ELSEVIER

Comparative Biochemistry and Physiology Part A 119 (1998) 895–903

CBP

The blood respiratory, haematological, acid-base and ionic status of the Port Jackson shark, *Heterodontus portusjacksoni*, during recovery from anaesthesia and surgery: a comparison with sampling by direct caudal puncture

Ashley R. Cooper *, Stephen Morris

School of Biological Sciences (A08), University of Sydney, Sydney, NSW 2006, Australia

Received 11 July 1997; received in revised form 28 October 1997; accepted 30 October 1997

Abstract

The effects of caudal cannulation on the blood physiology of the Port Jackson shark, *Heterodontus portusjacksoni*, were investigated in sharks given between 4 and 72 h to recover from surgery. Neither the $Pa_{O_2}-Pv_{O_2}$ difference nor the $Ca_{O_2}-Cv_{O_2}$ difference of cannulated sharks fluctuated throughout the sampling period. The plasma acidosis exhibited 4 h after surgery was partially compensated after 24 h by a respiratory (hyperventilatory) alkalosis and after 72 h by a marked metabolic alkalosis. Whilst *H. portusjacksoni* exhibited some cell swelling after surgery the haematological status of cannulated sharks generally varied little throughout the recovery period. In contrast, marked changes in plasma and erythrocyte ion concentrations were indicative of increased branchial and erythrocyte ion permeability. The blood status of *H. portusjacksoni* given 72 h to recover from surgery was also compared with sharks sampled by caudal puncture. The respiratory and acid-base status of sharks sampled by caudal puncture was comparable to that of cannulated sharks. In contrast, the plasma ion concentrations of the cannulated sharks were markedly elevated and the erythrocyte ion concentrations concomitantly reduced when compared with punctured sharks. The apparent increase in the water and ion permeability of cannulated sharks was reflected by the reduced [Hb] and mean cell haemoglobin concentrations (MCHC). Blood sampling by caudal puncture appeared to reduce the haematological and ionic perturbations that resulted from surgery and thus provided a less invasive and reliable method for obtaining samples from 'non-disturbed' elasmobranchs.

Keywords: Acid-base; Haematology; Ions; Respiration; Shark; Surgery; Heterodontus

1. Introduction

Handling and surgical procedures perturb the blood chemistry of teleost and elasmobranch fishes [4,9,12,16,17,21,34]. The reduction in gill ventilation and the concomitant cessation of breathing during anaesthesia can result in significant hypoxia and/or hypercapnia despite constant irrigation of the gills with aerated seawater [9,21,22,35].

Elevated blood haematocrit (Hct) and mean cell haemoglobin concentrations (MCHC) occur in marine elasmobranchs and teleosts following capture [44,52], cannulation [4,14,20,23,33] and exercise [39,47]. These increases have been attributed to various mechanisms, e.g. red blood cell count (RBC) swelling due to elevated venous P_{CO_2} [47]; plasma fluid loss and/or RBC mobilization from the spleen [39,45]; changes in membrane permeability resulting in fluid shifts from the plasma to the erythrocytes [50]; and/or water loss by increased diuresis [46]. In contrast, the increase in branchial permeability [11] and blood water volume [32] observed

* Corresponding author. Tel.: +61 2 93512433; fax: +61 2 93514119; e-mail: ashley@bio.usyd.edu.au

1095-6433/98/$19.00
PII S1095-6433(98)00014-2

in many freshwater teleosts after capture or cannulation [13,15,31,47,48] results in reduced Hct and RBC count.

Changes in fluid volume resulting from surgery not only perturb the haematological status but also results in the concentrations of the major inorganic plasma ions approaching those in the surrounding water. The handling and cannulation of marine elasmobranchs and teleosts can result in elevated plasma osmotic pressure (O.P.) and [NaCl] [5,7,11,26,39,44,49] as well as increased [K], [Ca] and [Mg] [7,12,34] whilst some freshwater teleosts exhibit a reduction in plasma ions [13,40,42]. Marked elevations in plasma [K] can disrupt elasmobranch myocardial function [24] whilst altered plasma [Mg] and [Ca] can impair muscle contraction and neuromuscular nerve transmission, respectively [7].

The current investigation examined the respiratory, acid-base, haematological and ionic status of the elasmobranch, *Heterodontus portusjacksoni*, following surgery. Whilst cannulation procedures can cause marked perturbations in blood chemistry recovery is usually complete within 72 h [22]. Thus, a comparison of sharks sampled 72 h after surgery with sharks sampled by caudal puncture was performed to examine the nature of the response to surgery and acute sampling and thus determine if the potentially stressful and lengthy procedure of surgery and recovery prior to sampling is necessary to provide 'resting values'.

2. Materials and methods

Male and female Port Jackson sharks (0.7–2.0 kg) were caught by long-line off Bermaguii, NSW and transported to Sydney Aquarium, Darling Harbour. The sharks were transferred to the closed-system aquaria at the University of Sydney at least 1 week prior to experimentation and acclimated in full-strength seawater (33–35 g l^{-1}) maintained at 19.0 ± 0.5°C. The P_{O_2} of the aquaria seawater was maintained above 18.5 kPa and the sharks fasted at least 3 days prior to experimentation.

2.1. Blood sampling procedures

2.1.1. Caudal cannulation

Sharks were anaesthetised in a bath of aerated seawater containing a 100 mg l^{-1} solution of MS222 until ventilatory activity ceased (~ 5 min). The sharks were transferred to an operating table, placed dorsal side up and covered with a damp cloth during surgery to prevent desiccation and to protect the eyes from light. The gills were irrigated with a continuous flow of aerated seawater containing 50 mg l^{-1} of MS222. Cannulation of the caudal artery and vein was based on a similar procedure outlined by Watters and Smith [41]. The caudal artery was punctured by a 22-gauge needle into which was then inserted a length of sterilized piano wire. A 10 cm length of polyethylene (P.E.) tubing (I.D. 0.36 mm, O.D. 0.58 mm) was placed over the piano wire and inserted into the artery and connected to a 30 cm length of P.E. tubing (I.D. 0.58 mm, O.D. 0.96 mm). The cannulae was flushed with heparinized (100 U ml^{-1} lithium heparin, Sigma, St. Louis, MO, Cat. no. H-0878) shark Ringers [30] and sealed by a 3-way tap. The caudal vein was cannulated using the same procedure outlined above with the vessel located 2–3 mm ventral to the caudal artery. The time required to induce anaesthesia and cannulate the arterial and venous vessels (32–40 min, $n = 18$) did not differ significantly between sharks measured 4, 24 or 72 h after surgery. Unlike teleost fish which tend to swim against the current, the Port Jackson shark frequently changes direction and becomes agitated if restrained, or prevented from turning. Thus, after surgery the sharks were placed in metabolism cages made from PVC pipe of 1 m length and 300 mm dia. and closed at each end with 1 cm^2 grid plastic mesh. The 3-way taps fixed to the end of the cannulae lead through a 1-cm channel cut in the top of the pipes and were attached to styrofoam blocks which could slide along the channel as the shark moved within the tube. The metabolism cages were orientated within the aquarium to allow a constant inflow of seawater at a P_{O_2} of > 18 kPa.

2.1.2. Caudal puncture

The sharks required for caudal puncture were placed in individual plastic tanks at least 24 h prior to blood sampling. Each individual tank had 25 l of recirculating filtered and aerated seawater taken directly from the acclimation aquaria. At the sampling time the sharks were quickly removed from their individual tanks and up to 1 ml of blood was taken by caudal arterial or venous puncture (20–30 s) using 22-gauge hypodermic needles attached to the pre-chilled heparinized syringes. The sharks were maintained quiescent during handling by placing a damp towel over their heads.

2.1.3. Measurement of respiratory and acid-base status

Blood samples of up to 1 ml were removed using pre-chilled 1-ml gas-tight glass syringes (Suzuki) pre-rinsed with heparinized solution. Whole blood P_{O_2} and P_{CO_2} were measured with a BMS Mk2 blood micro system (Radiometer) thermostatted to 19°C and connected to a PHM73 analyser/meter. The O_2 electrode was calibrated with an O_2 free mixture (CO_2/N_2) and air-saturated water. The CO_2 electrode was calibrated with analytical grade humidified gases containing 0.34 and 2.15% CO_2. Whole blood oxygen content (C_{O_2}) was measured using the modified Tucker chamber method [37]. The electrode was connected to an O_2 meter (Strathkelvin, model 781) and pen recorder. The maximum O_2 carrying capacity of the blood was determined

by equilibrating the blood with humidified air of known P_{O_2} (>20 kPa) and measuring the C_{O_2}.

Whole blood CO_2 content (C_{CO_2}) was measured with a 25-μl sample transferred to a gastight syringe (Hamilton) for injection into a CO_2 analyser (Ciba-Corning 965) calibrated daily with a 15 mmol l^{-1} $NaHCO_3$ standard solution. Whole blood and erythrocyte pH were measured with a capillary electrode (G229a) mounted within the BMS Mk2 and calibrated with Radiometer precision buffers. Erythrocyte pH was measured after the blood was centrifuged for 10 min at 6500 rpm and the plasma decanted. The erythrocytes were freeze-thawed three times in liquid nitrogen and the erythrocyte slurry centrifuged and the supernatant measured for pH. Bicarbonate concentrations ($[HCO_3^-]$) were calculated from the Henderson-Hasselbach equation using values for pK and αCO_2 obtained from Albers and Pleschka [1] and Pleschka and Wittenbrock [30]. Plasma and erythrocyte L-lactate concentrations were measured in duplicate with a test kit (Boehringer Mannheim, Cat. no. 139084).

Ventilation frequency (f_r) was determined using an impedance method [3]. Electrodes were implanted under the skin of the first gill flap on each side of the shark and fixed into place with histoacryl glue. The remaining length of electrode wire was tied to the front dorsal fin spine and attached to the styrofoam blocks securing the blood cannulae. The electrodes were connected to an impedance meter (University of Sydney Workshop) and pen recorder.

2.1.4. Haematology

Haematocrit (Hct) was determined on 9-μl blood samples in heparinized capillary tubes centrifuged for 3.5 min at $11000 \times g$. Whole blood haemoglobin concentration ([Hb]) was measured spectrophotometrically by the cyanomethaemoglobin method (Sigma Cat. no. 525-A) and RBC were determined using a haemocytometer on blood samples diluted 1:100 with the shark Ringers solution. MCHC and mean cell volume (MCV) were calculated as described by Stoskopf [36].

2.1.5. Osmotic pressure and ion analysis

Whole blood osmotic pressure was measured using a vapour pressure osmometer (Wescor, 5100C) calibrated with 290 and 1000 mOsm standards. Plasma and erythrocyte [Na], [Ca], [K] and [Mg] were measured on deproteinised samples using an atomic absorption spectrophotometer (GBC, 906A). Plasma and erythrocyte [Cl] were measured using a CMT 10 Chloride Titrator (Radiometer) routinely calibrated with a 100 mmol l^{-1} NaCl standard solution. Plasma and erythrocyte urea were determined using an assay test kit (Boehringer Mannheim, Cat. no. 542 946). Trimethylamine oxide (TMAO) was determined using a modified version of Bystedt et al. [6] supplied by Barnes and Blackstock [2]. The extent of any plasma inclusion in the erythrocyte fluid was determined from sharks ($n=3$) injected with Cr-EDTA (1 μl g^{-1} wet weight) (CJ-13P, Amersham). The sharks were sampled 24 h after injection by caudal puncture and the plasma and erythrocyte fluids treated as outlined above. The ^{51}Cr isotope was measured in duplicate 50 μl plasma and erythrocyte samples using a gamma counter (1272 Clini Gamma, LKB). The percent of plasma fluid contamination of erythrocyte samples was 9.4 ± 1.2 and the measured erythrocyte ion concentrations corrected accordingly.

2.1.6. Statistical analysis

All values are expressed as means $\pm$ S.E.M., unless otherwise stated, with a probability (P) value $0 \leq 0.05$ considered significant. For those symbols without error bars, the S.E.M. was smaller than the size of the symbol. Homogeneity of variances was determined using Bartletts χ^2 test. In cases where variances were heterogeneous, log or square root transformations were performed prior to further analysis. Data from the 4, 24 and 72 h cannulated groups were compared using one factor ANOVA and post-hoc testing was performed using either contrast analysis or Tukey's multiple means comparison analysis. Sharks sampled 72 h after surgery and those sampled by caudal puncture were compared using Student's t-test.

3. Results

3.1. Respiratory, acid-base and haematological response to caudal cannulation

Neither the Pa_{O_2} nor the Ca_{O_2} differed between sharks allowed to recover for 4, 24 or 72 h after surgery. With the exception of a marked elevation in Pv_{O_2} exhibited 72 h after surgery, neither the Pv_{O_2}, Cv_{O_2} nor the maximal O_2 content (saturated C_{O_2}) changed between 4 and 72 h (Table 1). Similarly, neither $[Hb\text{-}O_2]_a$, $[Hb\text{-}O_2]_v$ nor the maximal $[Hb\text{-}O_2]$ (saturated $[Hb\text{-}O_2]$) differed between 4, 24 or 72 h sampled sharks (Table 1). The ventilation rates exhibited by sharks recovered for 24 and 72 h after cannulation were $\sim 40\%$ lower than those of sharks sampled only 4 h after surgery (Table 1). Whole blood Hct and [Hb] remained constant between 4 and 72 h. The MCHC was lowest 24 h after surgery despite the RBC and MCV remaining constant throughout the sampling period (Table 1).

The Pa_{CO_2} and Pv_{O_2} of sharks sampled either 24 or 72 h after surgery were approximately 50 and 30% lower, respectively, than those of sharks allowed only 4 h to recover. The Ca_{CO_2} was also reduced in sharks sampled either 24 or 72 h after surgery when compared with sharks allowed to recover for only 4 h whilst the

Table 1
The respiratory status of *H. portusjacksoni* sampled 4, 24 or 72 h after surgery or by caudal puncture

	4 h	24 h	72 h	Caudal puncture
Pa_{O_2} (kPa)	11.6 (2.1)	13.9 (0.28)	13.2 (1.2)	13.0 (0.59)
Pv_{O_2} (kPa)	4.95 (0.38)	4.53 (0.09)[c]	5.73 (0.33)[d]	3.31 (0.75)
Ca_{O_2} (mmol l^{-1})	1.28 (0.05)	1.39 (0.06)	1.16 (0.11)	1.37 (0.26)
Cv_{O_2} (mmol l^{-1})	1.10 (0.21)	1.32 (0.04)	0.94 (0.13)	0.77 (0.14)
Saturated C_{O_2} (mmol l^{-1})	1.57 (0.07)	1.62 (0.10)	1.48 (0.14)	1.58 (0.25)
$[Hb\text{-}O_2]_a$ (mmol l^{-1})	0.97 (0.22)	1.16 (0.30)	0.86 (0.21)	1.25 (0.26)
$[Hb\text{-}O_2]_v$ (mmol l^{-1})	0.60 (0.17)	0.73 (0.19)	0.74 (0.20)	0.74 (0.14)
Saturated [Hb-O_2] (mmol l^{-1})	1.36 (0.07)	1.46 (0.14)	1.27 (0.13)	1.29 (0.25)
Hct (%)	19.0 (0.57)	20.7 (1.01)	19.0 (0.63)	20.0 (0.94)
Hb (g dl^{-1})	2.88 (0.31)	2.66 (0.24)	2.71 (0.09)[d]	3.69 (0.19)
MCHC (g l^{-1})	152 (14)[a]	127 (5)[c]	144 (4)[d]	187 (4)
RBC (10^{-6} μl)	0.26 (0.02)	0.23 (0.04)	0.23 (0.01)	0.23 (0.02)
MCV (f_L)	780 (35)	867 (38)	804 (35)	853 (102)
f_r (beat min^{-1})	56 (8)[ab]	39 (3)	42 (3)	—

Standard errors in brackets ($n = 6$).
[a] 4 h differs significantly from 24 h.
[b] 4 h differs significantly from 72 h.
[c] 24 h differs significantly from 72 h
[d] 72 h differs significantly from caudal puncture.

Cv_{CO_2} did not vary (Table 2). The plasma pH_a and pH_v of sharks sampled either 24 or 72 h after surgery were both elevated by ~ 0.1 pH units when compared to sharks sampled 4 h after surgery. In contrast, neither the erythrocyte pH_a nor pH_v varied between 4 and 72 h (Table 2). Plasma and erythrocyte L-lactate concentrations remained low and constant (Table 2).

The plasma [Cl] of sharks measured 4 h after surgery was elevated when compared with sharks allowed either 24 or 72 h to recover from surgery but the whole blood osmotic pressure and plasma [Na], [Ca], [K] and [Mg] varied little throughout the sampling period (Table 3). The erythrocyte [Na] of sharks measured 72 h after cannulation was $\sim 20\%$ lower than that observed in sharks sampled at 4 and 24 h (Table 3). Erythrocyte [Cl] was 18% higher and [K] was 30% lower in sharks recovered for 24 h when compared with sharks measured either 4 or 72 h after surgery. In contrast, erythrocyte [Ca] and [Mg] were both maximal 4 h after surgery (Table 3). The plasma strong ion difference (S.I.D.) as defined and determined by Maxime et al. [25] remained constant throughout the recovery from surgery with a mean cationic excess of -70 mmol l^{-1}. In contrast, the erythrocyte S.I.D. of sharks 4 h after surgery, despite being similar to that observed in the plasma, was markedly elevated when compared with sharks sampled 24 h (due to increased [Cl]) or 72 h (due to reduced [Na]) after surgery (Fig. 1). The plasma and erythrocyte urea concentrations of sharks recovered for 24 and 72 h after surgery were $\sim 30\%$ higher than that of sharks measured 4 h after surgery. In contrast, TMAO remained constant throughout the recovery period (Table 3).

3.2. The differences in the blood status of sharks sampled by caudal puncture as opposed to sharks sampled 72 h after surgery

With the exception of the Pv_{O_2}, which was 40% lower in punctured sharks, the respiratory status of sharks sampled by caudal puncture did not differ from that of sharks allowed 72 h to recover from cannulation (Table 1). The Hct of cannulated and punctured sharks did not differ but the MCHC, and as a consequence the [Hb], were markedly lower in cannulated sharks. While the 23% reduction in MCHC was indicative of cell swelling neither the RBC nor MCV differed between cannulated and punctured sharks (Table 1). The pH_a of punctured sharks was ~ 0.6 units lower than that of cannulated sharks despite plasma and erythrocyte L-lactate concentrations being $\sim 68\%$ lower in punctured sharks (Table 2).

Similar to the respiratory status, however, the acid-base status of sharks sampled by either caudal cannulation or puncture did not differ markedly. In contrast, the plasma and erythrocyte ionic status of cannulated and punctured sharks differed quite markedly due mainly to alterations in the concentrations of the major osmolytes Na, Cl and urea. The plasma [Na] of cannulated sharks was $\sim 18\%$ higher, and the erythrocyte [Na] $\sim 30\%$ lower, when compared with punctured sharks. The erythrocyte [Cl] of the cannulated sharks was also $\sim 25\%$ lower than that observed in punctured sharks (Table 3). The increased cationic strength (elevated [Na]) of the cannulated sharks when compared with punctured sharks resulted in a ~ 70 mmol l^{-1}

Table 2
The blood acid–base status of *H. portusjacksoni* sampled 4, 24 or 72 h after cannulation or by caudal puncture

	4 h	24 h	72 h	Caudal puncture
Pa_{CO_2} (kPa)	0.42 (0.03)[ab]	0.28 (0.01)	0.28 (0.03)	0.32 (0.04)
Pv_{CO_2} (kPa)	0.41 (0.04)[ab]	0.31 (0.02)	0.31 (0.02)	0.25 (0.03)
Plasma pH_a	7.77 (0.04)[b]	7.82 (0.01)	7.88 (0.02)[d]	7.82 (0.01)
Erythrocytic pH_a	7.07 (0.03)	7.06 (0.01)	7.12 (0.04)	7.10 (0.02)
Plasma pH_v	7.70 (0.03)[b]	7.76 (0.01)	7.83 (0.01)	7.82 (0.03)
Erythrocytic pH_v	7.06 (0.02)	7.10 (0.03)	7.09 (0.04)	7.12 (0.02)
Ca_{CO_2} (mmol l^{-1})	8.20 (0.73)[ab]	6.27 (0.31)	6.48 (0.50)	7.18 (0.21)
Cv_{CO_2} (mmol l^{-1})	6.70 (0.37)	6.27 (0.15)	6.76 (0.43)	6.90 (0.22)
$[HCO_3^-]_a$ (mmol l^{-1})	19.3 (1.8)[a]	14.1 (0.6)	16.0 (2.0)	15.1 (1.3)
$[HCO_3^-]_v$ (mmol l^{-1})	15.7 (1.0)	13.7 (1.0)	14.8 (0.8)	12.7 (2.1)
Plasma L-lactate (mmol l^{-1})	0.47 (0.11)	0.38 (0.08)	0.28 (0.04)[d]	0.17 (0.02)
Erythrocyte L-lactate (mmol l^{-1})	1.12 (0.27)	1.47 (0.26)	1.47 (0.26)[d]	0.48 (0.12)

Standard errors in brackets ($n = 6$).
[a] 4 h differs significantly from 24 h.
[b] 4 h differs significantly from 72 h.
[c] 24 h differs significantly from 72 h.
[d] 72 h differs significantly from caudal puncture.

difference in plasma S.I.D. (Fig. 1). In contrast, the ~ 15 mmol l^{-1} elevation in the erythrocyte S.I.D. of the cannulated sharks when compared with punctured sharks (Fig. 1) was due primarily to the reduction in the [Cl] of the cannulated sharks (Table 3). The concentrations of plasma and erythrocyte urea and plasma TMAO in cannulated sharks were markedly lower than those of punctured sharks whilst erythrocyte TMAO concentrations did not differ between cannulated or punctured sharks (Table 3).

4. Discussion

4.1. The effects of cannulation on blood chemistry

Despite the fluctuations in Pv_{O_2} exhibited by *H. portusjacksoni* after surgery the $Pa_{O_2}-Pv_{O_2}$ difference did not alter. The small differences in blood P_{O_2} had no significant effect on the $Ca_{O_2}-Cv_{O_2}$ difference, which did not change, and thus any changes in O_2 extraction following surgery would be due to altered blood perfusion. In contrast, cannulation of the dogfish, *Scyliorhinus canicula*, caused a severe internal hypoxia, which despite the recovery of the Pa_{O_2} and Ca_{O_2} within 24 h of surgery, still exhibited plasma (L-lactate) exceeding 5 mmol l^{-1} [9]. Clearly, any surgery induced hypoxia in *H. portusjacksoni* was thus relatively slight since at no time did plasma (L-lactate) exceed 0.5 mmol l^{-1}. A different investigation of *S. canicula* by Butler et al. [4] found that the Pv_{O_2} and Cv_{O_2} of sharks sampled 24 and 72 h after cannulation remained constant and that L-lactate did not exceed 0.3 mmol l^{-1}. The plasma (L-lactate) observed in the present study and by Butler et al. [4] are an order of magnitude lower than those observed for *S. canicula* [9] and *S. stellaris* [29] 'recovered' from surgery. Differences in surgery and post-surgery methodology can thus result in 'resting' fish, even of the same species, being in a markedly different physiological state.

Unlike the plasma pH, the erythrocyte pH_a and pH_v of *H. portusjacksoni* did not vary after surgery. The acute plasma acidosis exhibited by *H. portusjacksoni* 4 h after surgery, when compared with sharks sampled after 24 or 72 h (Fig. 2A), was a similar response to that observed in the trout, *Salmo gairdneri* [21,35] and *S. canicula* [9] following cannulation. The respiratory component of the acidosis was relieved within 24 h by the respiratory (hyperventilation) alkalosis. Hyperventilation appears to be the general response by elasmobranchs [9,29] and teleosts [39] to post-surgery acidosis. The metabolic component of the acidosis was removed by the marked metabolic alkalosis observed between 24 and 72 h (Fig. 2A). The metabolic acidosis is generally more prolonged than the respiratory component and may persist for up to 72 h after surgery [29,39].

The general maintenance of blood haematology by *H. portusjacksoni* throughout the 72 h post-cannulation period was indicative of the general absence of any major respiratory or acid-base changes. *H. portusjacksoni* exhibited a transient cell swelling 24 h after surgery due most likely a delayed response in erythrocyte water influx resulting from surgery induced changes in membrane permeability [8,26]. Whilst cell swelling in teleosts is usually associated with elevated plasma catecholamines elamsobranchs apparently lack the necessary red blood cell β-adrenergic Na^+/H^+ exchangers [38,51]. Consequently, elevated catecholamine levels have been found to have no effect on elasmobranch RBC water volume [38].

The use of anaesthetics such as MS222 during cannulation can perturb ionic status [21]. MS222 is cleared

Table 3
The plasma and erythrocyte ionic status of *H. portusjacksoni* sampled 4, 24 or 72 h after cannulation or by caudal puncture

	4 h	24 h	72 h	Caudal puncture
Whole blood osmotic pressure (mOsm)	987 (13)	963 (2)	986 (4)	960 (2)
Plasma (mmol l^{-1})				
[Na]	359 (4)[ab]	337 (10)[c]	350 (9)[d]	286 (3)
[Cl]	310 (3)[ab]	276 (7)	281 (4)	286 (3)
[Ca]	5.1 (0.1)	4.9 (0.2)	5.3 (0.1)	4.7 (0.2)
[K]	4.4 (0.1)[a]	5.5 (0.6)	4.6 (01.)	4.4 (0.3)
[Mg]	1.2 (0.1)	1.3 (0.2)	1.3 (0.2)	1.4 (0.1)
Urea	269 (16)[ab]	388 (6)[c]	356 (10)[d]	419 (6)
TMAO	57 (12)	46 (10)	78 (15)[d]	121 (7)
Erythrocytic (mmol l^{-1})				
[Na]	5 (5)	13 (7)[c]	8 (1)[d]	33 (10)
[Cl]	43 (6)[a]	68 (4)[c]	46 (5)[d]	81 (14)
[Ca]	0.09 (0.01)[ab]	0.15 (0.01)	0.20 (0.01)[d]	0.09 (0.05)
[K]	81 (4)[a]	52 (2)[c]	76 (4)[d]	61 (2)
[Mg]	13 (2)[b]	10 (2)	6 (2)[d]	11 (1)
Urea	208 (15)[ab]	299 (8)	312 (6)[d]	367 (16)
TMAO	70 (18)	73 (9)	77 (14)	76 (10)

Standard errors in brackets ($n = 6$).
[a] 4 h differs significantly from 24 h.
[b] 4 h differs significantly from 72 h.
[c] 24 h differs significantly from 72 h.
[d] 72 h differs significantly from caudal puncture.

from the blood of teleosts within 8 h and from the urine within 24 h of surgery [18,19] and it thus appears that changes in ion levels are more likely the result of handling stress [35]. The plasma cations of rainbow trout were only elevated if anaesthesia was preceded by removal from the water for 20 s when compared with fish in which the anaesthetic was added directly to their water bath [35]. Elasmobranchs also exhibit elevated plasma osmotic pressure and inorganic ions following handling and capture stress [9,11,24,44]. The changes in ionic strength observed in cannulated *H. portusjacksoni* are thus more likely due to the effects of handling and surgery than to MS222. The increase in plasma [Na] following handling and cannulation procedures is due to increased Na permeability [10,26,28]. An increase in Na influx and reduction in efflux (due to reduced urinary loss) were observed in handled *S. canicula* [26] and may explain the similar elevation in plasma [Na] observed in cannulated *H. portusjacksoni* 4 h after surgery. Interestingly, the plasma [NaCl] of *H. portusjacksoni* changed inversely with that of erythrocyte [NaCl] which also suggests changes in erythrocyte membrane ion permeability. The marked reduction in urea observed 4 h after cannulation cannot be explained by simple dilution and must therefore be due to a decrease in reabsorption and/or increased branchial permeability. Urea is readily diffusible through the erythrocyte membrane which would account for the comparable reduction in both plasma and erythrocyte concentrations. In contrast, TMAO was strongly conserved, a response similar to that observed in *H. portusjacksoni* transferred into diluted seawater (Cooper and Morris, in preparation).

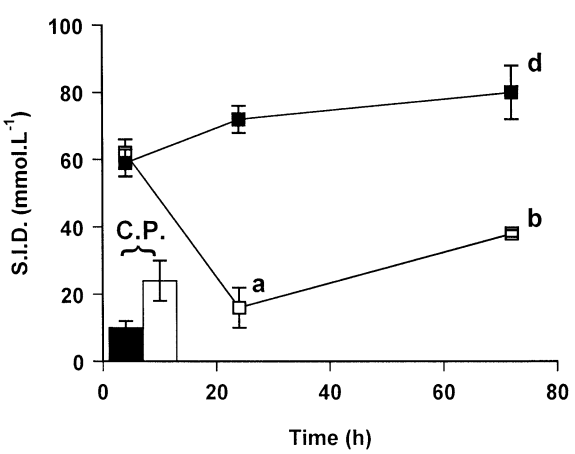

Fig. 1. Changes in the plasma (■) and erythrocyte (□) strong ion difference of *H. portusjacksoni* sampled by either cannulation or caudal puncture (C.P.). S.I.D. = ([Na] + [Ca] + [K] + [Mg])-([Cl] + [L-lactate]) as determined by Maxime et al. [25]. (a) 4 h differs significantly from 24 h; (b) 4 h differs significantly from 72 h; (c) 24 h differs significantly from 72 h; (d) 72 h differs significantly from caudal puncture.

4.2. Does caudal puncture cause greater stress than cannulation?

There was little difference in the respiratory status of punctured *H. portusjacksoni* when compared with

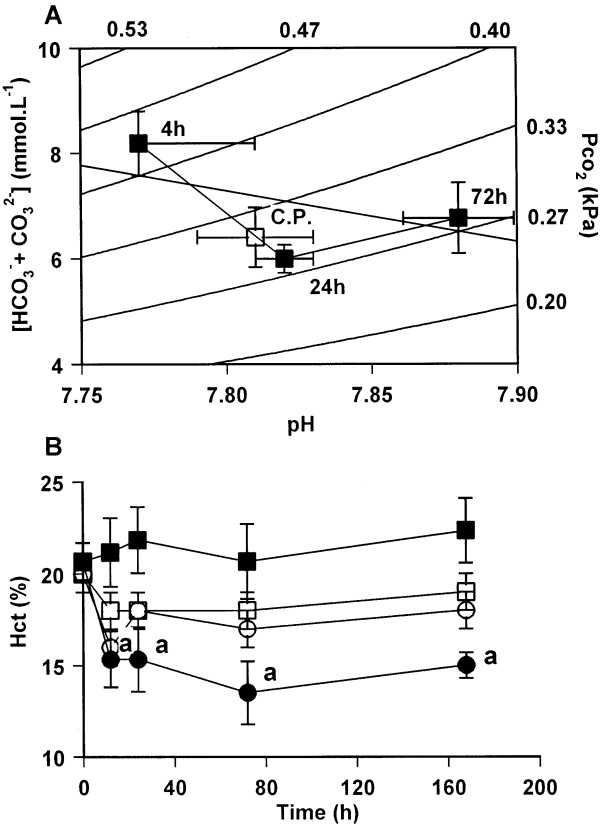

Fig. 2. (A) The pH-bicarbonate diagram illustrating changes in the acid-base status of *H. portusjacksoni* recovering from surgery (■) and after caudal puncture (□). The non-bicarbonate buffer line was constructed using tonometered 80 μl whole blood samples equilibrated to various P_{CO_2} values for 20 min using gas mixtures forwarded from Wosthoff pumps and measured for C_{CO_2} and pH. (b) Changes in the Hct of *H. portusjacksoni* sampled by either cannulation (solid symbols; 100% seawater (■) and 75% seawater (●)) or by caudal puncture (open symbols; 100% seawater (□) and 75% seawater (○)). (a) Cannulated sharks transferred to 75% seawater differ significantly from cannulated control sharks in 100% seawater (data from Cooper and Morris, in preparation).

sharks having 'recovered' for 72 h from surgery. There was evidence that the blood P_{O_2} in punctured sharks was lower than in sharks 72 h post-cannulation (Table 1). Nonetheless, both the $Pa_{O_2}-Pv_{O_2}$ and the $Ca_{O_2}-Cv_{O_2}$ differences were of similar magnitude in the two groups suggesting that if both groups had similar blood perfusion then O_2 extraction from the blood was also constant. The pH_a of cannulated sharks was slightly alkalotic when compared with punctured sharks due to a relative metabolic alkalosis most likely in response to the marked changes in strong ion difference (S.I.D.) (Figs. 1 and 2A).

The Hct, RBC and MCV of cannulated *H. portusjacksoni* were comparable to those of punctured sharks but the [Hb] and MCHC were significantly lower. A similar reduction in [Hb] and MCHC of cannulated *S. canicula* was the result of cell swelling [9]. In contrast, whilst the haematology of the ice-fish, *Pagothenia borchgrevinki*, sampled by caudal puncture or 24 h after surgery did not differ, the Hct and [Hb] was elevated and the MCHC reduced when compared with fish sampled 72 h after surgery [43]. In teleost fish, the elevation in circulating catecholamines following caudal puncture leads to a relative increase in MCHC when compared with fish cannulated for 24–48 h. Recent studies by Perry and Gilmour [27] have shown that plasma catecholamine concentrations in *S. acanthias* do not alter during severe hypoxia and hypercapnia. Furthermore, injection of catecholamine into *S. acanthias* did not alter Hct, [Hb] or MCHC. Interestingly, cannulated *H. portusjacksoni* transferred to diluted seawater (75% SW) exhibit a marked reduction in Hct which was not recovered after 1 week (Fig. 2B). In contrast, the Hct of sharks transferred to 75% SW and sampled by caudal puncture returned to control values within 12 h (Fig. 2B). The haematological perturbations observed in *H. portusjacksoni* after cannulation thus appears to be a direct response to surgery induced changes in water permeability. Without a large number of studies it is difficult to conclude whether the variety of handling and surgical techniques can account for the variability of response or if it is truly a species dependent phenomenon.

There were marked differences in the [NaCl], urea and TMAO concentrations of cannulated and punctured *H. portusjacksoni*. The relative disequilibrium between the intra- and extracellular inorganic ion levels were observed in the changes in S.I.D. (Fig. 1). The elevated plasma [Na] of cannulated sharks was most likely via increased Na permeability. An increase in branchial Na^+/H^+ exchange would also explain the elevated plasma pH of sharks allowed 72 h to recover from surgery since the changes in $[HCO_3^-]$ were negligible.

There was no appreciable difference between the respiratory and acid-base status of cannulated and punctured *H. portusjacksoni*. Clearly, for *H. portusjacksoni*, it is possible to sample by caudal puncture with reasonable assurance of obtaining 'non-disturbed' respiratory and acid-base values. Blood sampling by caudal puncture appeared to reduce haematological and ionic perturbations associated with surgery. Thus if serial sampling is not required, for elasmobranchs at least, blood sampling by caudal puncture could provide 'resting' values for the majority of blood parameters.

Acknowledgements

We are indebted to Mr Alan Broadhurst for the collection of the sharks and Darling Harbour Aquarium for facilities and help provided. This research was carried out while AC was in receipt of an Australian Post-Graduate Research Award. The research was car-

ried out with approval of the University of Sydney Animal Ethics Committee under the protocol LO4/9-94/3/1079.

References

[1] Albers C, Pleschka K. Effect of temperature on CO_2 transport in elasmobranch blood. Respir Physiol 1967;2:261–73.

[2] Barnes H, Blackstock J. The separation and estimation of free amino acids, trimethlamine oxide, and betaine in tissues and body fluids of marine invertebrates. J Exp Mar Biol Ecol 1974;16:29–45.

[3] Bridges CR, Taylor AC, Morris S, Grieshaber MK. Eco-physiological adaptations in *Blennius pholis* (L) blood to intertidal rockpool environments. J Exp Mar Biol Ecol 1984;77:151–67.

[4] Butler PJ, Taylor EW, Davison W. The effect of long term, moderate hypoxia on acid-base balance, plasma catecholamines and possible anaerobic end products in the unrestrained dogfish *Scyliorhinus canicula*. J Comp Physiol 1979;132:297–303.

[5] Byrne JM, Beamish FW, Saunders RL. Influence of salinity, temperature and exercise on plasma osmolality and ionic concentrations in Atlantic salmon (*Salmo salar*). J Fish Res Bd Can 1972;29:1217–20.

[6] Bystedt J, Swenne L, Aas HW. Determination of trimethylamine oxide in fish muscle. J Sci Fd Agric 1959;10:301–4.

[7] Cliff G, Thurman GD. Pathological and physiological effects of stress during capture and transport in the juvenile dusky shark, *Carcharhinus obscurus*. Comp Biochem Physiol 1984;78:167–73.

[8] DeVries AL, Ellory JC. The effect of stress on ion transport in fish erythrocytes. J Physiol 1981;324:55–63.

[9] Duthie GG, Tort L. Effects of dorsal aortic cannulation on the respiration and haematology of Mediterranean living *Scyliorhinus canicula* L. Comp Biochem Physiol 1985;81:879–83.

[10] Eddy FB, Bath RN. Effects of lanthanum on sodium and chloride fluxes in the goldfish *Carassius auratus*. J Comp Physiol 1979;129:145–9.

[11] Eddy, FB. Effects of stress on osmotic and ionic regulation in fish. In: Pickering AD, editor. Stress and Fish. New York: Academic Press, 1981:77–102.

[12] Fletcher GL. The effects of capture 'stress' and storage of whole blood on red blood cells, plasma proteins, glucose and electrolytes of the winter flounder (*Pseudopleuronectes americanus*). Can J Zool 1975;53:197–206.

[13] Hattingh HJ, van Pletzen AJJ. The influence of capture and transportation on some blood parameters of freshwater fish. Comp Biochem Physiol 1974;49A:607–9.

[14] Holeton GF, Randall DJ. The effect of hypoxia upon the partial pressure of gases in the blood and water afferent and efferent to the gills of the rainbow trout. J Exp Biol 1967;46:317–27.

[15] Houston AH, DeWilde MA, Madden JA. Some physiological consequences of aortic catheterization in the brook trout, *Salvelinus fontinalis*. J Fish Res Bd Can 1969;26:1847–56.

[16] Houston AH, Madden JA, Wards RJ, Miles HM. Some physiological effects of handling and tricaine methansulphonate anaesthetization upon brook trout *Salvelinus fontinalis*. J Fish Res Bd Can 1971;28:625–33.

[17] Houston AH, Madden JA, Wards RJ, Miles HM. Variations in blood and tissue chemistry of brook trout *Salvelinus fontinalis* subsequent to handling, anaesthesia and surgery. J Fish Res Bd Can 1971;28:635–42.

[18] Houston AH, Wards RJ. Blood concentration of tricaine methanesulphonate in brook trout *Salvelinus fontinalis* during anaesthetization, branchial irrigation and recovery. J Fish Res Bd Can 1972;29:1344–6.

[19] Hunn JB, Schoetlger RA, Willford WA. Turnover and urinary excretion of free and acetylated MS222 by rainbow trout *Salmo gairdneri*. J Fish Res Bd Can 1968;25:25–31.

[20] Irving L, Black EG, Safford V. The influence of temperature upon the combination of oxygen with the blood of trout. Biol Bull 1941;80:1–17.

[21] Iwama GK, McGeer JC, Pawluk MP. The effects of five fish anaesthetics on acid-base balance, hematocrit, blood gases, cortisol, and adrenaline in rainbow trout. Can J Zool 1989;67:2065–73.

[22] Iwama GK, Ishimatsu A. Cannulation of blood vessels. Tech Fish Immunol 1994;3:1–16.

[23] Khan SH, Siddigau AQ. Effect of asphyxiation on the blood constituents of the murrel, *Ophicephalus punctatus* Bloch. Broteria (Cienc Nat) 1970;39:187–95.

[24] Martini FF. Effects of capture and fasting confinement on an elasmobranch, *Squalus acanthias*. Unpublished Ph.D. Thesis, New York: Cornell University, 1974.

[25] Maxime V, Peyraud-Waitzenegger M, Claireaux G, Peyraud C. Effects of rapid transfer from sea water to fresh water on respiratory variables, blood acid-base status and O_2 affinity of haemoglobin in Atlantic salmon (*Salmo salar* L.). J Comp Physiol 1990;160:31–9.

[26] Payan P, Maetz J. Branchial sodium transport mechanisms in *Scyliorhinus canicula* Evidence for Na^+/NH_4^+ and Na^+/H^+ exchanges and for a role of carbonic anhydrase. J Exp Biol 1973;58:487–502.

[27] Perry SF, Gilmour KM. Consequences of catecholamine release on ventilation and blood oxygen transport during hypoxia and hypercapnia in an elasmobranch (*Squalus acanthias*) and a teleost (*Oncorhynchus mykiss*). J Exp Biol 1996;199:2105–18.

[28] Pic P. A comparative study of the mechanism of Na^+ and Cl^- excretion by the gill of *Mugil capito* and *Fundulus heteroclitus*: effects of stress. J Comp Physiol 1978;123:155–62.

[29] Piiper J, Meyer M, Drees F. Hydrogen ion balance in the elasmobranch *Scyliorhinus stellaris* after exhausting activity. Respir Physiol 1972;16:290–313.

[30] Pleschka K, Wittenbrock I. The solubility of carbon dioxide in elasmobranch plasma between 10°C and 22°C. Pflugers Arch Ges Physiol 1971;329:186–90.

[31] Rall DP, Sheldon W. Transport and organic dyes by the isolated choroid plexus of the spiny dogfish, *Squalus acanthias*. Biochem Pharmac 1961;11:169.

[32] Sleet RB, Weber LJ. Blood volume of a marine teleost before and after arterial cannulation. Comp Biochem Physiol 1983;76:791–4.

[33] Soivio A, Nyholm K. Notes on haematocrit determinations on rainbow trout, *Salmo gairdneri*. Aquaculture 1973;2:31–5.

[34] Soivio A, Oikari A. Haematological effects of stress on a teleost, *Esox lucius* L. J Fish Biol 1976;8:397–411.

[35] Soivio A, Nyholm K, Huhti M. Effects of anaesthesia with MS222, neutralised MS222 and benzocaine on the blood constituents of the rainbow trout, *Salmo gairdneri*. J Fish Biol 1977;10:91–101.

[36] Stoskopf MK. Clinical pathology of sharks, skates and rays. In: Stoskopf MK, editor. Fish Medicine. Philadelphia, PA: WB Saunders, 1993: 754–757.

[37] Tucker VA. Method for oxygen content and dissociation curves on microliter blood samples. J Appl Physiol 1967;23:410–4.

[38] Tufts BL, Randall DJ. The functional significance of adrenergic pH regulation in fish erythrocytes. Can J Zool 1989;67:235–8.

[39] Turner J D, Wood C M, Hobe H. Physiological consequences of severe exercise in the inactice benthic flathead sole (*Hippoglossoides elassodon*). A comparison with the active pelagic rainbow trout (*Salmo gairdneri*). J Exp Biol 1983;104:269–88.

[40] Umminger BL. Death induced by injection stress in cold-acclimated goldfish, *Carassius auratus*. Comp Biochem Physiol 1973;45:883–7.

[41] Watters KW, Smith LS. Respiratory dynamics of the starry flounder *Platichthys stellatus* in response to low oxygen and high temperature. Mar Biol 1973;19:133–48.
[42] Wedemeyer G. Some physiological consequences of handling stress in the juvenile steelhead trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*). J Fish Res Bd Can 1972;30(29):1780–3.
[43] Wells RMG, Tetens V, DeVries AL. Recovery from stress following capture and anaesthesia of antarctic fish: haematology and blood chemistry. J Fish Biol 1984;25:567–76.
[44] Wells RMG, McIntyre RH, Morgan AK, Davie PS. Physiological stress responses in big gamefish after capture: observations on plasma chemistry and blood factors. Comp Biochem Physiol 1986;84:565–71.
[45] Wells RMG, Weber RE. The spleen in hypoxic and exercised rainbow trout. J Exp Biol 1990;77:141–55.
[46] Wood CM, Randall DJ. The influence of swimming activity on water balance in the rainbow trout in vivo. J Exp Biol 1973;87:247–70.
[47] Wood CM, McMahon BR, McDonald DG. An analysis of changes in blood pH following exhausting activity in the starry flounder, *Platichthys stellatus*. J Exp Biol 1977;69:173–85.
[48] Wood CM, McMahon BR, McDonald DG. Respiratory gas exchange in the resting starry flounder, *Platichthys stellatus*: a comparison with other teleosts. J Exp Biol 1979;78:167–79.
[49] Wood CM, Turner JD, Graham MS. Why do fish die after severe exercise? J Fish Biol 1983;22:189–201.
[50] Wood CM, Perry SF. Respiratory, circulatory and metabolic adjustments to exercise in fish. In: Gilles R, editor. Circulation, Respiration and Metabolism. Berlin: Springer-Verlag; 1985: 2–22.
[51] Wood CM, Perry SF, Walsh PJ, Thomas S. HCO_3-dehydration by the blood of an elasmobranch in the absence of a Haldane effect. Respir Physiol 1994;98:319–37.
[52] Young PS, Cech JJ. Physiological stress responses to serial sampling and confinement in the young-of-the-year striped bass, *Morone saxatilis* (Walbaum). Comp Biochem Physiol 1993;105:239–44.