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**Response of cardiac function to environmental contaminants
and its significance for energy metabolism in salmonids**

by

Julie C. Brodeur

**A thesis
presented to the University of Waterloo
in fulfilment of the
thesis requirement for the degree of
Doctor of Philosophy
In
Biology**

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ABSTRACT

The objectives of this study were to examine the responses of teleost cardiac function to selected environmental contaminants, and to determine how changes in cardiac output relate to energy metabolism. When exposed for 24 h to concentrations representing 50 % and 25 % of the 96 h-LC50 of pentachlorophenol (PCP) and tetrachloroguaiacol (TCG), rainbow trout (*Oncorhynchus mykiss*) exhibited a reduction of oxygen consumption to about 50-60 % of basal levels while their cardiac output remained stable. The absence of variation in cardiac output probably originated from the fact that arterial oxygen content was maintained stable during the impairment of oxygen utilization by the chlorophenols; there was therefore no stimulus or need for an increase in convective oxygen delivery. In another series of experiments, adult Atlantic salmon (*Salmo salar*) were exposed for 48 h to water from the acidified (pH 5.2) Fossbekk River (Norway) with or without a nominal concentration of either 50, 75, 100, or 125 $\mu\text{g}\cdot\text{L}^{-1}$ of aluminium (Al) added as AlCl_3 , or to circumneutral water (pH 6.6) from Ims River (Norway). Most of the fish died before the end of the 48-h exposure period when exposed to Fossbekk River water + 125 $\mu\text{g Al}\cdot\text{L}^{-1}$, while no mortality was observed in the other treatments. Exposure to Fossbekk River water with 75, 100 and 125 $\mu\text{g}\cdot\text{L}^{-1}$ of Al caused a reduction of cardiac output to about 70-75 % of basal levels, primarily through a decrease in stroke volume. A large elevation of heart rate was observed at 125 $\mu\text{g Al}\cdot\text{L}^{-1}$, but such a tachycardia was not evident with the other concentrations of Al. The incapacity of the tachycardia to elevate cardiac output and the subsequent death of the fish at the highest Al concentration suggest that this response is more of a maladaptative reaction than a compensatory or adaptative reaction. Cardiac output returned to basal levels within 36 h when the water pH was raised to 5.8 by adding CaCO_3 at the end of the 48-h exposure period. By that time, plasma concentrations of sodium and chloride had started to return to basal levels and haematocrit was back to normal, but none of the other altered blood parameters (pH, HCO_3^- , potassium, glucose) had started to recover. The comparative dynamics of the alterations in blood chemistry and cardiac output suggest that the decrease in cardiac output was the result of the increase in haematocrit and the reduction in plasma volume resulting from the osmotic shifts associated with the ionic losses. In a different

experiment, the effects of Al and acidic water on cardiac output were examined after fish had time to acclimate to the presence of Al and their haematocrit and plasma ion concentration had returned to baseline. Atlantic salmon (*Salmo salar*) were exposed for 36 days to water from the Fossbekk River (pH 5.2) with 50 $\mu\text{g}\cdot\text{L}^{-1}$ of Al added as AlCl_3 , or to circumneutral water from Ims River (pH 6.6). The resting cardiac output of the fish exposed to Fossbekk River water + Al was not significantly different from the fish exposed to Ims River water. The growth rate of the fish exposed to acidic water was reduced after their food consumption had returned to normal levels, and their swimming activity was increased throughout the exposure to acidic water and Al. These results suggest that basal metabolic rate was not affected by the exposure and that the decrease in growth rate was predominantly the result of the increase in swimming activity. Another possible explanation is that the increase of basal metabolism, if present, was mainly the result of an increase in blood oxygen extraction rather than cardiac output. The last series of experiments indeed showed that the relative contribution of cardiac output and blood oxygen extraction to increases in oxygen consumption can vary depending on the factors affecting metabolic rate. This was concluded after examining the correlation of cardiac output, heart rate, and tissue blood oxygen extraction to oxygen consumption in fish subjected to either an acute increase or decrease in water temperature, or to an increase in swimming speed.

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GENERAL INTRODUCTION

The role of the circulatory system is the convective delivery of oxygen and nutrients to the cells and the transport of their wastes toward the excretory organs. Its functioning relies on the heart that receives blood by expansion and pumps it out through the blood vessels by contraction. Although the general principles governing the cardiovascular system are similar in all vertebrates, the nuances of fish cardiovascular function are considerable and attempts to describe this system from a mammalian perspective are tenuous at best (Olson, 1998). Our understanding of cardiovascular physiology specific to fish has, however, improved considerably over the last 20 years, and much of this information is now gathered in a number of review articles and monographs (Farrell, 1984; 1991; Satchell, 1991; Hoar *et al.*, 1992; Olson, 1998).

Nevertheless, very little attention has been devoted to the effects of environmental contaminants on fish cardiac function despite the variety of ways through which toxicants can alter the cardiovascular system. First, the function of the circulatory system is intimately related to the process of respiratory gas exchange and the control mechanisms for this system are largely based on the detection of changes in blood oxygen. Thus, the numerous toxicants such as zinc, chlorine, and aluminium that alter the process of oxygen uptake can be expected to affect circulation (Heath, 1995). This is not to exclude the possibility of some chemical acting directly on the heart or the vascular system and thereby impairing their normal functioning. Furthermore, adrenergic stimulation, a stereotypic response to a variety of stressors including toxic exposure (Mazeaud *et al.*, 1977), will have marked effects on the cardiac function.

Contaminants can also affect the heart of a fish by increasing its demands for metabolic energy. Indeed, the various defense and repair mechanisms used by an organism to resist the damaging effects of toxic chemicals are generally costly in terms of energetic resources and cause metabolic rate to increase (Calow, 1991). Such an elevation of metabolic rate may in turn be expected to influence cardiac function since, according to the Fick principle, cardiac

output and blood oxygen extraction are the only two parameters controlling oxygen consumption (Thorarensen *et al.*, 1996a).

The few studies that have examined the responses of the cardiac function to the presence of toxicants have also generally only looked at heart rate, although it is now known that many fish species increase their cardiac output mainly through an increase in stroke volume rather than heart rate (Farrell and Jones, 1992). As such, determination of cardiac output in fish is preferable to the measurement of heart rate alone, as it provides more instructive information about cardiac function.

The best technique actually available to measure cardiac output in teleost fish is the placement of cuff-type Doppler flow probes around the ventral aorta. The Doppler flow probe uses a piezoelectric transducer to emit a continuous sound signal. Due to Doppler shift, when the signal hits and is reflected from a moving object in the blood (e.g. a red blood cell), a shift in the signal frequency is observed. This shift in frequency depends on the speed of the moving object and represents blood velocity. Cardiac output, heart rate and stroke volume can subsequently be calculated from the data obtained on velocity. Cardiac output is the average velocity of the blood leaving the ventral aorta over time, heart rate is the frequency of peaks of velocity over time, and stroke volume is the integration of the instantaneous velocities of blood flow over a heart beat.

This study was designed to examine the responses of teleost cardiac function to selected environmental contaminants, and to determine how changes in cardiac output relate to energy metabolism. In the first three chapters, the Doppler flow technique is used to assess the impacts of acute exposures to chemicals on cardiac output, heart rate and stroke volume. The first chapter deals with acute exposures of rainbow trout (*Oncorhynchus mykiss*) to two chlorophenolic compounds: pentachlorophenol and tetrachloroguaiacol. Chlorophenols are a category of chemicals known to cause an uncoupling of oxidative phosphorylation, so that oxygen is used but the formation of ATP is reduced (Hanstein, 1976). Oxygen consumption was measured in combination with the cardiac parameters to provide the first observations on

the role of the heart in the dramatic increase of oxygen consumption associated to the uncoupling of oxidative phosphorylation.

The second and third chapters respectively report the cardiac responses of Atlantic salmon (*Salmo salar*) to lethal and sublethal exposures to acidic water containing elevated aluminium (Al) concentrations. Both the ionic loss and the hypoxia usually associated with an exposure to Al in low pH water are likely to induce compensatory responses by the cardiovascular system. Milligan and Wood (1982) proposed that fish death in acidic water is due to circulatory collapse resulting from the increase in cardiac workload associated with ionic loss. The second chapter of this manuscript examines if signs of increased workload are indeed exhibited by the cardiac function when fish are dying from acidic water and Al. The third chapter focuses on the causes of alterations in cardiac output by comparing its variations in time to the variations of a variety of blood parameters during an exposure to sublethal concentrations of Al in acidic water, and after the elevation of the pH to circumneutral levels.

The fourth chapter examines the effects of a subchronic exposure to Al and low pH on the bioenergetics of Atlantic salmon by assessing swimming activity, cardiac output, growth rate and food consumption. The objective of this study was to examine if a sublethal exposure to acidic water and Al imposes a metabolic load on fish, and if such an increase in metabolism is expressed by an elevation of cardiac output in resting fish.

Finally, in the fifth chapter, the correlation of cardiac output, heart rate and tissue blood extraction to oxygen consumption was examined in rainbow trout subjected to either an acute increase or decrease in water temperature, or to an increase in swimming speed. The objective of this experiment was to determine if cardiac output is a better predictor of metabolic rate than heart rate in fish. Heart rate has indeed been previously shown to be a poor indicator of metabolic rate in fish because its relationship to oxygen consumption can be described by numerous curves (Thorarensen *et al.*, 1996a). The capacity to predict oxygen consumption from cardiac output would allow estimating the metabolic costs associated with exposure to contaminants by simply monitoring cardiac output.

CHAPTER I

INHIBITION OF OXYGEN CONSUMPTION BY PENTACHLOROPHENOL AND TETRACHLOROGUAIACOL IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

ABSTRACT

Rainbow trout (*Oncorhynchus mykiss*) were exposed for 24 h to concentrations representing 100 %, 50 % and 25 % of the 96 h-LC50 of pentachlorophenol (PCP) and tetrachloroguaiacol (TCG), and their oxygen consumption, cardiac output, heart rate and stroke volume were measured at regular intervals. When fish were exposed to the 96 h-LC50 of each chemical, their oxygen consumption either remained stable at basal levels (PCP), or increased by up to 130 % of basal levels (TCG). However, oxygen consumption decreased to about 50-60 % of basal levels when fish were exposed to concentrations of PCP and TCG representing 50 and 25 % of the 96 h-LC50 of each chemical. This decrease in oxygen consumption did not affect cardiac function since variations in cardiac output, heart rate and stroke volume were not significantly different from the control group. This study is the first to report an inhibition of oxygen consumption by PCP and TCG in fish. The response typically observed is an uncoupling of oxidative phosphorylation and an increase in oxygen consumption. This study shows that the effects of PCP and TCG on fish metabolism are similar and vary depending on the concentration.

1. INTRODUCTION

Pentachlorophenol (PCP) is a broad-spectrum biocide used principally as a wood preservative in the pressure treatment of utility poles. PCP acts primarily by abolishing the coupling of substrate oxidation to ATP synthesis in the mitochondria (Weinbach, 1954). It converts the potential energy generated within the electron transport system into heat instead of ATP, which causes substrate oxidation, uninhibited by respiratory control, to proceed at maximal rate (Hanstein, 1976). As a result, fish exposed to PCP commonly exhibit increases in oxygen consumption (Crandall and Goodnight, 1962; Peer *et al.*, 1983; Kim *et al.*, 1996) and reductions in stored lipids and growth (Holmberg *et al.*; Webb and Brett, 1973; Hickie *et al.*, 1989; Samis *et al.*, 1994).

Chloroguaiacols are another form of chlorinated phenolic compounds formed from lignin residues during chlorine bleaching of wood pulp (Rosemarin *et al.*, 1990). Much less is known about the toxic effects of chloroguaiacols, but their chemical similarity to chlorophenols has led many workers to suggest that they may exert similar biological effects. However, Yang and Randall (1997) recently failed to observe an increase in oxygen consumption when exposing rainbow trout (*Oncorhynchus mykiss*) to tetrachloroguaiacol (TCG), suggesting the presence of a different mode of action.

The objective of this study was to compare the effects of PCP and TCG on fish metabolism by exposing rainbow trout to concentrations representing 100 %, 50 % and 25 % of the 96 h-LC50 of each chemical. The effects of exposure to both chemicals on cardiac function were also evaluated by monitoring cardiac output and the two parameters controlling it: heart rate and stroke volume. According to the Fick principle, cardiac output and blood oxygen extraction are the only two parameters controlling oxygen consumption (Thorarensen *et al.*, 1996a). Thus, it is reasonable to suggest that the dramatic increase in oxygen consumption observed when PCP uncouples oxidative phosphorylation will affect cardiac function. However, no studies have yet examined the effects of PCP or TCG on the fish heart.

2. METHODS

2.1. Experimental animals

Rainbow trout (*Oncorhynchus mykiss*) were obtained from Rainbow Springs Hatchery (Thamesford, Ontario) and held at 12 °C in a 2 x 2 m tank. Average body weight and total length of the fish were 467 ± 177 g and 33.6 ± 4.3 cm (mean \pm S.D.). Fish were not fed for 48 h before use to avoid the influence of food on cardiac output and oxygen consumption.

2.2. Surgical procedures

Anesthesia was induced by immersing the fish in an aqueous solution of clove oil (120 mg·L⁻¹) as described by Anderson *et al.* (1997). Fish were maintained under anesthesia during surgery by continuously irrigating the gills with a solution of clove oil (30 mg·L⁻¹). A Doppler flow probe was installed around the ventral aorta of the fish to allow blood velocity to be measured, and cardiac output, heart rate, and stroke volume to be calculated from these data. To install the Doppler flow probe, the operculum and gills were gently lifted and held in an “open” position with a specially designed plastic pad. Connective tissue covering the ventral aorta was carefully teased away on a section approximately 4 mm long, and a cuff-type Doppler flow probe (Iowa Doppler Products, Iowa City, IA, 20 MHz) was placed around the blood vessel. The cuffs were selected to match the diameter of the vessel. Cuffs with an internal diameter of 1.3 to 1.7 mm were used. The lead wire from the probe was sutured to the fish’s skin at one location on the edge of the opercular cavity and at three locations on the body wall. The surgery lasted about 15 min.

2.3. Respirometry

Immediately after surgery, fish were placed in a 120-L blazka-type respirometer. Water was pumped from a 400-L reservoir through the respirometer, and back into the reservoir. The reservoir was oxygenated and continuously supplied with an inflow of fresh water at a rate of 3 L·min⁻¹. Water characteristics were as follows (mean \pm S.D.): total hardness, 329 ± 7.9 mg·L⁻¹ as CaCO₃; alkalinity, 278 ± 4.5 mg·L⁻¹ as CaCO₃; pH, 7.8 ± 0.1 ; and temperature ranged between 16.5 and 17.5 °C. A slow water current (0.5 m·s⁻¹) was maintained in the respirometer. In this current, the fish usually oriented themselves against the water flow but showed little swimming activity.

Dissolved oxygen concentration in the respirometer were measured in a parallel external circuit where water was pumped out of the respirometer and brought back into it after flowing over the electrode of a YSI oxygen meter (Yellow Springs, OH). Fish oxygen consumption was estimated by sealing the respirometer (by shutting valves on the inflow and outflow tubes) and measuring the decrease in dissolved oxygen concentration over a 15 min period.

2.4. Preparation of chemicals and exposure

Fish were let to recover from surgery overnight and the exposure started the following morning. Exposures to three concentrations of PCP (40, 20 and 10 $\mu\text{g}\cdot\text{L}^{-1}$) and TCG (370, 185 and 93 $\mu\text{g}\cdot\text{L}^{-1}$), and control group (no chemical added) were performed. These concentrations were selected to represent 100 %, 50 % and 25 % of the 96 h-LC50 of rainbow trout as reported for each chemical by Davis and Hoos (1975) and Johansen *et al.* (1994). No chemical was added to the water of the control group.

PCP was obtained from Sigma-Aldrich Canada (Oakville, Ontario), and TCG was purchased from Helix Biotech Corp. (Richmond, British Columbia). A stock solution of 20 $\text{g}\cdot\text{L}^{-1}$ was prepared for both chemicals by dissolving 2 g of PCP or TCG in a mixture of 4 mL of 100 % ethanol and 4 mL of 1 N NaOH, and completing the solution to 100 mL with distilled water. The stock solution was stored in the dark at 4 °C. At the beginning of exposure, an appropriate volume of the stock solution was added to the reservoir of the respirometer to bring the concentration of PCP or TCG to the desired nominal concentration. The concentration was maintained during exposure by the continuous addition (via peristaltic pump) of PCP or TCG stock solution of appropriate concentration to the 3 $\text{L}\cdot\text{min}^{-1}$ freshwater-renewal inflow to the reservoir.

2.5. Cardiac measurements

Blood velocity in the ventral aorta and oxygen consumption were measured simultaneously every 2 h during the first 12 h of exposure, and then after 24 h of exposure. Blood velocity was measured by recording Doppler signals at 20 Hz using a pulsed Doppler flowmeter

(545C-4, Department of Bioengineering, University of Iowa, IA). Cardiac output, heart rate and stroke volume were calculated from the Doppler signal for each 6 s of recording, and the average value for the 15 min recording was calculated for each parameter.

2.6. Statistical analysis

For each parameter measured, the variation in time resulting from each treatment was compared to the control group using the univariate test for repeated measurements available in the Systat statistical package. Thus, the probabilities indicated in the text illustrate the probability that the variation in time observed for a given treatment is different from the control group.

3. RESULTS

3.1. Control group

Average basal values obtained for oxygen consumption, cardiac output, heart rate, and stroke volume (mean \pm SE) in all the fish were $3.68 \pm 1.21 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$, $47.3 \pm 18.8 \text{ mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$, $74.8 \pm 13.7 \text{ beats}\cdot\text{min}^{-1}$, and $0.68 \pm 0.31 \text{ mL}\cdot\text{kg}^{-1}$, respectively. All the parameters remained stable at basal levels over the first 12 h in the control group (Fig. 1.1). After 24 h of exposure, oxygen consumption, cardiac output, and stroke volume were slightly elevated and heart rate was lowered, but none of these variations were statistically significant.

3.2. Exposure to PCP

Oxygen consumption increased with time when fish were exposed to $40 \mu\text{g PCP}\cdot\text{L}^{-1}$ (Fig. 1.2a), but the variation was not significantly different from the control group. However, oxygen consumption decreased to 49 and 58 % of basal levels when fish were exposed to 20 and $10 \mu\text{g PCP}\cdot\text{L}^{-1}$ (Fig. 1.2 b,c; $p = 0.002$ and 0.027 , respectively).

Cardiac output tended to increase with time in fish exposed to both 40 and $20 \mu\text{g PCP}\cdot\text{L}^{-1}$, (Fig. 1.2 a,b) but the increase was not significantly different from the one observed in the control group. The increase of cardiac output was the result of an elevation in stroke volume;

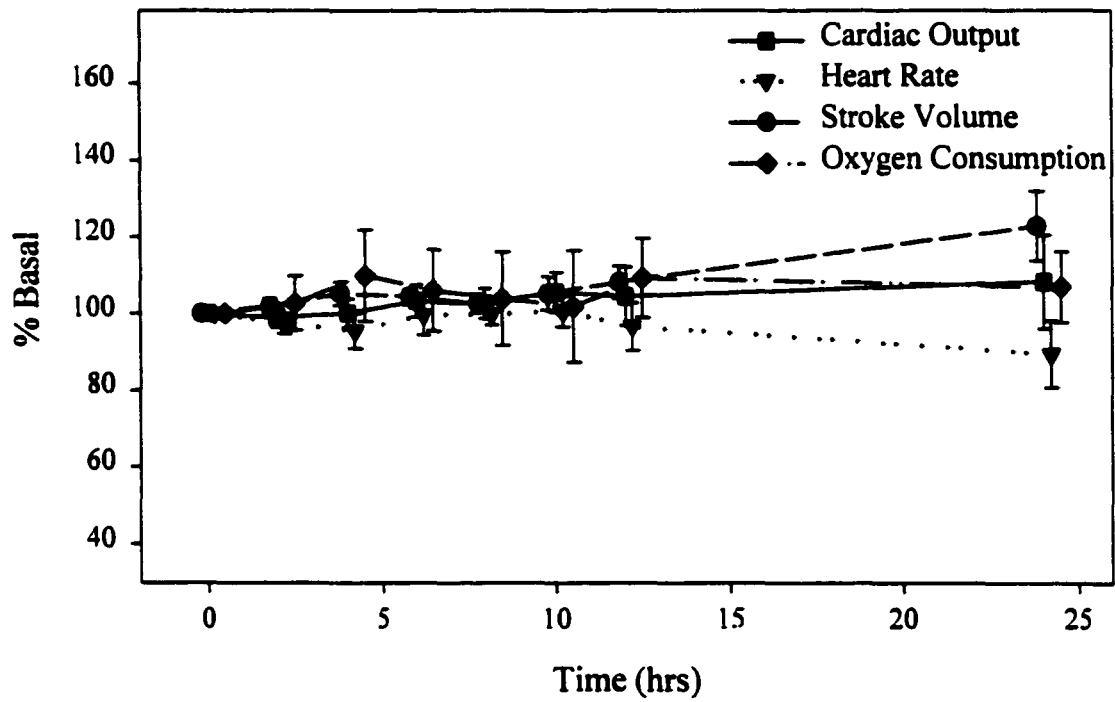


Fig. 1.1. Cardiac parameters and oxygen consumption (mean \pm S.E.) of control rainbow trout ($n = 7$) maintained in the respirometer for 24 h.

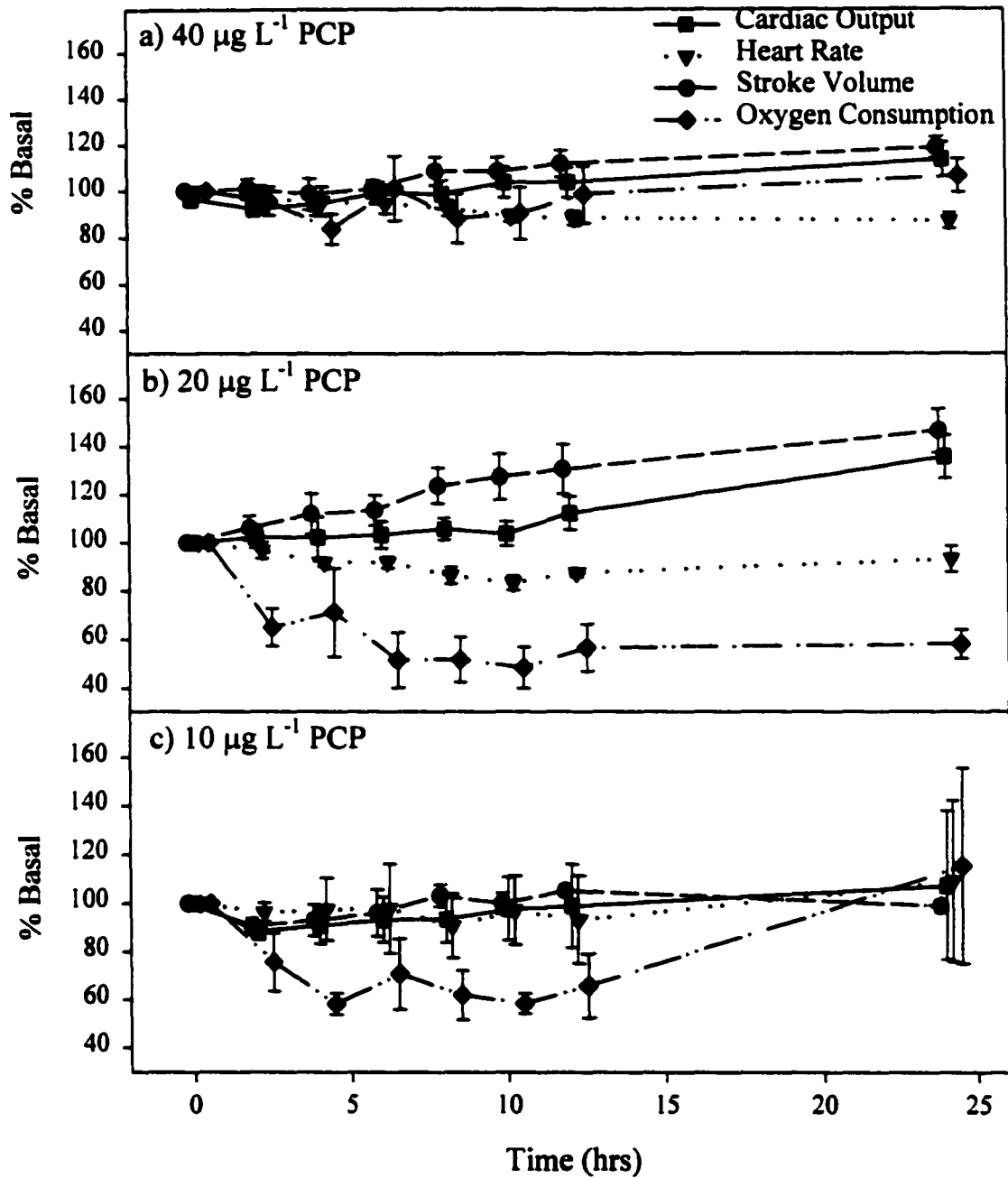


Fig. 1.2. Cardiac parameters and oxygen consumption (mean \pm S.E.) of rainbow trout exposed to 40 ($n = 8$), 20 ($n = 5$) and 10 ($n = 3$) $\mu\text{g}\cdot\text{L}^{-1}$ of PCP for 24 h.

heart rate remained stable (Fig. 1.2 *a,b*). Cardiac output, heart rate and stroke volume remained at basal levels throughout the exposure to 10 $\mu\text{g PCP}\cdot\text{L}^{-1}$ (Fig. 1.2*c*).

3.3. Exposure to TCG

Oxygen consumption increased up to 130 % of basal levels when fish were exposed to 370 $\mu\text{g TCG}\cdot\text{L}^{-1}$ (Fig. 1.3*a*; $p = 0.048$), but it decreased down to 64 and 53 % of basal levels in fish exposed to 185 and 93 $\mu\text{g TCG}\cdot\text{L}^{-1}$, respectively (Fig. 1.3 *b,c*; $p = 0.04$ and 0.001, respectively).

The increase in oxygen consumption observed at the highest concentration of TCG (Fig. 1.3*a*) was associated with a small but not statistically significant increase in cardiac output which resulted from a slight increase of both heart rate and stroke volume. Neither cardiac output, heart rate or stroke volume were significantly affected by the reduction of oxygen consumption observed at the two lowest concentrations of TCG (Fig. 1.3 *b,c*).

4. DISCUSSION

The action of uncoupling phosphorylation from oxidation was first described with chlorophenols, which is why these chemicals are best known as uncouplers of oxidative phosphorylation (Heytler, 1979). Until now, most studies with fish were in agreement with the idea that PCP uncouples oxidative phosphorylation, as they showed increases in oxygen consumption (Crandall and Goodnight, 1962; Peer *et al.*, 1983; Kim *et al.*, 1996), decreases in growth (Holmberg *et al.*, 1972; Webb and Brett, 1973; Hickie *et al.*, 1989; Samis *et al.*, 1994), or increases in the activity of various enzymes of the citric acid cycle, respiratory chain and the pentose phosphate shunt (Boström and Johansson, 1972).

However, the data obtained in this study are not consistent with an uncoupling of oxidative phosphorylation by PCP since oxygen consumption remained stable with 40 $\mu\text{g PCP}\cdot\text{L}^{-1}$, and decreased below basal levels with 20 and 10 $\mu\text{g PCP}\cdot\text{L}^{-1}$ (Fig. 1.2). Although it is much less discussed than its uncoupling action, PCP has also been found to inhibit oxygen consumption when present at high concentrations. Such an inhibition of oxidation has been observed in

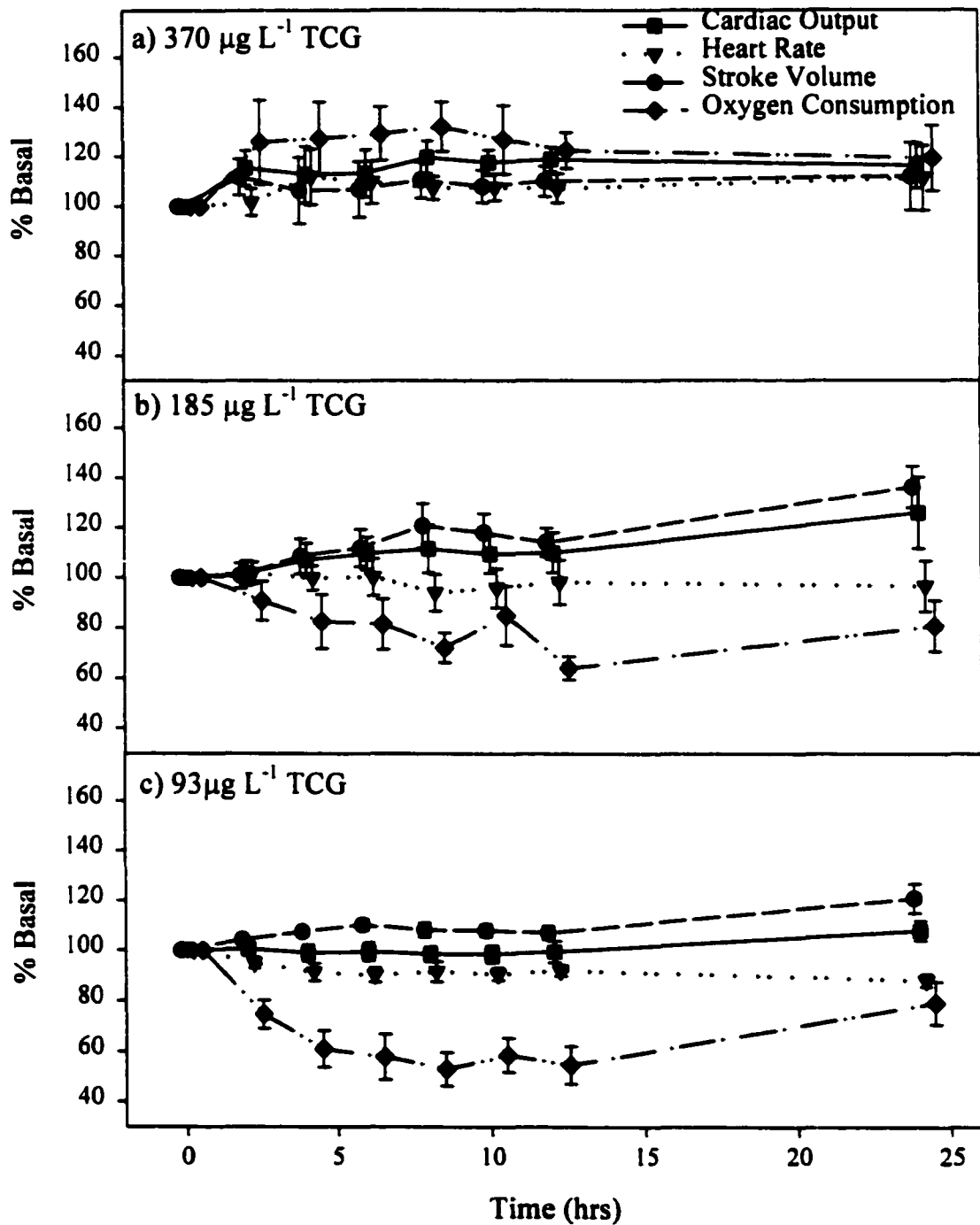


Fig. 1.3. Cardiac parameters and oxygen consumption (mean \pm S.E.) of rainbow trout exposed to 370 ($n = 8$), 185 ($n = 8$) and 93 ($n = 8$) $\mu\text{g}\cdot\text{L}^{-1}$ of TCG for 24 h.

oxidative phosphorylation (Chappell, 1964; Crestea and Gurban, 1964; Ishak *et al.*, 1972). However, other workers have concluded that a direct inhibition of succinate dehydrogenase by PCP is more likely (Wilson and Merz, 1969; Stockdale and Selwyn, 1971).

This study is the first to create conditions that induce inhibition of oxygen consumption by PCP in fish. However, only a small number of studies have previously measured oxygen consumption in fish exposed to PCP, and they have employed either very high concentrations (McKim *et al.*, 1987) or sublethal exposures (Crandall and Goodnight, 1962; Peer *et al.*, 1983; Kim *et al.*, 1996; Farrell *et al.*, 1998). It is likely that the inhibition observed in the present study results from the same mechanisms previously described in studies on other animals. However, the absence of an inhibition at the highest concentration of PCP is contradictory with these studies since they usually observe a stimulation of oxygen consumption at low concentrations and an inhibition at high concentrations. A possible explanation could be that there are three levels to the effects of PCP on fish metabolism. PCP could, consistent with studies on other organisms, uncouple oxidative phosphorylation at low concentrations and inhibit oxidation at higher concentrations. However, at acutely lethal concentrations, the release of catecholamines and its stimulating effects on metabolism could mask the inhibition of respiration created by PCP and cause oxygen consumption to remain stable, or even increase. Evidence for a release of catecholamines in the presence of PCP have been previously reported (Boström and Johansson, 1972) and could explain the stable levels of oxygen consumption seen in the present study when fish were exposed to a concentration of PCP equivalent to the 96 h-LC50.

Cardiac output did not vary in a manner significantly different from the control group despite the decrease of oxygen consumption to around 50 % of basal levels when fish were exposed to 10 and 20 $\mu\text{g PCP}\cdot\text{L}^{-1}$. This is not surprising since the arterial oxygen content of the fish most likely remained elevated as a result of the impairment of oxygen utilization by PCP, and there was therefore no stimulus or need for an increase in cardiac output.

The effects of TCG on oxygen consumption and cardiac function were much the same as the ones observed with PCP (Fig. 1.3). Indeed, fish exposed to the two lowest concentrations of

TCG exhibited a decrease in oxygen consumption that was not associated with variation in cardiac output. As with PCP, this inhibition of oxygen consumption was not observed when fish were exposed to a concentration of TCG equivalent to the 96 h-LC50. However, in the case of TCG, oxygen consumption and, to a smaller extent, cardiac output actually increased over basal levels when fish were exposed to the 96h-LC50.

In conclusion, this study has shown that PCP and TCG act in a similar fashion and can inhibit the oxygen consumption of fish when present in appropriate concentrations. Cardiac function is not affected when such an inhibition of oxygen consumption occurs. However, the response of the heart when PCP uncouples oxidative phosphorylation remains to be examined as the concentrations used in the present study did not result in such an effect.

CHAPTER II

INCREASE OF HEART RATE WITHOUT ELEVATION OF CARDIAC OUTPUT IN ADULT ATLANTIC SALMON (*SALMO SALAR*) EXPOSED TO ACIDIC WATER AND ALUMINIUM*

ABSTRACT

Adult Atlantic salmon (*Salmo salar*) were exposed for 48 h to water from acidified (pH 5.2) Fossbekk River (Norway), with and without 125 µg aluminium (Al)·L⁻¹ added as AlCl₃, and to water from circumneutral (pH 6.7) Ims River (Norway) (controls). Cardiac output, heart rate, and stroke volume were monitored throughout the exposure period with Doppler flow probes placed around the ventral aorta of the fish. Fish exposed to Fossbekk River water without added Al showed few physiological disturbances. When 125 µg Al·L⁻¹ was added to Fossbekk River water, most of the fish died before the end of the 48-h exposure period, and a large elevation in heart rate was observed together with a decrease in plasma chloride concentrations and an increase in haematocrit, plasma glucose and plasma cortisol levels. Cardiac output was maintained at basal levels during the first 24 h of exposure because the tachycardia was accompanied by a concomitant reduction of stroke volume. Signs of arrhythmia appeared after 32 h of exposure and were associated with a further decrease in stroke volume that caused cardiac output to decrease below basal levels. The incapacity of the tachycardia to elevate cardiac output and the subsequent death of the fish suggest that this response to low pH and Al is more of a maladaptation reaction than a compensatory or adaptative reaction.

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1. INTRODUCTION

Gradual losses or reductions of fish populations have been correlated with water acidification in a number of studies from Scandinavia and eastern North America (Beamish and Harvey, 1972; Hesthagen and Hansen, 1991). Acidification of freshwater causes a leaching of aluminum (Al) from soil and sediments (Cronan and Schofield, 1979), and the mortality of fish appears to be determined primarily by Al rather than acidity (Baker and Schofield, 1982).

Inorganic monomeric Al precipitates on fish gills and causes ionoregulatory and respiratory disturbances (Exley *et al.*, 1991). Fish exposed to Al in acid water exhibit a loss of plasma ions (sodium, chloride), a decrease in blood O₂ tension and pH, and an increase in blood CO₂ tension and lactate concentration (Neville, 1985; Witters, 1986; Malte and Weber, 1988; Sayer *et al.*, 1991; Rosseland and Staurnes, 1994). Both the ionic loss and the hypoxia associated with an exposure to Al and low pH are likely to induce compensatory responses by the cardiovascular system. Hypoxia is indeed known to modify fish heart rate and stroke volume (Randall, 1982), and the ionic loss causes an increase in arterial pressure and cardiac workload that may result in a circulatory collapse (Wood, 1989). Despite its major adaptive and compensatory functions, only a few studies have been concerned with the effects of Al and low pH on the fish cardiovascular system. The few studies conducted (Milligan and Wood, 1982; Giles *et al.*, 1984; Laitinen and Valtonen, 1995) have also only examined heart rate, although it is known that many fish species increase their cardiac output mainly through an increase in stroke volume rather than heart rate (Farrell and Jones, 1992).

The objective of the present study was to monitor cardiac output, heart rate, and stroke volume as well as the changes in blood chemistry of adult Atlantic salmon (*Salmo salar*) acutely exposed to Al and low pH. Rapid reductions of water pH executed in the present study can be experienced by fish during heavy rain and snowmelt (Reader and Dempsey, 1989). The Atlantic salmon may be particularly affected by such acidic episodes, since it is one of the most sensitive salmonid species to Al and low pH (Rosseland and Skogheim, 1984).

2. METHODS

2.1. Experimental animals

Adult Atlantic salmon reared at the research station of the Norwegian Institute for Nature Research (NINA) in Ims, southern Norway, were used for this study. Average body weight and total length of the fish (mean \pm SD) were 1518 ± 381 g and 51.5 ± 3.9 cm, respectively. Fish were starved for at least 72 h before use to avoid influence of food on blood parameters and cardiac output.

2.2. Surgical procedures

Fish were anaesthetized by immersion in an aqueous solution of clove oil ($50 \text{ mg}\cdot\text{L}^{-1}$) as described by Anderson *et al.* (1997). Anaesthetized fish were placed supine in a wet-sponge operating sling and quickly fitted with a dorsal aortic cannula (PE 50, Intramedic, Clay-Adams, Sparks, Md.) as described by Smith and Bell (1964). The cannula was filled with 0.9% NaCl saline containing $25 \text{ IU heparin}\cdot\text{mL}^{-1}$ (Apothekernes Laboratorium A.S., Oslo, Norway). After being cannulated, the fish were turned on their side and their gills were irrigated with cold water while a Doppler flow probe was installed around their ventral aorta.

To install the Doppler flow probe, the operculum and gills were gently lifted and held in an “open” position with a specially designed plastic pad. Connective tissue covering the ventral aorta was carefully teased away on a section about 4 mm long, and a cuff-type Doppler flow probe (20 MHz, Iowa Doppler Products, Iowa City, Iowa) was placed around the blood vessel. The cuffs were selected to match the diameter of the vessel. Cuffs with an internal diameter of 1.5–2.0 mm were used. The lead wire from the probe was sutured to the fish’s skin at one location on the edge of the opercular cavity and at three locations on the body wall.

2.3. Experimental protocol

Immediately after surgery, fish were placed in a submerged black Plexiglas box (115 cm long \times 20 cm wide \times 25 cm high; 1 fish/box). The box had a black Plexiglas lid to minimize light entry and plastic grids at both ends to facilitate water circulation. Fish were allowed to recover from surgery overnight and the exposure began the morning of the following day.

The physical and chemical properties of the water used in the three experimental treatments are described in Table 2.1. The control group was maintained in water from Ims River, a Norwegian river with circumneutral pH. A second group of fish was exposed to water from Fossbekk River, an acidified river of southern Norway, while a third group of salmon was exposed to Fossbekk River water with a nominal concentration of $125 \mu\text{g Al}\cdot\text{L}^{-1}$ added as AlCl_3 . One flow-through tank of 1 m^3 (1000 L) containing four Plexiglas boxes was assigned to each group, so 12 fish (four per group) were exposed simultaneously during each experiment. Exposures were replicated six times between 11 October and 4 November 1997.

The fish were maintained in Ims River water for at least 72 h before the beginning of the experiment, and they were all placed back in Ims River water after the surgery and during the overnight recovery. At the beginning of the exposure, the inflow of Ims River water was turned off and replaced by an inflow of Fossbekk River water in both acid water treatment tanks (Fossbekk River water and Fossbekk River water + Al). The flow of Ims River water was maintained in the control tank. It took about 30 min for the water in the acid water treatment tanks to reach the pH of the incoming Fossbekk River water. It was only at this moment that the Fossbekk River water inflow was turned off in the high-Al concentration treatment tank, and Fossbekk River water with added Al was pumped in a closed system from a 3000-L reservoir tank. The flow of pure Fossbekk River water was maintained in the second acid water treatment tank.

The pH was first decreased before adding Al to avoid the formation of highly toxic Al polymers that occurs when neutral water and Al-rich acid water are mixed (Verbost *et al.*, 1995; Witters *et al.*, 1996). To ensure that the water quality of the closed system was not degrading during the exposure, the water coming out of the holding tank was oxygenated before going back to the reservoir tank, and water from a new reservoir tank was used after 24 h of exposure. Dissolved O_2 concentrations were also monitored, and no decrease was observed during the exposure period. The water in both reservoir tanks was prepared at least 18 h before being used to give time for the different forms of bound and

Table 2.1. Chemistry and Al concentrations (mean±S.E.) of the water used in the three treatments ($n = 8$).

Treatment	Ims River	Fossbekk River	Fossbekk+Al
pH	6.71±0.10	5.15 ±0.05	5.15±0.04
Temperature (oC)	8.8±0.2	7.1±0.2	10.5±0.2
Turbidity (FTU)	0.69±0.39	1.21±0.13	1.51±0.10
Color (mg Pt/L)	13.5±2.27	32.75±2.90	30.8±2.3
Conductivity (µS/cm)	72.5±0.44	48.1±1.17	49.7±0.64
Alkalinity (µequiv/L)	143±6	3.32±1.26	4.99±1.39
Ca (mg/L)	3.66±0.08	1.07±0.04	1.08±0.02
Nitrate (µg/L)	658±46	47.0±9.1	45.3±7.4
T-Al (µg/L)	50±35	237±20	330±12
Tm-Al (µg/L)	13.4±1.9	112±7	153±4
Om-Al (µg/L)	13±2	78.6±5.9	94.6±4.2
Im-Al (µg/L)	0.38±1.06	33.1±3.0	58.8±5.3
Pc-Al (µg/L)	36.6±34.1	125±18	177±13

T-Al=Total Al, Tm-Al=Total Monomeric Al, Om-Al=Organic Monomeric Al, Im-Al=Inorganic Monomeric Al, Pc-Al=Polymeric Colloidal Al

ionic Al to stabilize. The tanks receiving Ims River water and pure Fossbekk River water were both in a flow-through open system.

Basal levels of cardiac output and blood chemistry were measured by recording the Doppler signal for 10 min and taking a blood sample (0.3 mL) through the cannula with a 1-mL heparinized syringe immediately before the beginning of the treatment. The exposure lasted 48 h. Blood samples (0.3 mL) were taken through the cannula each 12 h, and blood flow in the ventral aorta was measured by recording the Doppler signal for 10 min each 4 h during the first 12 h of exposure, each 4 h from 24 to 36 h of exposure, and 48 h after the beginning of the exposure. Doppler signals were recorded with a pulsed Doppler flowmeter (545C-4, Department of Bioengineering, University of Iowa, Iowa City, Iowa) connected to a PCMCIA data acquisition card (DAQ-Card-AI-16E-4, National Instruments, Austin, Tex.). Cardiac output, heart rate, and stroke volume were automatically calculated from the Doppler signal for each 5 s of recording with a program written with LabView (National Instruments), and the average value for the 10-min recording period was calculated for each parameter. At the end of the exposure, the first gill arch was collected and frozen at -20°C until gill Al content was measured.

2.4. Analytical techniques

Total water Al was fractionated into organic monomeric, inorganic monomeric and polymeric colloidal Al using a modified version of Driscoll's (1984) method. Gills were freeze-dried, weighed, and digested in a solution of HNO_3 and H_2O_2 before total Al content was measured by inductive coupled plasma emission spectroscopy. Plasma glucose was measured on whole blood immediately after sampling using a Medisense Precision QID sensor, and haematocrit was read after centrifugation of blood in capillary tubes (5 min, 3500 rpm). The plasma of the remaining blood was separated by centrifugation (5 min, 5000 rpm) and frozen. Plasma chloride concentrations were measured by coulometric titration using a Radiometer CMT 10 chloride titrator. Total plasma cortisol concentrations were estimated by radioimmunoassay as described by Simensen *et al.* (1978) and modified for fish by Olsen *et al.* (1992). The detection limit of the assay was $1.33 \pm 0.73 \text{ nmol}\cdot\text{L}^{-1}$. Intraassay variation was less than 4.2 %, interassay variation was less than 8.3 %, and nonspecific binding varied

from 0.6 to 2.0 % of the total activity. Recovery was 93, 96, 100, and 97 % at 4, 17, 34, and 69 nmol cortisol·L⁻¹, respectively.

2.5. Statistical analysis

For each parameter measured, the statistical significance of the variation in time observed within a group was tested using the univariate test for repeated measurements in the PROC GLM function of the SAS statistical package. The significance levels were determined using *F* values adjusted with the Huynh and Felt epsilon to protect against a violation of the assumption of compound symmetry. Gill Al contents were compared using the Kruskal–Wallis test and Dunn’s test for multiple comparisons.

3. RESULTS

3.1. Gill Al content

The amount of Al measured in the gills of the fish after 48 h of exposure (Fig. 2.1) was related to the concentration of Al present in the water (Table 2.1). Fish exposed to Fossbekk River water + 125 µg Al·L⁻¹ had significantly more Al in their gills than fish exposed to both Ims River water and Fossbekk River water only, and the gill Al content of fish exposed to Fossbekk River water was significantly higher than the gill Al content of fish exposed to Ims River water.

3.2. Heart function

Basal values of cardiac output, heart rate, and stroke volume (mean ± SE) were 10.6 ± 1.8 mL·min⁻¹·kg⁻¹, 46.6 ± 2.7 beats·min, and 0.24 ± 0.02 mL·kg, respectively. Cardiac output of the fish from the control group (Ims River water) increased gradually (*p* = 0.0001) during the exposure, reaching a maximum of 152 % of basal level after 48 h of exposure (Fig. 2.2a). This increase in cardiac output resulted entirely from an increase in stroke volume (189 % of basal level after 48 h, *p* = 0.0001), since heart rate showed a slight but significant (*p* = 0.0008) decrease throughout the exposure period (85 % of basal after 48 h).

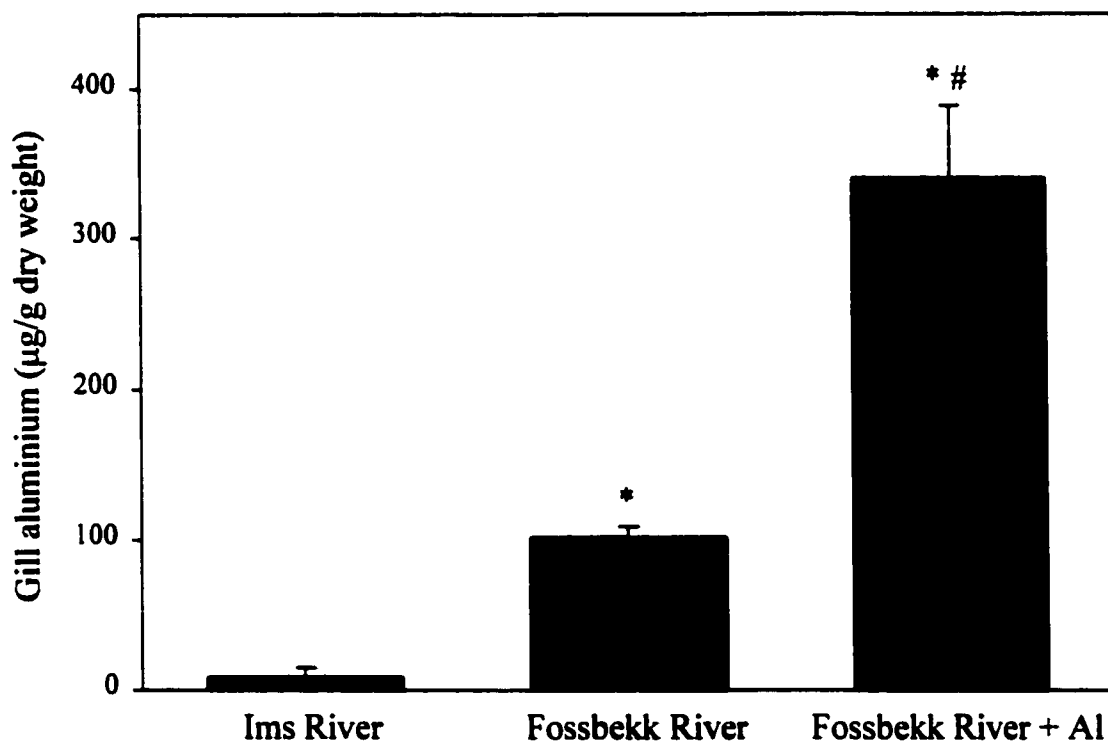


Fig. 2.1. Gill Al concentrations in Atlantic salmon exposed for 48 h to water from Ims River ($n = 15$), Fossbekk River ($n = 19$), or Fossbekk River + $125 \mu\text{g Al}\cdot\text{L}^{-1}$ ($n = 15$). *Significantly different from control ($p < 0.05$); # significantly different from Fossbekk River ($p < 0.05$).

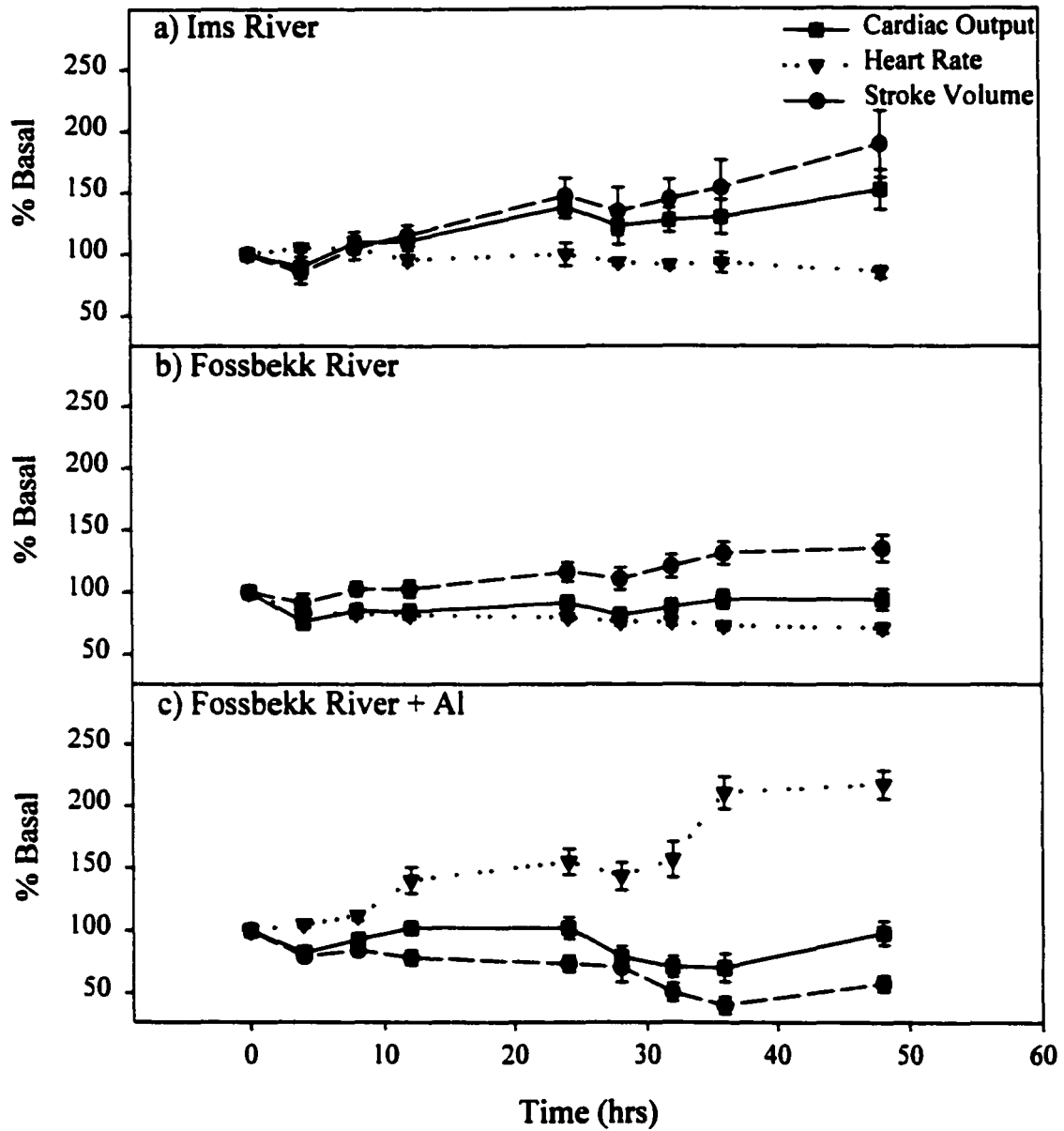


Fig. 2.2. Cardiac output, heart rate, and stroke volume in Atlantic salmon exposed for 48 h to (a) Ims River water ($n = 13$), (b) Fossbekk River water ($n = 13$), or (c) Fossbekk River water + $125 \mu\text{g Al}\cdot\text{L}^{-1}$ ($n = 17$).

Fish exposed to Fossbekk River water exhibited a slight decrease in cardiac output to about 80 % of basal level 4 h after the beginning of the exposure (Fig. 2.2*b*). This decrease in cardiac output was mainly the result of a reduction in heart rate. Stroke volume increased gradually afterward ($p = 0.0001$), reaching 125 % of basal level after 48 h of exposure. The increase in stroke volume did not, however, significantly increase cardiac output (p of time effect = 0.1449), since heart rate continued to decrease during the rest of the exposure period ($p = 0.0001$), reaching 66 % of basal level after 48 h of exposure.

Only three of the 17 fish exposed to Fossbekk River water + Al survived the total 48-h exposure period. The majority of the fish died between 36 and 48 h of exposure. An increase in heart rate (140–150 % of basal level) was apparent 12 h after the beginning of the exposure (Fig. 2.2*c*; p of time effect = 0.0001), but cardiac output remained at basal values because stroke volume was reduced to 75 % of basal level (p of time effect = 0.0001). After 32 h of exposure (i.e., about 4 h before fish typically started to die), signs of arrhythmia appeared (Fig. 2.3) and resulted in a further increase in heart rate to 200 % of basal level after 36 h of exposure. Cardiac output, however, decreased to 70 % of basal level (p of time effect = 0.0191) because of a concomitant reduction of stroke volume to 40 % of basal level (Fig. 2.2*c*).

3.3. Blood chemistry

Haematocrit (Fig. 2.4*a*) of the fish exposed to Fossbekk River water + Al was greatly increased (p of time effect = 0.0005) after 36 and 48 h of exposure (about 37 % after 36 and 48 h of exposure compared with 27 % at the beginning of the exposure). In contrast, the haematocrit of the fish exposed to Ims River water (controls) and Fossbekk River water decreased slightly ($p = 0.0138$ and 0.0427, respectively) throughout the exposure period due to repetitive blood sampling (from 27 to 20 % in Ims River water and from 30 to 26 % in Fossbekk River water).

Plasma chloride concentrations (Fig. 2.4*b*) of fish exposed to Fossbekk River water and Fossbekk River water + Al showed a significant decrease throughout the exposure period ($p = 0.0052$ and 0.0003, respectively). The loss of plasma chloride was greater when Al was

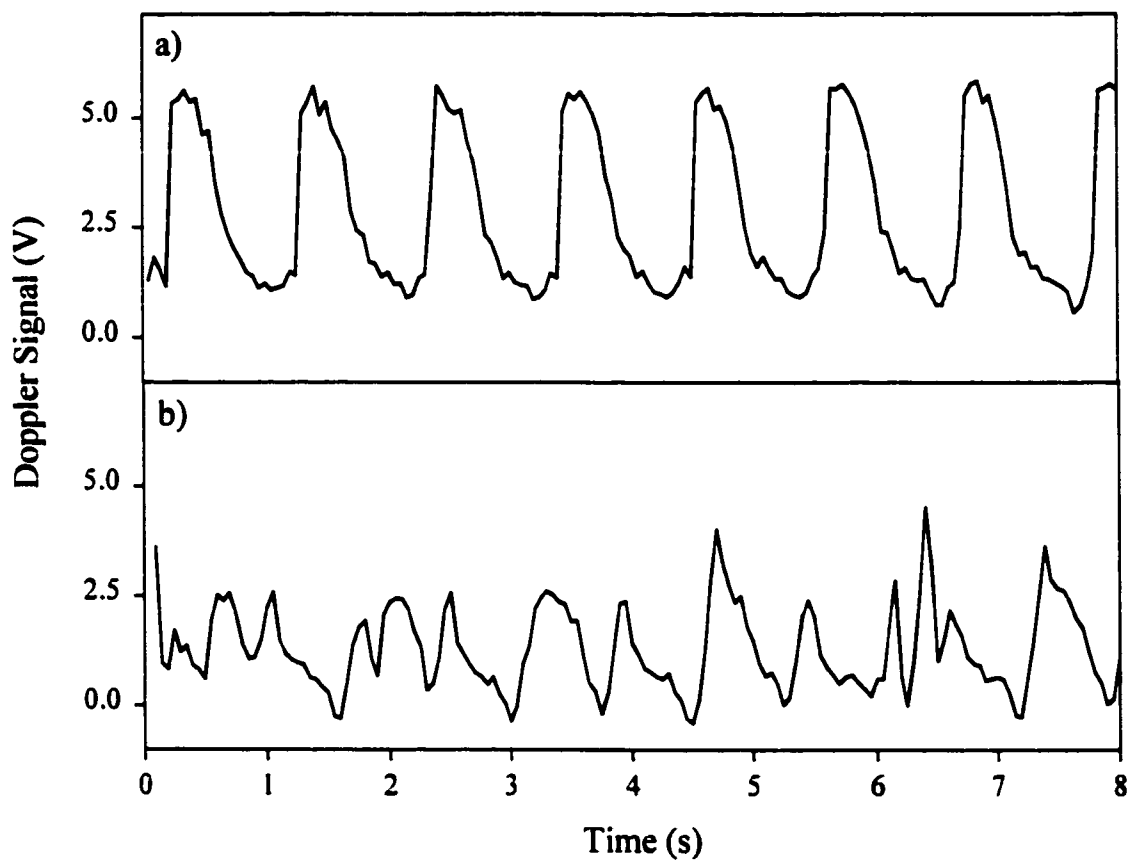


Fig. 2.3. Doppler signal of an individual Atlantic salmon (*a*) before the beginning of the exposure (heart rate = 53.3 beats·min⁻¹) and (*b*) after 36 h of exposure to Fossbekk River water + 125 µg Al·L⁻¹ (heart rate = 102.9 beats·min⁻¹). Signs of arrhythmia are clearly visible toward the end of the exposure.

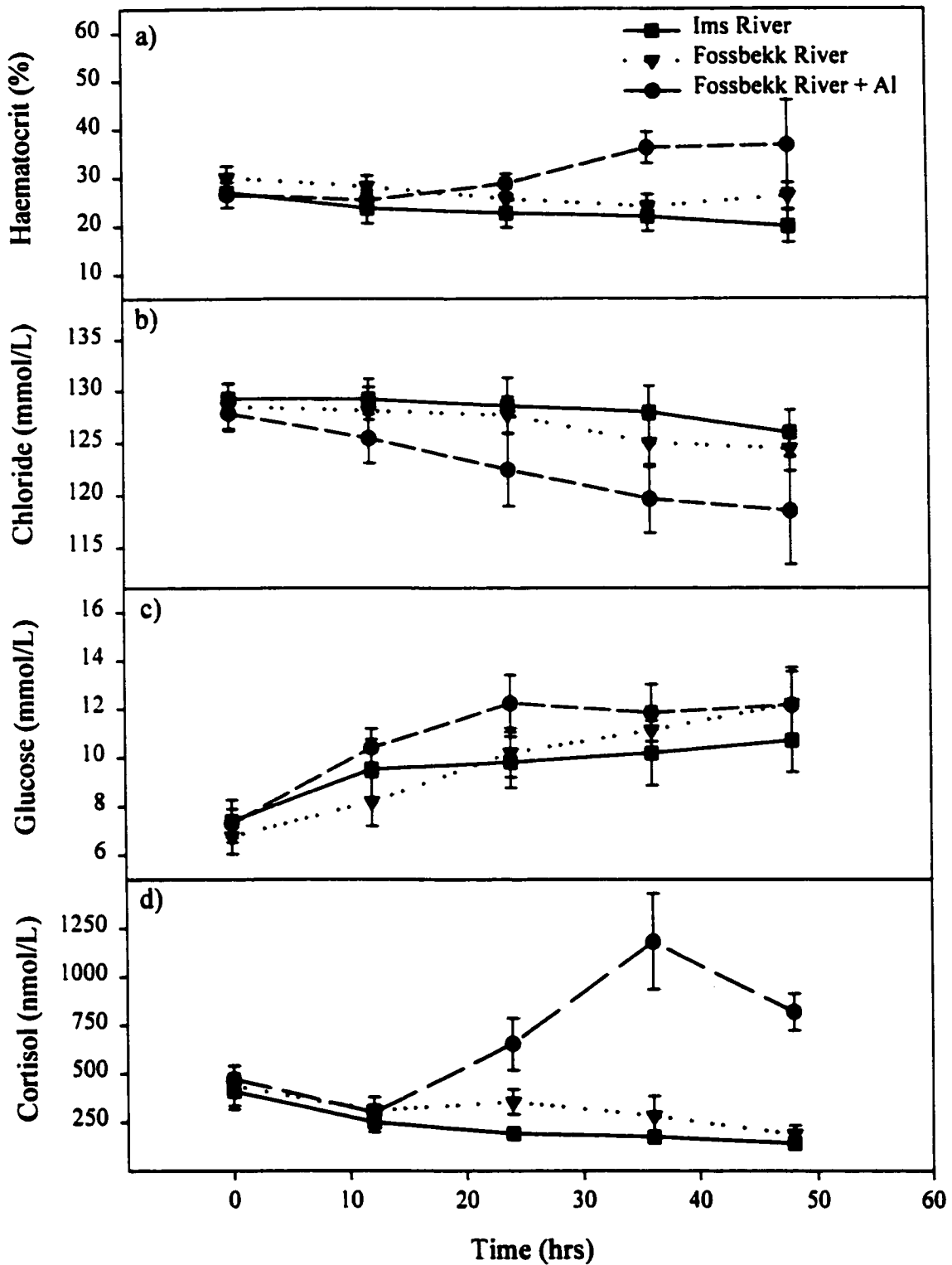


Fig. 2.4. (a) Haematocrit, (b) plasma chloride, (c) plasma glucose, and (d) plasma cortisol in Atlantic salmon exposed for 48 h to water from Ims River ($n = 13$), Fossbekk River ($n = 11$), or Fossbekk River + $125 \mu\text{g Al}\cdot\text{L}^{-1}$ ($n = 11$).

added: from 128 to 119 mmol·L⁻¹ in Fossbekk River + Al, and from 128 to 124 mmol·L⁻¹ in Fossbekk River water alone. Plasma chloride concentrations of fish exposed to Ims River water (controls) did not vary significantly with time (*p* of time effect = 0.3410).

All treatments caused significant increases in plasma glucose concentrations (Fig. 2.4c). Plasma glucose of fish exposed to Fossbekk River water + Al reached 12 mmol·L⁻¹ after 24 h of exposure (*p* of time effect = 0.0001) and remained stable at this level until the end of the exposure. Plasma glucose also reached 12 mmol·L⁻¹ (*p* of time effect = 0.0006) in fish exposed to Fossbekk River water alone, but the increase was slower in these fish, and this concentration was only reached after 48 h of exposure. Plasma glucose concentrations of fish exposed to Ims River water (controls) remained elevated at 8–9 mmol·L⁻¹ throughout the exposure period (*p* of time effect = 0.0002).

Plasma cortisol concentrations (Fig. 2.4d) of fish exposed to Fossbekk River water + Al decreased during the first 12 h of exposure (from 476 to 312 nmol·L⁻¹) but subsequently exhibited a substantial increase, reaching a maximum of 1088 nmol·L⁻¹ after 36 h of exposure (*p* of time effect = 0.0084). Plasma cortisol concentrations of fish exposed to Fossbekk River water decreased in a similar way during the first 12 h of exposure, remained stable at about 315 nmol·L⁻¹ from 12 to 24 h of exposure, and then kept on decreasing to reach 187 nmol·L⁻¹ at the end of the exposure (*p* of time effect = 0.0014). In fish exposed to Ims River water (controls), plasma cortisol decreased from 408 to 192 nmol·L⁻¹ during the first 24 h of exposure but only decreased from 192 to 141 nmol·L⁻¹ during the second half of the exposure (*p* of time effect = 0.0146).

4. DISCUSSION

Adult Atlantic salmon were exposed to low pH and Al for 48 h to examine the compensatory adaptations exhibited by the heart when ionoregulatory and respiratory disturbances are induced. Exposure to both Fossbekk River water and Fossbekk River water + 125 µg Al·L⁻¹ created a dose-dependent accumulation of Al on fish gills. However, major physiological

alterations were only observed in the high-AI treatment, and the exposure to Fossbekk River water alone caused only minor disturbances.

Haematocrit and plasma chloride slightly decreased with time in fish exposed solely to Fossbekk River water, but these effects are likely attributable to repetitive blood sampling, since they were also observed in the control group. Cardiac output was also decreased below basal levels during the entire exposure, but this was most likely related to the fact that Fossbekk River water temperature was always 2°C lower than Ims River water, which was present during the recording of basal levels. A reduction in water temperature is indeed known to cause a decrease in cardiac output through a lowering of heart rate (Farrell and Jones, 1992), and Laitinen and Valtonen (1995) observed a similar increase in the heart rate of brown trout (*Salmo trutta*) when water temperature was increased by only 3°C.

Fish exposed to Fossbekk River water also differed from the control group (Ims River) by the absence of a large increase in cardiac output during the last 24 h of exposure. This increase in cardiac output in the control group most likely indicates that the holding system was causing discomfort to the fish and inducing a stress response. Indeed, although plasma cortisol levels decreased during the first 24 h of exposure in the control group, they afterward stabilized at about 170 nmol·L⁻¹, which is much higher than the basal levels typically observed in salmonids (Wendelaar Bonga, 1997). The time course of plasma cortisol probably illustrates an initial recovery from the major stress of surgery followed by the maintenance of a lower level of stress caused by the confinement of the fish in the Plexiglas boxes. The maintenance of elevated plasma glucose levels is additional evidence of a stress response in the control group.

The smaller amplitude of the increase in cardiac output and stroke volume observed in the fish exposed to Fossbekk River water only may be due to the slightly colder temperatures (about 2°C lower) of this river, since, as mentioned previously, the difference in temperature was important enough to cause a reduction in cardiac output. There is indeed no reason to believe that the fish exposed to Fossbekk River water were less stressed than the fish exposed to Ims River water (controls), since their plasma cortisol and glucose levels were slightly

higher than those of the control group. The presence of higher levels of plasma cortisol and glucose in the fish exposed to Fossbekk River water indicates that the acid water created some additional stress to the fish.

Thus, Fossbekk River water alone caused only minor disturbances to the fish, although it contained $237 \mu\text{g Al}\cdot\text{L}^{-1}$. The low toxicity of the Al is most likely related to the high dissolved organic matter content of Fossbekk River (as suggested by water color), which binds Al and reduces the proportion of toxic ionic species. However, when $125 \mu\text{g Al}\cdot\text{L}^{-1}$ was added to Fossbekk River water, the concentration of toxic inorganic monomeric Al was raised enough to greatly increase the toxicity of the acidic water and induce death within 48 h in most of the fish. Death was preceded by large increases in plasma glucose and cortisol levels (Fig. 2.4) typical of acute exposures to toxic chemicals (Fu *et al.*, 1990; Hontela *et al.*, 1996). An increase in haematocrit and a reduction in plasma chloride concentrations were also observed, indicating an alteration of ionoregulatory mechanisms. Heart rate was increased considerably, but cardiac output remained stable and even decreased toward the end of the exposure because of a reduction in stroke volume. Similar increases in heart rate have been previously observed in rainbow trout (*Oncorhynchus mykiss*) and brown trout exposed to acid water with or without Al (Milligan and Wood, 1982; Giles *et al.*, 1984; Laitinen and Valtonen, 1995). This is, however, the first study to show that cardiac output itself does not increase during an exposure to low pH and Al, in spite of increased heart rate.

In fish, an increase in heart rate is an inefficient way to elevate cardiac output because the reduction in filling time and force of contraction involved provoke a reduction in stroke volume (Farrell and Jones, 1992). The induction of tachycardia is thus a rather strange strategy for a fish to cope with the increase in metabolic rate observed by others during an exposure to acidity and Al (Neville, 1985; Malte, 1986; Wood and McDonald, 1987), since the fish need to increase their cardiac output to maintain an elevated metabolic rate. Fish exposed to acid water and Al have also been shown to experience hypoxia (Malte, 1986; Malte and Weber, 1988; Playle *et al.*, 1989), and the typical response of a fish to hypoxia is usually a bradycardia rather than a tachycardia. The bradycardia results in a longer residence

time of the blood in the gill lamellae, which allows the diffusion of oxygen into the blood to proceed further towards completion (Satchell, 1991).

As the heart was trying to maintain tachycardia in hypoxic conditions, blood pressure and cardiac workload must have increased because of the elevation in blood viscosity associated with the increase in haematocrit observed (Milligan and Wood, 1982). The demand on the heart must then have been very important, and the appearance of arrhythmia a few hours before death likely resulted from myocardial hypoxia brought about by the maintenance of tachycardia under hypoxic and high blood pressure conditions. Associated with the appearance of arrhythmia were a further decrease in stroke volume and a reduction in cardiac output below basal levels, which eventually led to cardiac failure.

It is thus possible to question the benefits obtained by the fish from the tachycardia observed, and this phenomenon even appears to illustrate more of a maladaptation reaction than a compensatory or adaptative reaction. Milligan and Wood (1982) showed that the increase in heart rate was the result of increased adrenergic stimulation to the heart and not a withdrawal of vagal cholinergic tone in rainbow trout exposed to low pH without Al. A similar increase in heart rate was also observed in rainbow trout exposed to a lethal concentration of copper and was proposed as part of a general acute toxicity syndrome that may be common to many toxic metals (Wilson and Taylor, 1993).

In conclusion, this study has shown that the exposure to water from the acidified Fossbekk River caused few physiological disturbances to adult Atlantic salmon but that the addition of $125 \mu\text{g Al}\cdot\text{L}^{-1}$ led to death within 48 h. Death was preceded by an increase in plasma glucose, cortisol, and haematocrit, together with a reduction in plasma chloride concentrations and an arrhythmia. The arrhythmia caused cardiac output to decrease below basal levels and followed a long-lasting tachycardia during which cardiac output was maintained at basal levels.

CHAPTER III

SUBLETHAL CONCENTRATIONS OF ALUMINIUM IN ACIDIC WATER REDUCE CARDIAC OUTPUT OF ADULT ATLANTIC SALMON (*SALMO SALAR*) MAINLY THROUGH REDUCED STROKE VOLUME

ABSTRACT

Adult Atlantic salmon (*Salmo salar*) were exposed for 48 h to water from the acidified (pH 5.2) Fossbekk River (Norway) with a nominal concentration of either 50, 75 or 100 $\mu\text{g}\cdot\text{L}^{-1}$ of Al added as AlCl_3 , or to circumneutral water (pH 6.6) from Ims River (Norway). Cardiac function and blood chemistry were monitored throughout the exposure period to assess the effects of sublethal exposures to acidic water and Al on the heart. The exposure to both the intermediate and the high concentrations of Al caused a reduction of cardiac output to about 75 % of basal levels, primarily through a decrease in stroke volume. Cardiac output returned to basal levels within 36 h when the water pH was raised to 5.8 by adding CaCO_3 at the end of the 48 h exposure. By that time, plasma concentrations of sodium and chloride had started to return to basal levels and haematocrit was back to normal, but none of the other altered blood parameters (pH, HCO_3^- , potassium, glucose) had started to recover. The comparative dynamics of the alterations in blood chemistry and cardiac output suggest that the decrease in cardiac output was the result of the increase in haematocrit and the reduction in plasma volume resulting from the osmotic shifts associated with the ionic losses.

1. INTRODUCTION

It is now well established that aluminium (Al) in acidic soft water causes ionoregulatory and respiratory disturbances in fish due to the deposition of Al on the gills (Playle and Wood, 1991; Poléo, 1995). Fish exposed to Al in acidic water exhibit a loss of plasma sodium and chloride, a decrease in blood O₂ tensions and pH, and an increase in blood CO₂ tensions and lactate concentrations (Neville, 1985; Malte and Weber, 1988; Playle *et al.*, 1989). Milligan and Wood (1982) proposed that fish death in acidic water is due to a circulatory collapse resulting from the increase in cardiac workload associated with the ionic loss. Recent work by our group has supported this view by showing a decrease in cardiac output in adult Atlantic salmon (*Salmo salar*) dying from acidic water and Al (Brodeur *et al.*, 1999).

However, it has not yet been established whether the ionic loss is the cause of the decrease in cardiac output. It is also unknown whether sublethal concentrations of Al also reduce cardiac output, or if cardiac function is only affected at lethal levels of Al. A limited capacity for elevating cardiac output could be particularly damaging in fish subjected to sublethal concentrations of Al, since it could impair their capacity to generate the extra energy needed to rectify their homeostasis.

The current study was undertaken in an attempt to: 1) elucidate the cause of the decrease in cardiac output previously observed in fish exposed to acidic water and elevated Al, and 2) determine whether the fish cardiac function is affected by sublethal concentrations of Al in acidic water. The overall experimental design included four experimental groups of adult Atlantic salmon. The control group was exposed to water from the Ims River, a Norwegian river with circumneutral pH. The three other groups of fish were exposed to water from the acidic Fossbekk River (Norway) with a nominal concentration of either 50, 75 or 100 µg·L⁻¹ of Al added as AlCl₃. All fish were fitted with a cannula in the dorsal aorta and a Doppler flow probe around the ventral aorta before beginning the exposure. The fish were exposed for 48 h, and cardiac output, heart rate and stroke volume were estimated every 12 h by measuring blood velocity in the ventral aorta with the Doppler flow probe. A blood sample was also taken through the cannula every 24 h and an extensive number of blood parameters were measured. At the end of the exposure period, the pH of the treatment with the highest

concentration of Al was raised to 5.8 by adding calcium carbonate (CaCO_3) to the water. Cardiac function and blood chemistry were then monitored for an extra 36 h to determine if the physiological alterations caused by low pH and elevated Al can be reversed when fish are placed back into circumneutral water. The rapid reductions of water pH used in this study can be experienced by fish during heavy rain and snowmelt (Reader and Dempsey, 1989). The Atlantic salmon may be particularly affected by such acidic episodes, since it is one of the most sensitive salmonid species to Al and low pH (Rosseland and Skogheim, 1984; Poléo *et al.*, 1997).

2. METHODS

2.1. Experimental animals

Adult Atlantic salmon (*Salmo salar*) reared at the Norwegian Institute for Nature Research (NINA) in Ims, southern Norway, were used for this study. The average body weight and length of the fish were 1258 ± 295 g and 46.6 ± 3.4 cm (mean \pm S.D.). Fish were not fed for at least 72 h before use to avoid the influence of food on cardiac output and blood chemistry.

2.2. Surgical procedures

Fish were anaesthetized by immersion in an aqueous solution of clove oil ($120 \text{ mg}\cdot\text{L}^{-1}$) as described by Anderson *et al.* (1997). Anaesthetized fish were placed supine in a wet-sponge operating sling and quickly fitted with a dorsal aortic cannula (PE 50; Intramedic, Clay-Adams, Sparks, MD) as described by Smith and Bell (1964). The cannula was filled with 0.9% NaCl saline containing $25 \text{ IU}\cdot\text{mL}^{-1}$ of heparin. After cannulation, the fish were turned on their side and their gills were irrigated with a $30 \text{ mg}\cdot\text{L}^{-1}$ solution of clove oil while a Doppler flow probe was installed around the ventral aorta.

To install the Doppler flow probe, the operculum and gills were gently lifted and held in an "open" position with a specially designed plastic pad. The connective tissue covering the ventral aorta was then carefully teased away on a section approximately 4 mm long, and a cuff-type Doppler flow probe (Iowa Doppler Products, Iowa City, IA, 20 MHz) was placed around the blood vessel. The cuffs were selected to match the diameter of the vessel. Cuffs

with an internal diameter of 1.4 to 1.8 mm were used. The lead wire from the probe was sutured to the skin at one location on the edge of the opercular cavity and at three locations on the body wall.

2.3. Experimental protocol

After surgery, fish were held individually in an enclosed section (65 cm long x 65 cm wide x 30 cm high) of a 1 m³ tank. The tank was divided in four equal sections by plastic grids to allow water circulation. Fish were allowed to recover from surgery overnight and the exposure started the following morning. The physical and chemical properties of the water used in the four experimental treatments are given in Table 3.1. The control group was maintained in water from the Ims River, a Norwegian river with circumneutral pH. The three other treatments consisted of water from the acidic Norwegian River Fossbekk with a nominal concentration of either 50, 75 or 100 µg·L⁻¹ of Al added as AlCl₃. Water temperature was 10.8 ± 1.8 °C (mean ± S.D.).

The fish were maintained in Ims River water for at least 72 h before the beginning of the experiment, and they were all put back into Ims River water after the surgery and during the overnight recovery. At the beginning of the exposure, the inflow of Ims River water was turned off and replaced by an inflow of pure Fossbekk River water in the acidic water treatment tanks. The flow of Ims River water was maintained in the control tank. It took approximately 30 to 40 min for the water in the acidic water treatment tanks to reach the pH of the incoming Fossbekk River water. At this point, the inflow of pure Fossbekk River water was turned off, and Fossbekk River water with added Al was pumped into each treatment tank from a 3000-L reservoir. Water pH was first decreased before the addition of Al to avoid the formation of highly toxic Al polymers that occurs when neutral and Al-rich acidic water are mixed (Verbost *et al.*, 1995, Witters *et al.*, 1996).

The water of the reservoir was renewed at a rate of 20 L·min⁻¹ with fresh Fossbekk River water to which a solution of AlCl₃ was added via peristaltic pump to achieve the concentrations required by the design. To ensure that the dissolved oxygen concentrations did not decrease during the exposure, the water coming out of the holding tanks was

Table 3.1. Chemistry and Al concentrations (mean \pm S.E.) of the water used in the five treatments.

Treatment	Control <i>(n = 5)</i>	Low Al <i>(n = 7)</i>	Intermediate Al <i>(n = 3)</i>	High Al <i>(n = 5)</i>	High Al + CaCO₃ <i>(n = 3)</i>
pH	6.62 \pm 0.02	5.38 \pm 0.08	5.16 \pm 0.01	5.28 \pm 0.08	5.83 \pm 0.22
Turbidity (FTU)	1.39 \pm 0.45	2.43 \pm 0.76	1.05 \pm 0.03	1.42 \pm 0.29	1.13 \pm 0.34
Color (mg Pt L⁻¹)	16.8 \pm 0.6	51.0 \pm 8.8	33.8 \pm 0.6	44.6 \pm 2.7	47.0 \pm 6.4
Conductivity (μS cm⁻¹)	62.1 \pm 2.3	30.2 \pm 3.2	33.8 \pm 0.6	31.4 \pm 3.5	38.2 \pm 2.4
Alkalinity (μequiv L⁻¹)	149 \pm 4	17.2 \pm 4.5	4.7 \pm 0.5	9.5 \pm 3.4	31.7 \pm 10.2
Ca (mg L⁻¹)	3.61 \pm 0.11	0.98 \pm 0.11	0.79 \pm 0.02	0.83 \pm 0.09	1.67 \pm 0.03
Nitrate (μg L⁻¹)	650 \pm 28	130 \pm 55	42 \pm 2	58 \pm 10	269 \pm 152
T-Al (μg L⁻¹)	38.6 \pm 4.0	228 \pm 29	254 \pm 5	277 \pm 28	267 \pm 19
Tm-Al (μg L⁻¹)	13.4 \pm 0.4	83.6 \pm 11.0	124.7 \pm 0.3	120 \pm 16	84.7 \pm 2.8
Om-Al (μg L⁻¹)	11.2 \pm 0.4	72.0 \pm 10.6	93.7 \pm 0.3	92.2 \pm 7.2	76.7 \pm 2.0
Im-Al (μg L⁻¹)	2.2 \pm 0.4	11.6 \pm 1.8	31 \pm 0	28.2 \pm 7.2	8.0 \pm 3.8
Pc-Al (μg L⁻¹)	25.2 \pm 3.9	144 \pm 22	129 \pm 4	157 \pm 16	182 \pm 21

T-Al=total Al, Tm-Al= total monomeric Al, Om-Al=organic monomeric Al, Im-Al=inorganic monomeric Al, Pc-Al=polymeric colloidal Al

oxygenated before going back into the reservoir tank. Dissolved oxygen concentrations were also monitored, and no decrease was observed during the exposure period. The water of the reservoir tank was initially prepared at least 18 h before use to give time for the different forms of bound and ionic Al to stabilize. The control tanks receiving Ims River water were in an open system throughout the study.

Basal levels of cardiac output, heart rate, stroke volume and blood chemistry were measured by recording the Doppler signal for 10 min and taking a blood sample (0.3 mL) through the cannula with a 1 mL heparinized syringe immediately before the beginning of the treatment. During the exposure, blood samples (0.3 mL) were taken through the cannula every 24 h, and blood velocity in the ventral aorta was measured by recording the Doppler signal for 10 min every 12 h. The Doppler signal was recorded at 20 Hz using a pulsed Doppler flowmeter (545C-4, Department of Bioengineering, University of Iowa, IA). Cardiac output, heart rate and stroke volume were calculated from the Doppler signal every 6 s of recording, and the average value for the 10 min recording period was calculated for each parameter.

At the end of the 48-h exposure period, the fish were anaesthetized by immersion in an aqueous solution of clove oil, killed by cutting the neural cord behind the brain, and the first gill arch was collected and frozen at -20°C until gill Al content was measured. This was done for the controls and the fish exposed to the lowest and the intermediate concentrations of Al. For the treatment with the highest concentration of Al, calcium carbonate (CaCO_3) was added to the water at the end of the 48-h exposure period to buffer the protons and raise the pH of the water to 5.8. Cardiac function and blood chemistry were then monitored according to the same protocol for an additional 36 h to determine if the physiological alterations caused by low pH and elevated Al can be reversed when fish are placed back into circumneutral water. The fish were then sacrificed and their first gill arch was collected as described earlier for the other treatments.

2.4. Analytical techniques

Total water Al was fractionated into organic monomeric, inorganic monomeric, and polymeric colloidal Al using a modified version of Driscoll's (1984) method. Gills were

weighed and digested in a solution of HNO₃ and H₂O₂ before total Al content was measured by inductive coupled plasma emission spectroscopy. Haematocrit, blood gas partial pressures and pH, as well as concentrations of plasma ions, glucose, and lactate were measured on whole blood immediately after sampling using an automated blood analyzer (Stat Profile 9+, Nova Biomedicals, Waltham, MA). The analyzer uses the capability of the different blood parameters to create electrical potentials (on their own or after an enzymatic reaction) to measure their concentrations with specific electrodes. The analyzer also calculates the plasma bicarbonate concentrations (HCO₃⁻) using the equation:

$$\text{Log}_{10} [\text{HCO}_3^-] = \text{pH} + \text{Log}_{10} \text{pCO}_2 - 7.604$$

2.5. Statistical analysis

For each parameter measured, the statistical significance of the variation in time observed within a group over the exposure period was tested using the univariate test for repeated measurements available in the Systat statistical package. The significance levels were determined using F values adjusted with the Huynh and Felt epsilon to protect against a violation of the assumption of compound symmetry. The effect of the addition of CaCO₃ was also tested by executing an univariate test for repeated measurements using the last data point measured before the addition of CaCO₃ and the data points measured after 24 and 36 h of exposure to CaCO₃. Gill Al contents were compared using the Kruskal-Wallis test and Dunn's test for multiple comparisons.

3. RESULTS

3.1. Gill Al content

The amount of Al measured in the gills of the fish at the end of the exposure (Fig. 3.1) was related to the concentration of Al present in the water for the controls and the treatments with the lowest and intermediate concentrations of Al. However, the amount of Al measured in the gills of the fish exposed to the highest concentration of Al was equivalent to the values found in fish exposed to the lowest concentration of Al. This probably results from the fact that the fish exposed to the highest concentration of Al were maintained for an extra 36 h at

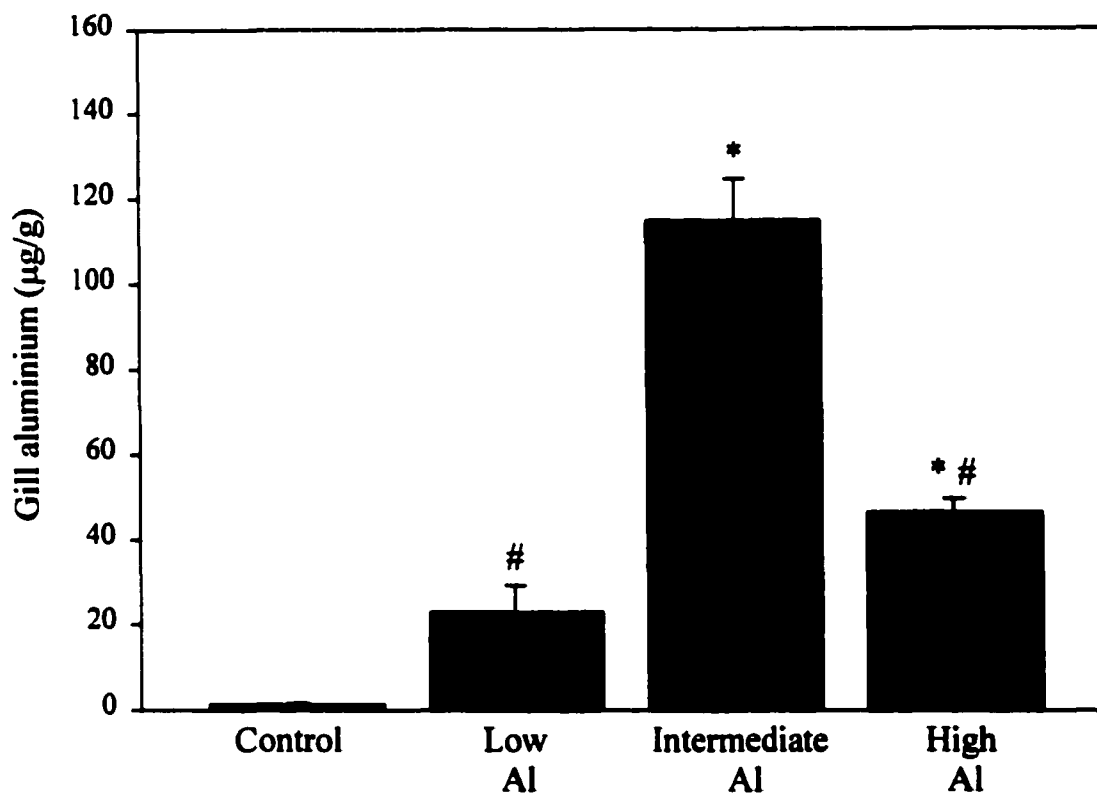


Fig. 3.1. Gill Al content of Atlantic salmon exposed to circumneutral control water ($n = 7$) or to acidic water with low ($n = 15$), intermediate ($n = 15$) or high ($n = 20$) concentrations of Al. * Significantly different from the controls ($p < 0.05$); # significantly different from the group with intermediate Al ($p < 0.05$).

circumneutral pH, which apparently give time to the gills to eliminate part of the Al they had accumulated in acidic water.

3.2. Respiratory and acid-base status

Arterial oxygen (P_{aO_2}) and carbon dioxide (P_{aCO_2}) tensions (Fig. 3.2 *a,b*) were stable over the 48 h exposure period in fish exposed to the lowest and highest concentrations of Al (p of time effect = 0.287 and 0.189 for P_{aO_2} and P_{aCO_2} , respectively at low Al; p of time effect = 0.399 and 0.319 for P_{aO_2} and P_{aCO_2} , respectively at high Al). At the intermediate concentration of Al, P_{aO_2} was elevated while P_{aCO_2} was decreased after 48 h of exposure (p of time effect = 0.027 and 0.003, respectively). Similarly, when $CaCO_3$ was added to the treatment with the highest concentration of Al to raise the pH of the water to 5.8, P_{aO_2} increased significantly while P_{aCO_2} decreased (p of time effect = 0.028 and < 0.001 , respectively).

Blood pH (Fig. 3.2*c*) decreased by approximately 0.12 units over the 48 h exposure period in both the intermediate and high Al exposures ($p < 0.001$ at both concentrations), while it remained stable in fish exposed to the lowest concentration of Al (p of time effect = 0.231). Plasma bicarbonate concentrations (HCO_3^-) varied in a similar fashion (Fig. 3.2*d*), showing a significant decrease from about 7.5 to 5.7 $mmol \cdot L^{-1}$ at both the intermediate and high concentrations of Al (p of time effect < 0.001 at both concentrations), and remaining stable over the course of the experiment at the lowest concentration of Al (p of time effect = 0.884). The addition of $CaCO_3$ to the treatment with the highest concentration of Al did not cause either blood pH or plasma HCO_3^- to return to basal levels (Fig. 3.2 *c,d*). Instead, plasma HCO_3^- continued to decrease over the first 24 h of exposure (p of time effect < 0.001), while blood pH remained at low levels (p of time effect = 0.089).

3.3. Ionoregulatory status

Plasma chloride (Cl^-) concentrations (Fig. 3.3*a*) increased significantly with time in the control group ($p = 0.013$); most likely because the fish were still recovering from the surgery. However, plasma Cl^- levels remained stable throughout the exposure period in fish exposed to the low and intermediate concentrations of Al (p of time effect = 0.330 and 0.312,

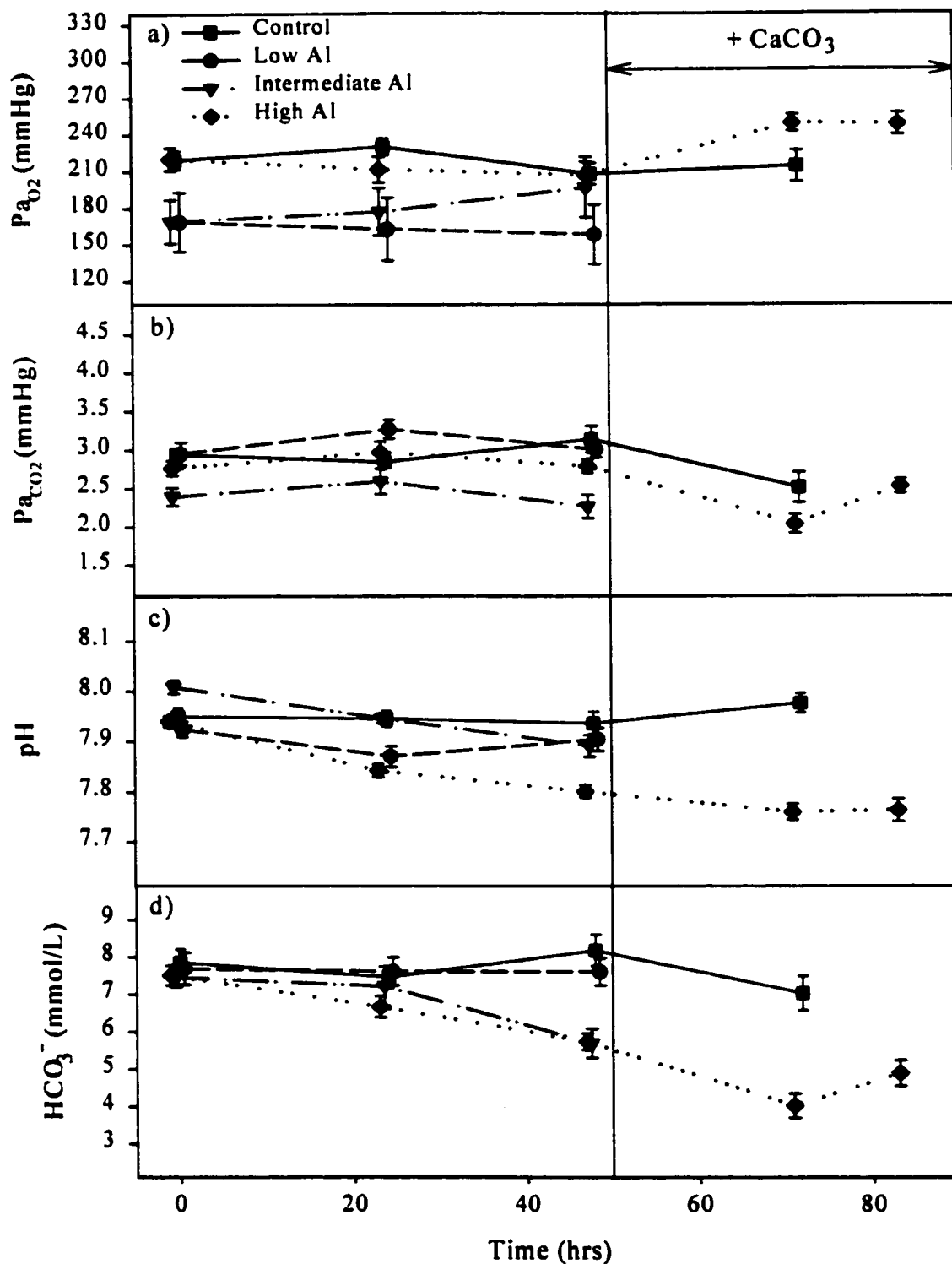


Fig. 3.2. Blood (a) PaO₂, (b) PaCO₂, (c) pH, and (d) HCO₃⁻ (mean ± S.E.) of Atlantic salmon exposed for 48 h to circumneutral control water (*n* = 18) or to acidic water with low (*n* = 18), intermediate (*n* = 15) or high (*n* = 22) concentrations of Al. At the end of the exposure, CaCO₃ was added to the high Al treatment to raise the pH of the water to 5.8, and measurements were taken for an extra 36 h.

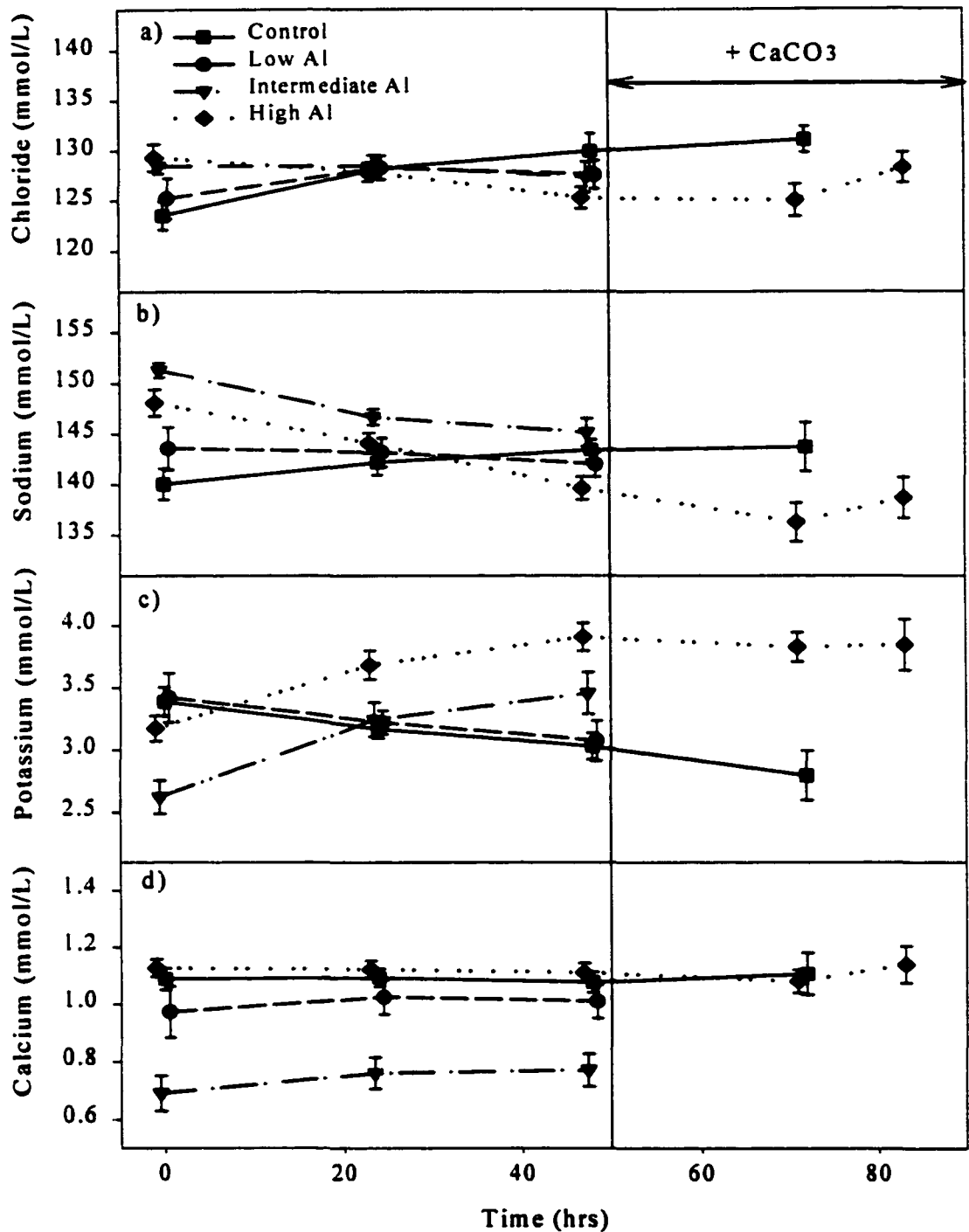


Fig. 3.3. Plasma (a) Cl^- , (b) Na^+ , (c) K^+ , and (d) Ca^{2+} (mean \pm S.E.) of Atlantic salmon exposed for 48 h to circumneutral control water ($n = 18$) or to acidic water with low ($n = 18$), intermediate ($n = 15$) or high ($n = 22$) concentrations of Al. At the end of the exposure, CaCO_3 was added to the high Al treatment to raise the pH of the water to 5.8, and measurements were taken for an extra 36 h.

respectively), while they decreased by 3.2 % in the group treated with the highest concentration of Al ($p = 0.018$). Plasma sodium (Na^+) levels (Fig. 3.3*b*) also decreased by 4.1 and 5.7 % in fish exposed to the intermediate and high concentrations of Al, respectively ($p < 0.001$ at both concentrations), but remained stable in the controls and the group treated with the lowest concentration of Al (p of time effect = 0.296 and 0.822, respectively).

Plasma potassium (K^+) concentrations (Fig. 3.3*c*) increased by 24 and 18.7 % over the course of the exposure in the intermediate and high Al treatments, respectively ($p < 0.001$ for both groups). However, the control and low Al groups showed a decrease in plasma K^+ with time, although this decrease was not statistically significant in the low Al treatment ($p = 0.044$ and 0.897 for the controls and low Al treatment, respectively). Plasma calcium (Ca^{2+}) concentrations (Fig. 3.3*d*) were not affected by any of the treatments (p of time effect = 0.807, 0.725, 0.460 and 0.981 for the controls, and low, intermediate and high Al treatments, respectively).

The addition of CaCO_3 to the treatment with the highest concentration of Al caused plasma Cl^- and Na^+ levels (Fig. 3.3 *a,b*) to start recovering after 36 h of exposure, although the changes were not statistically significant (p of time effect = 0.060 and 0.494 for Cl^- and Na^+ , respectively). Plasma concentrations of K^+ (Fig. 3.3*c*) remained elevated despite the return of water pH to neutral levels (p of time effect = 0.898).

3.4. Blood Chemistry

Haematocrit (Fig. 3.4*a*) decreased gradually in both the controls and the low Al treatment due to repetitive blood sampling ($p = 0.001$ and 0.017, respectively). However, haematocrit increased by 12.9 % in fish exposed to the highest concentration of Al ($p < 0.001$), and tended to be elevated after 48 h in the intermediate Al treatment although the variation was not statistically significant (p of time effect = 0.304).

Plasma glucose concentrations (Fig. 3.4*b*) increased significantly with time in fish exposed to all three concentrations of Al ($p = 0.039$ at low Al, and $p < 0.001$ at both the intermediate and high concentrations of Al). The elevation of plasma glucose levels equaled 22.2, 49.6, and

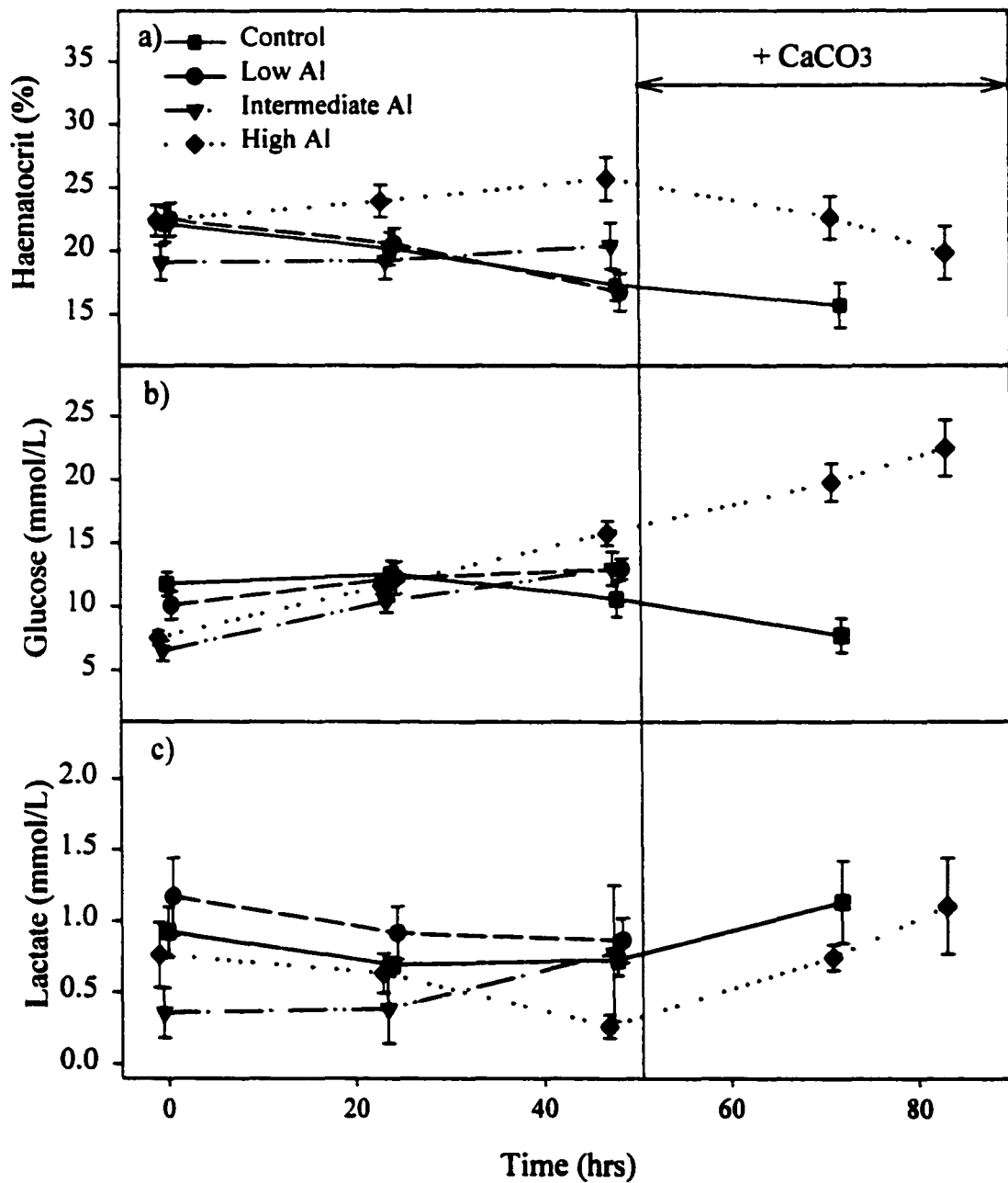


Fig. 3.4. (a) Haematocrit, and plasma (b) glucose, (c) lactate, and (d) cortisol (mean \pm S.E.) of Atlantic salmon exposed for 48 h to water from circumneutral control water ($n = 18$) or to acidic water with low ($n = 18$), intermediate ($n = 15$) or high ($n = 22$) concentrations of Al. At the end of the exposure, CaCO_3 was added to the high Al treatment to raise the pH of the water to 5.8, and measurements were taken for an extra 36 h.

52.1 % in fish exposed to the low, intermediate and high concentrations of Al, respectively. Plasma lactate (Fig. 3.4c) did not vary significantly in fish exposed to the low and intermediate concentration of Al, but was decreased by 65.1 % after 48 h in fish exposed to the highest concentration of Al (p of time effect = 0.606, 0.132 and 0.028, respectively).

The addition of CaCO₃ to the high Al treatment caused haematocrit (Fig. 3.4a) to decrease back to basal levels (p = 0.003) while plasma glucose (Fig. 3.4b) kept on increasing (p < 0.001). Plasma lactate (Fig. 3.4c) also returned to basal levels (p = 0.038) after the elevation of water pH to circumneutral values.

3.5. Cardiac Function

Cardiac output of the fish in the control group (Fig. 3.5a) increased gradually during the exposure, reaching 123 and 137.2 % of basal level after 48 and 72 h of exposure, respectively (p = 0.019). This increase in cardiac output resulted entirely from an increase in stroke volume (127.8 and 128.5 % of basal level after 48 and 72 h, respectively; p = 0.028), since heart rate did not vary significantly during the exposure period (p of time effect = 0.329).

Cardiac output was elevated to 109.4 % of basal level after 36 h of exposure to the lowest concentration of Al (Fig. 3.5b), but was back to basal levels by 48 h of exposure (p of time effect = 0.032). The variations in cardiac output followed the changes in stroke volume which also decreased back to 110.2 % of basal level at the end of the exposure after reaching a maximum of 120.3 % of basal level at 36 h (p of time effect = 0.025). Heart rate was not significantly affected at this concentration of Al (p of time effect = 0.188).

The exposure to the intermediate concentration of Al (Fig. 3.5c) caused a gradual decrease of cardiac output to 75 % of basal level after 48 h of exposure (p = 0.048). This decrease of cardiac output resulted from a slight decrease of both heart rate and stroke volume (p of time effect = 0.039 and 0.555, respectively). Stroke volume was reduced throughout the exposure period, but heart rate first increased up to 112.8 % of basal level before decreasing below basal levels.

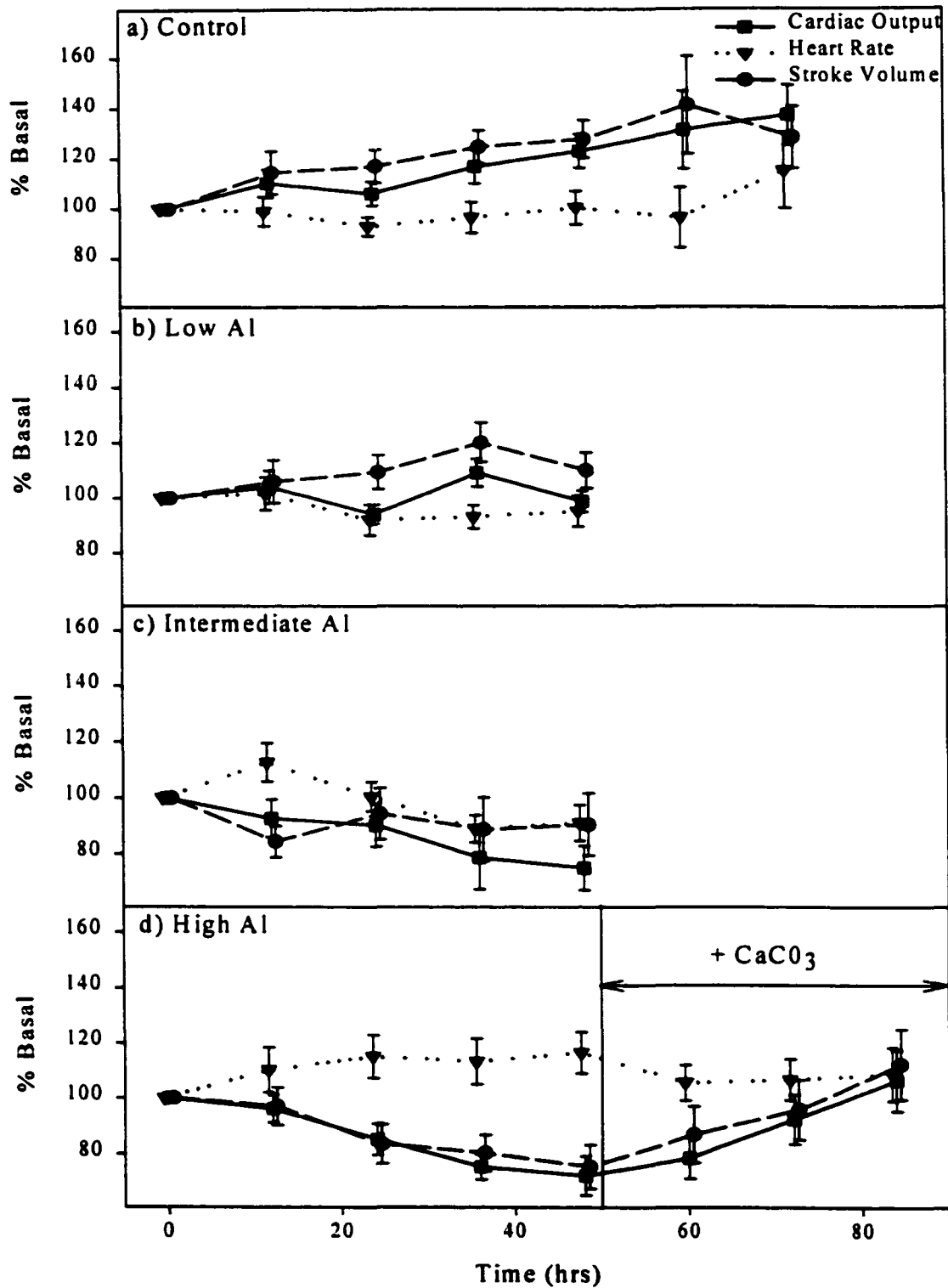


Fig. 3.5. Cardiac output, heart rate, and stroke volume (mean \pm S.E.) of Atlantic salmon exposed for 48 h to (a) circumneutral control water ($n = 17$), or to acidic water with (b) low ($n = 19$), (c) intermediate ($n = 11$), or (d) high ($n = 22$) concentrations of Al. At the end of the exposure, CaCO₃ was added to the high Al treatment to raise the pH of the water to 5.8, and measurements were taken for an extra 36 h.

Cardiac output also gradually decreased in fish exposed to the highest concentration of Al (Fig. 3.5d), reaching 72.4 % of basal level after 48 h of exposure ($p < 0.001$). The decrease of cardiac output was the result of a gradual decrease of stroke volume to 75.6 % of basal level ($p = 0.001$). Heart rate was slightly elevated throughout the exposure period but the variation was not statistically significant (p of time effect = 0.844). The addition of CaCO_3 to elevate the pH of the water caused cardiac output, heart rate and stroke volume to all return to basal levels within 36 h ($p = 0.000, 0.863, \text{ and } 0.007$, respectively).

4. DISCUSSION

The lowest concentration of Al used slightly affected the fish since their plasma glucose increased and their plasma Cl^- levels remained stable instead of decreasing as in the control group. The fish exposed to the two highest concentrations of Al exhibited more important alterations of blood chemistry, but the changes observed were quite similar between the two groups. This is probably related to the fact that the two treatments produced fairly similar levels of inorganic monomeric Al (Table 3.1), which is the fraction believed to cause physiological disturbances in fish (Exley *et al.*, 1991).

The two highest concentrations of Al produced the characteristic responses generally observed in fish exposed to Al in acidic water; namely an elevation of haematocrit and a decrease of plasma concentrations of Na^+ and Cl^- . The displacement of Ca^{2+} by Al^{3+} causes a weakening of the tight junctions of the gills, which results in paracellular losses of Na^+ and Cl^- . Parallel to this, the inhibition of Na^+, K^+ -ATPase decreases the influx of ions through the gills (Battram, 1988; Booth *et al.*, 1988; Witters *et al.*, 1992). The reduction of plasma ion concentrations induces an osmotic swelling of the red blood cells and a decrease in plasma volume, which explains the elevation in haematocrit observed. The release of red blood cells from the spleen also contributes to the increase in haematocrit (Witters *et al.*, 1990; Milligan and Wood, 1982).

Fish exposed to the two highest concentrations of Al also exhibited a decrease in blood pH and HCO_3^- concentrations, as well as an increase in plasma K^+ . The absence of an increase

in pCO₂ and plasma lactate concentrations indicates that neither metabolic nor respiratory acidosis was present. Thus, the decrease in blood pH was most likely the result of an uptake of H⁺ from the water, and HCO₃⁻ concentrations decreased along with blood pH because HCO₃⁻ was titrated to CO₂ by the extra protons. Similarly, the observed elevations in plasma K⁺ concentrations may have been the result of the K⁺/H⁺ exchange mechanisms used to maintain intracellular pH (Ladé and Brown, 1963). Decreases in intracellular K⁺ supporting this hypothesis have been previously observed in the epaxial and ventricular muscles of fish exposed to acidic water (Fugelli and Vislie, 1980; McDonald and Wood, 1981). The reduction of plasma volume was also likely a factor in the increase of plasma K⁺ concentrations.

The exposure to both the intermediate and high concentrations of Al resulted in a gradual decrease of cardiac output to about 75% of basal level after 48 h. At the low concentration of Al, cardiac output fluctuated around basal level as opposed to the gradual increase observed in the control group (Fig. 3.5). This increase of cardiac output in the control group may illustrate the presence of a stress response resulting from the maintenance of the fish in captivity. However, the larger enclosures used in the present experiment were apparently less stressful to the fish compared to the smaller boxes used the year before in a similar experiment (Brodeur *et al.*, 1999). In this experiment, cardiac output was elevated to 123 % of basal level after 48 h of exposure as opposed to 152 % of basal level the year before. Plasma glucose levels also decreased with time in the current experiment (Fig. 3.5b), whereas they remained elevated the previous year. Allowing the fish more space for movement thus constituted an improvement to the protocol since it decreased the level of stress in the control group. The slow decrease in haematocrit caused by the repetitive blood sampling may also have contributed to the increase in cardiac output observed in the control group. Gallagher *et al.* (1995) observed such an elevation of cardiac output in anemic fish to compensate for the reduction in arterial oxygen concentrations.

Cardiac output returned to basal levels within 36 h when the water pH of the high Al treatment was raised to 5.8 by adding CaCO₃. By that time, plasma levels of Na⁺ and Cl⁻ had started to recover and haematocrit was back to basal levels, but none of the other altered

blood parameters had began to recover. The fact that haematocrit was the only blood parameter to have fully recovered when cardiac output was back to normal levels suggests that its increase played an important role in the decrease of cardiac output observed during the exposure to acidic water and Al. An increase in haematocrit elevates vascular resistance by increasing the viscosity of the blood (Bushnell *et al.*, 1992). Eventually, the increase in vascular resistance impairs venous return and thereby causes a decrease in stroke volume and therefore cardiac output. Such a decrease of cardiac output has been previously observed by Gallagher *et al.* (1995) in fish with induced polycythemia (elevated haematocrit). The effect of the increase in haematocrit may also have been aggravated by the simultaneous occurrence of a reduction in plasma volume, which also decreases venous return. The partial correction of plasma Cl^- and Na^+ levels after the addition of CaCO_3 probably helped correct the osmotic shifts from which the alterations in haematocrit and plasma volume originated.

Together, this study and a previous experiment conducted by our group (Brodeur *et al.*, 1999), provide the first evidence to support the hypothesis of Milligan and Wood (1982) that fish death in acidic water is the result of circulatory failure. In our previous work, we showed that fish dying from acidic water and Al exhibit a reduction in cardiac output. In this study, we show that the decrease in cardiac output also occurs at sublethal concentrations of Al, and that it is likely the result of the increase in haematocrit and reduction of plasma volume resulting from the osmotic shifts associated with the ionic losses. In our previous work where the Al exposure was lethal, the reduction of cardiac output was concomitant with a large increase in heart rate (Brodeur *et al.*, 1999). Such a major increase in heart rate was not seen in this study, indicating that this is a symptom associated with lethality and it is not the sole cause of the decrease in cardiac output.

The lowest level of cardiac output observed in the previous study when fish were dying was 70 % of basal (Brodeur *et al.*, 1999), a level similar to the levels observed this year with sublethal concentrations of Al (75 and 72 % of basal level at the intermediate and high Al concentrations, respectively). Thus, the decrease of cardiac output appears to be more of an all or nothing phenomenon that occurs when the ionic and osmotic alterations reach a certain level, rather than being dose-related. The threshold concentration at which cardiac output

starts to decrease seems to be situated somewhere between the lowest and the intermediate concentrations of Al used in this study, since cardiac output did not decrease below basal level in fish exposed to the lowest concentration of Al. However, the absence of an increase in cardiac output in this group as opposed to the control group suggests that some constraints might have already been acting on the heart.

This study showed that the ionoregulatory failure and the resulting osmotic alterations experienced by the fish during an exposure to acidic water and Al causes a reduction of cardiac output, primarily through a decrease in stroke volume. This means that the fish face a period of altered homeostasis with associated elevated energetic demands without the possibility of increasing their cardiac output to elevate their oxygen consumption. According to the Fick principle, the only other way for a fish to raise its oxygen consumption, beside elevating its cardiac output, is to increase the extraction of oxygen as the blood flows through the tissues (Thorarensen *et al.*, 1996a). When blood Pa_{O_2} levels are maintained as in this study, such an increase of blood oxygen extraction might be a possibility, but the extent of this increase may be limited in other situations where respiratory effects are seen and Pa_{O_2} decreases (Wood *et al.*, 1988b; Playle *et al.*, 1989). Such a limited capacity of elevating cardiac output and blood oxygen extraction may explain why fish appear to avoid elevating their metabolic rate when exposed to acidic water and Al (Walker *et al.*, 1991; Allin and Wilson, 1999).

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UMI

1. INTRODUCTION

Freshwater fish living in acidified soft waters are chronically exposed to elevated concentrations of aluminium (Al) as a result of the leaching of Al from soils and sediments (Cronan and Schofield, 1979). It is now well established that Al in acidic soft water causes acute ionoregulatory and respiratory disturbances in fish due to the deposition of Al^{3+} on the gills (Neville, 1985; Playle *et al.*, 1989; Poléo, 1995). However, chronic laboratory exposures have also shown that acclimation (i.e. increased resistance to lethal concentrations of Al) occurs with time and physiological disturbances eventually recover at sublethal doses (Wood *et al.*, 1988 *a,b*; McDonald *et al.*, 1991). The acclimation process is the result of a damage-repair phenomenon involving physiological, biochemical, and structural changes at the gills (McDonald and Wood, 1992).

These acclimatory mechanisms are costly for the organism in terms of metabolic resources and may thus alter the allocation of energy to functions such as growth and activity. Impaired growth has frequently been observed in fish exposed to sublethal concentrations of Al in acidic water, but a reduction of food intake was usually found to play a major role in these reduced growth rates (Wilson, 1996; Wilson *et al.*, 1994, 1996). The suppression of food consumption and growth was also relatively short-lived, and both parameters usually returned to normal within 10 to 12 days of exposure.

Allin and Wilson (1999) recently provided evidence for a loading influence of Al and low pH on metabolism by showing that juvenile rainbow trout (*Oncorhynchus mykiss*) reduce their swimming activity in order to maintain their routine metabolic rate at control levels when exposed to acidic water and Al. In the present study, the effects of a subchronic exposure to Al and low pH on the bioenergetics of Atlantic salmon (*Salmo salar*) were assessed by measuring swimming activity, cardiac output, growth rate and food consumption.

Cardiac output was measured in resting fish in an attempt to acquire information on the effects of Al and low pH on resting metabolic rate. Together with blood oxygen extraction (i.e. the amount of O_2 that is extracted from the blood as it passes through the tissues), cardiac output is one of the two parameters that control oxygen consumption and thus

metabolic rate (Thorarensen *et al.*, 1996a). The observation of an increase in cardiac output would consequently constitute evidence for the presence of an increase in resting metabolism. This study is the first to use radio-telemetry to obtain electromyograms (EMG) from the axial muscle to estimate swimming activity in acidic water. EMG telemetry is one of the best techniques presently available for measuring swimming activity as it allows continuous recordings to be made, and can detect energy-demanding changes in speed, direction and position (Wheatherley *et al.* 1982; Kaseloo *et al.* 1992).

2. METHODS

2.1. Preliminary experiment

To ensure that the Al exposure concentration selected for the 36-days study induced sublethal physiological alterations, a preliminary exposure was conducted in which 14 fish were exposed for four days to water from the acidified Fossbekk River with $50 \mu\text{g Al}\cdot\text{L}^{-1}$ added as AlCl_3 . The fish were divided into two subgroups by tying a cord around the base of the caudal fin of half the fish, and each subgroup was sampled every other day. The exposure conditions and experimental animals were similar to those described below for the subchronic exposure. Blood was sampled from a control group maintained in circumneutral Ims River water according to the same protocol. When sampled, the fish were captured with a dip net, anaesthetized by immersion in an aqueous solution of clove oil ($120 \text{ mg}\cdot\text{L}^{-1}$) as described by Anderson *et al.* (1997), and a blood sample was taken from the caudal vasculature with an heparinized syringe.

2.2. Subchronic exposure

2.2.1. Fish holding and exposure conditions

Atlantic salmon (*Salmo salar*) reared at the Norwegian Institute for Nature Research (NINA) in Ims, southern Norway were used in this study. The average body weight and length of the fish at the beginning of the experiment were $395 \pm 132 \text{ g}$ and $33.4 \pm 3.1 \text{ cm}$ (mean \pm S.D.). The fish were tagged with Carlin tags and randomly divided into two groups of 130 fish. Each group was held in a circular 3000-L tank. Both tanks were first supplied with circumneutral Ims River water for 10 days to allow the fish to adapt to the holding conditions

and recover from tagging. The water inflow to one of the two tanks was then switched to Fossbekk River water, an acidified river of southern Norway. The other group of fish acted as controls and remained in Ims River water throughout the exposure. The two tanks were in a flow-through open system, and small pumps were fixed onto their sides to create a current against which the fish oriented themselves. A peristaltic pump continuously brought an acidified AlCl_3 stock solution into the tank containing Fossbekk River water to add $50 \mu\text{g}\cdot\text{L}^{-1}$ of Al to the water. Water temperature slowly decreased from about 14°C at the beginning of the exposure to around 9°C at the end. The physical and chemical properties of the water used in the two experimental treatments are given in Table 4.1.

2.2.2. Feeding regime, growth measurement and blood sampling

Fish were fed to satiation everyday with sinking food pellets. Small amounts of food were offered to the fish at regular intervals until they stopped feeding, and the mass of food consumed was measured by weighing the food bowl before and after feeding the fish.

Growth was evaluated by weighing the fish at the beginning of the experiment, and after 12 and 36 days of exposure. When weighed, the fish were removed from the tanks in small groups of 8-10 fish, slightly anaesthetized by immersion in an aqueous solution of clove oil ($120 \text{mg}\cdot\text{L}^{-1}$), weighed, and then temporarily placed in a tank containing Fossbekk River water. When all the fish were weighed, the awakened fish were captured with a dip net and returned to their original holding tank. The Carlin tag allowed keeping individual records on body weight and estimating growth on each individual.

Variations in blood chemistry were monitored throughout the exposure by taking a blood sample from 10 fish every 6 days. Fish were captured from each tank with a dip net, anaesthetized by immersion in an aqueous solution of clove oil ($120 \text{mg}\cdot\text{L}^{-1}$), and a blood sample (0.3 mL) was taken from their caudal vasculature with an heparinized syringe. The fish were returned to their original holding tank after being blood sampled.

Table 4.1. Chemistry and Al concentrations (mean \pm S.E.) of the water used in the two treatments.

Treatment	Ims River (<i>n</i> = 5)	Fossbekk+Al (<i>n</i> = 7)
pH	6.62 \pm 0.02	5.23 \pm 0.21
Turbidity (FTU)	1.39 \pm 0.45	1.80 \pm 0.05
Color (mg Pt L⁻¹)	16.8 \pm 0.6	51.2 \pm 5.9
Conductivity (μS cm⁻¹)	62 \pm 2	36 \pm 3
Alkalinity (μequiv L⁻¹)	149 \pm 4	15.4 \pm 7.6
Ca (mg L⁻¹)	3.61 \pm 0.11	0.94 \pm 0.05
Nitrate (μg L⁻¹)	650 \pm 28	176 \pm 111
T-Al (μg L⁻¹)	39 \pm 4	275 \pm 24
Tm-Al (μg L⁻¹)	13.4 \pm 0.4	116 \pm 21
Om-Al (μg L⁻¹)	11.2 \pm 0.4	89 \pm 12
Im-Al (μg L⁻¹)	2.2 \pm 0.4	27 \pm 12
Pc-Al (μg L⁻¹)	25 \pm 4	158 \pm 10

T-Al=total Al, Tm-Al= total monomeric Al, Om-Al=organic monomeric Al,
Im-Al=inorganic monomeric Al, Pc-Al=polymeric colloidal Al

2.2.3. Cardiac output measurements

Resting levels of cardiac output were estimated on 15 fish from each group at the end of the exposure using Doppler flow probes. Fish used for cardiac output measurements were randomly captured with a dip net and anaesthetized by immersion in an aqueous solution of clove oil ($120 \text{ mg}\cdot\text{L}^{-1}$). The average body weight and length of the fish used was $431 \pm 68 \text{ g}$ and $34.9 \pm 2.1 \text{ cm}$ (mean \pm S.D.) for the control group, and $404 \pm 56 \text{ g}$ and $35.0 \pm 1.9 \text{ cm}$ for the group exposed to acidic water plus Al. Once anaesthetized, the fish were placed on their side on a wetted sponge, and their gills were irrigated with a solution of clove oil of $30 \text{ mg}\cdot\text{L}^{-1}$ while a Doppler flow probe was installed around their ventral aorta as previously described (Brodeur *et al.*, 1999).

After surgery, the fish were held individually in an enclosed section (65 cm long x 65 cm wide x 30 cm high) of a 1 m^3 tank. The tank was divided into four equal sections by plastic grids to allow water circulation. Fish were maintained in the same water they had been exposed to during the subchronic exposure. They were allowed to recover from surgery overnight and blood velocity in the ventral aorta was measured the following morning by recording the Doppler signal for 3.5 h. The Doppler signal was recorded at 20 Hz using a pulsed Doppler flowmeter (545C-4, Department of Bioengineering, University of Iowa, IA). At the end of the recording, an *in situ* calibration of the Doppler flow probe was performed on each fish to obtain absolute values of blood flow. To perform the calibration, the heart was exposed and the bulbus arteriosus was catheterized so that the ventral aorta could be perfused. An infusion pump (model 70-2000, Harvard Apparatus, Holliston, MA) was used to perfuse heparinized salmon blood through the ventral aorta at a range of flow rates while the Doppler signal was recorded. The linear relationship obtained between the flow rate in $\text{mL}\cdot\text{min}^{-1}$ and the Doppler signal in volts was used to transform the Doppler signals to absolute blood flow.

To obtain the best possible estimates of resting cardiac output, the data obtained over the 3.5 h recording period were graphed, and a section where cardiac output was at its lowest values for several minutes (20-30 min in general) was averaged. The fish were also removed from the holding tanks and operated upon before the other fish were fed, so that more than 36 h

had elapsed since the last feeding event when cardiac output was measured. This procedure ensured that the peak of the specific dynamic action (SDA) had passed (Jobling, 1983), and that feeding had a minimum effect on cardiac output. Water temperature during the recording of cardiac output was 9.1 ± 0.5 °C (mean \pm S.D.) for the control group, and 9.8 ± 0.2 °C for the fish in acidic water. At the end of the recording, the fish were anaesthetized in an aqueous solution of clove oil ($120 \text{ mg}\cdot\text{L}^{-1}$), and a blood sample was taken from their caudal vasculature with a heparinized syringe. The fish were then killed by section of the spinal cord and the Doppler flow probe was calibrated.

2.2.4. Activity measurements

Swimming activity was estimated by monitoring the electromyograms (EMGs) of two fish per group with EMG radio transmitters (Lotek Engineering Inc. Newmarket, Ontario, Canada). The fish selected for activity measurements were slightly bigger than the other fish to ensure that swimming was not impaired by the weight of the transmitters. The average body weight and length of the fish used was 1056 ± 125 g and 46.0 ± 2.5 cm (mean \pm S.D.). The transmitters were cylindrical (50×13 mm) and weighed 8.5 g in water (18.4 g in air). Two teflon-coated surgical stainless steel electrodes detected the EMG signals emitted from the musculature. A 14-carat gold rod (7×1 mm) was attached to each electrode to hook and hold the electrodes in position in the muscle during the experiment. Signals were processed through an integrator so that a radio pulse was transmitted when a threshold value of $150 \mu\text{V}$ was reached. Increasing electrical activity associated with increasing muscle activity thus resulted in a decreasing interval between successive radio pulses. The pulses were recorded and stored as intervals (ms) between successive radio signals by a combined receiver and data logger (SRX-400, Lotek Engineering Inc., Newmarket, Ontario, Canada).

To implant the transmitters, the fish were anaesthetized by immersion in an aqueous solution of clove oil ($120 \text{ mg}\cdot\text{L}^{-1}$), and placed supine in a wet-sponge operating sling. The fish gills were continuously irrigated with an aqueous solution of clove oil of $30 \text{ mg}\cdot\text{L}^{-1}$ during the operation. The transmitter was inserted into the abdomen through a 3-cm incision made on the ventral surface anterior to the pelvic fins. To place the antenna, a hollow needle

sharpened at one end was inserted into the incision and pushed through the body wall. The whip antenna was threaded into the needle before the needle was pulled completely through the body wall leaving the antenna in place. The two gold rods attached to the electrodes were implanted into the red musculature below the lateral line. The gold rods were inserted into a 21-gauge rod and placed parallel into the muscle, approximately 5 to 8 mm apart, after which the gauge rods were removed. The incision was closed using four to eight independent permanent silk sutures.

The fish were allowed to recover from surgery in circumneutral Ims River water for 9 days before the exposure began. EMG pulse intervals were recorded over the last 3 days of the recovery period to estimate basal levels of activity. The transmitter-implanted fish were kept together with the fish used to assess growth and food consumption. The holding and feeding condition were thus the same as described earlier. EMG pulse intervals were logged continuously over the 20 days of exposure. The pulse intervals were later averaged over 30-min periods for each day of recording. The 30 min intervals during which feeding occurred were removed from the data to ensure that differences in feeding behavior did not influence the results.

2.3. Analytical techniques

Total water Al was fractionated into organic monomeric, inorganic monomeric, and polymeric colloidal Al using a modified version of Driscoll's (1984) method. Haematocrit, and plasma concentrations of ions, glucose, and lactate were measured on whole blood immediately after sampling using an automated blood analyzer (Stat Profile 9+, Nova Biomedicals, Waltham, MA). The blood analyzer uses the capability of the different blood parameters to create electrical potentials (on their own or after an enzymatic reaction) to measure their concentrations with specific electrodes.

2.4. Statistical analysis

Differences in food consumption were analyzed using a two-way Anova followed by a Tukey test for multiple comparisons. Growth was compared between treatments and time intervals using a non-parametric Mann-Whitney rank sum test. Blood parameters were

compared between groups at each sampling time using a T-test on transformed or untransformed data, or using a non-parametric Mann-Whitney rank sum test when normality and equal variance could not be achieved by transformation of the data. Differences in cardiac output between the two groups were evaluated using a T-test, and the EMG pulse intervals observed during the exposure were compared to basal levels using a Mann-Whitney rank sum test.

3. RESULTS

3.1. Preliminary experiment

When fish were exposed to Fossbekk River water with $50 \mu\text{g}\cdot\text{L}^{-1}$ of Al added (Table 4.2), their plasma concentrations of chloride (Cl^-) and sodium (Na^+) remained significantly reduced during the four days of exposure. Plasma glucose levels on the other hand were continuously elevated, while haematocrit initially increased but returned to control levels on the second day of exposure. These blood alterations are typical of fish exposed to acidic water and Al, and indicate the presence of sublethal physiological alterations. This concentration of Al thus appeared appropriate to assess the bioenergetic effects of sublethal exposure to acidic water and Al, and was selected for the subchronic exposure.

3.2. Food consumption and growth rate

The amount of food consumed (Fig. 4.1) by the fish exposed to acidic water and Al was significantly lower than the control group during the first 12 days of exposure. However, food consumption recovered with time, and was not significantly different from controls during the rest of the exposure.

A significant decrease in body weight (Fig. 4.2) was concomitant with the reduction in food consumption observed in acidic water during the first 12 days of exposure. The fish also kept on losing weight during the remaining of the exposure despite the return of food consumption to control levels. However, the daily weight loss observed when appetite was back to normal was lower than during the first 12 days of exposure. Fish maintained in

Table 4.2. Preliminary experiment. Blood chemistry (mean \pm S.E.) of Atlantic salmon exposed for 4 days to Ims River water (control, $n = 7$), or to Fossbekk River water + 50 $\mu\text{g Al L}^{-1}$ (acidic water + Al, $n = 7$).

* = significantly different from the control group ($p < 0.05$).

	Day 1		Day 2		Day 3		Day 4	
	Control	Acid Water + Al	Control	Acid Water + Al	Control	Acid Water + Al	Control	Acid Water + Al
Haematocrit (%)	41 \pm 2	49 \pm 2*	42 \pm 1	45 \pm 2	32 \pm 3	32 \pm 4	36 \pm 1	34 \pm 3
Na ⁺ (mmol L ⁻¹)	152 \pm 0.8	141 \pm 4*	148 \pm 5	148 \pm 2	150 \pm 1	138 \pm 4*	154 \pm 1	146 \pm 1*
Cl ⁻ (mmol L ⁻¹)	136 \pm 2	123 \pm 9*	128 \pm 4	127 \pm 3	135 \pm 2	123 \pm 4*	137 \pm 6	124 \pm 2*
K ⁺ (mmol L ⁻¹)	2.9 \pm 0.3	3.5 \pm 0.5	3.7 \pm 0.1	3.6 \pm 0.2	3.5 \pm 1	3.5 \pm 0.2	3.1 \pm 0.3	3.0 \pm 0.3
Ca ²⁺ (mmol L ⁻¹)	1.4 \pm 0.1	1.3 \pm 0.1	1.3 \pm 0.1	1.4 \pm 0.1	1.4 \pm 0.1	1.3 \pm 0.1	1.5 \pm 0.1	1.5 \pm 0.1
Glucose (mmol L ⁻¹)	6.0 \pm 0.5	22 \pm 5*	7.8 \pm 3.1	16 \pm 4	9.0 \pm 1.4	28 \pm 5*	6.1 \pm 1.4	20 \pm 6*
Lactate (mmol L ⁻¹)	3.3 \pm 0.3	3.9 \pm 0.5	3.1 \pm 0.3	3.4 \pm 0.5	3.7 \pm 0.3	3.7 \pm 0.3	2.9 \pm 0.2	3.2 \pm 0.3

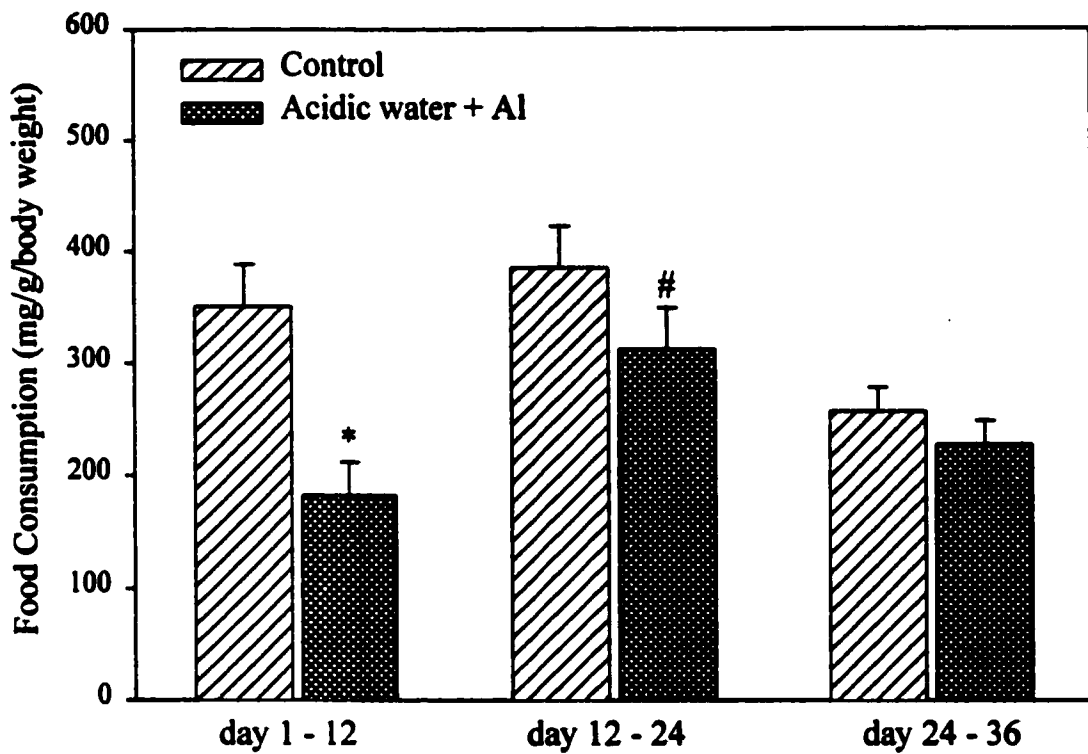


Fig. 4.1. Daily food consumption (mean \pm S.E.) of Atlantic salmon exposed for 36 d to circumneutral water (control), or to acidic water + Al. * = significantly different from the control group ($p < 0.01$). # = significantly different from day 1-12 ($p < 0.01$).

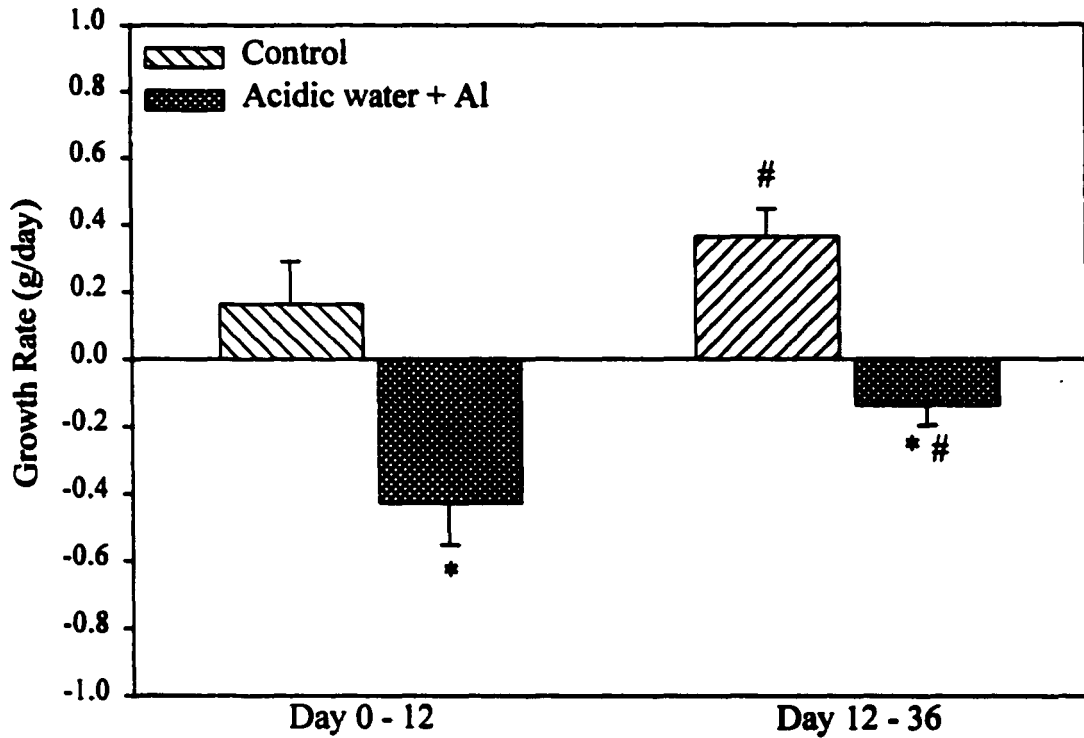


Fig. 4.2. Growth rate (mean \pm S.E.) of Atlantic salmon exposed for 36 d to circumneutral water (control, $n = 115$), or to acidic water + Al ($n = 115$). * = significantly different from the control group ($p < 0.01$). # = significantly different from day 1-12 ($p < 0.01$).

circumneutral water continuously gained weight, their growth rate being, however, higher during the last 24 days of exposure than during the first 12 days (Fig. 4.2).

3.3. Blood chemistry and cardiac output

Most blood parameters were unaltered in fish exposed to acidic water and Al from day 6 onward (Fig. 4.3-4.4). Plasma glucose was the only parameter that was consistently altered compared to the control group (Fig 4.4b). By 24 days of exposure, plasma glucose levels of the fish exposed to acidic water and Al were approximately twice the levels observed in the control group.

Resting cardiac output of fish in acidic water and Al was not significantly different from control fish after 36 days of exposure (14.6 ± 1.5 vs 17.9 ± 2.2 mL·min⁻¹·kg⁻¹, respectively; $n = 15$). Interestingly, when sampled after cardiac output measurements, fish maintained in acidic water with Al exhibited many blood alterations even though most of their blood parameters were normal before the surgery. After cardiac output measurements, haematocrit and plasma K⁺ and glucose levels were significantly elevated in fish maintained in acidic water with Al, while plasma concentrations of Na⁺, Cl⁻ and Ca²⁺ were significantly lower than the control group (Table 4.3).

3.4. Swimming activity

EMG pulse intervals decreased when fish were exposed to acidic water and Al, indicating that their swimming activity increased (Fig. 4.5 c,d). Indeed, the average pulse intervals of both treated fish were significantly lower when the fish were in acidic water plus Al compared to when they were in circumneutral water ($p < 0.001$). This reduction of the EMG pulse intervals indicates that muscle activity, and thus swimming activity, were increased when fish were exposed to acidic water and Al. The average EMG pulse intervals measured during the exposure in the two control fish were not significantly different from basal levels (Fig. 4.5 a,b).

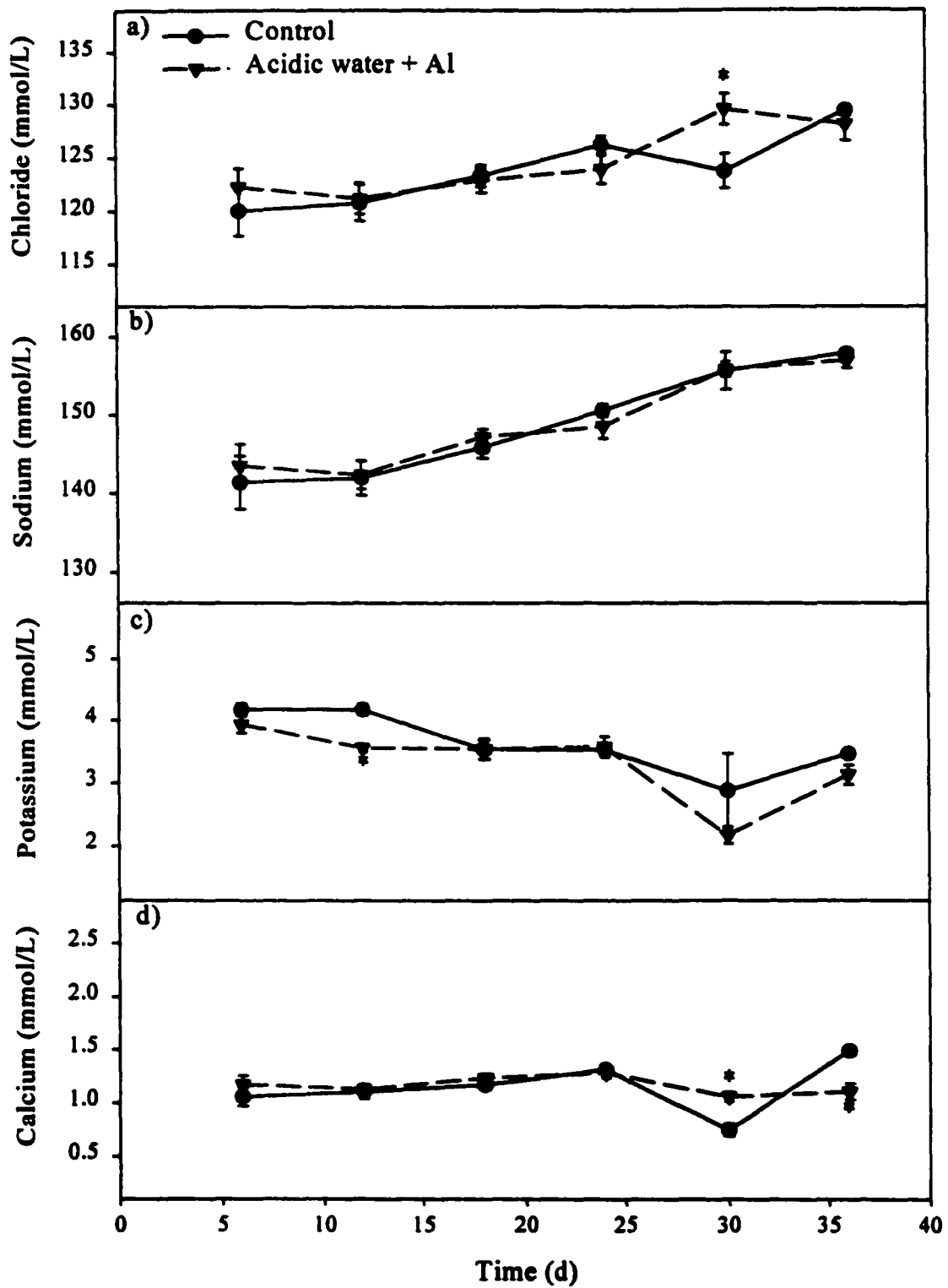


Fig. 4.3. Plasma concentrations of ions (mean ± S.E.) in Atlantic salmon exposed for 36 days to circumneutral water (control), or to acidic water + Al ($n = 10$). * = significantly different from the control group ($p < 0.05$).

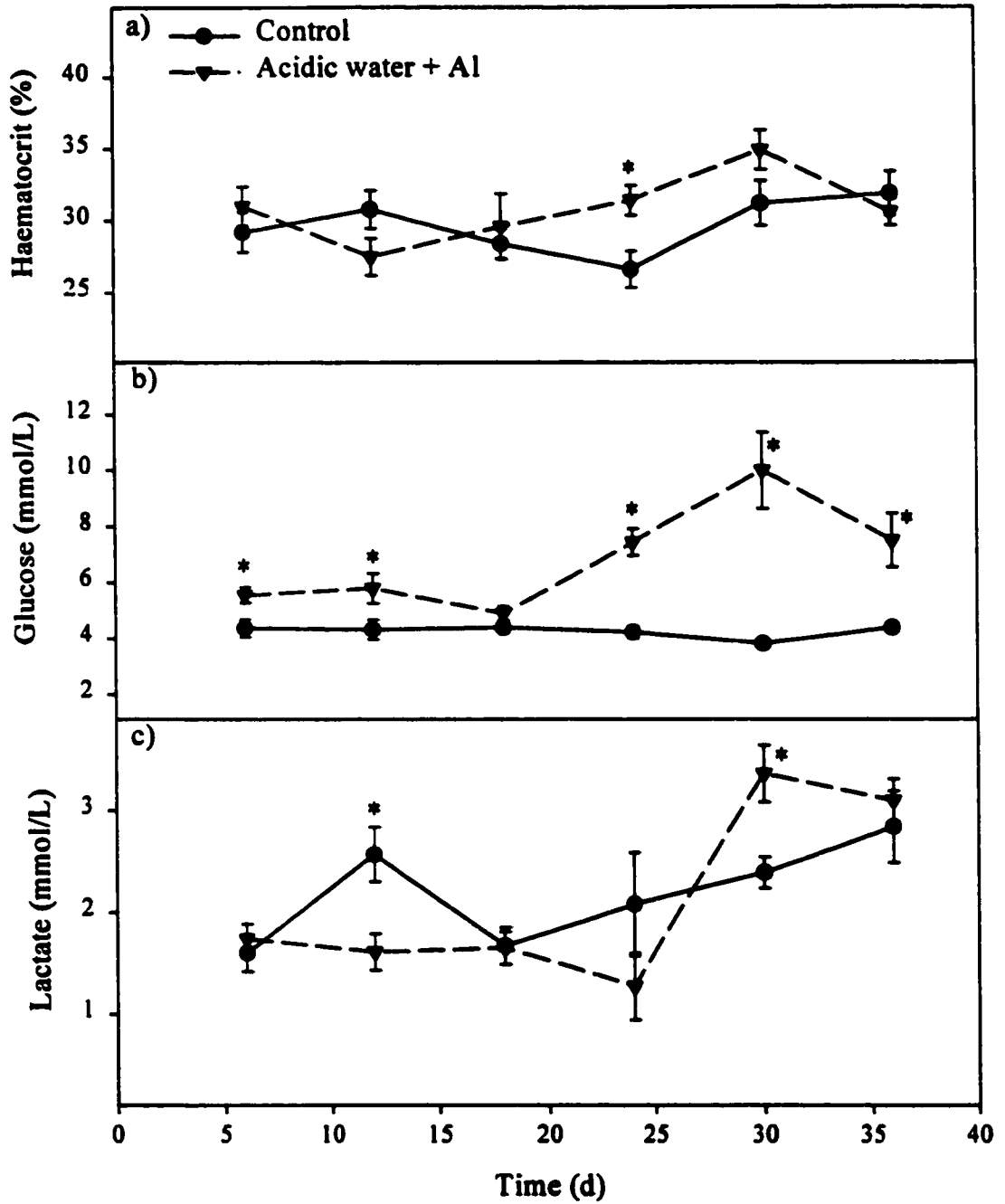


Fig. 4.4. Haematocrit (a) and plasma concentrations of glucose (b) and lactate (c) (mean \pm S.E.) in Atlantic salmon exposed for 36 days to circumneutral water (control), or to acidic water + Al ($n = 10$). * = significantly different from the control group ($p < 0.05$).

Table 4.3. Blood chemistry (mean \pm S.E.) of Atlantic salmon after surgery and recording of cardiac output ($n = 15$). * = significantly different from the control group ($p < 0.05$).

	Control	Acidic water + Al
Haematocrit (%)	29 \pm 1	34 \pm 2*
Na ⁺ (mmol L ⁻¹)	153 \pm 1	137 \pm 3*
Cl ⁻ (mmol L ⁻¹)	129 \pm 1	115 \pm 3*
K ⁺ (mmol L ⁻¹)	3.8 \pm 0.1	4.4 \pm 0.2*
Ca ²⁺ (mmol L ⁻¹)	1.25 \pm 0.03	1.09 \pm 0.04*
Glucose (mmol L ⁻¹)	8.2 \pm 0.4	20.1 \pm 1.5*
Lactate (mmol L ⁻¹)	1.5 \pm 0.2	1.6 \pm 0.1

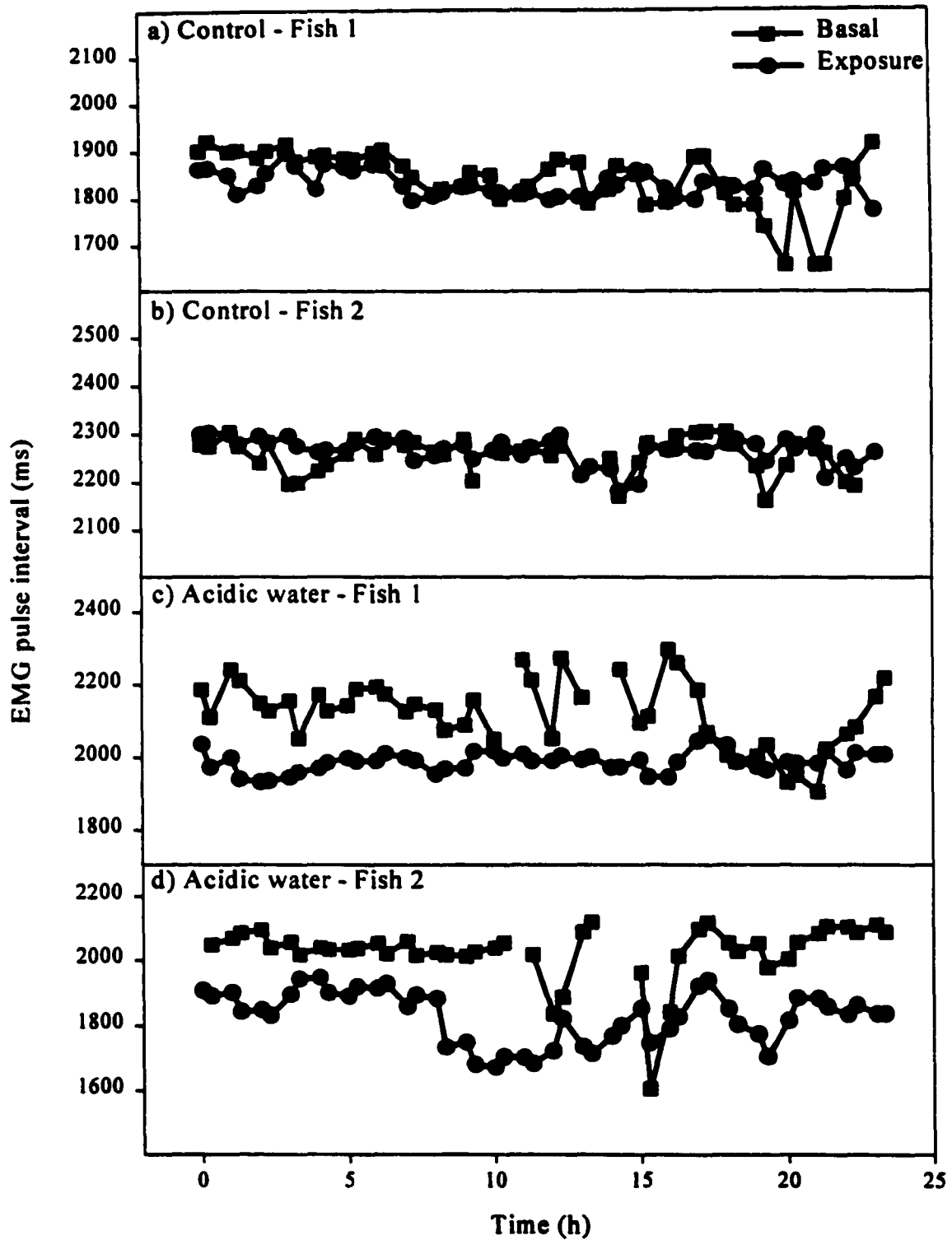


Fig. 4.5. Average daily pattern of EMG pulse interval of Atlantic salmon recorded over 3 days in circumneutral water (basal), and during the first 20 days following the exposition of the fish to acidic water and Al (acidic water fish), or their maintenance in circumneutral water (control fish).

4. DISCUSSION

The absence of alteration in plasma concentrations of Cl^- and Na^+ during the subchronic exposure indicates that the fish were able to correct the ionoregulatory disturbances caused by acidic water and Al, most likely through the damage-repair phenomenon previously described at the gill level (McDonald and Wood, 1992). Despite the ability to ionoregulate, the Al and low pH seem to have remained a burden on the fish throughout the exposure since they maintained elevated levels of plasma glucose (Fig. 4.4b), a typical secondary stress response (Pickering and Pottinger, 1995). Blood glucose was the only blood parameter to be consistently altered throughout the exposure, as all the other parameters measured were not significantly different from the control group from day 5 until the end of the exposure.

As observed in other studies (Wilson, 1996; Wilson *et al.*, 1994, 1996), the fish expressed a major reduction in appetite when first exposed to low pH and Al, but their food consumption was back to normal 12 days after the beginning of the exposure (Fig. 4.1). Allin and Wilson (1999) suggested that elevated levels of plasma glucose can act as a satiation signal and cause a reduction in appetite. However, in this study plasma glucose was still elevated when feeding rate returned to normal levels (Fig. 4.4b), suggesting that it was not the sole cause of the decrease in food consumption. Low pH has been found to reduce the electrophysiological response of the olfactory bulb to different amino acids (Thommesen, 1983; Klaprat *et al.*, 1988). Such an inhibition of the chemoreceptive process may also have been involved in the alteration of feeding behavior.

As previously reported, a decrease in growth rate accompanied the reduction in food consumption, but growth did not return to normal levels once appetite recovered, as opposed to what was seen in other studies (Wilson, 1996; Wilson *et al.*, 1996). Instead, the fish kept on losing weight during all of the exposure to acidic water and Al while the control fish significantly grew during the same period. However, the weight loss of the fish in acidic water and Al was less important after their food consumption had returned to normal, compared to when their appetite was reduced.

Cardiac output was measured in resting fish in an attempt to acquire information on the effects of Al and low pH on resting metabolic rate. Together with blood oxygen extraction (i.e. the amount of O₂ that is extracted from the blood as it passes through the tissues), cardiac output is one of the two parameters that controls oxygen consumption and thus metabolic rate (Thorarensen *et al.*, 1996a). The observation of an increase in cardiac output could consequently have provided evidence for the presence of an increase in metabolism. However, the resting cardiac output of the fish exposed to acidic water and Al was not significantly different from the control group when measured after 36 days of exposure, and it was even slightly lower. The absence of an increase in cardiac output indicates that, if an elevation in resting metabolic rate was present, it was totally dependent on an increase in blood oxygen extraction. However, since this last parameter was not measured in the present study, it is impossible to determine at this point what were the effects of acidic water and Al on resting metabolism. Allin and Wilson (1999) concluded that the basal metabolic rate of rainbow trout exposed to low pH and Al for 32 days must have been elevated for the routine metabolic rate to be at control levels despite a reduction in swimming activity. However, Walker *et al.* (1991) found no elevation of resting metabolic rate in brook trout (*Salvelinus fontinalis*) acutely exposed to low pH and Al.

An interesting observation is that the fish showed many alterations of their blood chemistry after the surgery to install a Doppler probe despite the fact that they appeared to have acclimated to the low pH and Al, and showed few physiological alterations during the exposure. These data indicate that fish living in acidic waters containing sublethal concentrations of Al may be more sensitive to stress even though they appear to be acclimated to the environmental conditions. Such a reduced resistance to stress could reduce the chances of survival of the fish in the wild.

Although we were limited to two control and two treated fish, Atlantic salmon were found to be more active in acidic water and Al than in circumneutral water (Fig. 4.5). The telemetry of EMG from the axial muscle used in this study is one of the best techniques presently available for measuring swimming activity as it allows continuous recordings to be made, and can detect energy-demanding changes in speed, direction and position (Wheatherley *et*

al., 1982; Kaseloo *et al.*, 1992). Although reductions of swimming activity are frequently observed in low pH water (Ogilvie and Stechey, 1983; Scherer *et al.*, 1986; Jones *et al.*, 1987; Allin and Wilson, 1999), there have also been reports of fish being more active in acidic water than in circumneutral water (Mount, 1973; McFarlane and Livingston, 1983). Thus, the effect of low pH and Al on swimming behavior appears to be variable and may depend on a variety of factors, one of them being the intensity of the toxic insult. Jones *et al.* (1985) provided evidence for such a dependency of the behavioral response on the level of toxicity by showing that arctic char (*Salvelinus alpinus*) are hyperactive when exposed to pH 5.0 but hypoactive at pH 4.75 and 4.5. Avoidance of acidic water by fish has been repeatedly reported (Gunn and Noakes, 1986; Pedder and Maly, 1986; Peterson *et al.*, 1989; Åtland and Barlaup, 1996), and the increase in swimming activity may illustrate an elevation of the exploratory activity associated with the avoidance behavior.

The results obtained in this study differ from the observations previously made by Allin and Wilson (1999) on juvenile rainbow trout. In their study, the trout reduced their swimming activity to counter for the loading influence of acidic water and Al without having to increase their total energy expenditure and impairing their growth rate. In the present study, the Atlantic salmon were on the contrary more active when exposed to acidic water and Al which caused them to lose weight because of the increased energy expenditure associated with swimming. These contradictory results indicate that fish may respond in different ways to sublethal levels of Al in acidic water. Many factors can account for these differences such as the relative level of toxicity of the acidic water, or some other species-specific or age-related variations. Atlantic salmon have been reported to be naturally more active than rainbow trout when maintained in enclosures (Phillips, 1985), which may explain why they may be more subject to elevate their exploratory activity when sensing deleterious water conditions. The trout used by Allin and Wilson (1999) were also smaller and younger than the salmon used in this study which may have played a role in the differences in response observed.

Although the responses observed were different, the present study and the study by Allin and Wilson (1999) agree in that sublethal levels of Al in acidic water can alter the bioenergetics

of salmonids. Regardless of whether the response of the fish is an increase or a decrease in swimming activity, the alteration in behavior can, in both cases, limit the chances for survival of a fish in the wild. Reduced activity can affect the ability of the fish to forage, avoid predation, migrate, and successfully reproduce (Allin and Wilson, 1999). An increase in activity like the one observed in this study might be beneficial in the wild if the fish can avoid the low pH water and find refuge in more favorable environments. However, if no escape is possible, the extra energy spent on swimming will, as seen in the laboratory, reduce the condition of the fish and may impair their capacity to face additional stressors.

CHAPTER V

ASSESSMENT OF CARDIAC OUTPUT AS A PREDICTOR OF METABOLIC RATE IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

ABSTRACT

The objective of this study was to determine if cardiac output is a better predictor of metabolic rate than heart rate in rainbow trout (*Oncorhynchus mykiss*). The correlation of cardiac output, heart rate, and tissue blood oxygen extraction to oxygen consumption was determined in fish subjected to either an acute increase or decrease in water temperature, or to an increase in swimming speed. When water temperature was increased from 12 to 27 °C at a rate of 2 °C·h⁻¹, both heart rate and blood oxygen extraction showed a strong positive correlation with oxygen consumption, but the relationship between cardiac output and oxygen consumption was variable and weak. The situation was reversed when water temperature was decreased from 21 to 12 °C at a rate of 0.5 °C·h⁻¹. Heart rate and cardiac output were correlated with oxygen consumption but blood oxygen extraction was not. When fish were forced to swim at increasingly faster swimming speeds, heart rate, cardiac output and blood oxygen extraction were all positively correlated to oxygen consumption. For both cardiac output and heart rate, the slope of the regression line with oxygen consumption was significantly more elevated when the fish were forced to swim at increasingly higher swimming speeds compared to when water temperature was increased or decreased. The variability of the regression lines found between cardiac output and oxygen consumption indicates that cardiac output presents few advantages over heart rate as a predictor of metabolic rate.

1. INTRODUCTION

The increase in popularity of bioenergetic modelling to address fisheries research problems has greatly accentuated the needs for information on the metabolic rate of free-living fish. Most recent attempts to estimate fish energy expenditure in the field have used a new generation of telemetric devices which allow researchers to monitor metabolic correlates such as heart rate and locomotor muscle electromyograms (EMGs) (Lucas *et al.*, 1991; Hinch *et al.*, 1996; Briggs and Post, 1997). However, most parameters so far measured have some limitations. For example, EMG telemetry can be used to estimate the metabolic cost of activity, but doesn't allow prediction of the influence of other factors such as feeding or environmental stress. Heart rate, although more likely to integrate all respiratory components, has also been criticized as an indicator of metabolism because its relationship to oxygen consumption can be described by numerous curves, depending on the physiological state of the fish and on environmental factors (Thorarensen *et al.*, 1996a).

This variability in the relationship between heart rate and oxygen consumption results from the fact that heart rate is only one of several cardiovascular variables that can be modified in response to increased tissue oxygen demand. This is shown by the Fick equation which relates oxygen consumption to the cardiovascular variables:

$$VO_2 = f_H \cdot SV_H \cdot EO_2$$

where VO_2 is oxygen consumption, f_H is heart rate, SV_H is stroke volume, and EO_2 is blood oxygen extraction, i.e. the amount of O_2 that is extracted from the blood as it passes through the tissues.

Thorarensen *et al.*, (1996a) suggested that more accurate estimates of metabolic rate could be obtained by monitoring cardiac output (the product of heart rate and stroke volume) rather than heart rate. Webber *et al.*, (1998) have recently supported this view by showing the presence of a strong relationship between cardiac output and oxygen consumption in the cod (*Gadus morhua*). This relationship was established by forcing the fish to swim at various speeds and taking simultaneous measurements of cardiac output and oxygen consumption

over several weeks. However, if cardiac output is to be measured telemetrically and used as an indicator of metabolic rate in wild fish, it is essential that the calibration for oxygen consumption be done rapidly without holding the animal for extended periods. Furthermore, it should be established whether cardiac output is a good indicator of metabolic rate when factors other than swimming modify fish metabolism, since this would represent the main advantage of a telemetric device measuring cardiac output over the currently available EMG transmitters.

The objective of this study was to determine whether cardiac output is a better correlate of oxygen consumption than is heart rate when the metabolic rate of rainbow trout is modified by either an acute increase or decrease in water temperature or by an increase in swimming speed. An effort was made to keep the procedures simple so that they could be used as a protocol to calibrate an eventual telemetric device for wild animals.

2. METHODS

2.1. Experimental animals

Rainbow trout (*Oncorhynchus mykiss*) were obtained from Rainbow Springs Hatchery (Thamesford, Ontario) and held at 12 °C in a 2 x 2 m tank. Average body weight and total length of the fish were 396 ± 45 g and 32.6 ± 1.3 cm (mean \pm S.D.). Fish were starved for at least 48 h before use to avoid the influence of food on cardiac output and oxygen consumption.

2.2. Surgical procedures

Anesthesia was induced by immersing the fish in an aqueous solution of clove oil ($120 \text{ mg}\cdot\text{L}^{-1}$) as described by Anderson *et al.* (1997). Fish were maintained under anesthesia during the surgery by continuously irrigating the gills with a $30 \text{ mg}\cdot\text{L}^{-1}$ solution of clove oil. A Doppler flow probe was installed around the ventral aorta of the fish to allow blood velocity to be measured and cardiac output, heart rate, and stroke volume to be calculated from these data. To install the Doppler flow probe, the operculum and gills were gently lifted and held in an "open" position with a specially designed plastic pad. Connective tissue covering the ventral

aorta was carefully teased away on a section approximately 4 mm long, and a cuff-type Doppler flow probe (Iowa Doppler Products, Iowa City, IA, 20 MHz) was placed around the blood vessel. The cuffs were selected to match the diameter of the vessel. Cuffs with an internal diameter of 1.2 to 1.5 mm were used. The lead wire from the probe was sutured to the fish's skin at one location on the edge of the opercular cavity and at three locations on the body wall. The surgery lasted about 15 min.

2.3. Respirometry

Immediately after surgery, fish were placed in a 120-L blazka-type respirometer and maintained at low swimming speed ($0.5 \text{ m}\cdot\text{s}^{-1}$) overnight to allow recovery from surgery and acclimation to the respirometer. Water was pumped from a 400-L reservoir through the respirometer, and back into the reservoir. The reservoir was oxygenated and continuously supplied with an inflow of fresh water.

Dissolved oxygen concentration in the respirometer were measured in a parallel external circuit where water was pumped out of the respirometer and brought back into it after flowing over the electrode of a YSI oxygen meter (Yellow Springs, OH). Fish oxygen consumption was estimated by sealing the respirometer (by shutting valves on the inflow and outflow tubes) and measuring the decrease in dissolved oxygen concentration over a 15 min period (swimming experiment) or over 30 min (temperature experiments). Oxygen consumption (VO_2) was adjusted to a standard body mass of 1kg using the mass exponent of 0.8 (Saunders, 1963):

$$\text{VO}_2 (1\text{kg}) = (1/\text{Mb})^{0.8} \text{VO}_2$$

where Mb is body mass.

2.4. Cardiac measurements

To determine the relationship existing between oxygen consumption and the cardiovascular parameters, blood velocity in the ventral aorta was sampled continuously with a Doppler flow probe over the 15 min (swimming experiment) or 30 min (temperature experiments)

during which oxygen consumption was measured. Doppler signals were recorded at 20 Hz using a pulsed Doppler flowmeter (545C-4, Department of Bioengineering, University of Iowa, IA). Cardiac output, heart rate and stroke volume were calculated from the Doppler signal for each 8 s of recording, and the average value for the 15 or 30 min recording was calculated for each parameter.

At the end of the experiment, an *in situ* calibration of the Doppler flow probe was performed on each fish to obtain absolute values of blood flow. After exposing the heart, the bulbus arteriosus was catheterized so that the ventral aorta could be perfused. An infusion pump (model 70-2000, Harvard Apparatus, Holliston, MA) was then used to perfuse calf blood through the ventral aorta at a range of flow rates while the Doppler signal was recorded. The linear relationship obtained between the flow rate in mL·min⁻¹ and the Doppler signal in volts was used to transform the Doppler signals to absolute blood flow.

Blood oxygen extraction (EO₂) was calculated from cardiac output (CO) and oxygen consumption (VO₂) using a modified version of the Fick equation:

$$EO_2 = VO_2 \cdot CO^{-1}$$

The contribution of cardiac output and blood oxygen extraction to the variations in oxygen consumption was calculated as the log of the factorial increase of the variable over the log of the factorial increase of oxygen consumption, e.g. for cardiac output:

$$100 \log(CO_{(2)}/CO_{(1)}) \log(VO_{2(2)}/VO_{2(1)})^{-1}$$

where (2) denotes the higher values and (1) denotes the lower values of CO and VO₂.

2.5. Experimental protocol

2.5.1. Increasing water temperature

After the fish had been allowed to recover from surgery overnight at 12 °C, the water reservoir supplying the respirometer was heated at a rate of 2 °C·h⁻¹. Oxygen consumption

and Doppler signal were measured simultaneously over a 30 min period when water temperature reached 12, 15, 18, 21, 24, and 27 °C. Swimming speed was maintained at 0.5 m·s⁻¹ during the experiment.

2.5.2. Decreasing water temperature

After introduction of the fish in the respirometer, the water of the reservoir was heated to 21 °C overnight at a rate of 0.5 °C·h⁻¹. On the next morning, the water temperature was reduced at a rate of 0.5 °C·h⁻¹ and measurements of oxygen consumption and Doppler signal were taken simultaneously over 30 min when water temperature reached 21, 18, 15, and 12 °C. Swimming speed was maintained at 0.5 m·s⁻¹ during the experiment.

2.5.3. Increasing swimming speed

On the morning following the surgery, swimming speed was increased by 0.33 m·s⁻¹ every 30 min up to 1.48 m·s⁻¹. Oxygen consumption and Doppler signal were measured simultaneously over the first 15 min the fish spent swimming at each speed. During the remaining 15 min, fresh oxygenated water was allowed to recirculate in the respirometer to avoid a depletion of dissolved oxygen concentrations.

2.6. Statistical analysis

Relationships between the different parameters measured were estimated using linear or exponential regressions, depending on the nature of the relationship. Regression lines were compared using an analysis of covariance.

3. RESULTS

3.1. Response to an increase in water temperature

An exponential increase of oxygen consumption ($\dot{V}O_2$) from 1.9 to 5.0 mg·kg⁻¹·min⁻¹ was observed when water temperature (T) was elevated from 12 to 27 °C at a rate of 2 °C·h⁻¹ (Fig. 5.1a; $\dot{V}O_2 = 0.724 * 1.073^T$, $r^2 = 0.588$, $p < 0.001$, $n = 9$). The increase of blood oxygen extraction from 0.07 to 0.13 mg·mL⁻¹ contributed most (62.4 ± 15.5 %, mean ± SE) to the elevation of oxygen consumption (Fig. 5.1a). The small increase of cardiac output from 30.9

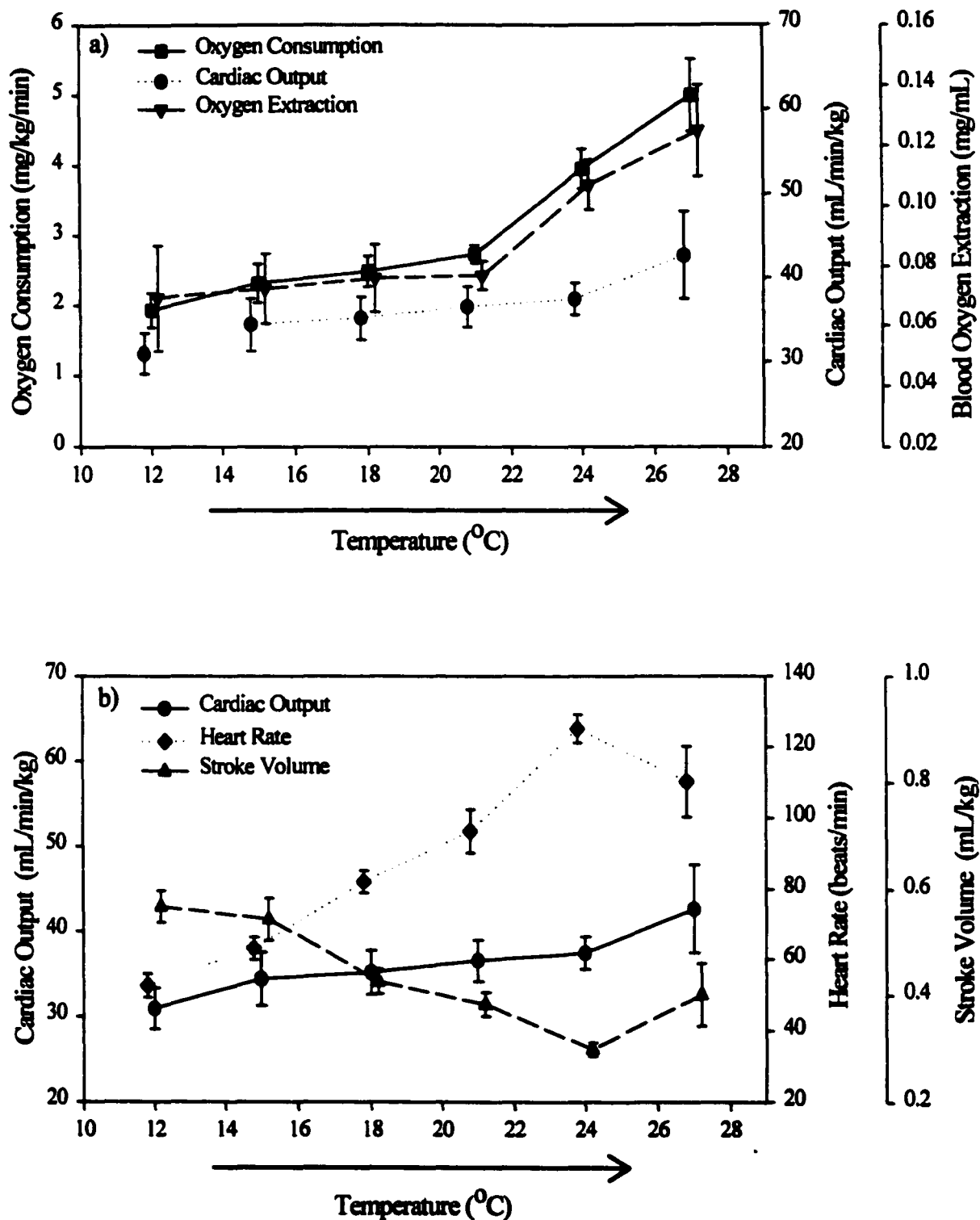


Fig. 5.1. Cardiovascular adjustments of rainbow trout ($n = 9$, mean \pm S.E.) during an acute increase of water temperature at a rate of $2\text{ }^{\circ}\text{C}\cdot\text{h}^{-1}$. (a) Interaction of cardiac output and blood oxygen extraction in the regulation of oxygen consumption. (b) Interaction of heart rate and stroke volume in the regulation of cardiac output.

to $42.7 \text{ mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ accounted for the rest of the increase in oxygen consumption (Fig. 5.1a). The increase of cardiac output was only moderate because a decrease in stroke volume was associated with the large elevation of heart rate from 52.7 to $110.3 \text{ beats}\cdot\text{min}^{-1}$ (Fig. 5.1b). Cardiac output (CO) was only weakly correlated to oxygen consumption (Fig. 5.2a; $\text{VO}_2 = 0.052 \text{ CO} + 1.246$, $r^2 = 0.132$, $p = 0.01$, $n = 9$), while both blood oxygen extraction (EO_2) and heart rate (f_H) were strongly correlated to oxygen consumption (Fig. 5.2 b,c; $\text{VO}_2 = 27.0 \text{ EO}_2 + 0.736$, $r^2 = 0.567$, $p < 0.001$, $n = 9$; $\text{VO}_2 = 0.030 f_H + 0.382$, $r^2 = 0.452$, $p < 0.001$, $n = 9$)

3.2. Response to a decrease in water temperature

Oxygen consumption decreased exponentially from 2.5 to $1.3 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ when water temperature was reduced from 21 to $12 \text{ }^\circ\text{C}$ at a rate of $0.5 \text{ }^\circ\text{C}\cdot\text{h}^{-1}$ (Fig. 5.3a; $\text{VO}_2 = 0.473 \cdot 1.082^T$, $r^2 = 0.685$, $p < 0.001$, $n = 9$). An analysis of covariance performed on log transformed data revealed that the slope of the regression line obtained between oxygen consumption and temperature when water temperature was increased did not significantly differ from the slope obtained when temperature decreased ($p = 0.492$). However, the intercepts of the two regression lines were significantly different ($p < 0.001$). Blood oxygen extraction only slightly decreased as water temperature was reduced, and it is the decrease of cardiac output from 57.2 to $34.3 \text{ mL}\cdot\text{min}^{-1}\cdot\text{kg}^{-1}$ that accounted for $84.3 \pm 14.8 \%$ of the decrease in oxygen consumption (Fig. 5.3a). The reduction of cardiac output was caused by a large decrease of heart rate from 82.9 to $39.3 \text{ beats}\cdot\text{min}^{-1}$. Stroke volume increased from 0.703 to $0.874 \text{ mL}\cdot\text{kg}^{-1}$ (Fig. 5.3b) but this increase was not sufficient to compensate for the large decrease in heart rate observed. Both cardiac output and heart rate showed a strong linear relationship with oxygen consumption (Fig. 5.4 a,b; $\text{VO}_2 = 0.035 \text{ CO} + 0.210$, $r^2 = 0.664$, $p < 0.001$, $n = 9$; $\text{VO}_2 = 0.023 f_H + 0.482$, $r^2 = 0.621$, $p < 0.001$, $n = 9$), but no correlation was found between blood oxygen extraction and oxygen consumption (Fig. 5.4c; $p = 0.105$).

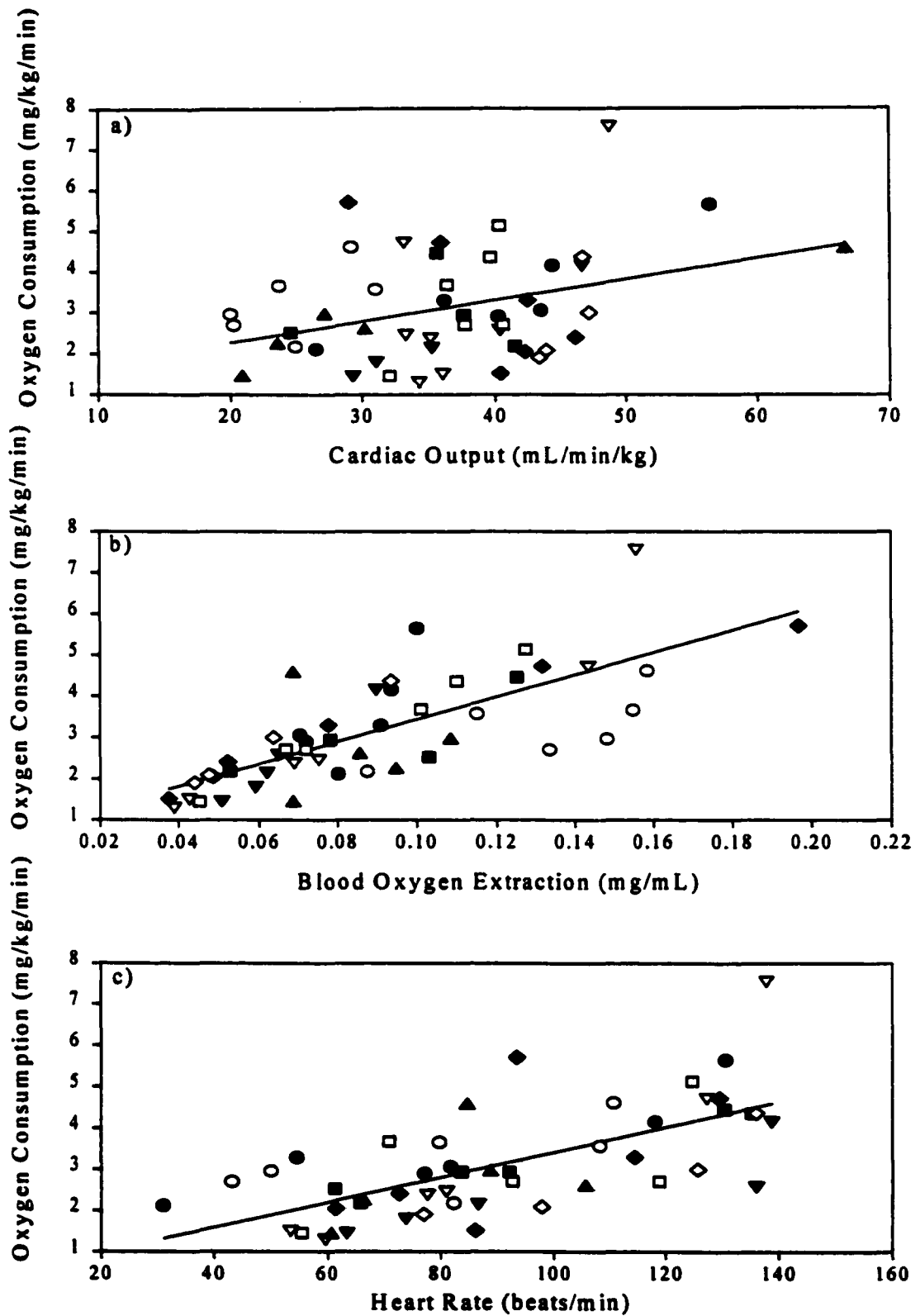


Fig. 5.2. Relationship between oxygen consumption and (a) cardiac output (b) blood oxygen extraction and (c) heart rate in rainbow trout submitted to an acute increase of water temperature at a rate of $2\text{ }^{\circ}\text{C}\cdot\text{h}^{-1}$. Each symbol represents an individual fish.

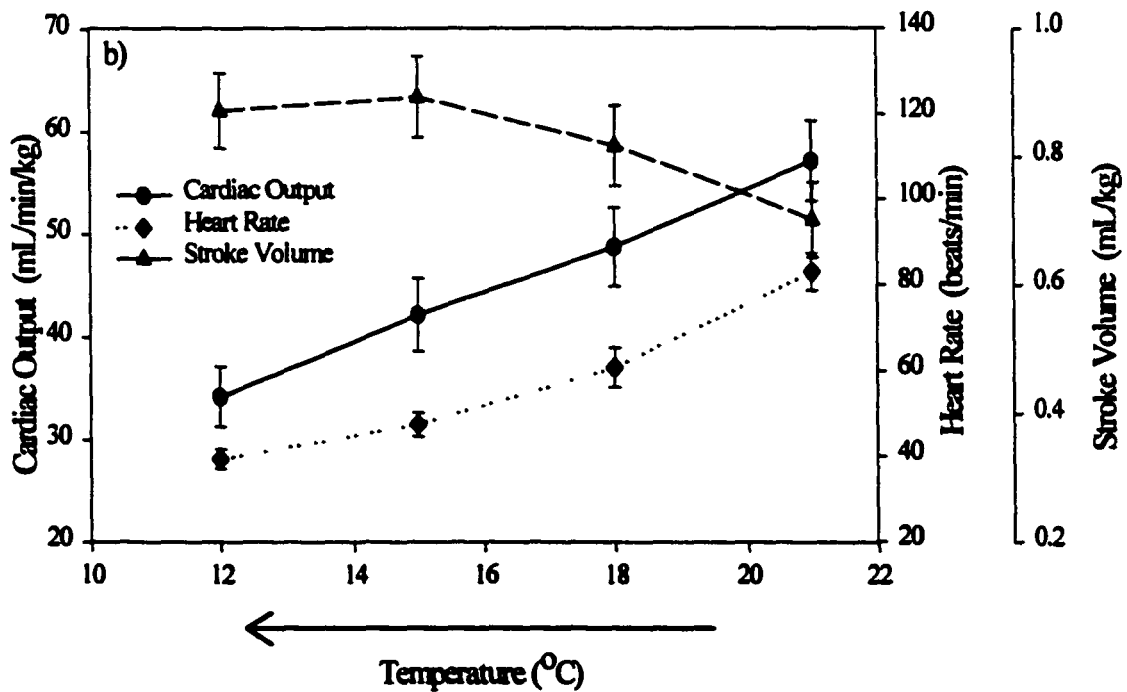
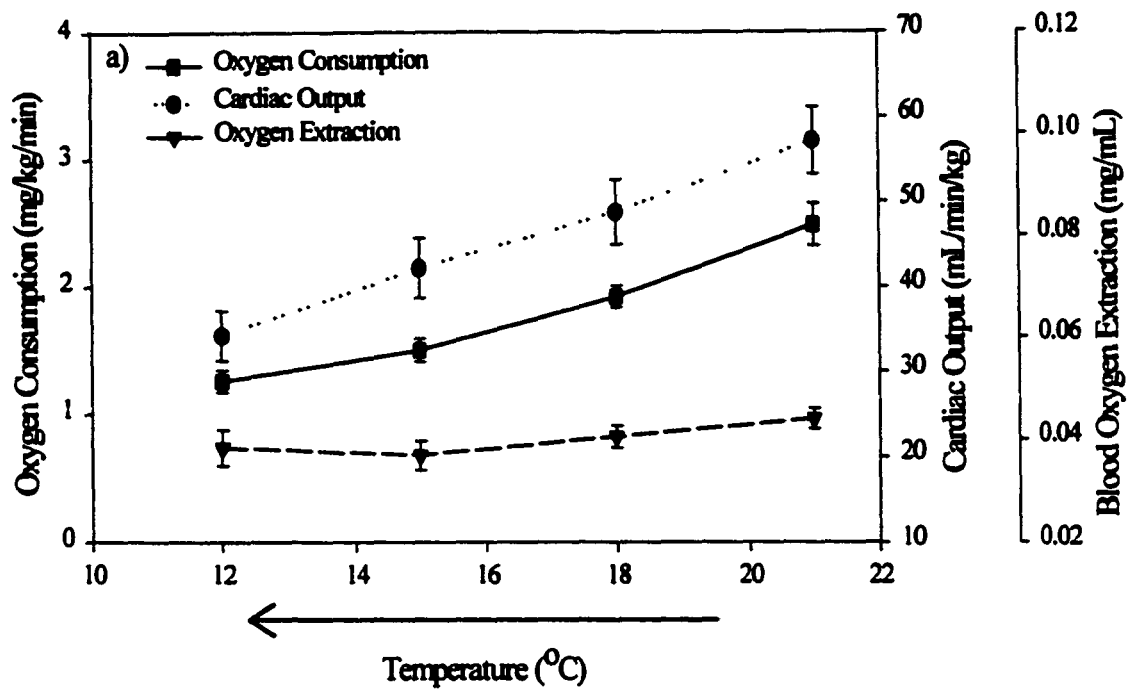


Fig. 5.3. Cardiovascular adjustments of rainbow trout ($n = 9$, mean \pm S.E.) during an acute decrease of water temperature at a rate of $0.5\text{ }^{\circ}\text{C}\cdot\text{h}^{-1}$. (a) Interaction of cardiac output and blood oxygen extraction in the regulation of oxygen consumption. (b) Interaction of heart rate and stroke volume in the regulation of cardiac output.

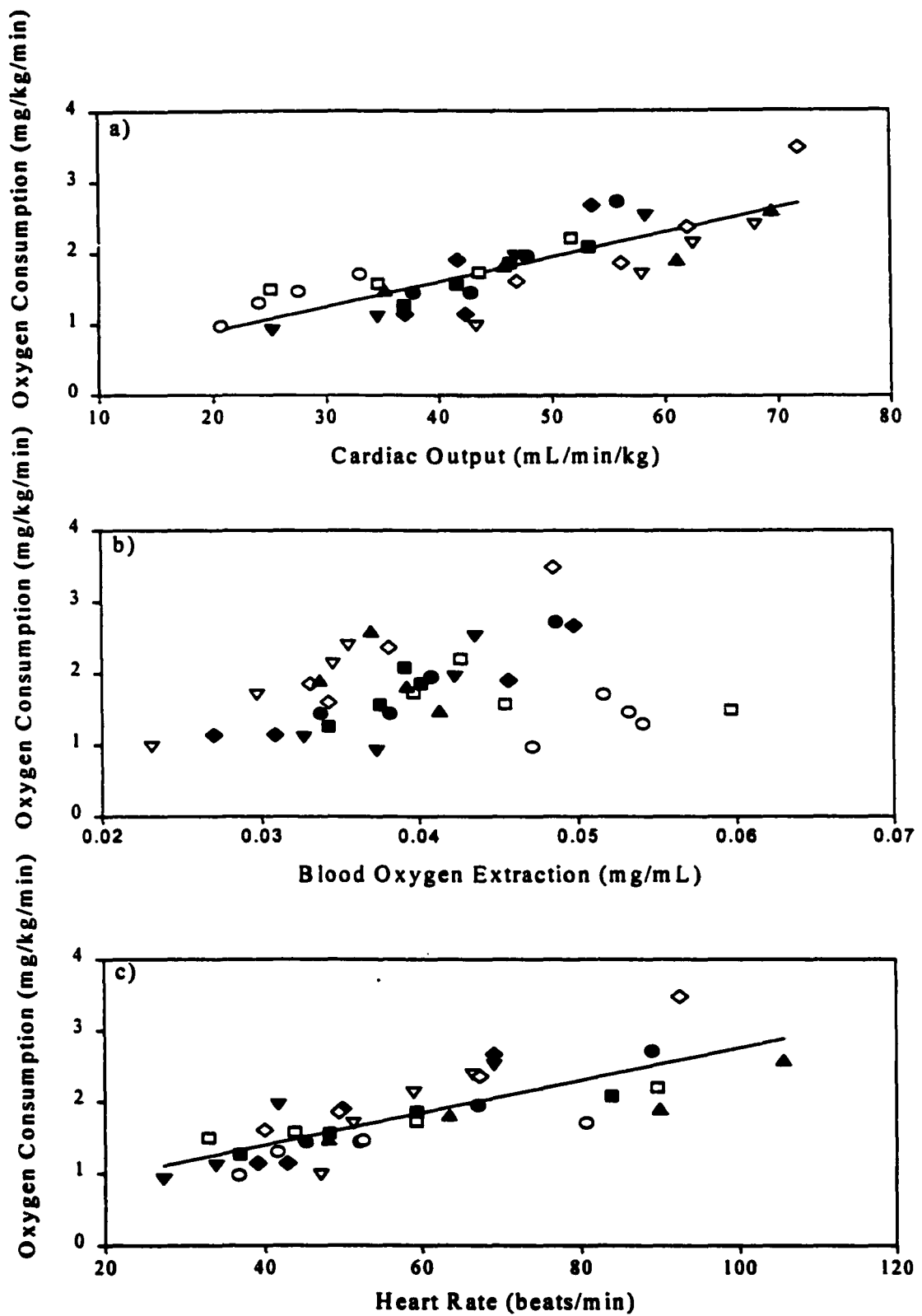


Fig. 5.4. Relationship between oxygen consumption and (a) cardiac output (b) blood oxygen extraction and (c) heart rate in rainbow trout submitted to an acute decrease of water temperature at a rate of $0.5\text{ }^{\circ}\text{C}\cdot\text{h}^{-1}$. Each symbol represents an individual fish

3.3. Response to increasing swimming speed

The gradual increase in swimming speed (SPD) caused oxygen consumption to increase exponentially (Fig. 5.5a; $VO_2 = 1.282 * 3.360^{SPD}$, $r^2 = 0.611$, $p < 0.001$, $n = 9$). The increase of blood oxygen extraction accounted for 66.9 ± 8.4 % of the elevation in oxygen consumption while the increase of cardiac output from 45.2 to 66.5 $mL \cdot min^{-1} \cdot kg^{-1}$ accounted for 32.8 ± 8.4 % of the increase (Fig. 5.5a). The elevation of cardiac output resulted mainly from the increase of heart rate from 50.0 to 72.6 $beats \cdot min^{-1}$ since stroke volume only slightly increased (Fig. 5.5b). Cardiac output, blood oxygen extraction and heart rate all showed a significant linear relationship with oxygen consumption (Fig. 5.6 a,b,c; $VO_2 = 0.086 CO - 0.391$, $r^2 = 0.537$, $p < 0.001$, $n = 9$; $VO_2 = 59.82 EO_2 - 0.186$, $r^2 = 0.517$, $p < 0.001$, $n = 9$; $VO_2 = 0.162 f_H - 4.96$, $r^2 = 0.709$, $p < 0.001$, $n = 9$). Heart rate was the parameter showing the best correlation coefficient because the regression lines obtained with cardiac output and blood oxygen extraction tend to vary considerably from fish to fish.

3.4. Variability of the regression lines between treatments

For both cardiac output and heart rate, the slope of the regression line with oxygen consumption did not differ significantly when water temperature was either increased or decreased (Fig. 5.7 a,c; $p = 0.389$ for cardiac output and $p = 0.513$ for heart rate). However, in both cases, the intercept was significantly higher when water temperature was decreased compared to when it was increased ($p < 0.001$ for both cardiac output and heart rate). Also, for both cardiac output and heart rate, the slope of the regression line with oxygen consumption was significantly more elevated when the fish were forced to swim at increasingly higher swimming speeds compared to when water temperature was increased or decreased (Fig. 5.7 a,c; $p = 0.017$ for cardiac output and $p < 0.001$ for heart rate). The slope of the regression line between oxygen consumption and blood oxygen extraction was also significantly higher when the fish were forced to swim than when water temperature was decreased (Fig. 5.7b; $p = 0.001$).

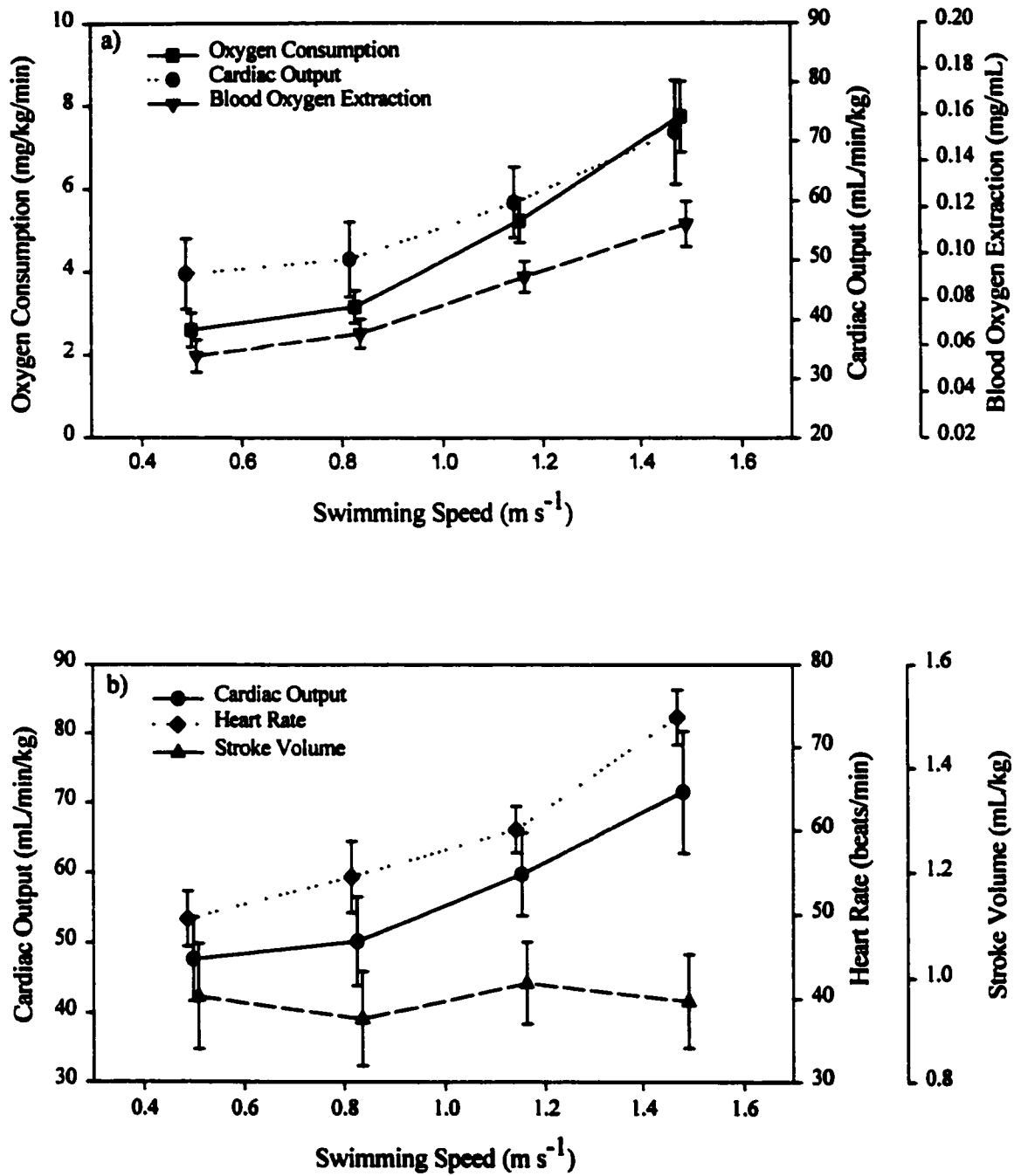


Fig. 5.5. Cardiovascular adjustments of rainbow trout ($n = 9$, mean \pm S.E.) during a gradual increase in swimming speed. (a) Interaction of cardiac output and blood oxygen extraction in the regulation of oxygen consumption. (b) Interaction of heart rate and stroke volume in the regulation of cardiac output.

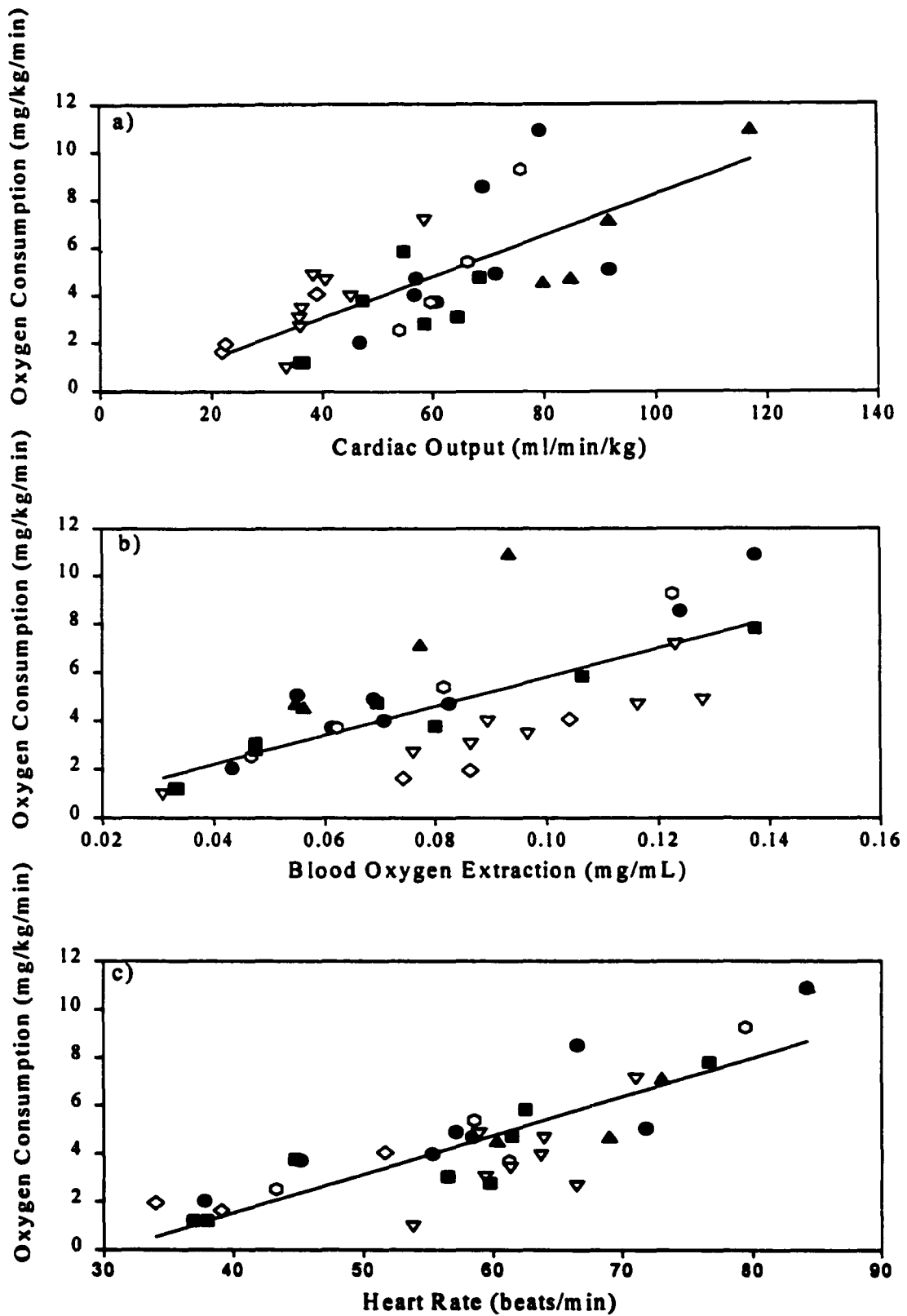


Fig. 5.6. Relationship between oxygen consumption and (a) cardiac output (b) blood oxygen extraction and (c) heart rate in rainbow trout submitted to a gradual increase in swimming speed. Each symbol represents an individual fish

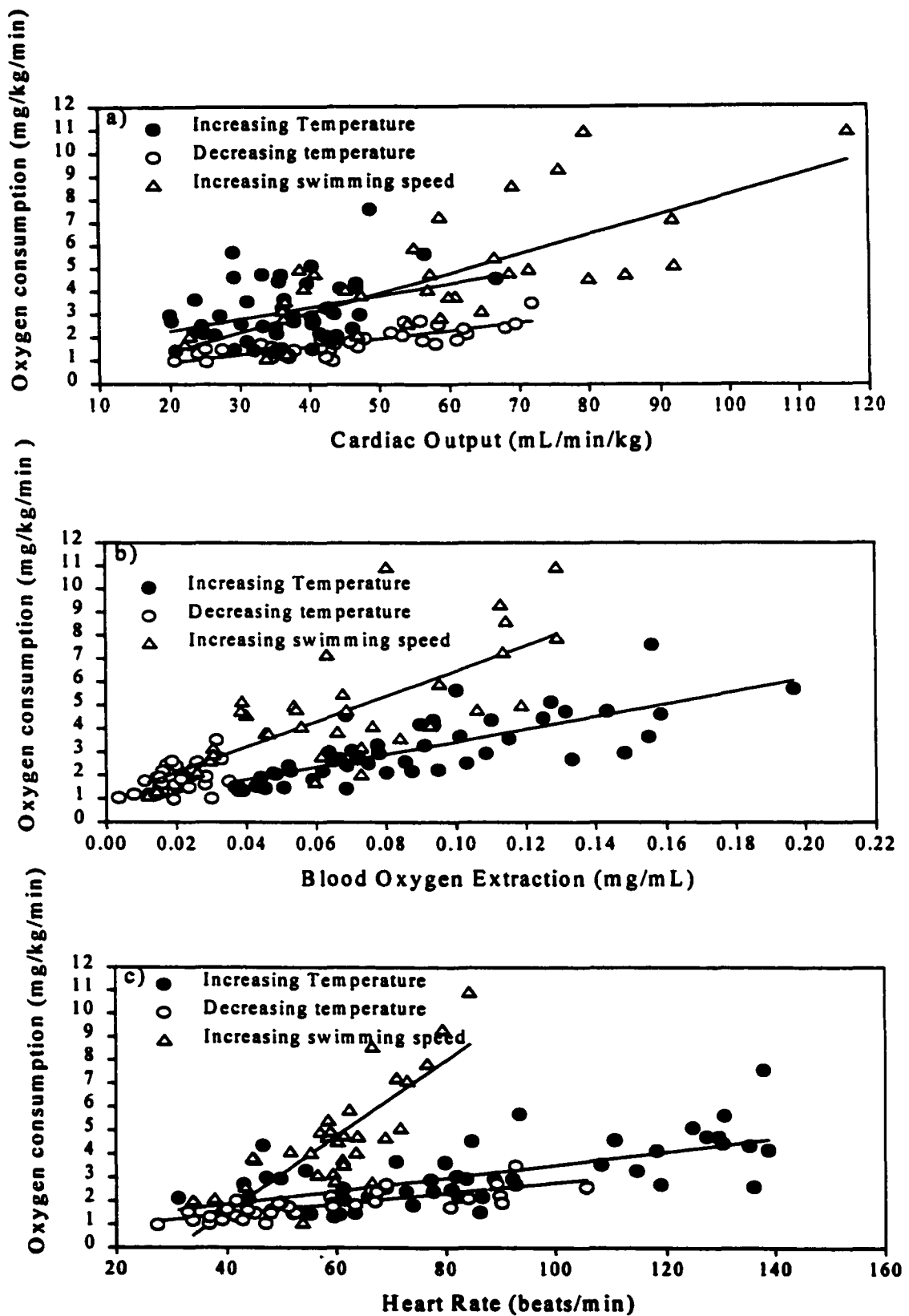


Fig. 5.7. Relationship between oxygen consumption and (a) cardiac output (b) blood oxygen extraction and (c) heart rate in rainbow trout subjected to an acute increase of water temperature, an acute decrease of water temperature or an increase of swimming speed.

4. DISCUSSION

As typically observed in fish (Jobling, 1994), oxygen consumption varied exponentially with water temperature. When water temperature was acutely increased, the elevation of oxygen consumption mainly resulted from an increase in blood oxygen extraction while cardiac output only slightly increased (Fig. 5.1a). The increase in cardiac output was only moderate because a decrease in stroke volume counteracted the large elevation in heart rate observed (Fig. 5.1b). The elevation of water temperature is believed to increase heart rate through a direct effect on the membrane permeability of the pacemaker fibers (Randall, 1970). In fish, such an elevation in heart rate causes stroke volume to decrease because it reduces both the filling time and the force of contraction (Farrell and Jones, 1992).

The elevation of blood oxygen extraction observed in this study is consistent with the results of Heath and Hughes (1973). An acute elevation of water temperature reduces both the oxygen affinity and the oxygen carrying capacity of fish blood (Eddy, 1971). A reduction of blood oxygen affinity can first benefit oxygen delivery by favoring oxygen unloading in the tissues, provided a full saturation of the blood can be maintained at the gills (Farrell, 1996). When the thermal shock is more important, a release of catecholamines like the one observed by Mazeaud *et al.* (1977) may contribute to the maintenance of high levels of blood oxygen extraction by improving blood oxygen affinity and carrying capacity. Catecholamines prevent blood oxygen affinity to decrease too drastically through the combined effects of red blood cell alkalization, decreased concentration of organic phosphates, and swelling of red blood cells (Walsh *et al.*, 1998). Catecholamines also cause the release of stored erythrocytes from the spleen (Randall and Perry, 1992) which elevates blood oxygen carrying capacity by increasing hemoglobin concentrations. Such an elevation of blood hemoglobin concentrations as been noted by Wedemeyer (1973) in juvenile steelhead trout (*Salmo gairdneri*) and coho salmon (*Oncorhynchus kisutch*) subjected to an acute increase in water temperature of 10 °C.

As opposed to what was observed when water temperature was increased, the decrease of oxygen consumption associated with the acute reduction of water temperature was mainly caused by a reduction in cardiac output while blood oxygen extraction only slightly

decreased (Fig. 5.3a). The inhibitory effect of the low temperatures on heart rate was responsible for the reduction of cardiac output.

As usually found in fish (Jobling, 1994), oxygen consumption increased exponentially with swimming speed. This elevation of internal oxygen convection was the result of an increase of both cardiac output and, to a larger extent, blood oxygen extraction (Fig. 5.5a). Interestingly, the elevation of cardiac output depended almost entirely on an increase in heart rate with almost no variation in stroke volume (Fig. 5.5b). This finding is surprising since the relative contribution of stroke volume to the increase of cardiac output has previously been found to be similar to or greater than that of heart rate (Farrell and Jones, 1992). The major difference that could explain the divergence in findings between previous studies and this one is the swimming speed attained by the fish. In the previous studies on swimming rainbow trout, the fish only reached a swimming speed of about $2 \text{ BL}\cdot\text{S}^{-1}$ (Kiceniuk and Jones 1977, Taylor *et al.*, 1996, Thorarensen *et al.*, 1996b) while the fish swam up to about $4.6 \text{ BL}\cdot\text{S}^{-1}$ in the present study. The larger amount of instrumentation carried by the fish in the other studies is most likely an important factor for the smaller swimming speeds attained. Critical swimming velocity in non-instrumented rainbow trout is usually around $4 \text{ BL}\cdot\text{S}^{-1}$ (Beamish, 1978). The physiological status of the highly instrumented fish or the sole fact of swimming more slowly may be responsible for the different cardiac response observed in previous studies. In cod, Webber *et al.* (1998) noted a variation in the relationship obtained between heart rate and oxygen consumption during swimming when fish were allowed to recover from a long surgery (30-60 min) for either less than 7 days or more than 7 days. This finding is coherent with the idea that highly instrumented fish subjected to a long surgery may present a different cardiac response to swimming than fish subjected to a short surgery (15 min or less) like in the present study. Thorarensen *et al.* (1996a) also noted that the contribution of heart rate to the increase of oxygen consumption is lower when fish are subjected to a more invasive surgery.

The objective of this study was to determine if cardiac output is a better indicator of metabolic rate than heart rate. The data obtained on rainbow trout show that although a strong relationship is often found between oxygen consumption and cardiac output (Fig. 5.4a

and 5.6a), there are situations where the variations in oxygen consumption are independent from cardiac output (Fig. 5.2a). It has also been observed that, when present, the relationship between oxygen consumption and cardiac output depends on the factor altering metabolic rate (Fig. 5.7a), and sometimes varies considerably between individuals (Fig. 5.6a). Thus, it appears that the problems of multiple regression lines and variability between individuals highlighted by Thorarensen *et al.* (1996a) about the use of heart rate as a predictor of metabolic rate are also of concern with cardiac output. Heart rate was even often more strongly correlated to oxygen consumption than cardiac output in this study, although the problem of scatter of data points (Fig. 5.2c) and variability of regression lines (Fig. 5.7c) was again observed.

For a fixed linear relationship to exist between cardiac output and oxygen consumption, blood oxygen extraction would have to be either constant or vary in a predictable manner with cardiac output as oxygen consumption changes. However, this study shows that the contribution of cardiac output and blood oxygen extraction to the modifications of oxygen consumption varies depending on the circumstances, which is why the relationship between oxygen consumption and cardiac output can be expressed by more than a single regression line. Cech *et al.* (1976) also observed variability in the contribution of cardiac output and blood oxygen extraction to the elevation of oxygen demand after an acute increase of temperature in the winter flounder (*Pseudopleuronectes americanus*). The cardiovascular adjustments changed from an increased blood oxygen extraction with no change in cardiac output at winter temperatures to an increased cardiac output and relatively unchanged blood oxygen extraction at summer temperatures.

In conclusion, this study showed that cardiac output presents very few advantages over heart rate as a predictor of metabolic rate in rainbow trout. The complex nature of the interactions existing between cardiac output, heart rate and blood oxygen extraction in the control of oxygen consumption calls for more research to be done before any cardiac parameter can be used to predict metabolic rate in free-swimming fish. A better understanding of the contributions of cardiac output and heart rate to the variation of oxygen consumption under various environmental conditions would maybe eventually allow to predict metabolic rate

from a set of regression lines, provided some information is known on the nature of the factors affecting metabolic rate. The problem of having to calibrate each fish individually may however remain if interindividual variability is always important. Some species other than rainbow trout could also be more suitable to the use of cardiac output as a predictor of metabolic rate since Webber *et al.* (1998) obtained a good relationship between cardiac output and oxygen consumption in the cod (*Gadus morhua*).

GENERAL DISCUSSION

Toxic chemicals are often expected to cause an elevation of cardiac output because of the increased demands for metabolic energy that they create. However, neither of the acute exposures to chlorophenols or Al and acidic water executed in this study caused an increase of cardiac output in salmonids. The case of the chlorophenols (chapter I) may be particular in that these chemicals impaired oxidation and decreased oxygen consumption at the concentrations used. It is thus not surprising that cardiac output did not increase under these circumstances since the energy demands and the need for convective delivery of oxygen were low.

In the case of the exposures to acidic water and Al (chapter II), the experiments have demonstrated that the observed decrease of cardiac output below basal level was most likely due to an impairment of the normal function of the heart. Indeed, by increasing haematocrit and reducing plasma volume, the exposure to Al in acidic water created an increase in vascular resistance that caused a reduction of stroke volume and therefore cardiac output. These experiments thus showed that Al in acidic water directly affects the cardiovascular system of the fish and prevents cardiac output to increase in order to account for the elevated energy demands.

The absence of an increase in cardiac output with the two types of contaminants used in this study should not be seen as an indication that cardiac output never contributes to the elevation of metabolic rate in the presence of toxicants. Chemicals can exert their toxicity through many different modes of action, and some are less likely to directly affect the heart or the respiratory enzymes than the one used in this study. If the normal function of the heart is not altered, then a fish may well elevate its cardiac output to support the increase in its energy metabolism.

However, although it does not affect the fact that some toxicants may elevate cardiac output, the data obtained in chapter IV show that cardiac output does not necessarily increase when heart function is not altered by the toxic exposure. In this chapter, the effects of Al and

acidic water on cardiac output were examined after the fish had been exposed for 36 days and had time to acclimate to the presence of Al. The haematocrit and plasma ion concentrations of these fish had essentially returned to baseline, so their capacity to increase cardiac output was likely unaffected. The resting cardiac output of these treated fish was not significantly different from the control group. These results show that, once they have acclimated to Al, the capacity of the fish to maintain their cardiac output is not reduced as was the case for acute exposure. However, these results also indicate that the metabolic alterations caused by Al in acidic water did not have any effects on cardiac output.

The subchronic exposure to acidic water and Al indeed altered the bioenergetics of the fish. Growth rate was reduced even after food consumption had returned to normal levels, and swimming activity was increased throughout the exposure. There are two potential explanations for the absence of an elevation of cardiac output. First, it may mean that the basal metabolic rate was not affected by the exposure and that the decrease in growth rate was entirely the result of the increase in swimming activity. In these conditions, the measurement of cardiac output would not show an increase in energy demands since it was executed under resting conditions. Another possible explanation for the absence of an effect on cardiac output is that the exposure to acidic water and Al did elevate the basal metabolic rate of the fish, but that the associated increase in oxygen consumption was mainly the result of an increase in blood oxygen extraction rather than cardiac output. The series of experiment described in chapter V indeed showed that the relative contribution of cardiac output and blood oxygen extraction to the increases of oxygen consumption can vary depending on the factors affecting metabolic rate.

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