

CONSEQUENCES OF ELECTROFISHING AND AIR EXPOSURE ON
TROUT

THE CONSEQUENCES OF PULSED DC ELECTROFISHING AND AIR
EXPOSURE ON RAINBOW TROUT

by

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ABSTRACT

Electrofishing, which is widely used for fish collection, is a procedure that is often followed by handling and air exposure before the fish are released. Although the consequences of electrofishing are not fully known, some studies suggest that physical damage such as skeletal injury and mortality can result. Physiological disturbances resulting from stimulation of the stress axis and impaired ventilation have also been reported following electroshock. In the present study, rainbow trout treated with pulsed DC electroshock, in fact, showed no evidence of direct mortality, and skeletal damage was only induced under the most severe conditions. Physiological disturbances in the absence of physical damage consisted of a lactacidosis and stimulation of the stress response. The lactacidosis was likely induced by tetany during immobilization and impaired ventilation during immobilization and early recovery. These disturbances, which persisted for at least 4h, were greater than those reported following DC electrofishing. They were, in fact, similar in magnitude and duration to 2-3 min bout of exhaustive exercise. Swim performance following pulsed DC electroshock was also impaired for at least 1 h. If fish were air exposed immediately following pulsed DC electroshock the stress response and lactacidosis tended to be more severe and swim performance was further impaired.

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LIST OF ABBREVIATIONS

AC:	alternating current
amp:	ampere
β :	non-bicarbonate buffering capacity of the blood
BL/s:	body lengths per second
$^{\circ}\text{C}$:	degree Celcius
Ca^{2+} :	calcium ion
Cl^- :	chloride ion
cm/s:	centimeter per second
CO_2 :	carbon dioxide
D.A.	dorsal aorta
DC:	direct current
g:	gram
h:	hour
ΔH^+_m :	metabolic acid load
HCO_3^- :	bicarbonate
Hg:	mercury
HPLC:	high pressure liquid chromatography
Hz:	Hertz
K^+ :	potassium ion
l:	liter
μl :	microliter
m:	meter
μm :	micromole
meq/l:	milliequivalents per liter
Mg^{2+} :	magnesium ion
min:	minute
mmol:	millimole
ml:	milliliter
MO_2 :	oxygen consumption
ms:	millisecond
N:	number
Na^+ :	sodium ion
ng:	nanogram
nmol:	nanomole
$\mu\text{ohms/cm}$:	micro-ohms per centimeter
pca:	perchloric acid
PCO_2 :	partial pressure of CO_2
pers. comm.:	personal communication
pmol:	picomole

PO ₂ :	partial pressure of oxygen
r ² :	correlation coefficient
s:	seconds
S.E.M.:	standard error measurement
T:	temperature
U _{crit} :	critical swimming velocity
v:	volts
wt:	weight

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CHAPTER 1

GENERAL INTRODUCTION

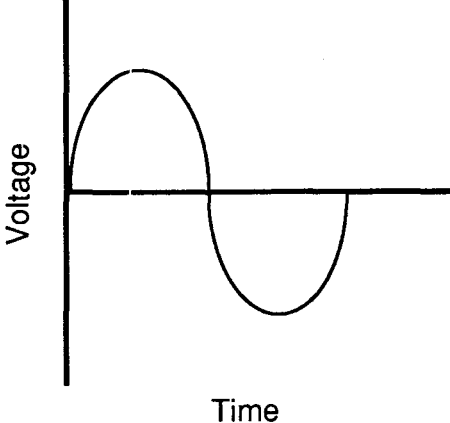
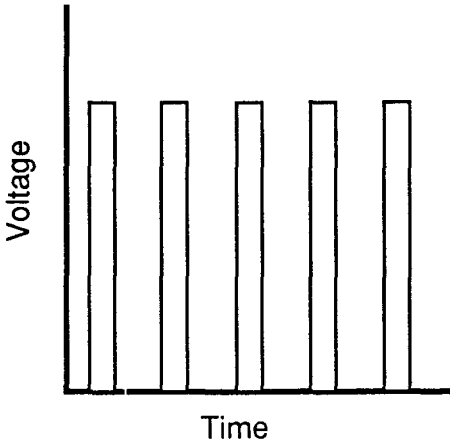
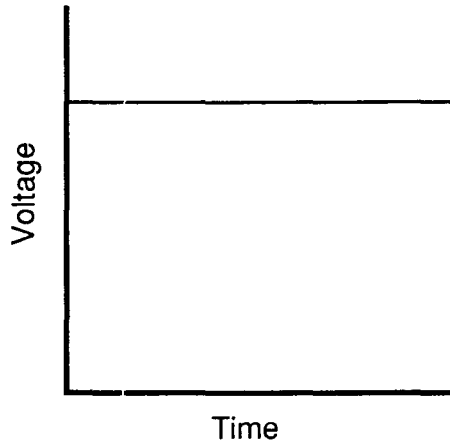
Electrofishing Gear and Uses

Since its invention in the 1930's, electrofishing has gained widespread acceptance as a viable technique for fish collection. Worldwide, electrofishing is used extensively by governmental and non-governmental agencies for the collection of brood stock and stock assessment, measurements of fish distribution, density and health, the removal of unwanted species and inventories of diversity (Lazauski and Malvestuto, 1990; Steinmetz, 1990). This work often involves tagging, length and weight measurements, scale sampling and the collection of eggs or milk for stocking programs (Hickley, 1990; G. Goodchild, pers. comm.). During this time fish are often air exposed for extensive periods of time before being released to their natural environment.

Three distinct types of electrofishing gear exists: alternating current (AC), direct current (DC) and pulsed DC (Figure 1.1). The type of gear used depends largely on the site and purpose of collection. AC, which is the most damaging type of electroshock, is known to tetanize fish and kill a high percentage of the catch (Lamarque, 1990). Therefore, its use is mainly

Figure 1.1. Summary of electrofishing techniques and the degree of potential damage associated with each technique. AC immobilizes fish by tetany and is the most damaging; pulsed DC also immobilizes fish with tetany, but is less damaging; DC immobilizes fish with galvanonarcosis then tetany and is the least damaging type of gear.

SUMMARY OF ELECTROFISHING TECHNIQUES

Type	Current	Mode Of Immobilization	Potential Damage
AC	 <p>A graph showing an alternating current (AC) waveform. The vertical axis is labeled 'Voltage' and the horizontal axis is labeled 'Time'. The waveform is a smooth sine wave that starts at the origin, rises to a peak, crosses the zero line, reaches a trough, and returns to zero.</p>	tetany	most damaging
Pulsed DC	 <p>A graph showing a pulsed direct current (Pulsed DC) waveform. The vertical axis is labeled 'Voltage' and the horizontal axis is labeled 'Time'. The waveform consists of five identical rectangular pulses of equal height and width, separated by equal intervals of zero voltage.</p>	tetany	intermediate
DC	 <p>A graph showing a direct current (DC) waveform. The vertical axis is labeled 'Voltage' and the horizontal axis is labeled 'Time'. The waveform is a single horizontal line at a constant positive voltage level, extending across the time axis.</p>	galvanonarcosis then tetany	least damaging

limited to low conductivity streams where it is the most efficient (Hudy, 1985). In fact, its use is no longer recommended under any conditions (Bohlin *et al.*, 1989), although use continues because of the great expense to replace equipment (Lazauski and Malvestuto, 1990). DC, which facilitates capture because fish are attracted to the anode, is the least damaging form and is especially good for densely covered, turbid or turbulent waters, and is used in many situations because of its less destructive nature (Bohlin *et al.*, 1989). The effects of pulsed DC gear are intermediate between AC and DC (Lamarque, 1990). Nonetheless, pulsed DC electrofishing gear is used most extensively in Ontario and throughout the United States because it is a more energy efficient technique than DC, requiring less voltage and therefore a smaller and less expensive electrical source (Reynolds, 1983). The pulse width, frequency and duration of pulses can all be varied with pulsed DC electrofishing gear, making it readily adaptable for various conditions. The frequency setting is typically chosen in relation to the desired fish species (Halsband, 1967) (e.g. 50 Hz for trout; W. Yerex, pers. comm.; R. Dalziel, pers. comm.), then the pulse width is appropriately adjusted to obtain the desired duty cycle (Lamarque, 1990). Duty cycle, which is equal to the frequency in Hz multiplied by pulse duration in seconds, represents the percentage of time the current is on (Bohlin *et al.*, 1989). Voltage adjustments are made to obtain the desired current according to conductivity of the water (Reynolds, 1983).

Responses of Fish to Electrofishing Equipment

During electrofishing, a current is passed between cathode and anode. Although the external symptoms of fish exposed to these currents are similar with different gear types, the physiological responses are very different. Fish exposed to AC are immobilized by tetany at great distances from the electrodes (Lamarque, 1990). Overexposure to a tetanizing current can lead to mortality immediately following electrofishing if breathing is not re-established (Lamarque, 1967, 1990). Initial simultaneous violent contractions on both sides of the vertebral column can result in dislocated or broken vertebrae which may rupture arteries and lead to mortality several days following electroshock (Lamarque, 1990). Increases in voltage gradients induced by pulsed DC electrofishing gear lead to a series of stepwise reactions in response to stimulation of sensory and motor fibers (Lamarque, 1990). Fish respond initially with short, rapid undulations towards the anode (electrotaxis), followed by tetanic contractions of the muscles which leads to immobilization (Halsband, 1967). Exposure to the current induced by pulsed DC electrofishing gear can lead to physical damage and/or mortality (Lamarque, 1990) in a similar manner to AC gear. DC electrofishing elicits a more varied stepped response than pulsed DC. Immobilization from exposure to this type of current is caused by a state of galvanonarcosis, where fish muscles are relaxed. If the voltage gradient is further increased, a second bout of electrotaxis occurs, which is finally followed by a second immobilization of tetanic origin (Lamarque, 1990). Physical damage and/or mortality may also occur as a result of DC electroshock, however, the

probability is much lower than with either AC or pulsed DC electrofishing gear (Spencer, 1967; Vincent, 1971; Lamarque, 1990).

A number of studies have indicated that rainbow trout (*Oncorhynchus mykiss*) severely shocked with all three types of electrofishing gear can experience a variety of disturbances including: spinal injuries (Hauck, 1949; McCrimmon and Bidgood, 1965; Spencer, 1967; Hudy, 1985; Sharber and Carothers, 1990), hemorrhage (Hauck, 1949; Horak and Klein, 1967; Sharber and Crothers, 1990) and occasionally death (Pratt, 1954; Horak and Klein, 1967; Hudy, 1985; Barrett and Grossman, 1988). Behavioural changes such as sluggish swimming and decreased aggression and feeding have been observed in cutthroat trout (*Oncorhynchus clarki*) and rainbow trout following DC and pulsed DC electrofishing (Bouck and Ball, 1966; Horak and Klein, 1967; Mesa and Schreck, 1989). Reduced growth rates have been observed following repetitive bouts of pulsed DC electroshock (Gatz *et al.*, 1986; Gatz and Adams, 1987); although single electroshock treatments are not severe enough to affect growth (Maxfield *et al.*, 1971; Gatz and Adams, 1987). The few physiological studies conducted, with DC electrofishing gear, have shown slight elevations in plasma cortisol (Schreck *et al.*, 1976; Woodward and Strange, 1987; Mesa and Schreck 1989) glucose (Schreck *et al.*, 1976) and whole blood lactate (Schreck *et al.*, 1976; Mesa and Schreck, 1989) and small decreases in plasma Cl⁻ (Woodward and Strange, 1987) concentrations. In addition, increased buccal pressure, violent coughing and irregular cardiac activity also occurred immediately following DC electroshock (Schreck *et al.*, 1976). These responses were attributed to a

combined effect of impaired ventilation and stimulation of the stress axis (Schreck *et al.*, 1976; Mesa and Schreck, 1989).

There are a number of important limitations to the existing data:

1. It is difficult to draw general conclusions from these studies as the observed responses to electroshock are highly variable, especially in the magnitude of the disturbance. In part, this is due to improvements in technology since the 1950's, leading to increases in both the safety and efficiency of electrofishing. But more important, is the variation in physiological response of fish to different currents (ie. immobilization by tetany with AC and pulsed DC and by galvanonarcosis with DC). Differences in other experimental techniques including: water quality (ie. conductivity, temperature), electrofisher settings (voltage, pulse duration, frequency), size of the fish, species and strain of fish (Mesa and Schreck, 1989; Woodward and Strange, 1989), orientation of the fish, distance of the fish from the electrodes, duration of the shock and repetitions could also contribute to these variations.
2. Studies to date have either focussed on the pathological or the physiological damage and have not attempted to link the two areas together.
3. Despite the increasing popularity of pulsed DC electrofishing the physiological response to this technique has not been examined.
4. Following electrofishing, fish are often handled extensively while air exposed before being released. Yet, the combined effects of electrofishing and handling have not been examined.

Thus, additional research is needed to examine the pathological and physiological response of fish following pulsed DC electrofishing and handling. This work should examine the stress response, parameters related to muscular exhaustion and low oxygen stress, as well as how these factors may affect performance once fish are released.

Objectives

The objectives of this thesis were to conduct, on rainbow trout, a comprehensive study of the pathological, physiological and swim performance effects of pulsed DC electrofishing and to assess the additional effects of air exposure. Chapter 2 focusses on the pathological and physiological consequences. This work tested the hypothesis, based on studies of DC electrofishing, that the responses of fish can be attributed to a combined effect of activation of the general stress response and impaired ventilation (Schreck *et al.*, 1976; Mesa and Schreck, 1989). Due to the mode of immobilization with this electrofishing technique, physiological parameters associated with muscular exertion were also examined. Results are compared to existing literature on the responses of fish to pulsed DC and DC electrofishing and exhaustive exercise. In addition, the physiological effects of handling/air exposure following electrofishing were also examined.

Chapter 3 focusses on the development of a swim performance test to simulate environmental challenges, such as predator avoidance, faced by fish once they are returned to the environment. This test was then used to assess possible performance impairment and recovery following various electrofishing and air exposure treatments.

CHAPTER 2

PHYSIOLOGICAL CONSEQUENCES OF PULSED DC ELECTROFISHING AND AIR EXPOSURE

INTRODUCTION

Electrofishing is used extensively by governmental and non-governmental agencies to collect fish for monitoring and conservation projects. Following electroshock, fish are often air exposed for extensive periods of time for processing, before being released. Since this work is often conducted on rare and sensitive populations, it is vital to establish what damage might be caused to the fish. A number of authors have reported direct mortality (Hauck, 1949; Pratt, 1954; Horak and Klein, 1967; Hudy, 1985; Barrett and Grossman, 1988) and spinal injury (Hauck, 1949; McCrimmon and Bidgood, 1965; Hudy, 1985; Sharber and Carothers, 1990) as a result of electrofishing. Physiological studies conducted on fish exposed to DC electroshock have reported stimulation of the stress axis (Schreck *et al.*, 1976; Mesa and Schreck, 1989) and impaired ventilation during and immediately following treatment (Schreck *et al.*, 1976). But despite its widespread use, a comprehensive study on the consequences of pulsed DC electrofishing has not been conducted. In addition, no studies, to my knowledge, have considered the combined effects of electrofishing and subsequent air exposure and handling.

This study assessed the potential physical damage, mortality and physiological disturbances experienced by rainbow trout as a result of capture with a pulsed DC backpack electrofisher and handling. To simulate handling during field processing, fish were air exposed for various times following electroshock treatment. Physical damage was assessed on the basis of spinal X-rays, and survival was monitored following various electroshock and air exposure regimes. The assessment of physiological disturbances tested the hypothesis, based on studies of DC electrofishing, that the responses of fish can be attributed to a combined effect of stimulation of the general stress response and impaired ventilation (Schreck *et al.*, 1976; Mesa and Schreck, 1989). Due to the mode of immobilization with this electrofishing technique (tetany opposed to galvanonarcosis; Lamarque, 1990) physiological parameters associated with muscle exertion were also examined. Results are compared to existing literature on the responses of fish to DC- and pulsed DC electroshock and to exhaustive exercise.

To fully document the stress response, it was decided to examine plasma cortisol, glucose, and Cl^- , which have commonly been examined (Mesa and Schreck, 1989; Woodward and Strange, 1987; Schreck *et al.*, 1976), and also plasma Na^+ and catecholamines as well as oxygen consumption and branchial Na^+ loss which are only rarely examined but are also disturbed by stress (Mazeaud *et al.*, 1977). As in previous electroshock studies, whole blood lactate was examined. In addition, acid-base disturbances (pH, total CO_2) were studied since they typically follow any severe muscular exertion.

Two blood sampling techniques, caudal severance and chronically implanted dorsal aorta (D.A.) catheters, were employed in different trials. Catheterization was necessary for measurements of catecholamine levels and acid-base status, but there are disadvantages such as the chronic stress associated with anaesthetization and surgery (Heisler, 1984; McDonald and Milligan, 1992). Consequently, blood was also collected by caudal severance, which eliminates the chronic stress, but is not considered appropriate for measurements of acid-base status and catecholamines (McDonald and Milligan, 1992). The MO_2 and ion losses were measured on fish not stressed by either sampling protocol.

MATERIALS AND METHODS

Experimental Animals

Rainbow trout (30-600 g) were obtained from Rainbow Springs Trout Farm in Thamesford, Ontario. Fish were held for at least two weeks before experimentation in well aerated, 400 l circular tanks, supplied continuously with dechlorinated Hamilton tap water. Fish were fed 3 times weekly with commercial trout pellets (Martins Feed Mills, Elmira, Ontario).

Electrofishing Procedure

All experiments were conducted in a 2.4 m x 0.6 m test tank; fish were confined in a volume of 56 l where they were at least 0.3 m from the cathode and 1.2 m from the anode (Figure 2.1). The temperature and conductivity varied between 8-20 °C and 240-270 $\mu\text{mhos/cm}$, respectively. Electrofishing was conducted with a Smith-Root VII pulsed DC backpack electrofisher at 200 V for a 20 s duration, with the pulse duration and frequency settings at 5 ms and 60 Hz, respectively, unless otherwise specified.

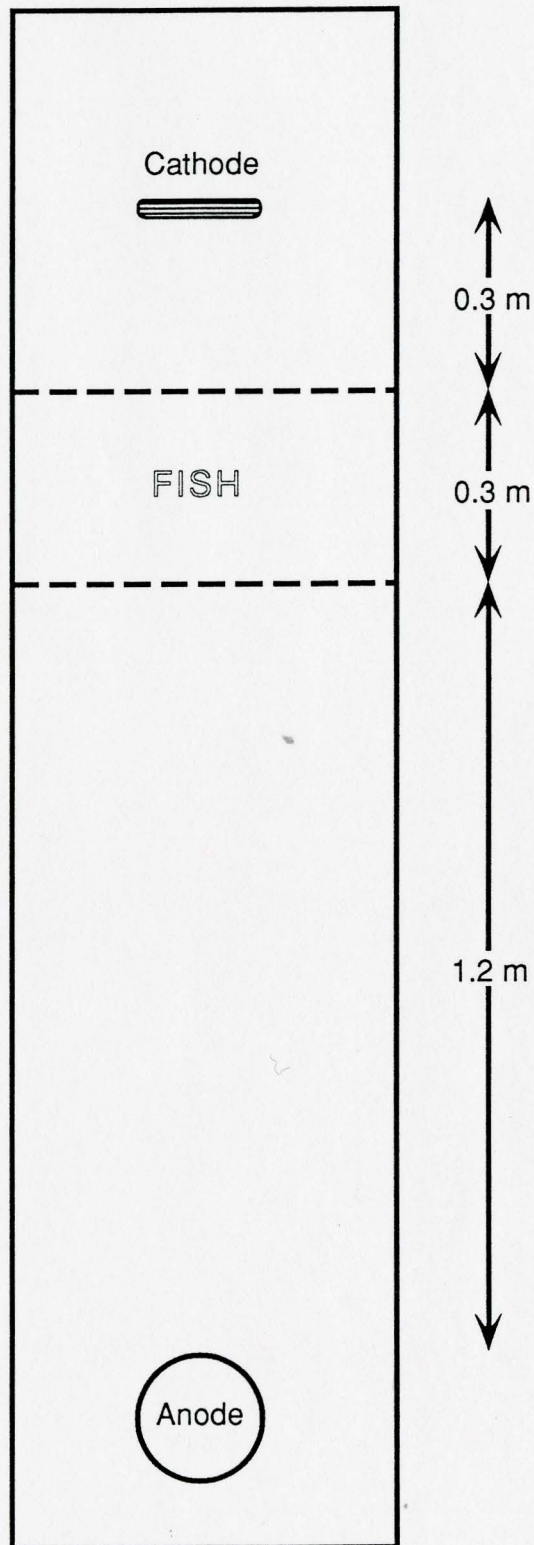
Experimental Protocols

1. Spinal Damage Assessment

Rainbow trout of three size classes (~ 50 g, ~ 100 g and ~ 600 g; $T = 7 \pm 1$ °C) were electroshocked at a constant distance from the electrodes as specified above. Four different electroshock treatments were used (200 V for

Figure 2.1. Electrofishing electrodes and fish positioning in the test tank (2.4 m x 0.6 m). In all experiments, fish were confined by nylon mesh dividers between the electrodes at a distance of 0.3 m from the cathode and 1.2 m from the anode.

ELECTROFISHING APPARATUS



20 s; 400 V for 20 s; 600 V for 20 s or 600 V for 40 s). Immediately following electroshock, fish were terminally anaesthetized in MS222 (2 g/l), then frozen for later examination. Both lateral and dorsal view X-rays were taken on Kodak Industrex M film with a Hewlett Packard, Faxitron 43805N X-ray system.

2. Survival

Survival following electrofishing and air exposure was monitored in two trials. In the first trial, fish (N = 10 per group, 4 groups; wt = 80-120 g) were fin clipped under water for identification, then acclimated to the test tank (Figure 2.1) for 3 days prior to experimentation. Fish were not fed during this time. Electrofishing was conducted using the standard procedure (see above). Immediately following electroshock, fish were netted into a container lined with moistened paper towels, where they were air exposed for 0, 1, 2, or 4 min then transferred into a partially covered circular 400 l holding tank where survival was monitored for 2 weeks. Temperature during treatment and recovery ranged between 15 and 20 °C. In the second trial, non-fin clipped trout (N = 20 per group, 4 groups; wt = 30-50 g) were treated as in the first trial. Following treatment each group was kept separate in partially covered, 50 l tanks at 13 ± 1 °C, where survival was monitored.

3. Behaviour

Response time and the duration of impaired ventilation was quantified in a separate trial. Free swimming rainbow trout and trout confined to 2 l darkened lucite boxes ($N = 22$; $wt = 125.7 \pm 7.5$ g; $T = 16$ °C) were electroshocked for 20 s, using the standard procedure (see above). Within this time period, the time required for fish to turn over was recorded and defined as response time. Following electroshock, the duration when ventilation was absent was also recorded.

4. Physiological Studies

Various trials were conducted in order to examine the physiological response of rainbow trout to pulsed DC electroshock with and without subsequent air exposure. Blood samples were collected with two different sampling techniques; caudal severance and D.A. catheter. The physiological response to electroshock and air exposure was examined using the D.A. cannulation technique only. MO_2 and ion loss were recorded on fish not disturbed by either blood sampling technique. Temperature for all physiological studies was 16 ± 1 °C unless otherwise stated.

a) Electroshock- sampled by caudal severance

Rainbow trout ($N = 70$; $wt = 113.3 \pm 3.6$ g) were acclimated to the test tank 24 h prior to treatment and were not fed during this time. Free swimming fish were electroshocked, as specified above, sacrificed with a blow to the head and terminally blood sampled (0, 1 h, 2 h, 4 h, 8 h, 24 h) via

caudal severance for measurements of hematocrit, blood lactate and plasma cortisol, glucose, Na^+ and Cl^- . From the blood collected (200-500 μl), 50 μl was spun for 5 min in heparinized capillary tubes to obtain hematocrit values, 50 μl of whole blood was diluted in 200 μl of ice cold 8 % perchloric acid (PCA; HClO_4) for later analysis of lactate. The remaining blood was centrifuged for 3 min, plasma was drawn off and immediately frozen in liquid nitrogen for later analysis of cortisol, glucose, Na^+ and Cl^- .

b) Electroshock- sampled by D.A. cannulae

Rainbow trout ($N = 7$; $\text{wt} = 355.3 \pm 20.7 \text{ g}$) were fitted with catheters in the dorsal aorta under MS222 anaesthesia (0.8 g/10 l). Fish were allowed at least 48 h to recover, unfed, from the surgery and anaesthesia in 2 l darkened lucite boxes before being treated. One day prior to treatment, fish were moved into the test tank, still physically confined by the boxes, but with a constant exchange of water (5 l/min) between the boxes and test tank. Electroshock was applied as above, to fish held in boxes. Blood collected from the catheter (0, 1 h, 2 h, 4 h, 8 h, 24 h) was analyzed for catecholamines, pH, total CO_2 and hemoglobin, in addition to those parameters measured by the caudal severance technique. During the experiment, blood samples were immediately analyzed for total CO_2 and pH. The remaining blood was treated as previously described for the caudal severance measurements.

c) Electroschock and Air Exposure- sampled by D.A. catheter

The physiological response of fish to electroschock and subsequent air exposure was assessed using the D.A. catheter technique only. The protocol for this experiment was similar to that just discussed, except immediately following electroschock the boxes were lifted out of the tank and inverted to permit water drainage (<10 s). The fish were then air exposed for 1 min before being returned to the test tank.

d) Oxygen Consumption and Na⁺ Loss

Rainbow trout (N = 15; wt = 59.9 ± 4.7 g; T = 14 ± 1 °C) were acclimated to the test tank one day prior to electroschock treatment and were not fed during this time. Immediately following electroschock (procedure as above) fish were rinsed in deionized water and transferred to cylindrical respirometers (370 ml volume, 5 cm diameter). Control fish were netted, rinsed and sampled as the electroschocked fish. Na⁺ free water (1mM CaCl₂, pH of 7.2) flowed through the respirometers at a rate of 100 ml/min. Water samples were taken 10 min, 30 min, 1 h and 2 h following treatment. Samples were immediately analyzed for PO₂ with a Radiometer E5046 PO₂ electrode connected to a Radiometer PHM 71 meter, and Na⁺ was measured with a Varian model 1275 atomic absorption spectrophotometer. Oxygen consumption and Na⁺ loss were calculated from the following equations:

$$MO_2 = (\text{water flow rate (ml/min)} \times (\Delta PO_2 \times \beta)) / \text{wt}$$

$$\text{Na}^+ \text{ efflux} = (\Delta \text{Na}^+ \times \text{water flow rate(ml/min)}) / \text{wt}$$

where β , is the oxygen solubility coefficient in nmol/ml·mm Hg and Δ represent differences in water PO_2 (in mm Hg) and Na^+ levels before and after flowing through the respirometer. The ion/gas ratio (pmol Na^+ /nmol O_2) was calculated by dividing Na^+ efflux (pmol/g/min) by the MO_2 (nmol/g/min).

Analytical Procedures

Arterial pH was determined on 50 μ l samples injected into a Radiometer pH microelectrode (type G297/G7) attached to a Radiometer PHM 71 Mk 2 acid-base analyzer. Total CO_2 was measured on 50 μ l of anaerobically obtained arterial blood in a Corning (model 965) CO_2 analyzer. Arterial CO_2 tension (PCO_2) and the metabolic acid load (ΔH^+_m) were then determined. PCO_2 was calculated using experimentally determined pK' and CO_2 solubility coefficients for trout blood (Boutilier *et al.*, 1984). ΔH^+_m was calculated over each time period (1 to 2) by the equation of McDonald *et al.* (1980):

$$\Delta H^+_m = [HCO_3^-]_1 - [HCO_3^-]_2 - \beta (pH_1 - pH_2)$$

where β , the non-bicarbonate buffering capacity of the blood, was estimated using the following formula (Wood *et al.*, 1982):

$$\beta = -1.1 \times [\text{hemoglobin}] - 2.5$$

Whole blood lactate was determined on 100 μ l aliquots of PCA extract using the lactate dehydrogenase method, with Sigma reagents. For determination of hemoglobin concentrations, 20 μ l whole blood in 5 mls Drabkins solution was read against hemoglobin standards on a Phillips PYE

Unicam PU 8600 UV/VIS spectrophotometer. Catecholamines were extracted from 100 μ l plasma aliquots by binding to alumina in a Tris/EDTA buffer at a pH of 8.6. The pellet was washed twice with double distilled water, then resuspended in 0.1 mol PCA. A 70 μ l aliquot of extract was separated by high performance liquid chromatography (HPLC; Waters Model 510) with a Waters M460 electrochemical detector. Individual catecholamines were identified and quantified by comparisons to extracted and unextracted catecholamine standards. Cortisol was measured on 20 μ l aliquots of plasma in a competitive binding radioimmunoassay (Kallestead Canada Inc. Quanticoat™ Cortisol RIA Kit 825). The standards were diluted by 50% to approximate fish serum protein levels (3.3 g/dl). Samples were counted on a gamma counter (Minaxi Auto-gamma® 5000 series). Plasma glucose concentrations were measured on 10 μ l aliquots using the hexokinase enzymatic assay and Sigma reagents. Plasma Na⁺ was determined with plasma diluted 1:1000 with double deionized water, then read at a wavelength of 589.6 nm on a Varian model 1275 atomic absorption spectrophotometer. Plasma Cl⁻ were determined by titration of 10 μ l plasma aliquots with a CMT 10 chloride titrator.

Statistical Analysis

All values are expressed as mean \pm 1 standard error (S.E.M.). Significance was determined by the Student's *t*-test at $p < 0.05$. Data obtained from D.A. catheterization trials was compared using the paired *t*-test whereas all other comparisons were made via the unpaired *t*-test.

RESULTS

Physical Damage and Mortality Studies

Through X-ray analysis, electroshock induced spinal damage was differentiated from no damage (Figure 2.2A) and naturally occurring, fused abnormalities (Figure 2.2B) by the appearance of misaligned vertebrae (Figure 2.2C). Such injuries were only observed in large trout (~600 g) treated with 600V for either 20 s or 40 s (Table 2.1). Each of these injuries involved 3 vertebrae. No injuries were observed in fish of smaller size or treated with smaller electroshock voltages.

Trout electroshocked for 20 s with 200 V, which were immediately treated with 0, 1, 2, or 4 min of air exposure, did not experience any mortality in the two week period following treatment.

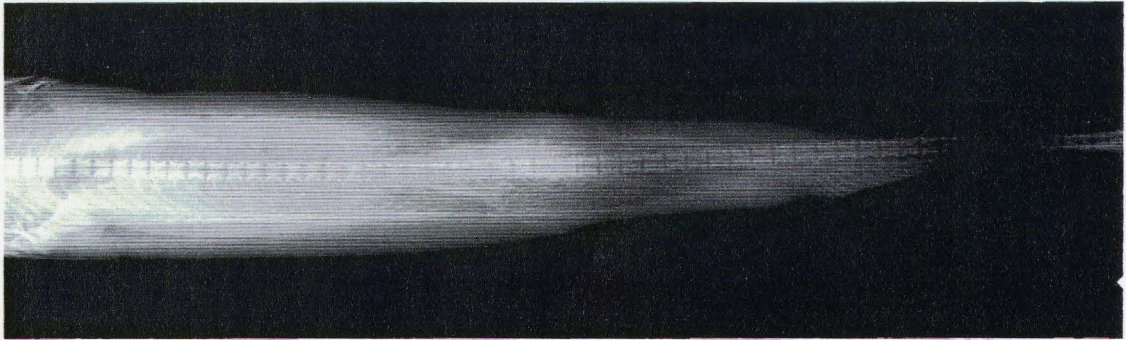
Behaviour

Free swimming trout responded to the pulsed DC current in 1.9 ± 0.3 s, whereas fish confined by lucite boxes required 2.9 ± 0.3 s to respond. However, the response times were not significantly different. Immediately following 20 s of electroshock, ventilation was absent in free-swimming fish for up to 3 min, with an average duration of 19 s. Unfortunately, ventilation behaviour could not be accurately observed for fish confined to boxes.

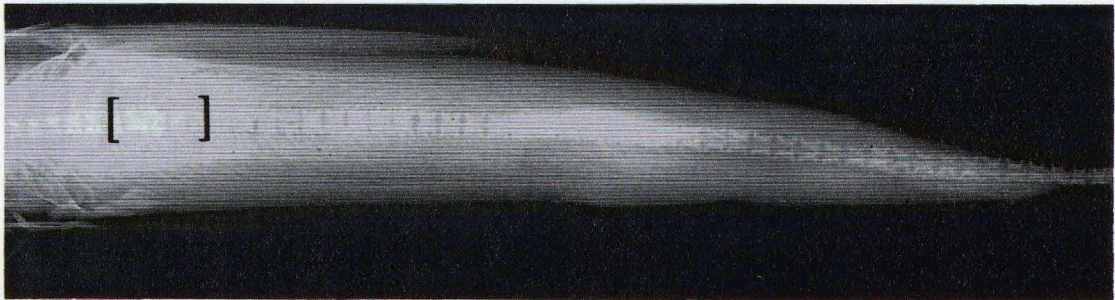
Figure 2.2. Representative dorsal X-rays of vertebral columns of rainbow trout, showing [in brackets] the types of vertebral abnormalities, (A) control, (B) natural abnormality and (C) pulsed DC injury.

3
REPRESENTATIVE DORSAL X-RAYS OF
VERTEBRAL COLUMNS

A



B



C

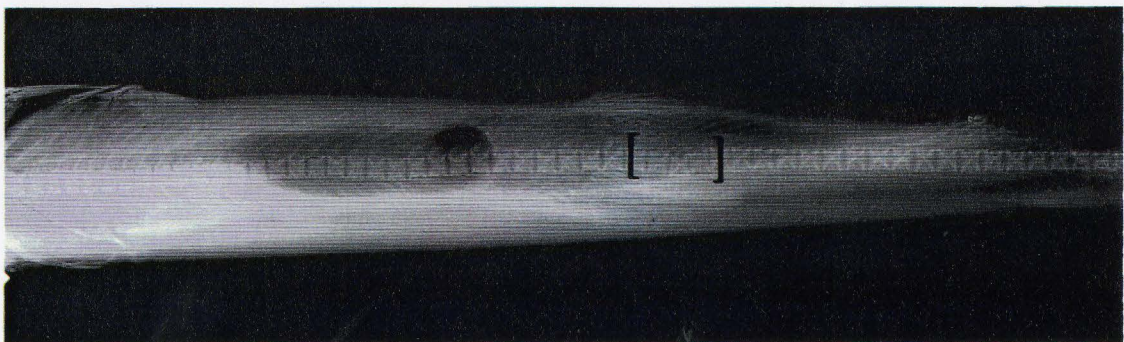


Table 2.1. Percentage of spinal damage induced by pulsed DC electroshock of 200 V, 400 V or 600 V for 20 s or 40 s durations on various fish sizes; number of fish per treatment group is given in brackets.

- indicates treatments that were not done.

Fish Size (g)	Electrofishing Treatment			
	200V 20 s	400V 20 s	600V 20 s	600V 40 s
47.2 ± 2.7	0 (5)	-	0 (20)	-
103.4 ± 6.1	0 (5)	0 (5)	0 (10)	-
593.6 ± 23.4	0 (5)	-	10 (10)	20 (5)

Physiological Studies

Plasma Cortisol

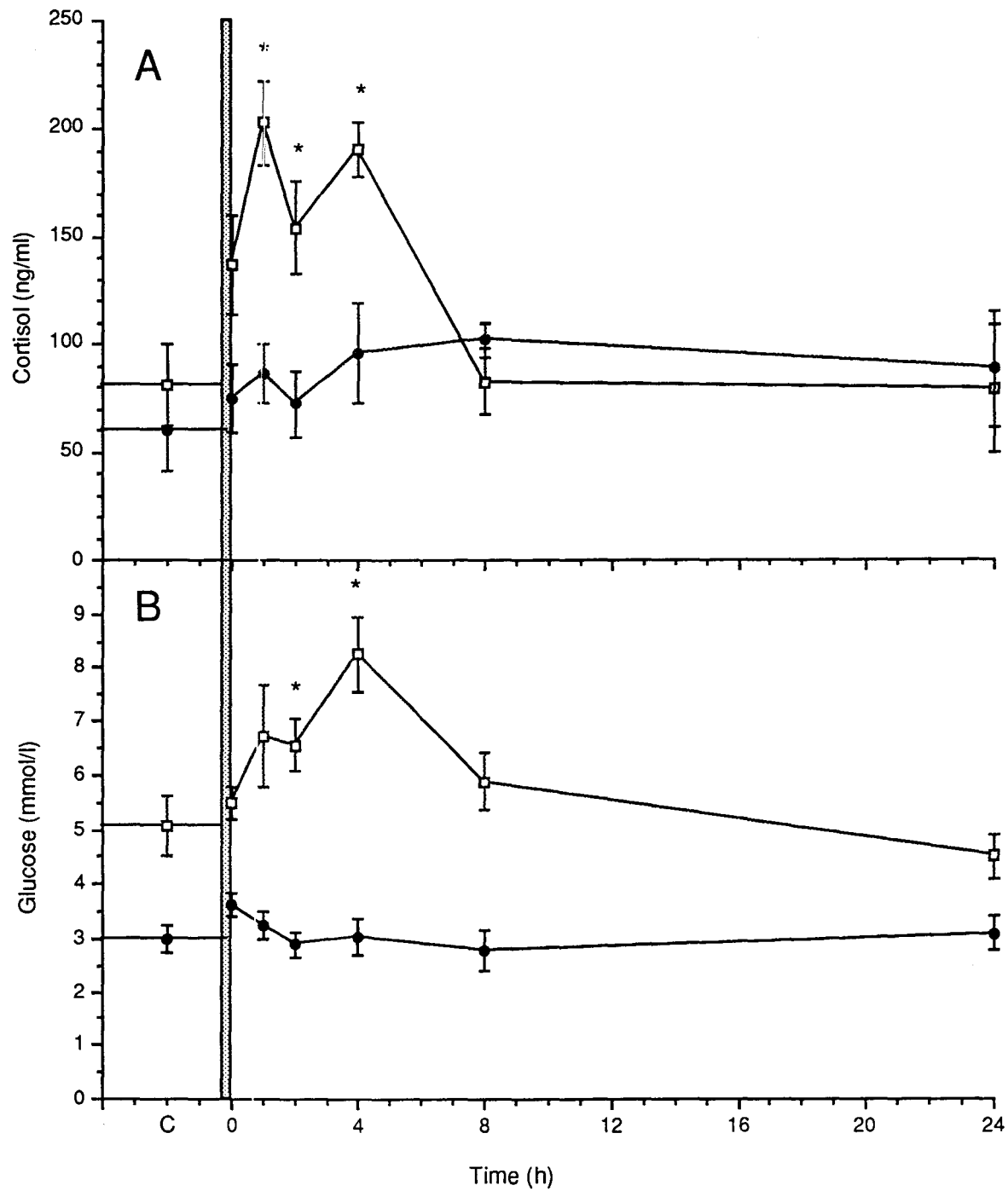
There was no difference in resting plasma cortisol between fish sampled via either D.A. catheter or caudal severance, with the average being 71.3 ± 12.4 ng/ml (Figure 2.3A). Immediately following 20 s of electroshock, cortisol concentrations in fish sampled by caudal severance began to rise. Cortisol was elevated by at least 2 fold between 1h and 4h after treatment, and did not recover until 8 h into the recovery period (Figure 2.3A). In contrast, fish fitted with D.A. catheters, showed no significant elevation following electroshock (Figure 2.3A).

Plasma Glucose

A significantly higher resting glucose (5.1 ± 0.5 mmol/l) was observed in fish sampled via caudal severance than in fish sampled via D.A. catheter (3.0 ± 0.3 mmol/l; Figure 2.3B). Plasma glucose concentration in caudal severance sampled fish, rose more slowly than cortisol, reaching a peak at 4 h post-electroshock then recovered by 8 h (Figure 2.3B). In contrast, fish fitted with catheters, showed no significant elevation following electroshock (Figure 2.3B).

Figure 2.3. The influence of electroshock on plasma (A) cortisol and (B) glucose sampled by (□) caudal severance and (•) D.A. catheter. The bar indicates the electroshock treatment. All values (N = 7-10) are expressed as mean \pm one S.E.M.; T = 17 °C; * indicates a significant difference from rest (p < 0.05).

PLASMA CORTISOL AND GLUCOSE CONCENTRATIONS FOLLOWING ELECTROSHOCK



Plasma Catecholamines

Under resting conditions, in catheterized fish, catecholamines were undetectable (ie. $< 2\text{-}3\text{ nmol/l}$). Immediately following electroshock both epinephrine and norepinephrine peaked at approximately 10 nmol/l and had fully recovered by 1 h into the recovery period (Figure 2.4A,B).

Plasma Electrolytes

A significantly higher resting plasma Cl^- concentration of 132 meq/l was observed in fish sampled via D.A. catheter than in fish sampled via caudal severance (121 meq/l ; Figure 2.5A). Initial changes from the resting plasma Cl^- in both trials were insignificant. However, there tended to be a slight elevation of 3 meq/l in Cl^- in the caudal severance trial immediately following electroshock. This was followed by a reduction in plasma Cl^- in both the caudal severance and catheterization trials (Figure 2.5A) to 117 meq/l 1 h after treatment and 128 meq/l 4 h after treatment, respectively. By 24 h following electroshock there was a significant increase in plasma Cl^- in fish sampled via caudal severance, whereas catheterized fish experienced a significant decrease in the plasma Cl^- concentration (Figure 2.5A).

Resting Na^+ for both electroshock trials were approximately 135 meq/l (Figure 2.5B). Plasma Na^+ was significantly elevated after treatment in both trials; to 141 meq/l in fish sampled via caudal severance and 142 meq/l in catheterized fish. Peak Na^+ concentrations for the two trials occurred immediately (caudal severance) or 1 h (D.A. catheter) following treatment.

Figure 2.4. Changes in plasma (A) epinephrine and (B) norepinephrine in catheterized electroshocked fish following (•) electroshock and (o) electroshock followed by 1 min of air exposure. The bar indicates the electroshock and air exposure treatment. All values (N = 4-7) are expressed as mean \pm one S.E.M.; T = 16 \pm 1 °C; * indicates a significant difference from rest (p < 0.05).

PLASMA EPINEPHRINE AND NOREPINEPHRINE CONCENTRATIONS FOLLOWING ELECTROSHOCK, AND ELECTROSHOCK & AIR EXPOSURE

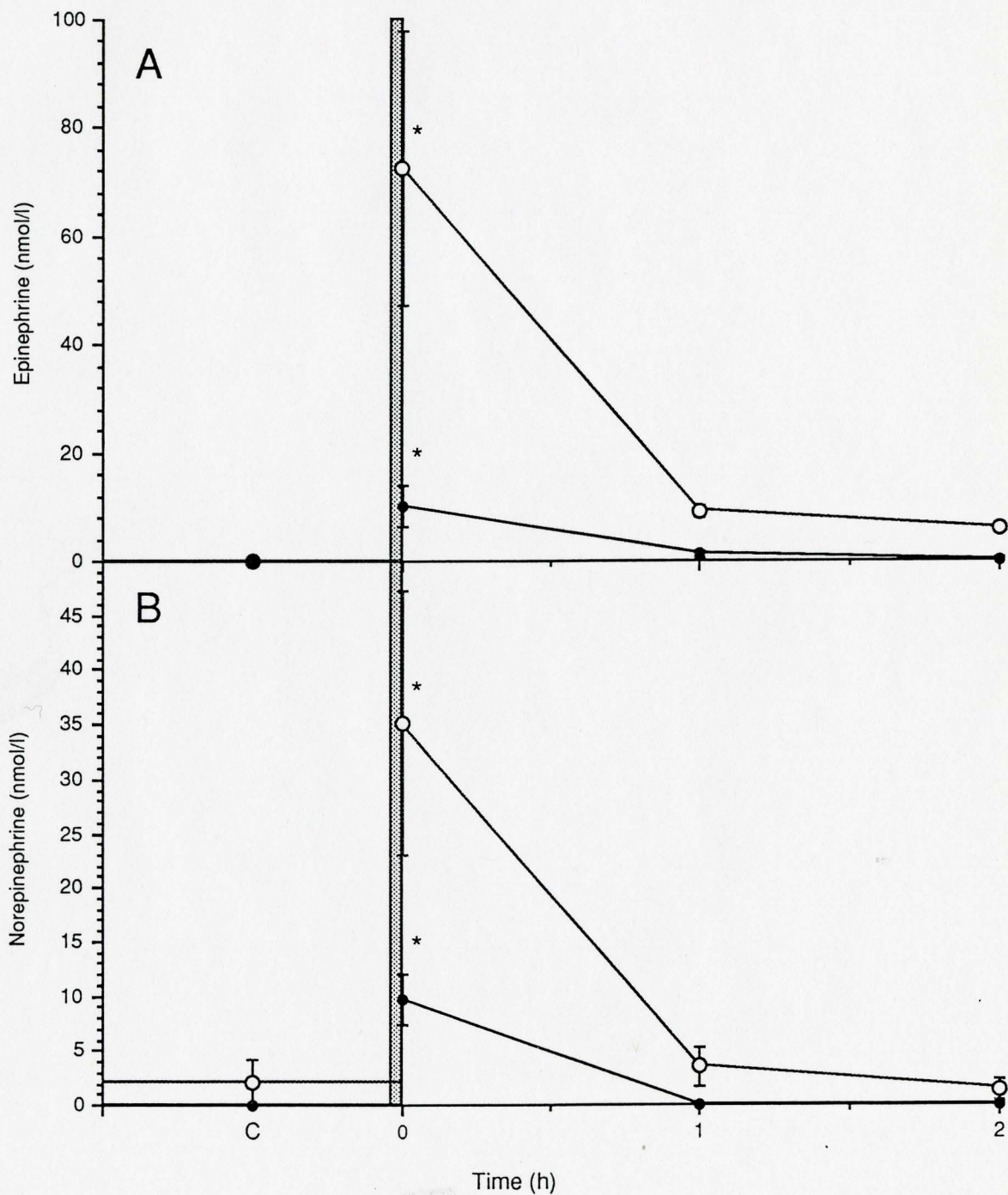
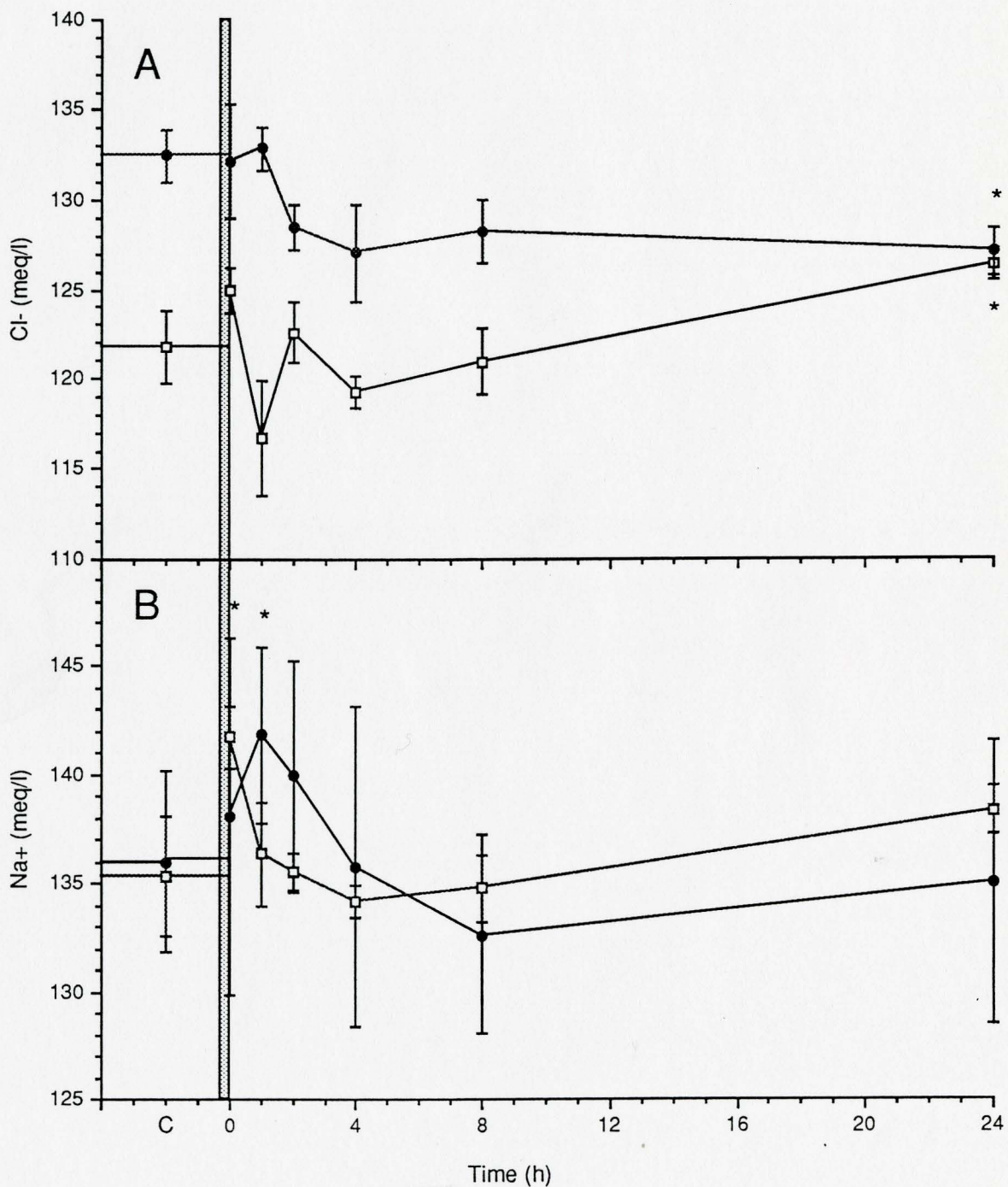


Figure 2.5. The influence of electroshock on plasma (A) chloride and (B) sodium concentrations, sampled by (□) caudal severance and (•) D.A. catheter . The bar indicates the electroshock treatment. All values (N = 7-10) are expressed as mean \pm one S.E.M.; T = 17 °C; * indicates a significant difference from rest (p < 0.05).

PLASMA CHLORIDE AND SODIUM CONCENTRATIONS FOLLOWING ELECTROSHOCK



Hematocrit

Significant differences in resting hematocrit values were observed between the two experiments. In the catheterized fish experiment, the pre-shock hematocrit of 16.7% was approximately one half of the hematocrit obtained via caudal severance (30.8%) (Figure 2.6A). Immediately following electroshock, hematocrit in fish sampled via caudal severance increased significantly to 39.7%. A similar but insignificant increase in hematocrit was observed in the catheterized fish. Due to repetitive blood sampling, these fish showed significant decreases in hematocrit from 2 h to the end of the experiment.

Whole Blood Lactate

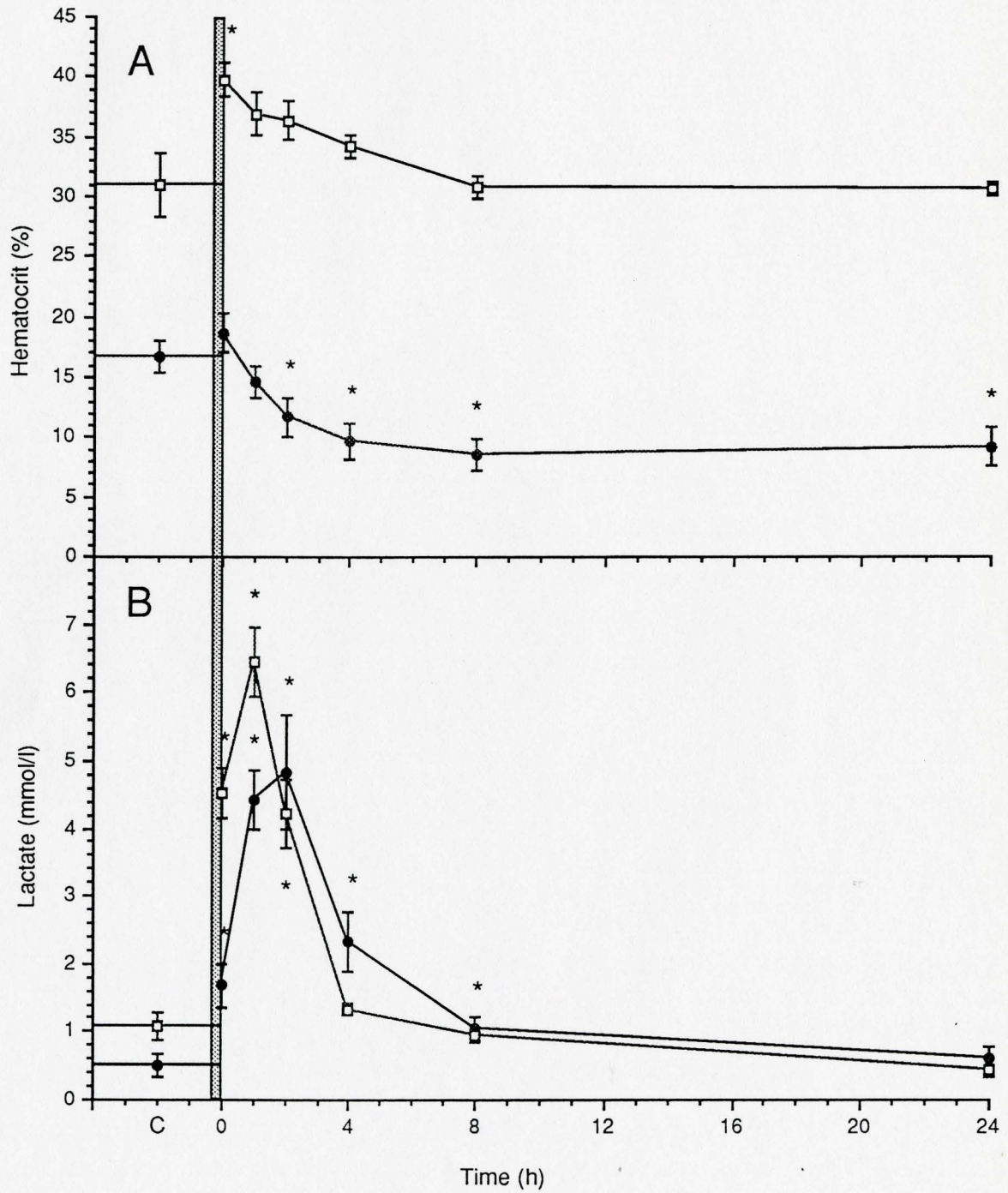
Resting whole blood lactate on blood collected via caudal severance (1.1 mmol/l) was double that from cannulated fish (0.5 mmol/l). In the caudal severance trial the lactate was elevated immediately following treatment and remained elevated for 2 h (Figure 2.6B). The peak lactate level of 6.5 mmol/l was recorded 1 h after the cessation of shock. In contrast, when blood was collected via D.A. catheter, lactate was elevated immediately following electroshock and remained elevated for 8 h after treatment (Figure 2.6B). However, there was not a significant difference in peak lactate values between the two trials.

Acid-Base Status

Substantial acid-base disturbances also occurred as a result of

Figure 2.6. The influence of electroshock on (A) hematocrit and (B) whole blood lactate concentration, sampled by (\square) caudal severance and (\bullet) D.A. catheter. The bar indicates the electroshock treatment. All values (N = 7-10) are expressed as mean \pm one S.E.M.; T = 17 °C; * indicates a significant difference from rest (p < 0.05).

HEMATOCRIT AND WHOLE BLOOD LACTATE FOLLOWING ELECTROFISHING



electroshock in fish fitted with D.A. catheters. Arterial pH immediately dropped 0.22 units from an initial pH of 7.80, but had recovered by 2 h with a overshoot of 0.04 pH units at 4 h (Figure 2.7A). Contributing to the pH depression were simultaneous increases in PCO_2 from 1.8 torr to 3.5 torr, and a metabolic acid load (ΔH^+_m) of 1.1 meq/l (Figure 2.7B,C). While PCO_2 levels returned to resting values by 1 h, ΔH^+_m was significantly reduced to -1.4 meq/l at 4 h following electroshock. The peak ΔH^+_m disturbance was 20 % of the peak Δ lactate.

Electroshock and Air Exposure

The additional stress of 1 min of air exposure immediately following electroshock tended to increase the degree of disturbance experienced by catheterized fish. Peak norepinephrine and epinephrine values were 35 and 70 nmol/l, respectively, compared to the 10 nmol/l catecholamine concentrations in fish that were electroshocked only (Figure 2.4A,B). Disturbances in pH, ΔH^+_m , PCO_2 and whole blood lactate also tended to be larger in fish subjected to 1 min of air exposure following electroshock (Figure 2.7A-D). However, none of these elevations were significantly higher than those produced by electroshock alone.

Oxygen Consumption and Na^+ Loss

Oxygen consumption in the two groups (handled, electroshocked and handled) were similar in the 2 h period following treatment (Figure 2.8B) with the peak MO_2 of electroshocked and control fish of 142.2 and 160.2 nmol/g/min, respectively recorded 10 min following treatment. In contrast,

Figure 2.7. Changes in plasma (A) pH, (B) PCO₂, (C) metabolic acid load and (D) Δ whole blood lactate in catheterized fish following (•) electroshock and (o) electroshock followed by 1 min. of air exposure. The bar indicates the electroshock and air exposure treatment. All values (N = 4-7) are expressed as mean \pm one S.E.M.; T= 16 \pm 1 °C; * indicates a significant difference from rest (p < 0.05); † indicates a significant difference between treatments.

WHOLE BLOOD ACID BASE STATUS FOLLOWING ELECTROFISHING, AND ELECTROFISHING & AIR EXPOSURE

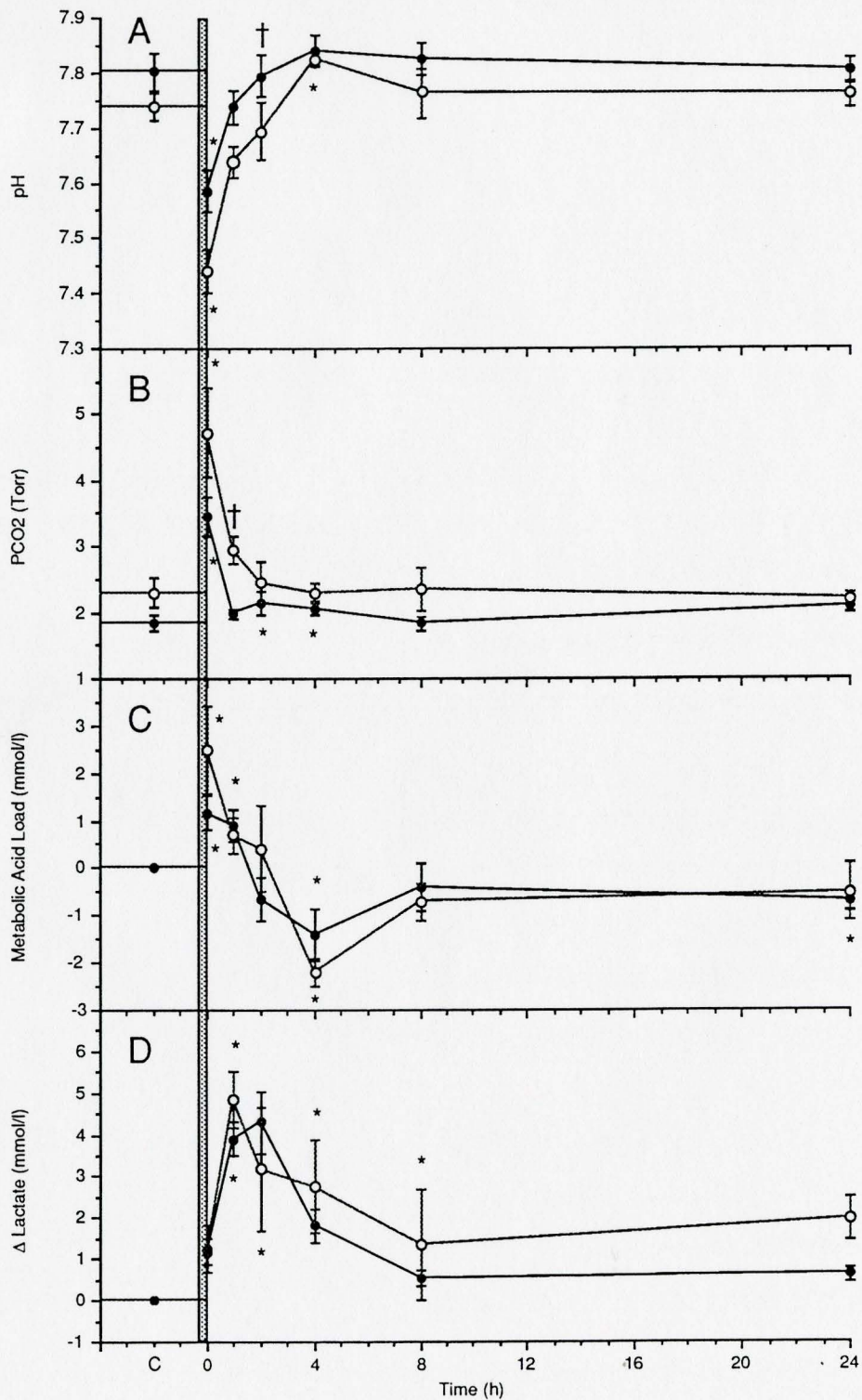
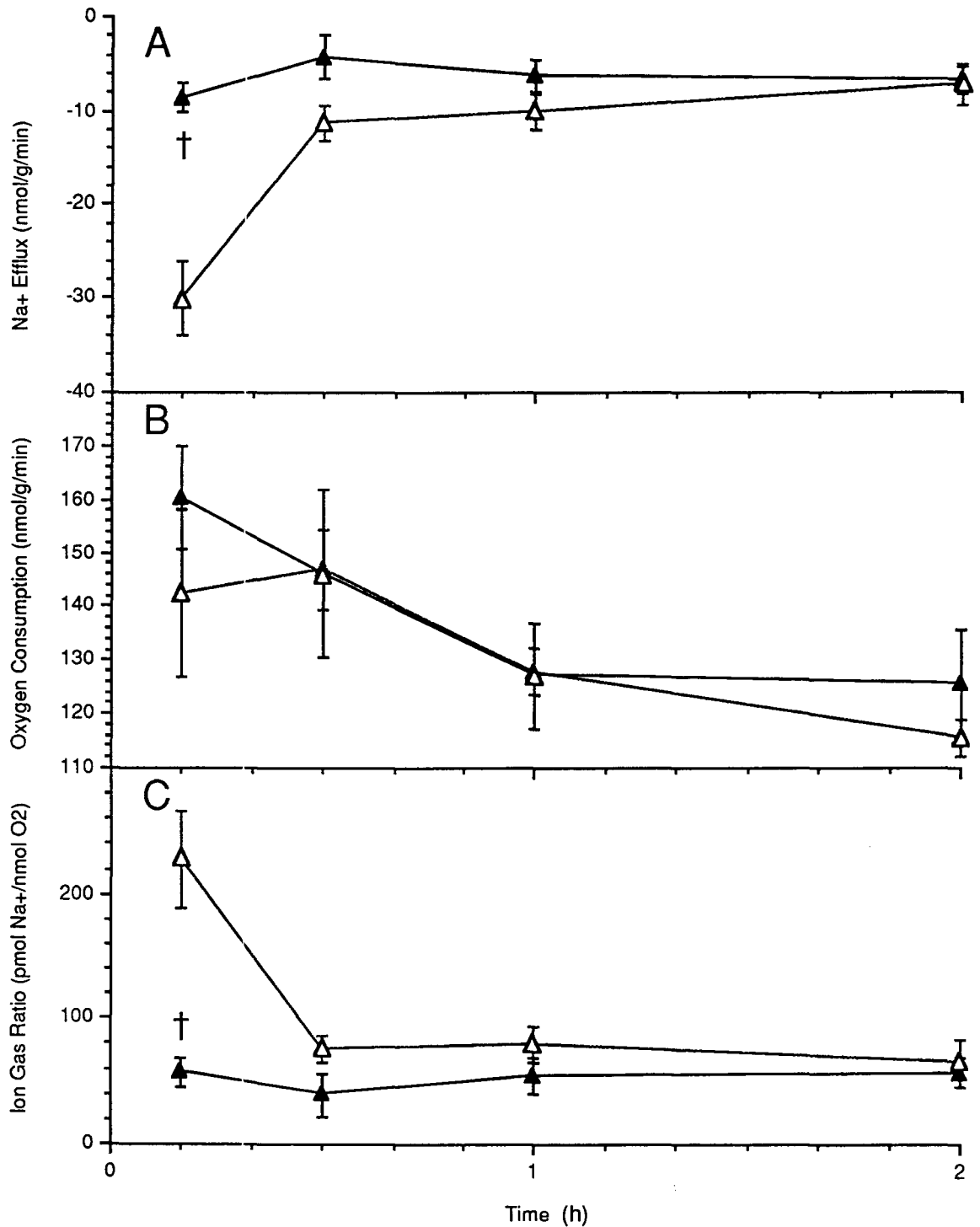


Figure 2.8. The influence of (Δ) handling or (\blacktriangle) pulsed DC electroshock and handling on (A) sodium efflux, (B) oxygen consumption and (C) ion/gas ratio. All values are expressed as mean \pm one S.E.M.; N = 9 for electroshock and handling and N = 6 for handling only; T = 14 °C; † indicates a significant difference between treatments (p < 0.05).

Na⁺ EFFLUX, O₂ CONSUMPTION AND ION/GAS RATIO FOLLOWING HANDLING AND ELECTROSHOCK & HANDLING



the Na⁺ efflux 10 min following electroshock and handling, of 8.53 nmol/g/min, was significantly less than in fish that were only handled (30.12 nmol/g/min; Figure 2.8A). Between 0.5 h and 2 h Na⁺ loss in both treatments stabilized around 7 nmol/g/min. As a result, the ion/gas ratio of 57 pmol Na⁺/nmol O₂, 10 min after electroshock, was significantly less than the ion/gas ratio of 228 pmol Na⁺/nmol O₂ in fish that were only handled (Figure 2.8C).

DISCUSSION

Physical Damage

Spinal misalignment induced by electroshock was only experienced by large, (~ 600 g) rainbow trout treated with 600 V for either 20 s or 40 s (Table 2.1). These results support the general trend that larger fish experience greater head to tail voltages than smaller fish (Ellis, 1975; Novotny, 1969; Reynolds, 1983), thus are influenced more quickly and by relatively smaller voltages (Bohlin *et al.*, 1989; Halband, 1967). The only previous spinal injury study as a result of pulsed DC electroshock was conducted by Sharber and Carothers (1990). Surprisingly, they found damage involving an average of 8 vertebrae, in 50% of the rainbow trout examined. Such high percentages have never before been reported. Even treatment with AC gear, which is known to be far more detrimental than pulsed DC or DC (Lamarque, 1990), yields lower incidence of physical damage (Hauck, 1949; Hudy, 1985; McCrimmon and Bidgood, 1965). However, the findings of Sharber and Carothers (1990) may have been influenced by the higher water conductivity, the large size of fish examined (30-56 cm in length) or perhaps their physical status. If the fish captured were decalcified by spawning or poor feeding, more vertebral dislocations would have occurred (Lamarque, 1990).

The presence of physical damage has been repeatedly implicated in post-electroshock mortalities (Hauck, 1949; Hudy, 1985; Lamarque, 1990; Schreck *et al.*, 1976). Thus, the lack of mortality in the present study may be partially attributed to the absence of spinal damage. But, even in the

presence of spinal damage, Horak and Klein (1967) reported no mortality following treatment with pulsed DC electrofishing gear. Whaley (1975) similarly reported negligible mortality following up to 15 s of pulsed DC electroshock. Most importantly, the absence of mortality and spinal damage with treatments of 200 V pulsed DC for 20 s are useful in categorizing the electroshock treatment used in the present study, as being above the threshold for immobilization, yet below that of physical damage and mortality.

Physiological Effects of Electroshock

The present study is the first to examine in detail the physiological consequences of pulsed DC electroshock. Blood samples were collected by caudal severance as in previous studies (Mesa and Schreck, 1989; Woodward and Strange, 1987; Schreck *et al.*, 1976); but were also obtained via D.A. catheters. The latter permitted a more detailed examination of the stress response as well as an examination of acid-base status. These data then permit detailed analysis of pulsed DC effects, a comparison of electrofishing gear types and an examination of the various hypotheses previously proposed regarding the physiological response of fish to electrofishing.

Pulsed DC electrofishing is thought to be more detrimental to fish than DC gear (Lamarque, 1990). This hypothesis is strongly supported by the comparison of plasma cortisol, glucose and whole blood lactate disturbances following pulsed DC electrofishing from the present study with changes reported in the literature for DC electrofishing (Table 2.2). Both cortisol and lactate disturbances following pulsed DC electrofishing are at least double

Table 2.2 Maximum disturbances of cortisol, glucose and lactate following pulsed DC and DC electrofishing in salmonids sampled by caudal severance. Electrofishing conditions are stated in parenthesis.

	Cortisol (ng/ml)	Glucose (mmol/l)	Lactate (mmol/l)	Reference
Pulsed DC	+ 130	+ 3.5	+ 5.5	Present Study (20 s, Rainbow trout)
DC	+ 47	+ 2.2	+0.9	Schreck <i>et al.</i> , 1976 (40 s, Rainbow trout)
	+ 90		+1.7	Mesa and Schreck, 1989 (4 s; Cutthroat trout)
	+40	+ 1.1		Woodward and Strange, 1987 (<5 s, Rainbow trout)

those of the largest reported values to date following treatment with DC gear (Mesa and Schreck, 1989; Woodward and Strange, 1987; Schreck *et al.*, 1976). Plasma glucose is also higher in fish treated with electrofishing gear. Thus, pulsed DC gear elicits a greater physiological disturbance than DC electrofishing. In fact, the stress response elicited by pulsed DC electrofishing is similar in magnitude to other acute stresses such as exhaustive exercise (peak glucose: 5 mmol/l; Milligan and Wood, 1986a; Ristori and Laurent, 1985) and handling (maximum cortisol: 45-140 ng/ml; Barton *et al.*, 1986; Barton *et al.*, 1985; Barton *et al.*, 1980; maximum glucose: 5-6 mmol/l; Barton *et al.*, 1986).

Catecholamines were also mobilized immediately following electroshock (Figure 2.4a,b). The stimulus for their release may have been a combination of the acidosis (Tang and Boutilier, 1988), a decrease in blood oxygen content (Perry *et al.*, 1989; Fievet *et al.*, 1990) and/or the psychological perception of stress acting through sympathetic innervation of the chromaffin tissue (Mazeaud and Mazeaud, 1981). No other studies, to my knowledge, have examined catecholamine concentrations following electroshock. However, numerous authors have reported epinephrine and norepinephrine values after bouts of exhaustive exercise. While reported levels are highly variable (increases in epinephrine of 27-210 nmol/l and norepinephrine of 26-75 nmol/l; Table 2.3); catecholamine mobilization following electroshock was generally of a smaller magnitude; 1/3 to 1/20 that of exhaustive exercise.

Table 2.3 Maximum disturbances of catecholamines, pH and lactate following pulsed DC electroshock and exhaustive exercise in rainbow trout sampled by D.A. catheter.

	Epinephrine (nmol/l)	Norepinephrine (nmol/l)	pH	Lactate (mmol/l)	Reference	
Pulsed DC Electroshock	+10	+10	-0.2	+4.5	Present study	
Exhaustive Exercise	+210	+75	-0.3	+3.4	Butler <i>et al.</i> , 1986 (2-3 min exercise)	
			-0.3	+9	Ferguson and Tufts, 1992 (10 min exercise)	
			-0.5	+13	Holeton <i>et al.</i> , 1983 (10 min exercise)	
				-0.5	+17	Milligan and Wood, 1986a (6 min exercise)
				-0.3	+19	Milligan and Wood, 1986b (6 min exercise)
				+27	+34	-0.3
	+36	+26	-0.3	+7	Primmatt <i>et al.</i> , 1986 (120 % Ucrit until exhausted)	
	+178	+47	-0.4		Tang and Boutilier, 1988 (10 min exercise)	
				-0.5	+13	Turner <i>et al.</i> 1983 (6 min exercise)
			-0.4	+12	Wood <i>et al.</i> 1983 (6 min exercise)	

Another insight into catecholamine mobilization can be obtained indirectly from the response of the ion/gas ratio to electroshock, since previous studies (Gonzalez and McDonald, 1992) have shown that the ion/gas ratio is particularly sensitive to elevated catecholamines. The ion/gas ratio 10 min after treatment was significantly greater for fish that were simply handled than fish that were both electroshocked and handled, largely because of a substantially larger Na^+ loss (Figure 2.8C). This suggests that electroshock inhibits catecholamine mobilization relative to that caused by handling alone.

As previously mentioned, losses of plasma Na^+ and Cl^- are often associated with catecholamine mobilization (Mazeaud and Mazeaud, 1981). However, following electroshock, the changes in ions were more similar to those induced by exhaustive exercise (increases in plasma Na^+ and reductions in plasma Cl^-) than to stress alone. Nonetheless, the disturbances resulting from pulsed DC electroshock were of a smaller magnitude and less prolonged than those resulting from exercise (peak Na^+ elevation of 16-30 mmol/l recovered by 4-8 h; Cl^- reduction 8-10 mmol/l recovered by 12 h; Holeton *et al.*, 1983; Turner *et al.*, 1983; Wood *et al.*, 1983). The elevation in plasma Na^+ is explainable by fluid shifts which occurred in response to the osmotic disturbance created by elevated intramuscular lactate levels, concentrating the red blood cells (Figure 2.6A) and other plasma constituents (Milligan and Wood, 1986a,b; Turner *et al.*, 1983, Holeton *et al.*, 1983). Despite this hemoconcentration, plasma Cl^- still appeared to drop slightly in both experiments. The likely explanation for this occurrence is Cl^- / lactate exchange between the muscle and plasma (Holeton *et al.*, 1983).

Efflux of the lactate into the bloodstream resulted in an immediate 4 fold increase of blood lactate levels, which remained elevated for at least 2 h in both the caudal severance and catheterization experiments (Figure 2.7B). The maximum increase in whole blood lactate observed in these studies was double that of the largest reported value to date following electrofishing (Table 2.2; Mesa and Schreck, 1989; Schreck *et al.*, 1976). In fact, numerous studies have reported no elevations in lactate (Lamarque, 1990). However, these previous studies have employed DC electrofishing gear, where fish immobilization is typically a result of galvanonarcosis and not tetany (Lamarque, 1990). The more pronounced elevation of lactate observed following pulsed DC electroshock was unlikely solely a result of impaired ventilation as hypothesized by Schreck *et al.* (1976) based on DC gear; the apnea in this study was no greater than in previous studies of DC electroshock, but lactate was twice as high. Thus, the lactacidosis induced by pulsed DC electroshock must be due to a combination of impaired ventilation with muscular tetany. This combined effect resulted in a pattern of whole blood lactate appearance and clearance similar to that of 2 to 3 minutes of exhaustive exercise, but slightly smaller and less prolonged than those induced by 5 to 6 min of exercise (Table 2.3).

The proton efflux to the blood from the muscle resulted in an immediate metabolic acidosis (Figure 2.7A,C). The fish concurrently experienced a respiratory acidosis, as was apparent by the elevation in PCO_2 (Figure 2.7b), exacerbating the overall reduction in arterial pH (Figure 2.7a). The latter may have been a result of gas exchange impairment during the absence of rhythmic ventilation throughout electroshock (Schreck *et al.*,

1976), but also could be simply due H⁺ titration of HCO₃⁻. Plasma pH recovered quickly from the acidosis, slightly overshooting resting pH values at 4 h due to overcompensation (Milligan and Wood, 1986a).

The metabolic and respiratory acidoses attributing to the depression in pH induced by electroshock, were similar to those induced by 2 to 3 min of exercise (Butler *et al.*, 1986), yet were slightly less than those resulting from 5 to 6 min of exhaustive exercise (Table 2.3).

Combined Effects of Electroshock and Air Exposure

Following electrofishing, fish are often air exposed for extensive periods of time for processing, before being released. During this time, the gill lamellae may have collapsed, inhibiting gas exchange (Boutilier, 1990; Ferguson and Tufts, 1992). Reduction or elimination of oxygen uptake and carbon dioxide loss during air exposure may have led to the slightly elevated lactate and acid-base disturbances observed following one minute of treatment (Figure 2.7A-D). Few studies have examined the physiological effects of air exposure alone. However, during severe hypoxia and anoxia, glycoytic rates are elevated which results in increased muscle lactate (Dunn and Hochachka, 1986) and reduced pH (Smith and Heath, 1980; Thomas and Hughes, 1982; Thomas *et al.*, 1986). This hypoxic stress also tends to increase plasma catecholamine levels (Tetens and Christensen, 1987; Butler *et al.*, 1978) (Figure 2.4A,B)). However, the combined treatment of electroshock and 1 min of air exposure did not significantly elevate any of these parameters above that of electroshock only.

The lack of a significant elevation in plasma catecholamines as a result of air exposure may simply have been due to the large variations in the individual catecholamine measurements. However, the relatively small additional accumulation of lactate is rather surprising given the likely absence of branchial oxygen uptake during air exposure. Furthermore, Ferguson and Tufts (1992) showed that 1 min of air exposure following exhaustive exercise additionally elevated whole blood lactate by 10 mmol/l above the post-exercise level of 10 mmol/l. A possible explanation for the lack of additional lactate accumulation is an inhibition of metabolism for a short duration of time immediately following electroshock. Mesa and Schreck (1989), similarly, showed no elevation in the lactate levels in cutthroat trout following treatment with DC electrofishing gear, anesthetization, weighing, measuring and marking, over treatment with DC electroshock only.

Despite these physiological disturbances rainbow trout can survive up to 4 min of air exposure following pulsed DC electroshock. Barrett and Grossman (1988) similarly found no mortality in mottled sculpin during the week following DC electroshock and approximately one minute of handling. Ferguson and Tufts (1992) however, reported elevated mortality in exercised rainbow trout followed by a only 30 s and 60 s periods of air exposure. Again, this may be due to differences in metabolic rates following treatment. In addition, this study was conducted on catheterized fish, which in itself is stressful, and may have accounted for the increased mortalities.

Caudal Severance vs D.A. Catheter Blood Sampling

Based on the size hypothesis, supported by the spinal damage experiment, one would have expected greater physiological disturbances in the catheterized fish, whose body weights averaged 355.3 ± 20.7 g compared to 113.3 ± 3.6 g in the caudal severance experiment. However, this was not the case. In fact, in catheterized fish, electroshock resulted in no cortisol (Figure 2.3A) or glucose (Figure 2.3B) disturbance, whereas fish sampled via caudal severance experienced elevations in both parameters. Part of the explanation for this difference may be the fact that catheterized fish were confined in lucite boxes, which may have partially insulated them from the electric shock. However, the response time to electroshock was only slightly, and not significantly greater. Furthermore, the lactate elevations in both experiments were similar (Figure 2.6B) indicating that both groups experienced similar amounts of muscle tetany.

The more likely explanation for the difference between caudal severance and catheterized fish is an attenuation of the stress response in the latter. This may be a consequence of the stresses associated with anaesthesia, surgery and chronic confinement which could take the form of a lowered psychological perception of stress (hence explaining both the lower cortisol and glucose mobilization) or depletion of interrenal and chromaffin tissue of cortisol and catecholamines respectively or a down regulation of receptors.

Conclusions

To summarize, the pulsed DC electrofishing technique used in this set of studies was above the threshold for immobilization, and did not induce physical damage or mortality. Treatment with such gear elicited a general stress response as well as a lactacidosis, as suggested by Schreck and colleagues for DC electrofishing gear (Mesa and Schreck, 1989; Schreck *et al.*, 1976). However, the more pronounced lactacidosis following pulsed DC electroshock is probably a combined result of the severe muscular exertion, tetany and irregular ventilation during and immediately following immobilization. Although both DC and pulsed DC electrofishing appear to elicit similar physiological responses, the magnitude of these disturbances are far greater for pulsed DC electrofishing (as is evident from glucose, cortisol and lactate measurements). This further supports the commonly accepted view that pulsed DC electroshock is more detrimental than DC electrofishing (Lamarque, 1990). In fact, the physiological disturbances elicited by 20 s of pulsed DC electroshock are more similar in pattern and magnitude to those resulting from 2 to 3 min bouts of exhaustive exercise. Electroshock also elicits a prolonged effect, up to minutes after the current is turned off (as evident from the ion/gas ratio and acid-base status following electroshock and subsequent handling and air exposure). During this time, response to additional stress such as handling or air exposure are minimized. All of this tends to support the previously held beliefs that pulsed DC electrofishing is a safe method of fish capture. However, due to the pronounced lactacidosis induced by pulsed DC gear, DC electrofishing probably poses fewer risks. This last point should be addressed in future studies. In addition,

catheterization appears to attenuate the stress component of electroshock but does not apparently affect the muscle tetany component.

CHAPTER 3

SWIM PERFORMANCE FOLLOWING ELECTROSHOCK, ELECTROSHOCK/AIR EXPOSURE AND EXERCISE

INTRODUCTION

Chapter 2 showed that treatment with pulsed DC electrofishing gear induced physiological disturbances similar to those produced by exhaustive exercise. Furthermore, these disturbances were slightly magnified by subsequent air exposure. Also, the probability of spinal damage and mortality following pulsed DC electrofishing was low which indicates its value as a catch and release capture technique. However, sluggish swimming has been repeatedly reported following electrofishing (Hauck, 1949; Horak and Klein, 1967; Hudy, 1985; Mesa and Schreck, 1989) and handling tends to increase the severity of the effect (Mesa and Schreck, 1989). Impaired swimming undoubtedly reduces the animals' ability to avoid predators and could increase the chance of mortality. However, the duration and magnitude of inhibited swim performance has not been critically examined. Thus, the focus of this chapter was to develop a performance test that simulates natural challenges such as predator avoidance, and to use this test to evaluate recovery from pulsed DC electroshock with and without subsequent air exposure.

Two types of tests have been commonly used to assess swim performance: endurance tests and burst tests. Endurance tests assess

sustained aerobic performance over relatively long durations (≥ 2 h), whereas burst tests measure anaerobic performance over very short periods (< 20 s). The standardized test for endurance is the critical swimming speed or maximum sustainable swimming speed (U_{crit}) determination. In this test, fish are subjected to stepwise increases in swimming speed (usually in a swim tunnel) until fatigue occurs. The test is designed in such a fashion to require at least 120 min and often up to 200 min (Beamish 1978). U_{crit} is then calculated from the maximum speed achieved prior to fatigue (Brett, 1964). This test, however, was judged unsuitable for two reasons. U_{crit} analyzes primarily aerobic performance which is not a key component in challenges such as predator avoidance. Secondly, during the long duration of time required to administer the test, fish would be recovering from the acute electroshock and exercise treatments. Therefore, a shorter, more natural, swim challenge was desired.

Rapid burst performance tests closely simulate predator avoidance and typically require only a few seconds to complete (< 20 s, and often < 1 s; Beamish, 1978). During such tests, maximum swim speeds are calculated on fish stimulated into movement or conditioned to race between two feeding points by underwater flashing lights (usually in raceways, swim tunnels or annular troughs) (Bainbridge, 1960, 1962; Ryland, 1963; Beamish, 1978). However, due to the short duration of time required to complete the test and the dependence upon training, subtle differences in performance are difficult to resolve.

Thus, a compromise between the endurance and burst performance tests was designed, in such a manner, that fish would exhaust between 1 and 10 min of the initiation of the challenge. By expanding the duration of the test, the resolving capability was increased, without greatly compromising the ability to simulate natural challenges such as predator avoidance.

Swim performance was examined following electroshock with and without air exposure (1-4 min) and following exhaustive exercise. The latter was analyzed to act as a control, since exercise induced similar physiological responses to pulsed DC electrofishing (see Chapter 2). In a second series of experiments, whole body lactate was examined as a measure of anerobic activity at two points following all treatments.

MATERIALS AND METHODS

Experimental Animals

Juvenile rainbow trout (3-12 g) were obtained from Rainbow Springs Trout Farm in Thamesford, Ontario. Fish were held in well aerated 400 l circular tanks supplied continuously with dechlorinated Hamilton tap water. Fish were feed daily with commercial trout pellets (Martins Feed Mills, Elmira, Ontario). One day prior to treatment, fish were acclimated to the test tank (2.4 m x 0.6 m) and were not fed during this time. The temperature and conductivity were 14 ± 2 °C and 255 ± 15 μ mhos/cm, respectively.

Electrofishing, Air Exposure and Exercise Procedures

In all electroshock trials, fish were confined in a volume of 56 l where they were at least 0.3 m from the cathode and 1.2 m from the anode (Figure 2.1). Electrofishing was conducted with a Smith-Root VII pulsed DC backpack electrofisher at 200 V for a 20 second duration, with the pulse duration and frequency set at 5ms and 60 Hz, respectively. Immediately following electroshock, fish were netted and placed into a container lined with moistened paper towels. Following 0, 1, 2 or 4 min of air exposure, fish were returned to the test tank.

Exercise was imposed by releasing fish (5-6 at a time) into a bucket filled with 6 l of water and then manually chasing and prodding for 5 min.

Swim Performance Test

Swim performance measurements were conducted in a 150 l Brett-type swim tunnel (Research Instruments Mfg. Co., Guelph, Ontario). Current was generated by a 3 hp (1 hp = 743 W) motor powering an impellor capable of producing water velocities up to 60 cm/s. A cooling jacket of anodized aluminum was used to maintain water temperature at 14 ± 1 °C. Following treatment, trout (N = 8-12 per group, 5 groups) were transferred and acclimated to the swim tunnel for 30 min at a water speed of 12 cm/s. At the end of the acclimation period, fish were exposed to a sudden increment in velocity. Various velocities were tested ranging between 36-54 cm/s to obtain a speed at which fish exhausted between 1 and 10 min. Exhaustion was defined at the point in time when the fish was overcome by the water speed, forced against the downstream screen and could not be further stimulated to swim by prodding. Fatigued fish were immediately removed unharmed by siphoning with a 4 cm diameter tube. This procedure permitted the remaining fish to continue swimming relatively undisturbed.

Time to exhaustion, total length (± 0.5 cm) and weight (± 0.1 g) were recorded. Swim performance was determined at 0.5 h, 1 h, 2 h, and 4 h following treatment and at 6 h for treatment groups not fully recovered by 4 h.

Whole Body Lactate

At two times following treatment (0.5 h and 4 h) trout were carefully netted to minimize struggling and immediately stunned with a rapid blow to

the head. Then fish were transferred to a bath of liquid nitrogen (-170 °C) and freeze-clamped with metal plates. All fish (N = 8-11 per group, 10 groups) were sampled within 5 min. Total lengths and weights were recorded, then whole fish were powdered in a liquid nitrogen cooled mortar and pestle. 8 ml of 8% PCA was immediately added to the powder and mixed, for 2 min on ice, with a Janke and Kunkel, Ultra-turrax[®] tissue homogenizer. Samples were centrifuged for 4 min, the supernatant was drawn off and frozen at -70 °C for further analysis. Samples were thawed and further diluted 1: 3 with 8% PCA. The PCA extracts were then assayed using the lactate dehydrogenase method with Sigma reagents. Lactate values were corrected for tissue weight and dilution and expressed as $\mu\text{mol/g}$ wet tissue mass.

Statistical Analysis

All values are expressed as mean \pm 1 S.E.M. Significance was determined by the unpaired Student's *t* -test ($p < 0.05$).

RESULTS

Swim Performance Test

Control fish varied in body weight and length between 2.9-8.4 g and 6.0-9.5 cm, respectively. Through trial and error, a velocity of 39 cm/s was found to produced exhaustion in the time frame desired (1.5-8.6 min). This velocity represented a swimming speed of 3.5-6.5 body length (BL)/s for the fish used, with the average being 4.5 BL/s. The usual approach to standardizing swimming data for size variations would be to express the time to exhaustion in terms of seconds per unit body length. Although time to exhaustion was, in fact, correlated to length ($r^2=0.47$), a better relationship was found between time to exhaustion and body weight ($r^2=0.59$; Figure 3.1). This may be partially due to the accuracy of each of the two measurements: length (± 0.5 cm) and weight (± 0.1 g). Consequently, to eliminate size effects amongst groups, time to exhaustion has been expressed per unit body weight. Thus, in control fish, time to exhaustion averaged 52.3 ± 3.9 s/g (N = 21; Figure 3.2A-E).

Effects of Treatments on Swim Performance

All treatments produced impaired performance in comparison to the control fish. Electroshocked fish experienced a 32 % decrease in swim performance by 0.5 h following treatment, then a further impairment to 53% of control by 1 h (Figure 3.2A). Swim performance had fully recovered by 2 h

Figure 3.1. Time to exhaustion of control fish was correlated to body weight ($r^2 = 0.587$). $T = 14 \pm 1$ °C, * indicates a significant correlation.

SWIM PERFORMANCE OF CONTROL FISH

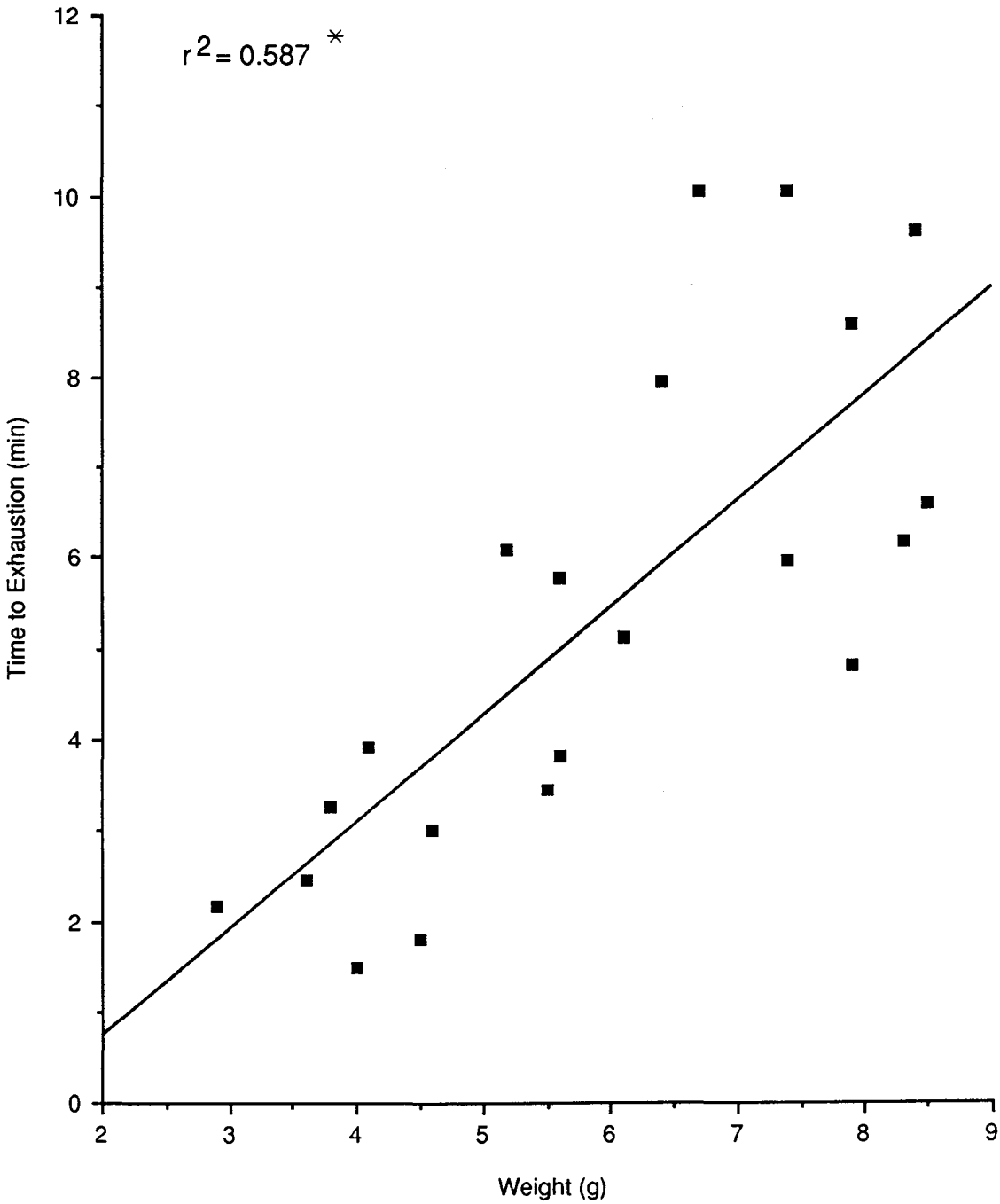
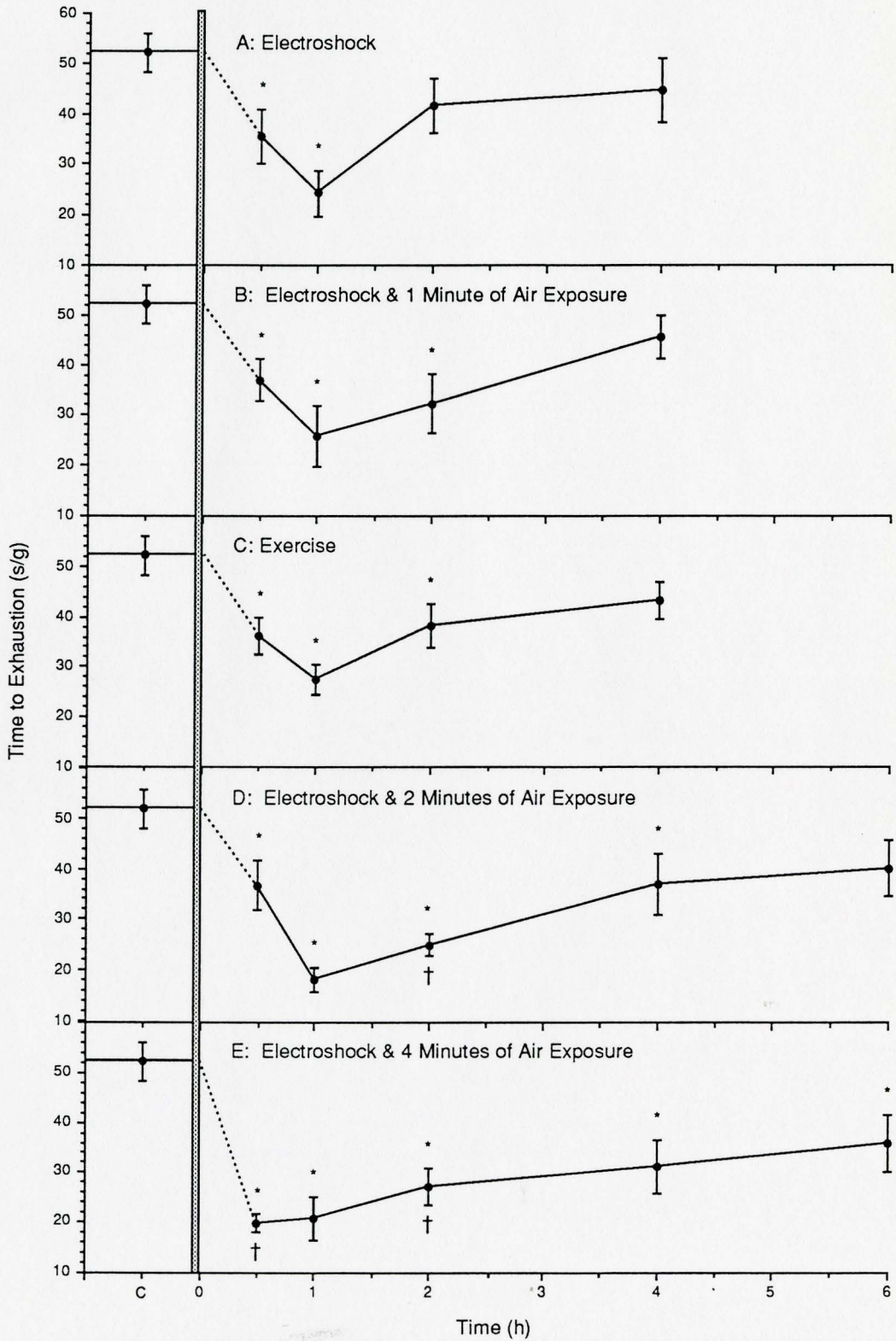


Figure 3.2. The influence of (A) electroshock, (B) electroshock and 1 min of air exposure, (C) a 5 min bout of exhaustive exercise, (D) electroshock and 2 min of air exposure and (E) electroshock and 4 min of air exposure on swim performance. Swim performance is expressed per unit weight. All values (N = 8-22) are expressed as mean \pm one S.E.M.; T=13 \pm 1 °C; * indicates a significant difference from rest (p < 0.05); † indicates a significant difference from electroshocked fish (p < 0.05).

SWIM PERFORMANCE FOLLOWING ELECTROSHOCK WITH AND WITHOUT AIR EXPOSURE, AND EXERCISE



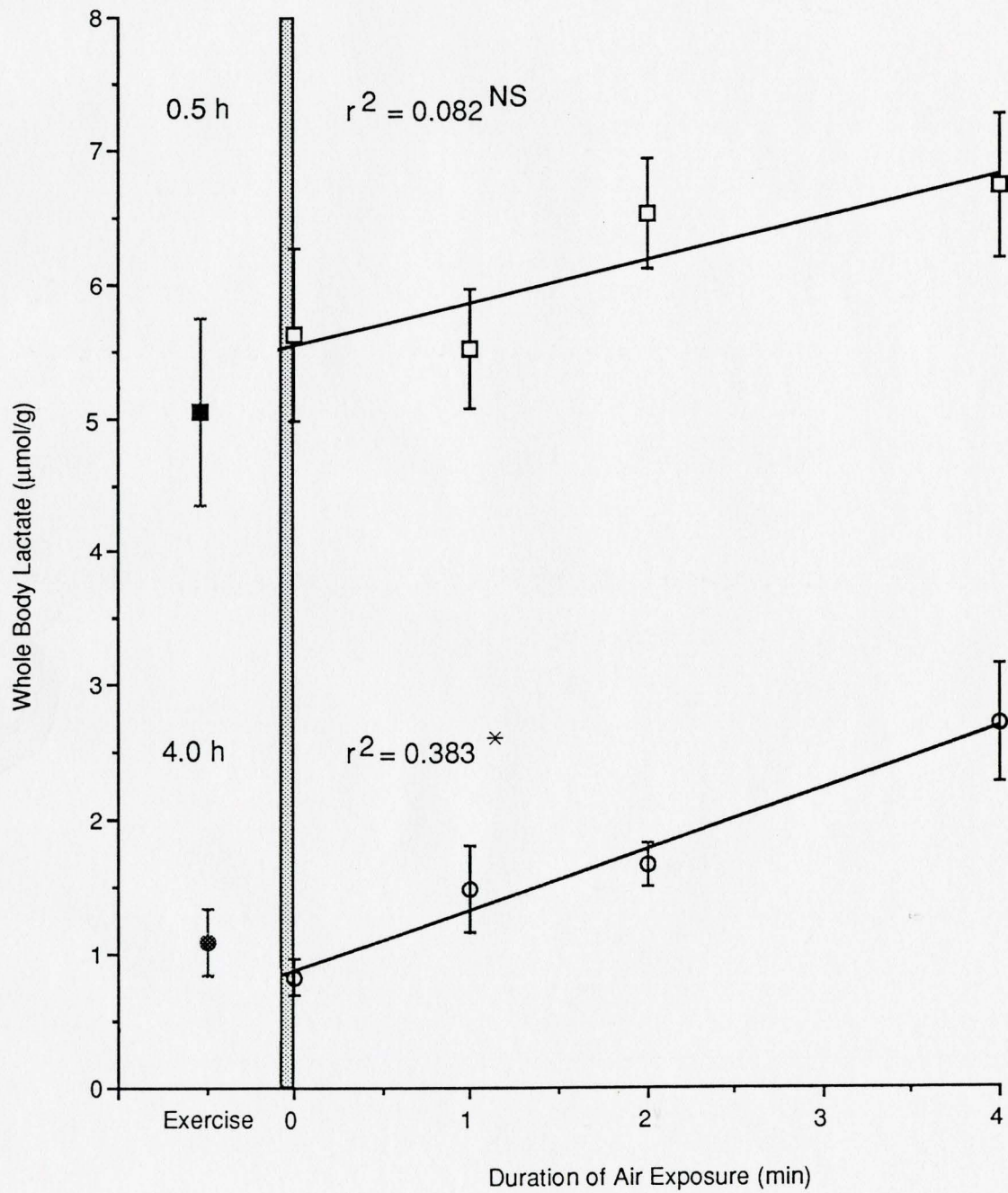
after the cessation of electroshock. Fish air exposed for 1 min following electroshock similarly experienced a depression in swim performance at 0.5 h with a maximum impairment of 51 % 1 h after treatment; however swim performance in these fish did not recover until 4 h following treatment (Figure 3.2B). Electroshocked fish which were air exposed for 2 min, 0.5 h after treatment experienced a swim performance depression similar to that induced by electroshock, but by 1 h experienced a further impairment to 65 % of rest, with full recovery not occurring until 6 h post-treatment (Figure 3.2D). Treatment with 4 min of air exposure following electroshock induced a 62 % reduction in swim performance 0.5 h to 1 h following treatment, and recovery required greater than 6 h (Figure 3.2E). Exercised fish also experienced impaired swimming with a pattern very similar to that induced by electroshock (Figure 3.2A,C); an initial impairment of 47 %, followed by a further reduction in performance 1 h to 53 % of control. Recovery of swim performance occurred by 4 h following treatment.

Whole Body Lactate

Following all treatments, whole body lactate was significantly elevated above control concentrations of $1.4 \pm 0.1 \mu\text{mol/g}$, at 0.5 h and had recovered by 4 h. At both 0.5 h and 4.0 h post-treatment the lactate burden in electroshocked fish ($5.6 \pm 0.6 \mu\text{mol/g}$ and $0.8 \pm 0.1 \mu\text{mol/g}$, respectively) was similar to that of exercised fish ($5.1 \pm 0.7 \mu\text{mol/g}$ and $1.1 \pm 0.3 \mu\text{mol/g}$, respectively; Figure 3.3). Additionally, at 0.5 h following treatment, the whole body lactate burden tended to relate to the amount of air exposure

Figure 3.3. The influence of (■ , ●) exercise and (□ , ○) electroshock, with and without subsequent air exposure on whole body lactate at 0.5 h ($y = 5.54 + 0.31x$) and 4 h ($y = 0.88 + 0.45x$) after treatment, respectively. All value (N = 8-11) are expressed as mean \pm one S.E.M.; $T = 8 \pm 2$ °C; * indicates a significant correlation ($p < 0.05$); NS indicates no significant correlation.

WHOLE BODY LACTATE 0.5 H AND 4.0 H FOLLOWING TREATMENT



experienced by the fish ($r^2 = 0.082$, $p < 0.05$; Figure 3.3). This effect persisted until 4 h, and indeed, it became more pronounced ($r^2 = 0.383$, $p < 0.05$; Figure 3.3).

DISCUSSION

Swim Performance Test

The swim performance test developed in this study, allows for a very quick or "snap shot" assessment of performance which relates directly to animals' ability to avoid predators in the wild. The validity of this test is shown in its reproducibility and in its ability to consistently resolve differences in severity amongst treatments used. This study clearly shows that swim performance was impaired by electroshock in a virtually identical fashion to that caused by exhaustive exercise. Furthermore, impairment was progressive with increasing duration of post-electroshock air exposure.

The impaired performance is undoubtedly related to a rapid development of muscle fatigue. Therefore, in order to understand the effects of electroshock/air exposure, it is necessary to understand the origin of muscle fatigue in fish. Although a variety of physiological and biochemical factors may contribute to the development of swimming fatigue, two prominent causes are the depletion of muscle substrates (e.g. ATP, creatine phosphate and glycogen) (Guyton, 1986; Hill and Wyse, 1989; Scarabello *et al.*, 1991b) and the development of extracellular and intracellular acidoses (Jones and Randall, 1978; Guyton, 1986; Blanchard and Solaro, 1984; Westerblad *et al.*, 1991). Although neither of these two factors have been critically examined following electroshock, they have been assessed following exhaustive exercise. Since exercise induces very similar physiological disturbances to pulsed DC electroshock, it is therefore possible to relate

impaired swim performance found in this study, to the extensive data available on fish exercise.

Muscle substrate levels are reduced following both bouts of exhaustive exercise (Milligan and Wood, 1986b; Scarabello *et al.*, 1991a,b) and hypoxia (Johnston, 1975; Dunn and Hochachka, 1986); the latter being a similar circumstance to that of air exposure. Although the degree of substrate depletion varies between experiments, following exercise ATP, creatine phosphate, and glycogen levels fall to 1/5, 1/3-1/7, and 2/3-1/10 of rest, respectively (Milligan and Wood, 1986b; Scarabello *et al.*, 1991a,b). Creatine phosphate and ATP are regenerated by 1 h to 4 h, while muscle glycogen requires 3 h to 12 h to recover to pre-exercise levels (Milligan and Wood, 1986b; Scarabello *et al.*, 1991a,b). Assuming a similar pattern of substrate depletion and recovery occurred with electroshock, recovery of swim performance may relate to recovery of substrate levels. Yet, the timing of maximal substrate depletion (immediately) and swim performance impairment (1 h) do not coincide, suggesting that other factors may also be involved.

An extracellular acidosis may impair hemoglobin oxygen transport by both Bohr and Root effects (Hill and Wyse, 1989). Following electroshock with pulsed DC gear, arterial pH was reduced (Figure 2.8A,C) and air exposure appeared to increase the degree of these disturbances experienced by catheterized fish, but again, the timing of maximal disturbances and recovery did not coincide with swim performance impairment (Figure 2.8A; Figure 3.3A,B). Similarly, the maximal arterial pH disturbance following

exhaustive exercise, is immediate and not 1 h (as with maximum swim performance impairment) following treatment (Holeton *et al.*, 1983; Wood *et al.*, 1983; Milligan and Wood, 1986a; Tang and Boutilier, 1988; McDonald *et al.*, 1989; Tang *et al.*, 1989; Ferguson and Tufts, 1992). Furthermore, MO_2 following electroshock was not impaired (Figure 2.9B). This suggests that the extracellular acidosis was not a key factor affecting swim performance.

Intramuscular pH is also reduced immediately following exhaustive exercise. However, the intracellular acidosis continues to develop until 1 h post-treatment, with full recovery requiring from 8 h to 12 h (Milligan and Wood, 1986a,b; Tang and Boutilier, 1991). Further reductions in intramuscular pH can be expected with air exposure. The continued development pattern of the intracellular acidosis to 1 h following exhaustive exercise, follows the same pattern of development of impaired swim performance following exercise. Therefore, intracellular pH was likely the major factor contributing to fatigue in this experiment.

Lactate is a metabolic end product co-produced with protons during anaerobic metabolism, which is often associated with fatigue (Hill and Wyse, 1989). Whole body lactate concentrations are maximal immediately to 1 h following bouts of exhaustive exercise, and return to resting levels by 3 h (Scarabello *et al.*, 1991a,b). Whole body lactate, in this study, was elevated following all treatments (exercise, electroshock and electroshock followed by 1-4 min air exposure) and in each case had fully recovered by 4 h (Figure 3.3). Air exposure did not further increase lactate production as much as one might expect, based on whole blood lactate values obtained following exercise

and air exposure (Ferguson and Tufts, 1992). As discussed in Chapter 2, this may be due to reductions in metabolic rate induced by electroshock. Nonetheless, the correlation between whole body lactate and the amount of air exposure became stronger with time. This implies that air exposure may impair lactate metabolism. This again may be a consequence of a more severe and prolonged intramuscular acidosis. Therefore, due to similarities in the recovery times and the timing of maximal lactate disturbances between groups, lactate burden does not appear to be directly related to fatigue.

Based on comparisons with studies on metabolic effects of exercise, the impaired test performance appears to be related with disturbances of intramuscular pH. With a knowledge of the basis of this performance test, the test can now be implemented for a variety of other uses, either in conjunction with, or instead of, the classical U_{crit} determination (Brett, 1964). U_{crit} has been used to simulate prolonged aerobic challenges experienced by fish in the wild such as migration or routine activity (Beamish, 1978); whereas, the aerobic/anaerobic performance assessed in the present study relates more closely to natural challenges such as predator avoidance, predation, angling and the negotiating of rapids and fish ladders. In addition, less than 1 h is required to fully complete the burst swim performance test, which is particularly useful in the examination of recovery from acute stresses (e.g. exercise, hypoxia) or the acclimation to chronic stressors (e.g. metals, pH).

Ecological Relevance

The 2 h inhibition of swim performance experienced by electroshocked rainbow trout (Figure 3.2A) was much less prolonged than the previously reported performance depressions determined via a modified U_{crit} test. Horak and Klein (1967) reported a reduced performance index (swimming stamina), 24 h following treatment with pulsed DC electrofishing gear. However, 39 % of the rainbow trout in their study experienced visible areas of internal bleeding which is often associated with spinal damage (Lamarque, 1990). Thus, Horak and Klein's study examined the chronic deterioration in swim performance induced by physical damage, while the present study assesses the acute impairment of swim performance induced by physiological disturbances.

Because the maximum impairment of swim performance and the time required for full recovery both increased with the duration of air exposure (Figure 3.3A-E), air exposure during post-electroshock handling has the potential to be more detrimental than the capture technique itself. Throughout a prolonged period of impaired swim performance, predator avoidance ability of the fish is likely also reduced. This undoubtedly leads to increased predation. Thus, when electrofishing is used in the field to collect juvenile or small fish, with the intent of returning them to the wild, air exposure during netting and processing should be minimized. Similarly, Barrett and Grossman (1988) and Mesa and Schreck (1989) concluded that handling following DC electrofishing may be more detrimental than the capture, based on mottled sculpin mortality studies and changes in feeding,

aggressive behaviour and physiological parameters (cortisol and lactate) of cutthroat trout, respectively. In addition, Ferguson and Tufts (1992) recommended that handling following angling be minimized.

Conclusions

The swim test developed in this study assesses a combination of aerobic and anaerobic capacity of fish, with fatigue likely resulting from an intramuscular acidosis. This study clearly shows that swim performance was impaired by electroshock in an identical fashion to exercise, and that this impairment was progressive with increasing durations of post-electroshock air exposure. Therefore, minimizing air exposure during post-electroshock handling and processing is crucial when the intent is to return fish to the wild unharmed. This is particularly vital when dealing with juvenile fish of rare or sensitive populations, for impaired swim performance undoubtedly leads to increased predation.

SUMMARY AND CONCLUSIONS

In the present study rainbow trout exposed to 20 s of 200 V pulsed DC electroshock, did not experience any mortality or spinal damage. However, severe electroshock conditions (~ 600 g fish, 600 V, 20-40 s) did induce vertebral misalignment. This indicates that standard electrofishing procedures in water of similar chemical composition should not elicit severe spinal damage leading to delayed mortality. However, maximum voltage and duration of time fish are in the current should be minimized.

Pulsed DC electroshock at 200 V for a 20 s duration resulted in elevated plasma catecholamines, cortisol and glucose due to stimulation of the stress axis. Fish also experienced a lactacidosis due to tetany during immobilization and impaired ventilation during immobilization and early recovery. The magnitude of these disturbances was greater than those reported for DC electrofishing gear. In fact, pulsed DC electrofishing elicited physiological disturbances more similar in magnitude and duration to a 2-3 min bout of exhaustive exercise. Swim performance is also impaired following pulsed DC electrofishing, for at least 1 h.

If electrofishing was immediately followed by 1 min of air exposure the general stress response and the lactacidosis tended to be more severe. With increasing duration of air exposure (1-4 min) the maximum magnitude and duration of impaired swim performance increased. The more rapid fatigue induced by electrofishing and air exposure was likely due to an intra-

muscular acidosis. Regardless of the cause of fatigue, it is crucial to minimize the duration of air exposure during post-electroshock handling, if the intent is to return the fish unharmed to the environment. This is particularly vital when electrofishing is used to collect juvenile or small fish, of rare or endangered population; since during prolonged periods of impaired swim-performance, predator avoidance ability of the fish is likely reduced. This, undoubtedly, leads to increased predation.

Minimizing air exposure while electrofishing in the field may be even more crucial than predicted by this study. This series of experiments were conducted on hatchery reared fish, but wild fish are known to exhibit greater stress responses and suffer more mortality than hatchery reared fish (Woodward and Strange, 1987). Thus, in the field, following capture by pulsed DC electrofishing and air exposure during handling, wild fish could experience increased delayed mortality due to the direct elicitation of more severe physical and physiological disturbances or to decreased predator avoidance ability. In addition, the effects of handling immediately following electrofishing, as in this study, are minimized due to a prolonged effect of the electroshock. Whereas, a majority of handling and air exposure in the field occurs minutes after capture (approximately 10-30 min). Thus, air exposure and handling at this time could elicit the full response, exacerbating the poor physiological condition of the fish, further impairing swim performance. Finally, this study only examined the air exposure portion of processing following electrofishing. Whereas, handling, tagging or scale sampling would all be more stressful and could elevate the probability of delayed mortality.

In summary, during pulsed DC electrofishing and fish processing maximum voltages and duration of time in the current and air exposed should be minimized. Yet, more studies are still needed to: 1) examine the effects of air exposure and handling minutes after electroshock; 2) verify the physiological origin of the more rapid fatigue following electrofishing and handling 3) compare this work to conditions experienced in the field (i.e.. wild fish, handling) and 4) directly compare the effects of pulsed DC gear to that of DC.

REFERENCES

- Bainbridge, R. (1960). Speed and stamina in three fish. *Journal of Experimental Biology* 37(1): 129-153.
- Bainbridge, R. (1962). Training, speed and stamina in trout. *Journal of Experimental Biology* 39: 537-555.
- Barrett, J. C. and G. D. Grossman (1988). Effects of direct current electrofishing on the Mottled Sculpin. *North American Journal of Fisheries Management* 8: 112-116.
- Barton, B.A., R.E. Peter and C.R. Paulencu (1980) Plasma cortisol levels of fingerling rainbow trout (*Salmo gairdneri*) at rest, and subjected to handling, confinement, transport, and stocking. *Canadian Journal of Fisheries and Aquatic Sciences* 37: 805-811.
- Barton, B.A., G.S. Weiner and C.B. Schreck (1985). Effect of prior acid exposure on physiological responses of juvenile rainbow trout (*Salmo gairdneri*) to acute handling stress. *Canadian Journal of Fisheries and Aquatic Sciences* 42: 710-717.
- Barton, B.A., C.B. Schreck and L.A. Sigismond (1986). Multiple acute disturbances evoke cumulative physiological stress responses in

juvenile chinook salmon. Transactions of the American Fisheries Society 115: 243-251.

Beamish, F. W. H. (1978). Swimming Capacity. In: *Fish Physiology. Volume VIII*. W. S. Hoar and D. J. Randall ed., New York, Academic Press, Inc. pp. 101-189.

Blanchard, E. M. and R. J. Solaro (1984). Inhibition of the activation and troponin calcium binding of dog cardiac myofibrils by acidic pH. Circulation Research 55: 382-391.

Bohlin, T., S. Hamrin, T. G. Heggberget, G. Rasmussen and S. J. Saltveit (1989). Electrofishing-Theory and practice with special emphasis on salmonids. Hydrobiologia 173: 9-43.

Bouck, G. R. and R. C. Ball (1966). Influence of capture methods on blood characteristics and mortality in the Rainbow Trout (*Salmo gairdneri*). Transactions of the American Fisheries Society 95: 170-176.

Boutilier, R. G., T. A. Heming and G. K. Iwama (1984). Physicochemical parameters for use in fish respiratory physiology. In: *Fish Physiology. Volume XA*. W. S. Hoar and D. J. Randall ed., Orlando, Academic Press, Inc. pp. 403-430.

- Boutlier, R. G. (1990). Control and co-ordination of gas exchange in bimodal breathers. In: *Advances in Comparative Environmental Physiology*. VI. R. G. Boutlier ed., Berlin, Springer-Verlag. pp. 280-346.
- Brett, J. R. (1964). The respiratory metabolism and swimming performance of young sockeye salmon. *Journal of the Fisheries Research Board of Canada* 21(5): 1183-1226.
- Butler, P. J., E. W. Taylor, M. F. Capra and W. Davison (1978). The effect of hypoxia on the levels of circulation catecholamines in the dogfish *Scyliorhinus canicula*. *Journal of Comparative Physiology* 127: 325-330.
- Butler, P.J., J.D. Metcalfe and S.A. Ginley (1986). Plasma catecholamines in the lesser spotted dogfish and rainbow trout at rest and during different levels of exercise. *Journal of Experimental Biology* 123: 409-421.
- Dunn, J. F., Hochachka, P. W. (1986). Metabolic responses of trout (*Salmo gairdneri*) to acute environmental hypoxia. *Journal of Experimental Biology* 123: 229-242.
- Ellis, J. E. (1975). Electrotaxic and narcotic responses of channel catfish to various electrical pulse rates and voltage amplitudes. *Progressive Fish-Culturist* 37(3): 155-157.

- Ferguson, R. A. and B. L. Tufts (1992). Physiological effects of brief air exposure in exhaustively exercised rainbow trout (*Oncorhynchus mykiss*): implications for "catch and release" fisheries. *Canadian Journal of Fisheries and Aquatic Sciences* in Press.
- Fievet, B., J. Caroff and R. Motais (1990). Catecholamine release controlled by blood oxygen tension during deep hypoxia in trout: effect on red blood cell Na⁺/H exchanger activity. *Respiration Physiology* 79: 81-90.
- Gatz Jr., A. J. and J. M. Adams (1987). Effects of repeated electroshocking on growth of Bluegill X Green sunfish hybrids. *North American Journal of Fisheries Management* 7(3): 448-450.
- Gatz Jr., A. J., J. M. Laar and G. F. Cada (1986). Effects of repeated electroshocking on instantaneous growth of trout. *North American Journal of Fisheries Management* 6: 176-182.
- Gonzalez, R. J. and D. G. McDonald (1992). The relationship between oxygen consumption and ion loss in a freshwater fish. *Journal of Experimental Biology* 163: 317-332.
- Guyton, A. C. (1986). *Textbook of Medical Physiology*. Philadelphia, W.B. Saunders Company.

- Halband, E. (1967). Basic principles of electric fishing. In: *Fishing with Electricity-its Applications to Biology and Management*. R. Vibert ed., Oxford, Fishing News Books. pp. 57-64.
- Hauck, F. R. (1949). Some harmful effects of the electric shocker on large rainbow trout. *Transactions of the American Fisheries Society* 47: 61-64.
- Heisler, N. (1984). Acid-base regulation in fishes. In: *Fish Physiology Volume XA*. W.S. Hoar and D.J. Randall ed., Orlando, Academic Press Inc. pp. 315-401.
- Hickley, P. (1990). Electric Fishing in Practice. In: *Fishing with Electricity: Applications in Freshwater Fisheries Management*. I. G. Cowx and P. Lamarque ed., Oxford, Fishing News Books. pp. 176-187.
- Hill, R. W. and G. A. Wyse (1989). *Animal Physiology*. Cambridge, Harper and Row Publishers.
- Holeton, G. F., P. Neumann and N. Heisler (1983). Branchial ion exchange and acid-base regulation after strenuous exercise in rainbow trout (*Salmo gairdneri*). *Respiration Physiology* 51: 303-318.

- Horak, D. L. and W. D. Klein (1967). Influence of capture methods on fishing success, stamina, and mortality of rainbow trout (*Salmo gairdneri*) in Colorado. *Transactions of the American Fisheries Society* 96: 220-222.
- Hudy, M. (1985). Rainbow trout and brook trout mortality from high voltage AC electrofishing in a controlled environment. *North American Journal of Fisheries Management* 5: 475-479.
- Johnston, I. A. (1975). Studies in the swimming musculature of the rainbow trout II. Muscle metabolism during severe hypoxia. *Journal of Fish Biology* 7: 459-467.
- Jones, D.R. and D.J. Randall (1978). The respiratory and circulatory systems during exercise. In: *Fish Physiology Volume VII*. W.S. Hoar and D.J. Randall ed., New York, Academic Press. pp.425-501.
- Lamarque, P. (1967). Electrophysiology of fish subject to the action of an electric field. In: *Fishing with Electricity-its Applications to Biology and Management*. R. Vibert ed., Oxford, Fishing News Books, Ltd. pp. 65-92.
- Lamarque, P. (1990). Electrophysiology of fish in electric fields. In: *Fishing with Electricity-Applications in Freshwater Fisheries Management*. I. Cowx and P. Lamarque ed., Oxford, Fishing News Books. 4-33.

- Lazauski, H. G. and S. P. Malvestuto (1990). Electric fishing: results of a survey on use, boat construction, configuration and safety in the USA. In: *Developments in Electric Fishing*. I. G. Cowx ed., Oxford, Fishing News Books. pp. 327-339.
- Maxfield, G. H., R. H. Lander and K. L. Liscom (1971). Survival, growth and fecundity of hatchery-reared rainbow trout after exposure to pulsating direct current. *Transactions of the American Fisheries Society* 100(3): 546-552.
- Mazeaud, M. M. and F. Mazeaud (1981). Adrenergic responses to stress in fish. In: *Stress in Fish*. A. D. Pickering ed., London, Academic Press. pp. 49-75.
- Mazeaud, M. M., F. Mazeaud and E. M. Donaldson (1977). Primary and secondary effects of stress in fish: some new data with a general review. *Transactions of the American Fisheries Society* 106(3): 201-212.
- McCrimmon, H. R. and B. Bidgood (1965). Abnormal vertebrae in the rainbow trout with particular reference to electrofishing. *Transactions of the American Fisheries Society* 94: 84-88.

- McDonald, D. G., H. Hobe and C. M. Wood (1980). The influence of calcium on the physiological responses of the rainbow trout, *Salmo gairdneri*, to low environmental pH. *Journal of Experimental Biology* 88: 109-131.
- McDonald, D.G. and C.L. Milligan (1992). Chemical properties of the blood. In: *Fish Physiology. Volume XIIB*. W.S. Hoar and D.J. Randall ed., New York, Academic Press, Inc. pp. 55-133.
- McDonald, D. G., Y. Tang and R. G. Boutilier (1989). The role of Beta-adrenoreceptors in the recovery from exhaustive exercise of freshwater-adapted rainbow trout. *Journal of Experimental Biology* 147: 471-491.
- Mesa, M. G. and C. B. Schreck (1989). Electrofishing mark-recapture and depletion methodologies evoke behavioural and physiological changes in cutthroat trout. *Transactions of the American Fisheries Society* 118: 644-658.
- Milligan, C. L. and C. M. Wood (1987). Regulation of blood oxygen transport and red cell pH_i after exhaustive activity in rainbow trout (*Salmo gairdneri*) and starry flounder (*Platichthys stellatus*). *Journal of Experimental Biology* 133: 263-282.

- Milligan, C. L. and C. M. Wood (1986a). Intracellular and extracellular acid-base status and H^+ exchange with the environment after exhaustive exercise in the rainbow trout. *Journal of Experimental Biology* 123: 93-121.
- Milligan, C. L. and C. M. Wood (1986b). Tissue intracellular acid-base status and the fate of lactate after exhaustive exercise in the rainbow trout. *Journal of Experimental Biology* 123: 123-144.
- Novotny, D. W. (1969). *Electrical principles of electrofishing*. University of Wisconsin.
- Perry, S. F., R. Kinkead, P. Gallagher and D. J. Randall (1989). Evidence that hypoxemia promotes catecholamine release during hypercapnic acidosis in rainbow trout (*Salmo gairdneri*). *Respiration Physiology* 77: 351-364.
- Pratt, V. S. (1954). Fish mortality caused by electrical shockers. *Transactions of the American Fisheries Society* 84: 93-96.
- Primmatt, D.R.N., D.J. Randall, M. Mazeaud and R.G. Boutilier. (1986). The role of catecholamines in erythrocyte pH regulation and oxygen transport in rainbow trout (*Salmo gairdneri*) during exercise. *Journal of Experimental Biology* 122: 139-148.

- Reynolds, J. B. (1983). Electrofishing. In: *Fisheries Techniques*. L. A. Nielson and D. L. Johnson ed., Bethesda, American Fisheries Society. pp. 147-163.
- Ristori, M. T. and P. Laurent (1985). Plasma catecholamines and glucose during moderate exercise in the trout: comparison with bursts of violent activity. *Journal of Experimental Biology* 44: 247-253.
- Ryland, J.S. (1963). The swimming speeds of plaice larvae. *Journal of Experimental Biology* 40: 285-299.
- Scarabello, M., G. J. F. Heigenhauser and C. M. Wood (1991a). The oxygen debt hypothesis in juvenile rainbow trout after exhaustive exercise. *Respiration Physiology* 84: 345-259.
- Scarabello, M., C. M. Wood and G. J. F. Heigenhauser (1991b). Glycogen depletion in juvenile rainbow trout as an experimental test of the oxygen debt hypothesis. *Canadian Journal of Zoology* 69: 2562-2568.
- Schreck, C. B., R. A. Whaley, M. L. Bass, O. E. Maughan and M. Solazzi (1976). Physiological responses of rainbow trout (*Salmo gairdneri*) to electroshock. *Journal of the Fisheries Research Board of Canada* 33: 76-84.

- Sharber, N. G. and S. W. Carothers (1990). Influence of electric fishing pulse shape on spinal injuries in adult rainbow trout. In: *Developments in Electric Fishing*. I. G. Cowx ed., Oxford, Fishing News Books. pp. 19-25.
- Smith, M. J. and A. G. Heath (1980). Responses to acute anoxia and prolonged hypoxia by rainbow trout (*Salmo gairdneri*) and mirror carp (*Cyprinus carpio*) red and white muscle: use of conventional and modified metabolic pathways. *Journal of Biochemical Physiology* 66B: 267-272.
- Spencer, S. L. (1967). Internal injuries of largemouth bass and bluegills caused by electricity. *Progressive Fish-Culturist* 29(3): 168-169.
- Steinmetz, B. (1990). Electric fishing: some remarks on its use. In: *Developments in Electric Fishing*. I. G. Cowx ed., Oxford, Fishing News Books. pp. 1-4.
- Tang, Y. and R. G. Boutilier (1988). Correlation between catecholamine release and degree of acidotic stress on trout. *American Journal of Physiology* 255: R395-R399.
- Tang, Y. and R. G. Boutilier (1991). White muscle intracellular acid-base and lactate status following exhaustive exercise: a comparison between

- freshwater- and seawater-adapted rainbow trout. *Journal of Experimental Biology* 156: 153-171.
- Tang, Y., D. G. McDonald and R. G. Boutilier (1989). Acid-base regulation following exhaustive exercise: a comparison between freshwater- and seawater-adapted rainbow trout (*Salmo gairdneri*). *Journal of Experimental Biology* 141: 407-418.
- Tetens, V. and N. J. Christensen (1987). Beta-adrenergic control of blood oxygen affinity in acutely hypoxia exposed trout. *Journal of Comparative Physiology B* 157: 667-675.
- Thomas, S., B. Fievet and R. Motais (1986). Effect of deep hypoxia on acid-base balance in trout: role of ion transfer processes. *American Journal of Physiology* 250: R319-R327.
- Thomas, S. and G. M. Hughes (1982). A study of the effects of hypoxia on acid-base status of rainbow trout blood using an extracorporeal blood circulation. *Respiration Physiology* 49: 371-382.
- Turner, J. D., C. M. Wood and D. Clark (1983). Lactate and proton dynamics in the rainbow trout (*Salmo gairdneri*). *Journal of Experimental Biology* 104: 247-268.

- Vincent, R. (1971). River electrofishing and fish population estimates. *Progressive Fish-Culturist* 36(3): 182.
- Westerblad, H., J. A. Lee, J. Lannergren and D. G. Allen (1991). Cellular mechanisms of fatigue in skeletal muscle. *American Journal of Physiology* 261: C195-C209.
- Whaley, R. A. (1975). Electrofishing: an evaluation of lethality and physiological consequences. Virginia Polytechnic Institute and State University.
- Wood, C.M., D.G. McDonald and B.R. McMahon (1982). The influence of experimental anaemia on blood acid-base regulation *in vivo* and *in vitro* in the starry flounder (*Platichthys stellatus*) and the rainbow trout (*Salmo gairdneri*). *Journal of Experimental Biology* 96:221-237.
- Wood, C. M. and S. F. Perry (1985). Respiratory, circulatory, and metabolic adjustments to exercise in fish. In: *Circulation, Respiration and Metabolism*. R. Gilles ed., Berlin, Springer-Verlag. pp. 1-22.
- Wood, C. M., J. D. Turner and M. S. Graham (1983). Why do fish die after severe exercise? *Journal of Fish Biology* 22: 189-201.

Woodward, C. C. and R. J. Strange (1987). Physiological stress response in wild and hatchery-reared rainbow trout. *Transactions of the American Fisheries Society* 116: 574-579.