account for at least part of the hypercapnic hyperventilation observed in the present study, in carp, the initial increase in V_f may be a direct response to the hypercapnic acidosis. Whereas there is no evidence for the existence of central CO_2/H^+ receptors in fish, branchial chemoreceptors appear to respond to both hypoxia and hypercapnia (Smatresk, 1994). In rainbow trout, some studies using modest P_wCO_2 elevations (<1·3 kPa) have reported a hypercapnic stimulation of branchial ventilation which could not be attributed to Bohr or Root effect reductions in O_2 content (Thomas *et al.*, 1983; Kinkead & Perry, 1991).

Overall the recovery times were considerably more variable than induction times. However, complete recovery times (recovery III) in non-stressed fish increased with higher P_wCO_2 levels. In coho and chinook salmon, recovery times from exposure to P_wCO_2 of approximately 18 to 44 mmHg were variable and not necessarily proportional to the anaesthetic level (Mechelen, 1985). In carp, although recovery from anaesthesia at a P_wCO_2 of 250 mmHg was on average three times longer than with a P_wCO_2 of 100 mmHg, the variable nature of the data prevented definitive conclusions (Yoshikawa *et al.*, 1991). Since CO_2 presumably exerts its anaesthetic action by decreasing the pH of the brain (Yoshikawa *et al.*, 1994), recovery of the cerebrospinal fluid pH, and therefore from CO_2 anaesthesia, depends on the overall rate of acid-base adjustment (Wood *et al.*, 1990). While the acid-base disturbances experience by the non-stressed fish during the anaesthetic treatment are primarily the result of a respiratory acidosis, a metabolic acidosis also develops gradually during the anaesthesia and becomes the principal contributing factor to acid-base disturbances during recovery (Fig. 3). The continual recruitment of anaerobic metabolism, as indicated by the large increase in plasma lactate during recovery, will contribute to the H^+ load of the blood. Unlike the respiratory acidosis, which is corrected rapidly during recovery, elimination of the H^+ load from the metabolic acidosis is limited by the net H^+ transfer rate to the environment (Heisler, 1993). Although rainbow trout anaesthetized with non-buffered hyperoxic hypercapnia (Iwama *et al.*, 1989) experienced a greater acidosis during the CO_2 treatment than the trout in this study, they also had a higher pH at stage III recovery. Hyperoxic conditions, by reducing the degree of hypercapnia induced hypoxaemia, probably reduces the metabolic component of the acidosis. Whereas one third of the trout exposed to a P_wCO_2 of 125 mmHg in this experiment did not recover from the anaesthesia, short exposure (30 min) to similar and higher levels of P_wCO_2 (100 to 250 mmHg) in hyperoxic carp did not cause any mortality (Yoshikawa *et al.*, 1991). In hyperoxic carp, mortality was observed only after chronic exposure (10 h) to a P_wCO_2 of 125 mmHg (Yoshikawa *et al.*, 1988*a*).

STRESS AND CO_2 ANAESTHESIA

The physiological response of the fish in the low+stress and medium+stress treatments to the 10 min period of partial water deprivation was characteristic of trout that undergo an acute hypoxic bout (Boutilier *et al.*, 1988; Claireaux *et al.*,

1988; Perry & Thomas, 1991). Although the stressed fish had significantly lower P_aO_2 values than their non-stressed counterparts at time 0, the numerous beneficial effects of the increased catecholamines on O_2 carrying capacity (Randall & Perry, 1992) maintained the O_2 content of the stressed fish close to saturation. The stress, however, was severe enough to lead to increased reliance on anaerobic glycolysis, resulting in increased lactic acid production and an associated metabolic acidosis.

In the low CO_2 + stress treatment, the metabolic acidosis may have reduced the induction time to anaesthesia stage I and II by augmenting the hypercapnic acidosis of the anaesthetic treatment. Meanwhile, the contribution of the stress-associated metabolic acidosis to the overall acidotic effects of the medium CO_2 + stress treatment may not have been great enough to further decrease the induction times. Also, the acute hypoxic stress, by increasing the overall recruitment of anaerobic metabolism, may be responsible for the extended period required for recovery. In general, the greater plasma lactate in the stress groups than in the non-stress grups at time 0 was maintained throughout the anaesthetic bout and into recovery. As stated above, the rate of recovery may depend primarily on the recovery of the acid-base disturbance associated with the metabolic acidosis. However, although the recovery time in the low CO_2 + stress treatment was 89% longer than in the low CO_2 treatment, and in the medium CO_2 + stress treatment it was 52% longer than in the medium CO_2 treatment, the variable nature of the recovery times precludes a firm conclusion.

The violent struggle of both quiescent and stressed trout upon introduction to the CO_2 anaesthetic water observed in this study, has been described previously by several authors (Mechelen, 1985; Bell, 1987; Loch, 1991). Hyperactivity was observed also in the non-buffered hyperoxic and hypercapnic conditions used to anaesthetize *C. carpio* (Yoshikawa *et al.*, 1988*a*). A comparison of the catecholamines released as a result of either the acute hypoxic stress or the anaesthetic treatment in the present study, shows clearly that the stress response elicited by the hypoxic bout is benign in comparison to the one elicited by the hypercapnic exposure. There is some evidence, however, that buffering the water and adding salts does reduce the irritation caused by CO_2 anaesthesia (Iwama & Ackerman, 1994). With *C. carpio*, low temperature is another sedative which can be used in combination with CO_2 to improve the overall effectiveness of the anaesthetic treatment and to reduce the hyperactive response of the fish (Yokoyama *et al.*, 1989). In the end, reducing handling stress, buffering the water, adding salts, and lowering the temperature of the anaesthetic bath may all be required to minimize the hyperactive response of fish that are anaesthetized with CO_2.

In conclusion, our results show that complete anaesthesia of rainbow trout cannot be achieved using normoxic and buffered hypercapnic conditions which have a P_wCO_2 ranging from 37 to 125 mmHg. Although increasing the P_wCO_2 of the anaesthetic water reduces the induction times to the early stages of anaesthesia, it also prolongs complete recovery and results in increasing hyperventilatory, hypoxaemic, and acid-base disturbances. Since complete anaesthesia of rainbow trout has been achieved previously using hyperoxic hypercapnia (Iwama *et al.*, 1989), future experiments should explore the potential benefits of using higher P_wCO_2 values with hyperoxic conditions in order to

optimize the sedative effects of CO_2 anaesthesia. Finally, even though the stress response elicited by CO_2 anaesthesia is very acute, handling and agitation stress prior to CO_2 anaesthesia should be avoided because they impair the sedative effects of the anaesthetic treatment by exacerbating its hypoxaemic effects.

We thank K. Ronalds and J. Harris for their invaluable technical assistance in the laboratory. Financial assistance for this project was provided by Yellow Island Aquaculture Ltd, and operating grants from the Natural Science and Engineering Research Council of Canada to DJR. A postgraduate scholarship (G.R.E.A.T.) was provided by the B. C. Science Council to NJB.

References

Bell, G. R. (1964). A guide to the properties, characteristics, and uses of some general anesthetics for fish. *Bulletin of the Fisheries Research Board of Canada* **148**.

Bell, G. R. (1987). An outline of anesthetics and anesthesia for salmonids, a guide for fish culturists in British Columbia. *Canadian Technical Report of Fisheries and Aquatic Sciences* **1534**.

Boutilier, R. G., Heming, T. A. & Iwama, G. K. (1984). Appendix: physiochemical parameters for use in fish respiratory physiology. In *Fish Physiology, Vol. XA* (Hoar, W. S. & Randall, D. J., eds), pp. 403–430. London: Academic Press.

Boutilier, R. G., Iwama, G. K., Heming, T. A. & Randall, D. J. (1985). The apparent pK of carbonic acid in rainbow trout plasma between 5 and 15° C. *Respiratory Physiology* **61,** 237–254.

Boutilier, R. G., Dobson, G., Hoeger, U. & Randall, D. J. (1988). Acute exposure to graded levels of hypoxia in rainbow trout (*Salmo gairdneri*): metabolic and respiratory adaptations. *Respiratory Physiology* **71,** 69–82.

Carpenter, Z. L. (1994). Guide to drug, vaccine, and pesticide use in aquaculture. Prepared by the Federal Joint Subcommittee on Aquaculture, Texas Agricultural Extension Service, The Texas A & M University System, College Station, Texas. 68 p.

Claireaux, G., Thomas, S., Fievet, B. & Motais, R. (1988). Adaptive respiratory responses of trout to acute hypoxia II. Blood oxygen carrying properties during hypoxia. *Respiratory Physiology* **74,** 91–98.

Fish, F. F. (1943). The anesthesia of fish by high carbon dioxide concentrations. *Transactions of the American Fisheries Society* **72,** 25–29.

Gilderhus, P. A. & Marking, L. L. (1987). Comparative efficacy of 16 anesthetic chemicals on rainbow trout. *North American Journal of Fisheries Management* **7,** 288–292.

Heisler, N. (1993). Acid-base regulation. In *The Physiology of Fishes* (Evans, D. H., ed.), pp. 343–378. Boca Raton: CRC Press.

Itazawa, Y. & Takeda, T. (1982). Respiration of carp under anesthesia induced by mixed bubbling of carbon dioxide and oxygen. *Bulletin of the Japanese Society of Scientific Fisheries* **48,** 489–493.

Iwama, G. K., McGeer, J. C. & Pawluk, M. P. (1989). The effects of five fish anesthetics on acid-base balance, hematocrit, blood gases, cortisol, and adrenaline in rainbow trout. *Canadian Journal of Zoology* **67,** 2065–2073.

Iwama, G. K. & Ackerman, P. (1994). Anesthetics. In *Biochemistry and Molecular Biology of Fishes, Vol. 3* (Hochachka, P. W. & Mommsen, T. P., eds), pp. 1–15. Amsterdam: Elsevier Science.

Kinkead, R. & Perry, S. F. (1991). The effects of catecholamines on ventilation in rainbow trout during hypoxia or hypercapnia. *Respiratory Physiology* **84,** 77–92.

Loch, J. J. (1991). Carbon dioxide as an anesthetic for handling steelhead collected at the Toutle River Fish Collection Facility, 1990. Report No. 90-18. Washington Department of Wildlife, Fisheries Management Division, 600 Capitol Way, No. GJ-11, Olympia, WA. 98501-1091.

Mechelen, J. A. N. van (1985). Report on a practical training at the Salmonid Enhancement Project, Department of Fisheries and Oceans, Canada. II. The use of carbon dioxide as an anesthetic for fish. Quinsam Hatchery, Department of Fisheries and Oceans. Box 467, Campbell River, B.C. V9W 5C1.

Mitsuda, H., Ueno, S., Mizuno, H., Ueda, T., Fujikawa, H., Nohara, T. & Fukada, C. (1982). Levels of CO_2 in arterial blood of carp under carbon dioxide anesthesia. *Journal of Nutritional Sciences and Vitaminology* **28,** 35–39.

Mitsuda, H., Ishida, Y., Yoshikawa, H. & Ueno, S. (1988). Effects of a high concentration of CO_2 on electrocardiograms in the carp, *Cyprinus carpio*. *Comparative Biochemistry and Physiology* **91A,** 749–757.

Perry, S. F. & Wood, C. M. (1989). Control and coordination of gas transfer in fishes. *Canadian Journal of Zoology* **67,** 2961–2970.

Perry, S. F. & Thomas, S. (1991). The effects of endogenous or exogenous catecholamines on blood respiratory status during acute hypoxia in rainbow trout (*Oncorhynchus mykiss*). *Journal of Comparative Physiology* **161B,** 489–497.

Perry, S. F. & McDonald, G. (1993). Gas exchange. In *The Physiology of Fishes* (Evans, D. H., ed.), pp. 251–278. Boca Raton: CRC Press.

Post, G. (1979). Carbonic acid anesthesia for aquatic organisms. *The Progressive Fish Culturist* **41,** 142–144.

Prince, A. M. J., Low, S. E., Lissimore, T. J., Diewart, R. E. & Hinch, S. G. (1995). Sodium bicarbonate and acetic acid: an effective anesthetic for field use. *North American Journal of Fisheries Management* **15,** 170–172.

Randall, D. J. (1990). Control and co-ordination of gas exchange in water breathers. In *Advances in Comparative and Environmental Physiology, Vol. 6* (Boutilier, R. G., ed.), pp. 253–278. Heidelberg: Springer-Verlag.

Randall, D. J. & Perry, S. F. (1992). Catecholamines. In *Fish Physiology, Vol. XIIB* (Randall, D. J., Hoar, W. S. & Farrell, A. P., eds), pp. 255–300. London: Academic Press.

Smatresk, N. J. (1994). Respiratory control in the transition from water to air breathing in vertebrates. *American Zoologist* **34,** 264–279.

Soivio, A., Nyholm, K. & Westman, K. (1975). A technique for repeated sampling of the blood of individual resting fish. *Journal of Experimental Biology* **62,** 207–217.

Thomas, S., Fievet, B., Barthelemy, L. & Peyraud, C. (1983). Comparison of the effects of exogenous and endogenous hypercapnia on ventilation and oxygen uptake in the rainbow trout (*Salmo gairdneri* R.). *Journal of Comparative Physiology* **151B,** 185–190.

Tucker, V. A. (1967). Method for oxygen content and dissociation curves on microliter blood samples. *Journal of Applied Physiology* **23,** 410–414.

Wolf, K. (1963). Physiological salines for fresh-water teleosts. *The Progressive Fish Culturist* **25,** 135–140.

Wood, C. M., Turner, J. D., Munger, R. S. & Graham, M. S. (1990). Control of ventilation in the hypercapnic skate *Raja ocellata*: II. Cerebrospinal fluid and intracellular pH in the brain and other tissues. *Journal of Experimental Biology* **80,** 279–298.

Woodward, J. J. (1982). Plasma catecholamines in resting rainbow trout, *Salmo gairdneri* Richardson, by high pressure liquid chromatography. *Journal of Fish Biology* **21,** 429–432.

Yokoyama, Y., Yoshikawa, H., Ueno, S. & Mitsuda, H. (1989). Applicaton of CO_2-anesthesia combined with low temperature for long-term anesthesia in carp. *Nippon Suisan Gakkaishi* **55,** 1203–1209.

Yoshikawa, H., Ishida, Y., Ueno, S. & Mitsuda, H. (1988*a*). Changes in depth of anesthesia of the carp anesthetized with a constant level of CO_2. *Nippon Suisan Gakkaishi* **54,** 457–462.

Yoshikawa, H., Ishida, Y., Ueno, S. & Mitsuda, H. (1988*b*). The use of sedating action of CO_2 for long-term anesthesia in carp. *Nippon Suisan Gakkaishi* **54,** 545–551.

Yoshikawa, H., Yokoyama, Y., Ueno, S. & Mitsuda, H. (1991). Changes of blood gas in carp, *Cyprinus carpio*, anesthetized with carbon dioxide. *Comparative Biochemistry and Physiology* **98A,** 431–436.

Yoshikawa, H., Kawai, F. & Kanamori, M. (1994). The relationship between the EEG and brain pH in carp, *Cyprinus carpio*, subjected to environmental hypercapnia at an anesthetic level. *Comparative Biochemistry and Physiology* **107A,** 307–312.