Fish Physiology and Biochemistry vol. 15 no. 5 pp 385-394 (1996)
Kugler Publications, Amsterdam/New York

Cortisol-induced changes in oxygen consumption and ionic regulation in coastal cutthroat trout *(Oncorhynchus clarki clarki)* parr

John D. Morgan and George K. Iwama
Department of Animal Science, University of British Columbia, 248-2357 Main Mall, Vancouver, B.C. V6T 1Z4, Canada

Accepted: March 7, 1996

Keywords: cortisol, oxygen consumption, salinity stress, ion regulation, gill Na^+,K^+-ATPase, plasma glucose, cutthroat trout parr

Abstract

The influence of cortisol on oxygen consumption and osmoregulatory variables was examined in coastal cutthroat trout *(Oncorhynchus clarki clarki)* parr kept in fresh water (FW) and transferred to seawater (SW). Intraperitoneal implants containing cortisol (50 $\mu g\ g^{-1}$) in vegetable oil resulted in elevated plasma cortisol titres similar to those observed in fish following a 24h SW exposure. Cortisol treatment significantly increased the oxygen consumption and plasma glucose levels of trout in FW, consistent with the glucocorticoid role of cortisol. Cortisol treatment did not cause any changes in plasma ion concentrations or gill Na^+,K^+-ATPase activity in FW after 10 days. Cortisol-implanted fish exposed to SW for 24h showed slightly improved ion regulatory ability compare to non-implanted controls. The results of this study suggest that during SW transfer in juvenile salmonids, increases in cortisol may act as both a mineralocorticoid and a glucocorticoid, depending on the developmental state of the fish (*e.g.*, smolt versus parr). Furthermore, the relative energetic costs of osmoregulation and that of the stress associated with SW transfer cannot be discerned using whole-animal oxygen consumption rates.

Introduction

Considerable attention has been given in recent years to the physiological aspects of seawater (SW) adaptation in salmonids. The transfer of juvenile salmonids from fresh water (FW) to SW is accompanied by a number of physiological changes, including increases in plasma cortisol levels, gill Na^+,K^+-ATPase activity and metabolism (see reviews by Folmar and Dickhoff 1980; McCormick and Saunders 1987; Hoar 1988). In a recent study, we found that oxygen consumption rates of rainbow and steelhead trout *(Oncorhynchus mykiss)* and fall chinook salmon *(Oncorhynchus tshawytscha)* fry were higher in SW compared to FW (Morgan and Iwama 1991). The data suggested that the energetic demands for osmoregulation increased in SW, but we also speculated that some of the increase in oxygen consumption rates in the SW fish may have been related to the secondary effects of increased cortisol production on other metabolic processes (Morgan and Iwama 1991). That assertion was supported indirectly by published evidence that plasma cortisol levels are elevated in the long term (2–3 wk) in juvenile salmonids after entry into SW (Redding *et al.* 1984a; Young *et al.* 1989; Avella *et al.* 1990), and that elevated cortisol levels have been associated with higher rates of oxygen consumption in FW fish (Chan and Woo 1978; Barton and Schreck 1987). In order to better understand the effects that elevated plasma cortisol levels may have on oxygen consumption rates in juvenile salmonids, we examined the effects of exogenous cortisol addition on oxygen consumption rates of coastal cutthroat trout *(Oncorhynchus clarki clarki)* parr. In the only other study of cortisol effects on oxygen consumption rate in fish (Chan and Woo 1978) Japanese eels *(Anguilla japonica)* were given a single intramuscular injection of cortisol, and oxygen

Table 1. Length, weight, and plasma cortisol and ion concentrations of cutthroat trout parr following a 24h seawater challenge test

Treatment	Fork-length (cm)	Body weight (g)	Plasma			
			Cortisol (ng ml^{-1})	[Na^+] (mM)	[Cl^-] (mM)	[K^+] (mM)
FW	16.3 (0.5)	45.6 (5.1)	59.2 (12.6)	156.5 (4.5)	130.3 (1.7)	4.0 (0.1)
SW	15.2 (0.2)	33.5 (1.5)	151.2 (12.6)*	184.4 (6.4)*	182.5 (2.7)*	4.0 (0.2)

Values are the mean (± SEM) of 10 fish per treatment; * indicates significant differences between treatments ($p < 0.05$).

consumption rates were determined over the following 24h. Plasma cortisol levels were not measured and may have been outside of the physiological range due to the mode of administration.

The primary objective of the present study was to achieve elevated cortisol levels similar to those observed in cutthroat trout parr following SW entry, and observe its effects on the rate of oxygen consumption. In addition, changes in plasma levels of glucose and major ions, as well as branchial Na^+,K^+-ATPase activity were measured to examine the effects of plasma cortisol elevation on glucose metabolism and SW adaptability in cutthroat trout parr. Cortisol treatment has been shown to stimulate hypo-osmoregulatory ability in several species of salmonids (*e.g.*, Madsen 1990a,b; Bisbal and Specker 1991), but comparable data are lacking for cutthroat trout.

Materials and methods

Animals

Sea-run cutthroat trout eggs were taken at the Chehalis River hatchery near Harrison, B.C. in March, 1993 and reared at the University of British Columbia Aquaculture Unit. The fish were maintained in 800l tanks continuously supplied with dechlorinated Vancouver City tap water (Na^+ = 1 mM, Cl^- = 1 mM, Ca^{2+} = 0.03 mM, seasonal temperature variation: 4–16°C) and the lighting was controlled to provide a simulated natural photoperiod. The fish were fed a diet of commercial salmon pellets (EWOS Canada, salt content = 0.9% NaCl). The cutthroat trout were 16-month-old parr (mean length = 15.6 cm, mean weight = 37.6 g) at the time of the experiment in the fall of 1994. Sea-run cutthroat trout juveniles typically spend 2 years in FW before migrating to the sea (Trotter 1989).

Determination of dose for cortisol implants

To determine plasma cortisol levels after SW entry and establish a dosage for the cortisol implants, the fish were subjected to a 24h seawater challenge (SWC) test (Blackburn and Clarke 1987). Ten fish were transferred into each of two 200l tanks, one supplied with FW (15°C) and one containing aerated SW (30 ppt, Instant Ocean®) with continuous filtration. After 24h, the fish were anaesthetized in neutralized MS222 (100 mg l^{-1}), killed by a blow to the head, and blood was collected from the caudal vessels into heparinized syringes. Blood was centrifuged and the plasma removed and frozen at –75°C for cortisol and ion analyses. From this preliminary experiment (Table 1), it was decided to use a dose for the slow-releasing cortisol implants that would achieve a chronic plasma cortisol concentration of about 150–200 ng ml^{-1} (see below).

Cortisol implantation

The cutthroat trout were injected with slow-releasing cortisol implants, using the method described by Specker *et al.* (1994). The cortisol (hydrocortisone, Sigma Chemical Co., St. Louis, MO) was dissolved in a 1:1 mixture (w/w) of coconut oil:vegetable oil heated to liquification (42°C), at a concentration of 10 mg cortisol per ml of oil. The injections were made while the mixture was still fluid (25°C) and the implant solidified in the ambient water temperatures. The injections were made using a 1 ml plastic syringe attached to a 21 gauge 1" needle, and the insertion point into the peritoneal cavity was on the ventral body surface, midway between the pelvic and pectoral fins.

To achieve plasma cortisol concentrations similar to those seen in the SWC, fish were implanted with a dose of 50 μg cortisol g^{-1} body weight. The

fish were lightly anaesthetized (50 mg l^{-1} MS222) prior to injection and weighed to determine injection volume (50 µl per 10 g). The treatment groups were: untreated controls; sham-implants (oil only); and cortisol-implanted fish. The three groups were held in separate 200l tanks at a stocking density of 40 fish per tank (8 g l^{-1}). The fish were fed once daily, and food was withheld 24h prior to sampling or being placed into the respirometer. Water temperature and dissolved oxygen were also monitored daily in the tanks and ranged from 14.0–15.5°C and 0.29–0.32 mmol l^{-1} (93–99% saturation), respectively, during the experiment.

Respirometry

Respirometry experiments were performed on fish from the three treatment groups in the following order: controls; cortisol implants; and sham implants. Measurements for the sham and cortisol implant groups began 1 and 3 days after injection, respectively. Oxygen consumption rates were measured in a Brett-type swimming respirometer (*e.g.*, Brett 1964; Steffenson *et al.* 1984; Gerhke *et al.* 1990), modified for use with juvenile salmonids. A swimming respirometer design was chosen to control the swimming speed of the fish and reduce variations in oxygen consumption caused by spontaneous activity (Brett and Glass 1973). The total volume of the respirometer was 5.3 l, and the transparent swimming section was 21.0 cm long and 6.5 cm in diameter. Water flow in the respirometer was generated by a stainless steel centrifugal pump (Jabsco), powered by a 0.25 HP electric motor (Reliance DC-1). Motor speed, and thus water velocity, was controlled by a variable speed controller. Velocities in the swimming section could be regulated up to 52 cm s^{-1} and were measured by a Signet flow transmitter (Signet Scientific Co., El Monte, CA) installed upstream of the swimming section. The respirometer apparatus was oriented in a horizontal plane and supported in a 200 l reservoir, with the exception of the pump and motor which were externally mounted. Water supplied to the reservoir was directed into the respirometer through a valve assembly, which allowed the system to be operated in a flow-through (acclimation) or recirculation (measurement) mode. Water temperature in the reservoir was controlled by an attached temperature control unit (Lauda RM6 circulator), and temperature was monitored using a thermistor (Cole Parmer, Chicago, IL) mounted inside the respirometer.

Water oxygen partial pressure (P_{O_2}) in the respirometer was measured using a Radiometer oxygen electrode (E-5046) mounted in a thermostatted jacket (D-616) and connected to an oxygen meter (manufactured by the Max Planck Institute for Experimental Medicine, Göttingen, Germany). The oxygen electrode was calibrated to zero with a sodium bisulphite solution and to saturation with FW equilibrated with air. A constant water flow from the respirometer was passed over the electrode, using a Piper peristaltic pump (Dungey Inc., Agincourt, ON) and gas impermeable tubing, before being returned back into the respirometer. Signals from the thermistor, oxygen and flow meters were transferred to an IBM-compatible microcomputer using a 12-bit analog-to-digital converter (PC-LabCard 812, Advantech Co., Sunnyvale, CA). Data acquisition was performed using the program Labtech Notebook version 7.1.1 (Laboratory Technologies Corp., Wilmington, MA).

Prior to each respirometry trial, individual fish from a treatment tank were immobilized with buffered MS222, measured for length, and then placed into the swimming section through a removable lid. The fish were placed in the respirometers in flow-through water for 24h and were not fed for 48h prior to testing to ensure a postabsorptive digestive state (Brett 1964). The respirometer was covered throughout this period and testing to shield the fish from visual disturbances. After acclimation, the swimming speed was set to 1 body length s^{-1} and the water flow to the respirometer was stopped. The subsequent decline in water P_{O_2} was then monitored for 90 min, with values recorded very 5 min. After a trial was completed, the fish was removed from the swimming chamber within 30 s, killed, weighed, and blood sampled for plasma cortisol and glucose as described above. Concurrent blood samples were also taken from fish maintained in the holding tank, to assess the effect of the respirometry trial on plasma cortisol and glucose levels. The fish were dissected to locate the implants, which formed a soft pellet and were generally found at the posterior end of the body cavity. The trials were conducted at approximately the same time each day to minimize diurnal variation

in metabolism due to entrainment to a feeding schedule or photoperiod (Brett and Zala 1975). Water temperature during the trials were kept similar to the holding tanks (mean 15°C). Background oxygen consumption was measured by running blanks (*i.e.*, no fish) throughout the experimental period, and a correction was applied to all measurements with fish.

Water P_{O_2} decreased at a constant rate and about 25% of the initial oxygen was consumed during each trial. Measured P_{O_2} values (mm Hg) were converted to oxygen content (mg l^{-1}) using the conversion tables found in Colt (1984). Oxygen consumption rates were estimated using linear regression analysis and expressed as mmol O_2 kg^{-1} of fish h^{-1}.

Seawater challenge

Ten days after implantation and 2 days after respirometry, control and cortisol-implanted trout were sampled in FW and then subjected to a SWC test, to determine the effect of exogenous cortisol addition on SW adaptability. Ten fish were transferred from the two treatment groups into a common 200l tank containing aerated SW (30 ppt, 15°C). The two groups were identified by adipose fin-clips. After 24h, the fish were sampled for plasma cortisol, glucose and ion determinations as described above. Immediately following blood collection, gill tissue was removed from the first branchial arch on the left side, blotted dry, and stored in 1 ml of SEI buffer (0.3 M sucrose, 0.2 M Na_2 EDTA, 0.1 M imidazole, pH 7.1) at –75°C for measurement of Na^+,K^+-ATPase activity. The criterion used for proper SW adaptation was the ability to maintain plasma sodium ion concentrations less than 170 mM, 1 day after transfer to SW (Clarke *et al.* 1981).

Analytical procedures

Plasma cortisol levels were determined using a [^{125}I] cortisol radioimmunoassay kit (Coat-a-Count, Diagnostic Products Corporation, Los Angeles, CA). Plasma glucose levels were measured using a modification of Trinder's (1969) glucose oxidase method (Sigma Procedure 315). Plasma sodium [Na^+] and potassium [K^+] concentrations were measured on an ion chromatograph (Shimadzu Model HIC-6A, Shimadzu Corporation, Kyoto, Japan). Plasma samples were deproteinated with acetonitrile, diluted with distilled deionized water and injected into the ion chromatograph with 5 mM nitric acid as the mobile phase. Plasma chloride concentrations [Cl^-] were determined by coulometric titration (Hakke Buchler Instruments digital chloridometer).

Gill Na^+,K^+-ATPase activity (μmoles of ADP mg protein^{-1} h^{-1}) measurements were made using the method described by McCormick (1993). In this kinetic assay, the ouabain-sensitive hydrolysis of ATP is enzymatically coupled to the oxidation of NADH, which is directly measured in 96-well microplates at 340 nm for 10 min. Na^+,K^+-ATPase activity in crude gill homogenates was determined at 25°C in a temperature-controlled plate reader (Thermomax, Molecular Devices Corp., Menlo Park, CA). Protein content of the gill homogenates was determined in the microplate reader using the bicinchoninic acid procedure (Smith *et al.* 1985).

Statistical analyses

Data are presented as means ± 1 SEM. When analysis of variance indicated significant differences, Student-Newman-Keuls multiple comparison test was used to identify significantly different treatment means ($p < 0.05$). Comparisons between two sample means were accomplished using a Student t-test ($p < 0.05$).

Results

Oxygen consumption rates

The average oxygen consumption rate of cortisol-implanted fish was 14.9±0.4 mmol O_2 kg^{-1} h^{-1}. This was significantly (53%) higher than measured for the control (9.5±0.6 mmol O_2 kg^{-1} h^{-1}) and sham (10.0±1.2 mmol O_2 kg^{-1} h^{-1}) groups (Fig. 1). Implantation with oil alone did not significantly affect the rate of oxygen consumption compared to the untreated controls.

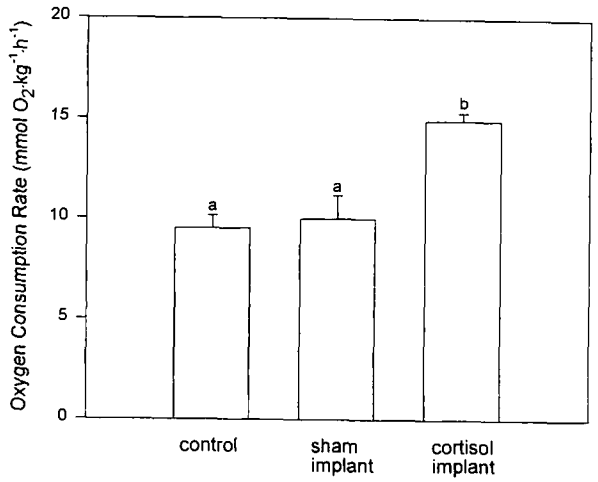
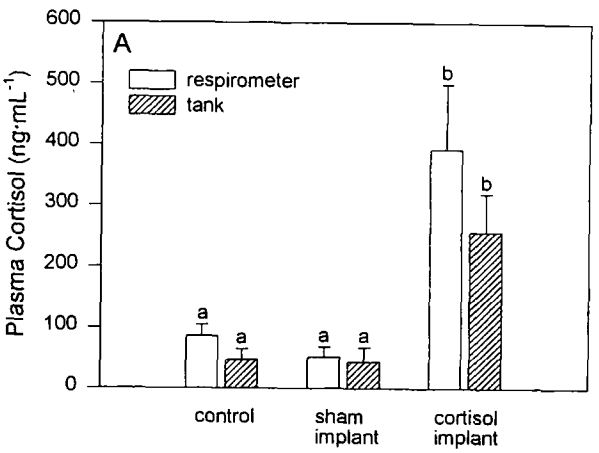
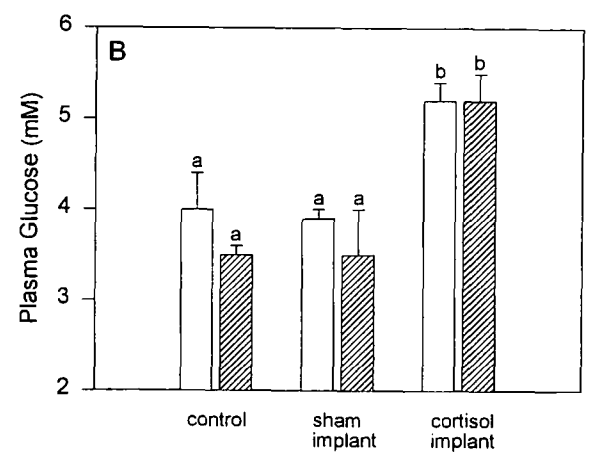


Fig. 1. The effect of cortisol treatment (50 μg g^{-1}) on oxygen consumption rate in cutthroat trout. Control fish were not treated, while sham fish received an oil implant only. Data are shown as means ± SEM (n = 6). Means with a common letter are not significantly different by Student-Newman-Keuls test ($p > 0.05$).





Plasma cortisol and glucose levels

During the respirometry trials, plasma cortisol levels in the cortisol-implanted fish averaged 257.0±62.9 ng ml^{-1}, and were about five times higher than measured for the control and sham groups (46.4±18.4 and 44.0±23.5 ng ml^{-1}, respectively). There was no significant difference in plasma cortisol titres between the control and sham groups (Fig. 2A). Plasma cortisol levels in fish sampled from the respirometer were slightly higher than the concurrently sampled tank fish, but the differences were not statistically significant. Following the 24h exposure to SW, plasma cortisol levels in the control group were significantly elevated, but there was no further increase in the cortisol-implanted group (Table 2).

Plasma glucose levels were significantly higher in the cortisol-implanted fish than in the control and sham groups, both in the respirometer and the holding tanks (Fig. 2B). In the SWC test, plasma glucose increased significantly in the control group, but showed no further increases in cortisol-implanted fish (Table 2).

Plasma ion concentrations

Cortisol treatment did not significantly affect plasma [Na^+], [Cl^-] or [K^+] of cutthroat trout parr in FW (Table 2). After the 24h SWC, plasma [Na^+] and [Cl^-] were significantly elevated in both groups, but plasma [Na^+] in the cortisol-implanted fish was significantly lower than the control fish, and below the 170 mM threshold value (Clarke *et al.* 1981). There were no changes in plasma [K^+] in control or cortisol-implanted fish following exposure to SW.

←

Fig. 2. Plasma cortisol and glucose concentrations in control, sham and cortisol-implanted (50 μg g^{-1}) cutthroat trout, sampled from the respirometer and holding tanks. Data are shown as means ± SEM (n = 6). Means for each group with a common letter are not significantly different by Student-Newman-Keuls test ($p > 0.05$). There were no significant differences between respirometer and tank values within a treatment ($p > 0.05$, t-test).

Table 2. Plasma cortisol, glucose and ion concentrations, and gill Na^+,K^+-ATPase activity in non-implanted and cortisol-implanted cutthroat trout parr, before and after a 24h seawater challenge test

Treatment	Plasma					Gill Na^+,K^+-ATPase (μmol ADP mg protein $^{-1}$ h^{-1})
	Cortisol (ng ml^{-1})	Glucose (mM)	$[Na^+]$ (mM)	$[Cl^-]$ (mM)	$[K^+]$ (mM)	
FW-Control	29.6 (8.6)	3.4 (0.1)	141.8 (4.0)	135.8 (1.1)	3.0 (0.1)	0.75 (0.06)
FW-Cortisol	203.8 (23.7)*	5.1 (0.2)*	134.4 (4.3)	135.0 (2.5)	2.7 (0.1)	0.69 (0.06)
SW-Control	282.3 (49.1)†	5.7 (0.4)†	197.1 (4.0)†	178.8 (4.2)†	3.1 (0.1)	0.90 (0.09)
SW-Cortisol	249.8 (36.3)	5.9 (1.6)	169.5 (7.2)*†	174.0 (3.1)†	3.0 (0.2)	1.18 (0.16)†

Values are the mean (± SEM) of 10 fish per treatment; * indicates significant difference between control and cortisol-implanted groups in fresh water or seawater ($p < 0.05$); † indicates significant difference between freshwater and seawater values within a treatment group ($p < 0.05$).

Gill Na^+,K^+-ATPase

Cortisol treatment for 10 days did not affect gill Na^+,K^+-ATPase activity of cutthroat trout parr in FW (Table 2). After the 24h SWC, only the cortisol implant group in SW showed a significant increase in enzyme activity compared to the cortisol implant group in FW.

Discussion

Plasma cortisol levels in the cutthroat trout parr following the two 24h SWC tests were quite high (means: 151 and 280 ng ml^{-1}) and resembled FW stenohaline fish rather than euryhaline fish during acclimation to SW. A number of studies with salmonids have shown that following an initial peak at 1–2h, plasma cortisol levels return to basal levels within 6–12h after SW entry (*e.g.*, Strange and Schreck 1980; Nichols and Weisbart 1985; Franklin *et al.* 1992). These cases generally involve smolt and post-smolt stages which can readily adapt to SW and may involve an increase in the clearance rate of cortisol in SW-adapted fish (Redding *et al.* 1984b; Nichols and Weisbart 1985; Patiño *et al.* 1987; Balm *et al.* 1995). In contrast, the plasma cortisol values obtained for cutthroat trout in this study were more comparable with other salmonid parr stages and non-salmonid species during exposure to saline environments. For example, Franklin *et al.* (1992) found that 15-month-old sockeye salmon *(Oncorhynchus nerka)* smolts which adapted to SW had a marked (to 231 ng ml^{-1}) but brief (6h) rise in plasma cortisol, while nine-month-old parr that did not fully acclimate had sustained increases in plasma cortisol levels (150–250 ng ml^{-1}). Carp *(Cyprinus carpio)* showed elevated plasma cortisol concentrations (120–190 ng ml^{-1}) for up to 3 days when transferred from FW to 15 ppt salinity (Abo Hegab and Hanke 1984). Goldfish *(Carassius auratus)* also showed a chronic (9 day) elevation of plasma cortisol (100–250 ng ml^{-1}) when exposed to 9 ppt salinity (Singley and Chavin 1975). These cyprinid species are considered to be FW stenohaline fish which do not normally encounter SW, and the increase in cortisol can be interpretated as being related to a stress response rather than having any osmoregulatory function (Strange and Schreck 1980). In fact, the plasma cortisol values in the SWC tests were very similar to those associated with acute handling stress in juvenile salmonids (see Barton and Iwama 1991). The increase in plasma glucose levels following SW exposure is also consistent with the concept of a stress response, and has been recorded previously for rainbow trout (Abo Hegab and Hanke 1986; Madsen 1990a). It is apparent then, that the cutthroat trout parr used in this study were stressed by the abrupt exposure to SW, and that the measured cortisol levels reflect a generalized stress response in addition to any osmoregulatory role.

The cortisol implant procedure employed in the present study proved effective in producing an elevation in plasma cortisol titres similar to those experienced by the cutthroat trout parr soon after SW entry. This increase in plasma cortisol concentration with the cortisol implant in FW fish resulted in increased plasma glucose levels and oxygen consumption rates. The increase in plasma glucose levels observed in the cortisol-implanted fish is

similar to results obtained in other studies (Chan and Woo 1978; Leach and Taylor 1982; Vijayan and Leatherland 1989) and probably resulted from a stimulatory effect of cortisol on gluconeogensis (Vijayan *et al.* 1994). In this study, the SWC test did not result in any further increases in plasma cortisol or glucose levels in cortisol-implanted fish. A similar result was obtained by Redding *et al.* (1984a) using coho salmon *(Oncorhynchus kisutch)*, and suggests that there may be an upper limit to the glucocorticoid response of juvenile salmonids to SW exposure.

The increases in oxygen consumption rate following cortisol implantation corroborate the findings of Chan and Woo (1978) using Japanese eels, and support the hypothesis that at least some of the increases in oxygen consumption rate in juvenile salmonid parr in SW may be caused by the glucocorticoid effects of cortisol, in addition to energy required to maintain ionic and osmotic homeostasis (*e.g.*, Morgan and Iwama 1991). As mentioned above, SW likely is a novel environment for life stages which are not preadapted for a marine existence and elicits a generalized stress response. The subsequent increase in cortisol production would stimulate several aspects of intermediary energy metabolism and result in an elevated rate of oxygen uptake. This type of response might be expected for life stages such as salmonid fry and parr (*e.g.*, Morgan and Iwama 1991; Franklin *et al.* 1992), salmonid smolts which have been held beyond the normal smolting 'window' prior to SW transfer and undergo parr-reversion (*e.g.*, Folmar *et al.* 1982; Avella *et al.* 1990), and other stenohaline FW species (*e.g.*, Toepfer and Barton 1992). The metabolic response of these fish to SW undoubtedly includes both stress and osmoregulation components, but the relative energetic demands of these processes cannot be discerned from whole-animal oxygen consumption measurements and requires further study. The metabolic cost of acute physical stress in juvenile salmonids has been described by Barton and Schreck (1987), who demonstrated a linear association between oxygen consumption rates and plasma cortisol levels in stressed juvenile steelhead trout kept in FW. In that study, a five fold increase in plasma cortisol levels was associated with a 70% increase in oxygen consumption rate; a response similar to that observed in the present study.

Given the apparent relationship between cortisol and oxygen consumption rate in fish presented here, it is also reasonable to propose that oxygen consumption rates would be elevated during the parr-smolt transformation in salmonids when there is a large transient increase in plasma cortisol levels (Specker and Schreck 1982; Hoar 1988). Resting oxygen consumption rates of Atlantic salmon *(Salmo salar)* smolts have been shown to be higher than parr of similar size (Higgins 1985).

Although cortisol plays an important role during SW acclimation in salmonids, it is unlikely that cortisol acts exclusively in causing an increase in oxygen consumption rate in SW. There are other osmoregulation hormones which show increases following SW entry and also have metabolic actions, most notably growth hormone (Sakamoto *et al.* 1993) and thyroid hormones (Dickhoff and Sullivan 1987), which are thought to interact with cortisol. Seddiki *et al.* (1995) have recently reported an increase in oxygen consumption in rainbow trout treated with trout recombinant growth hormone, both in FW and SW.

Gill Na^+,K^+-ATPase activity of untreated cutthroat trout parr in FW was similar to levels found for juvenile Atlantic salmon by McCormick (1993) in the fall, when activity of this enzyme is at its lowest level. Cortisol treatment did not significantly affect plasma ion concentrations or gill Na^+,K^+-ATPase activity in FW after ten days, contrary to the findings of Richman and Zaugg (1987), Madsen (1990a,b), and Bisbal and Specker (1991), but in agreement with Redding *et al.* (1984a), Langdon *et al.* (1984), and Eib and Hossner (1985). These differences among various studies may be due to interspecific differences, or changes in responsiveness of the gill tissue to cortisol treatment related to life history stage and season. McCormick *et al.* (1991), for example, found that coho salmon parr in the fall were not responsive to cortisol treatment in increasing gill Na^+,K^+-ATPase activity, compared to smolts in the following spring. Recent evidence has also indicated that cortisol can stimulate gill H^+-ATPase activity, which is linked to Na^+ uptake in FW fish (Lin and Randall 1993). If significant, this would result in an increase in plasma [Na^+] following cortisol treatment in FW, rather than a decrease normally associated with increased gill Na^+,K^+-ATPase activity (Madsen 1990a). H^+-ATPase activity was not measured in the present

study, but as mentioned above, cortisol did not significantly change plasma ion concentrations in FW.

Plasma [Na^+] and [Cl^-] of untreated fish were significantly elevated in both SWC tests, indicating that the cutthroat parr were not fully acclimated to SW. Similar plasma [Na^+] values following 24h SWC tests were reported for coastal cutthroat trout by Yeoh *et al.* (1991). Plasma [K^+] was unaffected by entry into SW, which was also observed for yearling coho salmon by Avella *et al.* (1990). The electrochemical gradient for K^+ between the blood and SW is much less than for Na^+ and Cl^- and does not appear to pose a problem for regulation (Evans 1993). Cortisol treatment resulted in lower plasma [Na^+] following SW exposure compared to untreated controls, similar to the findings of Madsen (1990a,b). This is consistent with the known stimulatory effects of cortisol on branchial ion excretion (McCormick and Bern 1989). The increase in plasma [Cl^-] after SW entry was not ameliorated by cortisol treatment. The reason for this is not clear, as the transport of Na^+ and Cl^- from the chloride cells into the external medium are thought to be linked to maintain electroneutrality (Zadunaisky 1984). Following the 24h SWC test, gill Na^+,K^+-ATPase activity showed a significant increase only in the cortisol-implanted fish. The relatively short exposure time may have prevented detection of this response, as increases in gill Na^+,K^+-ATPase associated with SW entry usually occur after a delay of several days (Folmar and Dickhoff 1980).

The present study demonstrates that oxygen consumption rates in juvenile salmonids may be elevated by cortisol concentrations similar to those found in pre-smolts following SW entry. The increases in cortisol which occur after SW transfer may be related to a stress response in addition to any mineralocorticoid role. The metabolic cost of the novel SW environment to the animal may be substantial, however, its impact on the energy demands for osmoregulation are not yet clear. It is apparent that studies which attempt to quantify the energetic cost of osmoregulation based on comparisons of oxygen consumption rates in fish acutely exposed to different salinities may be overestimating the true cost of this homeostatic process. Lastly, cortisol treatment did not significantly stimulate hypo-osmoregulatory mechanisms of cutthroat trout parr in FW, but it did appear to improve plasma Na^+ regulation following an acute (24h) exposure to SW.

Acknowledgements

Financial support for this research was provided by an NSERC operating grant to G.K.I. and a University of British Columbia Graduate Fellowship to J.D.M. We thank Dr. M.M. Vijayan for a review of the manuscript.

References cited

Abo Hegab, S. and Hanke, W. 1984. The significance of cortisol for osmoregulation in carp *(Cyprinus carpio)* and tilapia *(Sarotherodon mossambicus)*. Gen. Comp. Endocrinol. 54: 409–417.

Abo Hegab, S. and Hanke, W. 1986. Electrolyte changes, cell volume regulation and hormonal influences during acclimation of rainbow trout *(Salmo gairdneri)* to saltwater. Comp. Biochem. Physiol. 83A: 47–52.

Avella, M., Young, G., Prunet, P. and Schreck, C.B. 1990. Plasma prolactin and cortisol concentrations during salinity challenges of coho salmon *(Oncorhynchus kisutch)* at smolt and post-smolt stages. Aquaculture 91: 359–372.

Balm, P.H.M., Haenen, H.E.M.G. and Wendelaar Bonga, S.E. 1995. Regulation of interrenal function in freshwater and seawater adapted tilapia *(Oreochromis mossambicus)*. Fish Physiol. Biochem. 14: 37–47.

Barton, B.A. and Schreck, C.B. 1987. Metabolic cost of acute physical stress in juvenile steelhead. Trans. Am. Fish. Soc. 116: 257–263.

Barton, B.A. and Iwama, G.K. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Ann. Rev. Fish Dis. 1: 3–26.

Blackburn, J. and Clarke, W.C. 1987. Revised procedure for the 24 hour seawater challenge test to measure seawater adaptability of juvenile salmonids. Can. Tech. Rep. Fish. Aquat. Sci. 1515: 1–35.

Bisbal, G.A. and Specker, J.L. 1991. Cortisol stimulates hypo-osmoregulatory ability in Atlantic salmon, *Salmo salar*. J. Fish. Biol. 39: 421–432.

Brett, J.R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Bd. Can. 21: 1183–1226.

Brett, J.R. and Glass, N.R. 1973. Metabolic rates and critical swimming speeds of sockeye salmon *(Oncorhynchus nerka)* in relation to size and temperature. J. Fish. Res. Bd. Can. 30: 379–387.

Brett, J.R. and Zala, C.A. 1975. Daily pattern of nitrogen excretion and oxygen consumption of sockeye salmon *(Oncorhynchus nerka)* under controlled conditions. J. Fish. Res. Bd. Can. 32: 2479–2486.

Chan, D.O. and Woo, N.Y.S. 1978. Effect of cortisol on the metabolism of the eel, *Anguilla japonica*. Gen. Comp. Endocrinol. 35: 205–215.

Clarke, W.C., Shelbourn, J.E. and Brett, J.R. 1981. Effect of artificial photoperiod cycles, temperature, and salinity on growth and smolting in underyearling coho *(Oncorhynchus kisutch)*, chinook *(O. tshawytscha)*, and sockeye *(O. nerka)* salmon. Aquaculture 22: 105–116.

Colt, J. 1984. Computation of dissolved gas concentrations in water as functions of temperature, salinity, and pressure. Am. Fish. Soc. Spec. Publ. 14: 1–154.

Dickhoff, W.W. and Sullivan, C.V. 1987. Involvement of the thyroid gland in smoltification, with special reference to metabolic and developmental processes. Am. Fish. Soc. Symp. 1: 197–210.

Eib, D.W. and Hossner, K.L. 1985. The effects of cortisol and actinomycin D injections on chloride cells and branchial Na^+,K^+-ATPase in rainbow trout *(Salmo gairdneri)*. Gen. Comp. Endocrinol. 59: 449–452.

Evans, D.H. 1993. Osmotic and ionic regulation. *In* Physiology of Fishes. pp. 315–341. Edited by D.H. Evans. CRC Press, Boca Rotan.

Folmar, L.C. and Dickhoff, W.W. 1980. The parr-smolt transformation (smoltification) and seawater adaptation in salmonids. A review of selected literature. Aquaculture 21: 1–37.

Folmar, L.C., Dickhoff, W.W., Mahnken, C.V.W. and Wiknitz, F.W. 1982. Stunting and parr-reversion during smoltification of coho salmon *(Oncorhynchus kisutch)*. Aquaculture 28: 91–104.

Franklin, C.E., Forster, M.E. and Davison, W. 1992. Plasma cortisol and osmoregulatory changes in sockeye salmon transferred to sea water: Comparison between successful and unsuccessful adaptation. J. Fish Biol. 41: 113–122.

Gehrke, P.C., Fidler, L.E., Mense, D.C. and Randall, D.J. 1990. A respirometer with controlled water quality and computerized data acquisition for experiments with swimming fish. Fish Physiol. Biochem. 8: 61–67.

Higgins, P.J. 1985. Metabolic differences between Atlantic salmon *(Salmo salar)* parr and smolts. Aquaculture 45: 33–53.

Hoar, W.S. 1988. The physiology of smolting salmonids. *In* Fish Physiology. Vol. XIB, pp. 275–343. Edited by W.S. Hoar and D.J. Randall. Academic Press, San Diego.

Langdon, J.S. Thorpe, J.E. and Roberts, R.J. 1984. Effects of cortisol and ACTH on gill Na^+,K^+-ATPase, SDH and chloride cells in juvenile Atlantic salmon, *Salmo salar*. Comp. Biochem. Physiol. 77A: 9–12.

Leach, G.J. and Taylor, M.H. 1982. The effects of cortisol treatment on carbohydrate and protein metabolism in *Fundulus heteroclitus*. Gen. Comp. Endocrinol. 48: 76–83.

Lin, H. and Randall, D.J. 1993. H^+-ATPase activity in crude homogenates of fish gill tissue: inhibitor sensitivity and environmental and hormonal regulation. J. Exp. Biol. 180: 163–174.

Madsen, S.S. 1990a. Cortisol treatment improves the development of hypoosmoregulatory mechanisms in the euryhaline rainbow trout, *Salmo gairdneri*. Fish Physiol. Biochem. 8: 45–52.

Madsen, S.S. 1990b. The role of cortisol and growth hormone in seawater adaptation and development of hypoosomoregulatory mechanisms in sea trout parr *(Salmo trutta trutta)*. Gen. Comp. Endocrinol. 79: 1–11.

McCormick, S.D. and Saunders, R.L. 1987. Preparatory physiological adaptations for marine life of salmonids: osmoregulation, growth and metabolism. Am. Fish. Soc. Symp. 1: 211–219.

McCormick, S.D. and Bern, H.A. 1989. *In vitro* stimulation of Na^+,K^+-ATPase activity and ouabain binding by cortisol in coho salmon gill. Am. J. Physiol. 256: R707–R715.

McCormick, S.D., Dickhoff, W.W., Duston, J., Nishioka, R.S. and Bern, H.A. 1991. Developmental differences in the responsiveness of gill Na^+,K^+-ATPase to cortisol in salmonids. Gen. Comp. Endocrinol. 84: 308–317.

McCormick, S.D. 1993. Methods for nonlethal gill biopsy and measurements of Na^+,K^+-ATPase activity. Can. J. Fish. Aquat. Sci. 50: 656–658.

Morgan, J.D. and Iwama, G.K. 1991. Effects of salinity on growth, metabolism, and ion regulation in juvenile rainbow and steelhead trout *(Oncorhynchus mykiss)* and fall chinook salmon *(Oncorhynchus tshawytscha)*. Can. J. Fish. Aquat. Sci. 48: 2083–2094.

Nichols, D.J. and Weisbart, M. 1985. Cortisol dynamics during seawater adaptation of Atlantic salmon, *Salmo salar*. Am. J. Physiol. 248: R651–R659.

Patiño, R., Redding, J.M. and Schreck, C.B. 1987. Interrenal secretion of corticosteroids and plasma cortisol and cortisone concentrations after acute stress and during seawater acclimation in juvenile coho salmon *(Oncorhynchus kisutch)*. Gen. Comp. Endocrinol. 68: 431–439.

Redding, J.M., Schreck, C.B., Birks, E.K. and Ewing, R.D. 1984a. Cortisol and its effects on plasma thyroid hormone and plasma electrolyte concentrations in fresh water and during seawater acclimation in yearling coho salmon *(Oncorhynchus kisutch)*. Gen. Comp. Endocrinol. 56: 146–155.

Redding, J.M., Patiño, R. and Schreck, C.B. 1984b. Clearance of corticosteroids in yearling coho salmon, *Oncorhynchus kisutch*, in fresh water and seawater and after stress. Gen. Comp. Endocrinol. 54: 433–443.

Richman, N.H. and Zaugg, W.S. 1987. Effects of cortisol and growth hormone on osmoregulation in pre- and desmoltified coho salmon *(Oncorhynchus kisutch)*. Gen. Comp. Endocrinol. 65: 189–198.

Sakamoto, T., McCormick, S.D. and Hirano, T. 1993. Osmoregulatory actions of growth hormone and its mode of action in salmonids: A review. Fish Physiol. Biochem. 11: 1–6.

Seddiki, H., Maxime, V., Boeuf, G. and Peyraud, C. 1995. Effects of growth hormone on plasma ionic regulation, respiration and extracellular acid-base status in trout (*Oncorhynchus mykiss*) transferred to seawater. Fish Physiol. Biochem. 14: 279–288.

Singley, J.A. and Chavin, W. 1975. The adreno-cortical-hypophyseal response to saline stress in the goldfish, *Carassius auratus*. Comp. Biochem. Physiol. 51A: 749–756.

Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J. and Klenk, D.C. 1985. Measurement of protein using bicinchoninic acid. Anal. Biochem. 150: 76–85.

Specker, J.L. and Schreck, C.B. 1982. Changes in plasma corticosteroids during smoltification of coho salmon, *Oncorhynchus kisutch*. Gen. Comp. Endocrinol. 46: 53–58.

Specker, J.L., Portesi, D.M., Cornell, S.C. and Veillette, P.A. 1994. Methodology for implanting cortisol in Atlantic salmon and effects of chronically elevated cortisol on osmoregulatory physiology. Aquaculture 121: 181–193.

Steffensen, J.F., Johansen, K. and Bushnell, P.G. 1984. An automated swimming respirometer. Comp. Biochem. Physiol. 79A: 437–440.

Strange, R.J. and Schreck, C.B. 1980. Seawater and confinement

alters survival and cortisol concentration in juvenile chinook salmon. Copeia 1980: 351–353.

Toepfer, C. and Barton, M. 1992. Influence of salinity on the rates of oxygen consumption in two species of freshwater fishes, *Phoxinus erythrogaster* (family Cyprinidae), and *Fundulus catenatus* (family Fundulidae). Hydrobiologia 242: 149–154.

Trinder, P. 1969. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem. 6: 24.

Trotter, P.C. 1989. Coastal cutthroat trout: a life history compendium. Trans. Am. Fish. Soc. 118: 463–473.

Vijayan, M.M. and Leatherland, J.F. 1989. Cortisol-induced changes in plasma glucose, protein, and thyroid hormone levels, and liver glycogen content of coho salmon *(Oncorhynchus kisutch)*. Can. J. Zool. 67: 2746–2750.

Vijayan, M.M., Reddy, P.K., Leatherland, J.F. and Moon, T.W. 1994. The effects of cortisol on hepatocyte metabolism in rainbow trout: a study using the steroid analogue RU486. Gen. Comp. Endocrinol. 96: 75–84.

Yeoh, C.G., Kerstetter, T.H. and Loudenslager, E.J. 1991. Twenty-four-hour seawater challenge test for coastal cutthroat trout. Prog. Fish-Cult. 53: 173–176.

Young, G., Bjornsson, B.T., Prunet, P., Lin, R.J. and Bern, H.A. 1989. Smoltification and seawater adaptation in coho salmon *(Oncorhynchus kisutch)*: plasma prolactin, thyroid hormones, and cortisol. Gen. Comp. Endocrinol. 74: 335–345.

Zadunaisky, J.A. 1984. The chloride cell: the active transport of chloride and the paracellular pathways. *In* Fish Physiology. Vol. XB, pp. 129–176. Edited by W.S. Hoar and D.J. Randall. Academic Press, Orlando.