General and Comparative Endocrinology 308 (2021) 113769

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Effects of long-term cortisol treatment on growth and osmoregulation of Atlantic salmon and brook trout

L. Vargas-Chacoff [a,b,c,*], A.M. Regish [b], A. Weinstock [b], B.Th. Björnsson [d], S.D. McCormick [b,e]

[a] Instituto de Ciencias Marinas y Limnológicas, Laboratorio de Fisiología de Peces, Universidad Austral de Chile, Valdivia, Chile
[b] U.S. Geological Survey, Eastern Ecological Science Center, Conte Research Laboratory, Turners Falls, MA, USA
[c] Fondap-IDEAL, Universidad Austral de Chile, Valdivia, Chile
[d] Fish Endocrinology Laboratory, Department of Biological and Environmental Sciences, University of Gothenburg, Gothenburg, Sweden
[e] Department of Biology, University of Massachusetts, Amherst, MA 01003, USA

ARTICLE INFO

Keywords:
Brook trout
Atlantic salmon
Cortisol
Stress
Growth
Osmoregulation

ABSTRACT

Cortisol is the final product of the hypothalamic-pituitary-interrenal (HPI) axis and acts as a gluco- and mineralocorticoid in fish. Long-term elevations of cortisol have been linked to reduced growth in fishes, but the mechanism(s) and relative sensitivities of species are still unclear. We carried out experiments to examine the relative effects of cortisol on growth and gill NKA activity in two salmonids: Atlantic salmon (*Salmo salar*) and brook trout (*Salvelinus fontinalis*). Treatment with intraperitoneal cortisol implants for 30 days resulted in reduced growth in both species, but with greater sensitivity to cortisol in brook trout. Gill NKA activity was strongly upregulated by cortisol in Atlantic salmon, and weakly upregulated in brook trout but with no statistically significant effect. Cortisol treatment resulted in reduced plasma levels of insulin-like growth factor I and increased plasma growth hormone levels in Atlantic salmon. Our results demonstrate that there are species differences in the sensitivity of growth and osmoregulation to cortisol, even among species in the same family (Salmonidae).

1. Introduction

The stress response in fish, as in other vertebrates, is categorized into primary (release of catecholamines and cortisol), secondary (changes in metabolism, respiration rate, hydromineral balance, immune function and cellular responses) and tertiary (alteration in reproduction and growth, inhibition of resistance to disease and ultimately survival) responses (Barton, 2002; Donaldson et al., 2008; Vargas-Chacoff et al., 2014, 2015; Wendelaar-Bonga, 1997). Cortisol acts as both a gluco- and mineralocorticoid in fish (McCormick, 2001; Mommsen et al., 1999). As a glucocorticoid, it increases plasma levels of glucose, lactate, and other metabolites that are used as substrates in different tissues (Polakof et al., 2007; Vargas-Chacoff et al., 2016, 2017). In the short-term, cortisol has an adaptive role in shifting metabolic needs away from growth towards more immediate metabolic demands (Arjona et al., 2009; Laiz-Carrión et al., 2009; Oyarzún et al., 2020; Pickering, 1993; Vargas-Chacoff et al., 2015; Wendelaar-Bonga, 1997). It is widely accepted that long term elevations in cortisol in response to stressors results in reduced food intake and growth in fish, including rainbow trout (*Oncorhynchus mykiss*; Gregory and Wood, 1999; Madison et al., 2015), sea bass (*Dicentrarchus labrax*; Leal et al., 2011), channel catfish (*Ictalurus punctatus*; Peterson and Small, 2005), largemouth bass (*Micropterus salmoides*; O'Connor et al., 2011) and brown trout (*Salmo trutta*; Birnie-Gauvin et al., 2017). In addition, the mechanisms through which cortisol acts to affect feeding, metabolism, and growth, remain unclear (Bernier et al., 2004). Several studies have focused on the impact of cortisol on the GH/IGF-I system (Small et al., 2006; Breves et al., 2020; Kajimura et al., 2003; Peterson and Small, 2005; Pierce et al., 2005; Leung et al., 2008; Madison et al., 2015) due to it is critical role in controlling growth of fishes and other vertebrates, but a consistent picture of the impact of cortisol has yet to emerge.

The mineralocorticoid actions of cortisol help maintain internal ion and water homeostasis by affecting ionocytes and ion transport proteins (Laiz-Carrión et al., 2005; McCormick, 2013; McCormick et al., 2009, 2013). Regulation of Na^+,K^+-ATPase (NKA) by cortisol is a prime determinant of osmoregulatory capacity of euryhaline teleosts in seawater and has been widely investigated (Mancera and McCormick, 1999, 2007), but its role in regulating NKA in freshwater habitats is less clear. Cortisol plays a key role in smolt development of anadromous salmonids, a process which includes increased salinity tolerance. During

* Corresponding author at: Instituto de Ciencias Marinas y Limnológicas, Laboratorio de Fisiología de Peces, Universidad Austral de Chile, Valdivia, Chile.
E-mail address: luis.vargas@uach.cl (L. Vargas-Chacoff).

https://doi.org/10.1016/j.ygcen.2021.113769
Received 25 November 2020; Received in revised form 14 March 2021; Accepted 25 March 2021
Available online 29 March 2021

smolt development of Atlantic salmon (*Salmo salar*), plasma cortisol levels increase 5- to 10-fold during smolt development and decrease when it is complete (McCormick et al., 2007). Cortisol also has synergistic effects with growth hormone (GH) and insulin-like growth factor I (IGF-I) to regulate salinity tolerance (Hoar, 1988).

Brook trout and Atlantic salmon are economically, ecologically and culturally important species of salmonids. Hoar (1976) described the genus *Salvelinus* as having arisen earlier than *Salmo* and only rarely anadromous. In comparison to anadromous salmonids such as Atlantic salmon, brook trout have a lower seawater tolerance (McCormick and Naiman, 1984a, 1984b; McCormick et al., 1985). Possibly, such differences in salinity tolerance may be linked to differential osmoregulatory responses to cortisol. As with effects of cortisol on growth, differences in the magnitude and sensitivity of osmoregulatory parameters to cortisol have not been examined in closely related species. The aim of this study was to elucidate the effects of exogenous cortisol on growth and osmoregulation in Atlantic salmon and brook trout. To achieve these objectives, we implanted brook trout and Atlantic salmon with slow-release intraperitoneal cortisol implants at several doses and assessed growth, condition factor, gill NKA activity, plasma cortisol, chloride and glucose in both species. Plasma GH and IGF-I were assessed in both species, but assay validation could only be achieved for Atlantic salmon, and only those results are reported.

2. Material and methods

2.1. Animal source and rearing

Atlantic salmon parr were obtained from the U.S. Fish and Wildlife Service White River National Fish Hatchery (White River VT, USA) and brought to the Conte Anadromous Fish Research Center (Turners Falls, MA, USA) in autumn. Fish were reared in 1000 L tanks supplied with seasonally tempered river water at a flow rate of 4 L min^{-1} (1.4–15.9 °C) and provided with supplemental aeration. They were maintained under natural photoperiod conditions and fed *ad libitum* twice per day (Finfish Gold, Zeigler Bros, Gardners, PA, USA with a composition of 42% protein, 16% fat (minimum), 3% crude fiber, 12% moisture and 8% ash (maximum)).

Brook trout were obtained from Roger Reed Salmon Hatchery (Palmer, MA, USA) and maintained at the U.S. Geological Survey, Eastern Ecological Science Center, Conte Anadromous Fish Research Center (Turners Falls, MA, USA), they were maintained under natural photoperiod conditions and fed *ad libitum* twice per day (BioTrout, BioOregon, Westbrook, ME, USA with a composition of 47% protein, 24% fat (minimum), 2% crude fiber, 8.5% moisture and 12% ash (maximum)).

For acclimation experiments conducted in the winter, Atlantic salmon of mass 23 ± 2 g, and brook trout of mass 76 ± 11 g were placed in 1000 L tanks at 10 °C with flow, current, and continuous aeration in freshwater over a 30-day period. During the acclimation, fish were fed *ad libitum* once daily. To allow individual identification of experimental fish, they were tagged intraperitoneally with a 23 mm long, 3–4 diameter, 0.6 g passive integrated transponder (PIT) tag (Texas Instruments Dallas, TX, USA) as outlined in Zydlewski et al (2014).

2.2. Experiments

Atlantic salmon (n = 10–11 per treatment) were anaesthetized with MS-222, body mass (BM) and fork length (FL) measured, PIT-tagged, then were injected intraperitoneally (5 μL g BM^{-1}) with a semi-solid mixture of vegetable oil and shortening (1:1) with or without cortisol to achieve implants containing 0, 40 and 200 μg cortisol g BM^{-1} (Specker et al., 1994). A second 14 d experiment (with implants of 0 and 40 μg cortisol g BM^{-1}) was conducted on PIT tagged Atlantic salmon (n = 16 per treatment) to assess levels of plasma cortisol and the impact of cortisol on food consumption at this intermediate time point.

PIT tagged brook trout (n = 20 per treatment) were implanted intraperitoneally with semi-solid coconut oil (5 μL g BM^{-1}, Sigma C-1758), without or with cortisol to achieve implants containing 0, 8.25, 25 and 100 μg cortisol g BM^{-1}. Because of the mortality induced by 200 μg cortisol g BM^{-1} in Atlantic salmon, a lower and broader range of doses were chosen, but a range that still bracketed the 40 μg cortisol g BM^{-1} dose, allowing for direct comparison of dose responses in the two species. Prior to implantation, the coconut oil was warmed to facilitate the implant procedure. Fish were allowed to recover and transferred to experimental tanks.

All fish were captured, lightly anaesthetized with MS-222 and measured for BM and FL, as well as total body length (BL) at 0, 10, 20, and 30 days post-implantation (dpi). At these times, feeding rations were *ad libitum*.

Food was withheld for 24 h prior to physiological sampling of fish, which occurred between 10:00 and 12:00 h Eastern Standard Time. Atlantic salmon were sampled at 14 and 30 days post-implantation (dpi). Brook trout were sampled only at 30 dpi. Fish were anesthetized with MS-222 and their blood was drawn from the caudal vessels into a 1 mL ammonium heparinized syringe, within 5 min of capture. Blood was centrifuged at 3200*g* for 5 min at 4 °C and plasma aliquoted and stored at −80 °C. Four to six gill filaments were placed in 100 μL of ice-cold SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3) and frozen at −80 °C for measurement of NKA activity. For Atlantic salmon on day 14 the stomach and intestine were dissected and the ingested food contents were removed and weighed.

2.3. Plasma measurements

Plasma glucose was measured by enzymatic coupling with hexokinase and glucose 6-phosphate dehydrogenase (Stein, 1965). Plasma Cl^- was measured by silver titration using a digital chloridometer (Labconco, Kansas City, MO). Plasma cortisol was measured by a previously validated direct enzyme immunoassay (Carey and McCormick, 1998). Plasma growth hormone was measured by a radioimmunoassay validated for Atlantic salmon (Börnsson et al., 1988). Plasma IGF-I was measured by homologous radioimmunoassay as described by Moriyama et al. (1994). Neither the GH nor IGF-I assays that were utilized for Atlantic salmon could be validated for use in brook trout due to poor antibody-antigen cross reactivity. Limited volumes of plasma were available, so cortisol and IGF-I were measured first, followed by GH if there was sufficient volume for any given individual.

2.4. Gill NKA activity

Gill NKA activity was determined using the micro-assay method of McCormick (1993). In this assay, ouabain-sensitive ATPase activity was measured by coupling the production of ADP to NADH. Samples (10 μL) were run in duplicate in 96-well microplates at 25 °C and read at 340 nm for 10 min on a THERMOmax microplate reader using SOFTmax software (Molecular Devices, Menlo Park, CA, USA). Protein concentration of the homogenate was determined using a BCA protein assay (Pierce #23225).

2.5. Biometrics and statistics

Specific growth rate (SGR) was calculated as $[(\ln BM_f - \ln BM_i)/T] \times 100$, where BM_f is the final BM (g), BM_i is the initial BM (g) and T is the number of days (30) from the start until the end of the experiment. Condition factor was calculated as Fulton condition factor (K) = $(BM \times FL^{-3}) \times 100$. The liver was weighed to calculate the hepatosomatic index (HSI; [liver wet mass/BM] × 100). Gut contents were weighed to calculate the relative gut content mass = ([gut content wet mass/BM] × 100). Data were checked for normality, independence and homogeneity of variance before one, two-way analyses of variance (ANOVAs) and two-way mixed model ANOVA with missing values were carried out.

ANOVAs were conducted using doses or time-doses as factors of variance, followed by a Tukey test. Logarithmic transformations of the data were performed when necessary to fulfil the conditions of the parametric analysis of variance. Differences were considered to be significant at a level of $P < 0.05$. All data are presented as mean ± standard error (S.E.).

3. Results

3.1. Experiment 1a: Effects of cortisol on growth and osmoregulation in Atlantic salmon

There was no mortality in the control and 40 μg cortisol g BM^{-1} groups of Atlantic salmon over the 30-day study period, but 6 of 11 fish in 200 μg cortisol g BM^{-1} treated group died between days 20 and 30.

The effects of cortisol implants on body mass of Atlantic salmon juveniles are shown in Fig. 1. At the end of the experimental period, the control group (vehicle) of Atlantic salmon exhibited significantly greater body mass compared to initial mass, whereas no significant increase in body mass was detected in the 40 and 200 μg cortisol g BM^{-1} groups. Condition factor (K) at the end of 30 days was not significantly affected by cortisol treatment in Atlantic salmon (Fig. 2A). Due to the initial variability in mass present in all groups, individual specific growth rate (SGR) is a more robust way to examine the impact of cortisol on growth than examining changes in body mass in each group. The growth rate in Atlantic salmon injected with cortisol resulted in 40% and 36% of reduction compared to the control group, respectively (Fig. 2B).

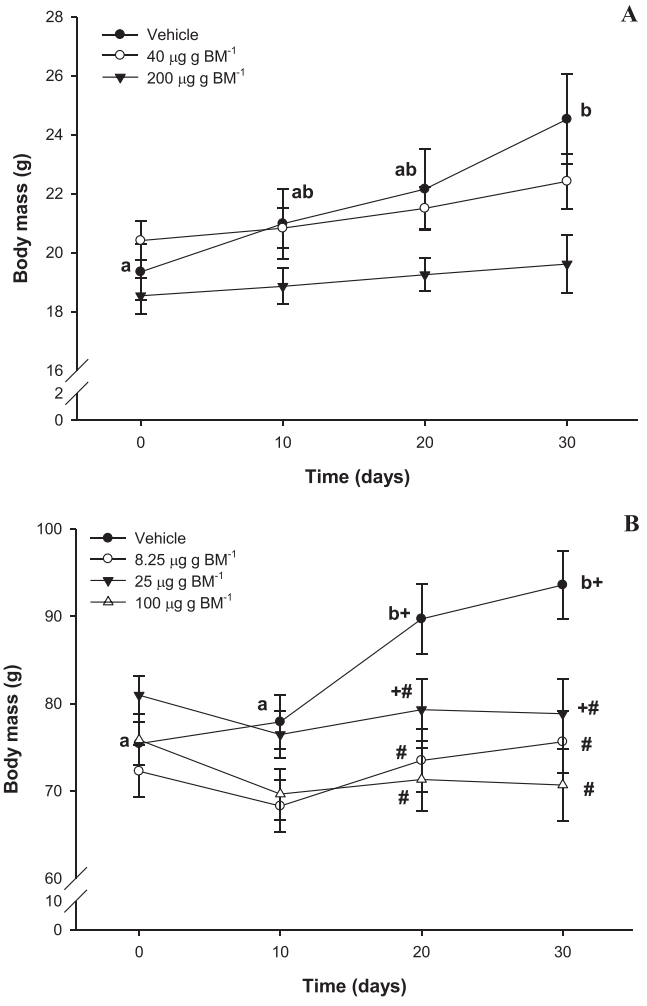

Fig. 1. Changes in body mass in (A) *Salmo salar* treated with 0, 40 or 200 μg cortisol g BM^{-1} (n = 10–11 per treatment). (B) *Salvelinus fontinalis* treated 0, 8.25, 25 or 100 μg cortisol g BM^{-1} over 30 days (n = 20 per treatment). Animals were sampled on days 10, 20 and 30 post-implantation. Mortality between days 20 and 30 in the 200 μg cortisol g BM^{-1} group resulted in sample size of 5 at the last time point. Lowercase letters indicate significant differences between days within each cortisol dose. Symbols (#, +) indicate significant differences among doses on the same day (Two-way ANOVA, Tukey-Test, P < 0.05).

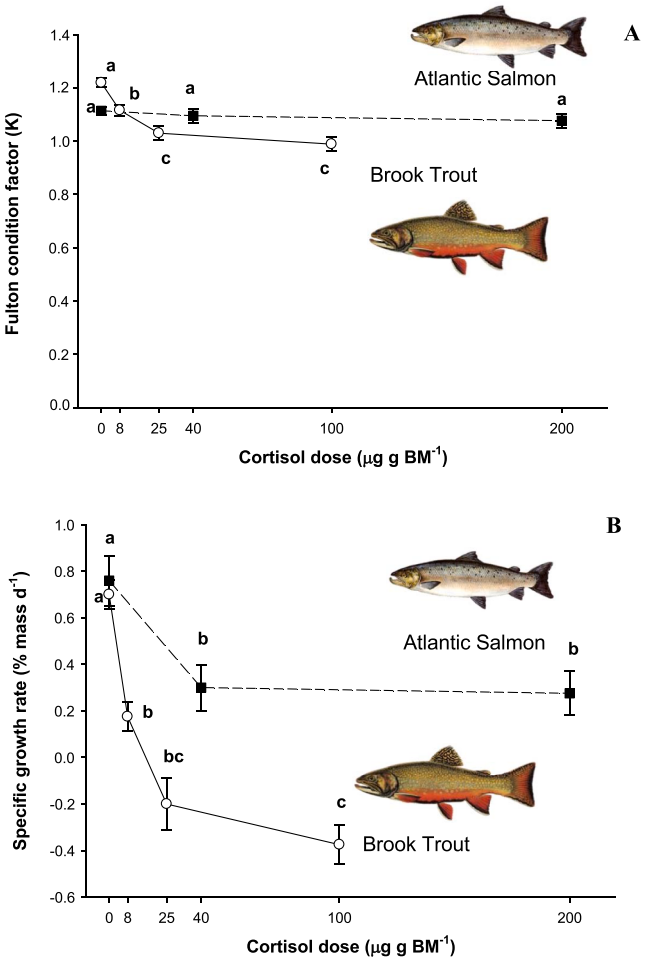

Fig. 2. Changes in Fulton condition factor (A) and growth rate (B) in Atlantic salmon treated with 0, 40 or 200 μg cortisol g BM^{-1} (n = 10 per treatment) and brook trout treated with 0, 8.25, 25 or 100 μg cortisol g BM^{-1} (n = 20 per treatment) for 30 d. Lowercase letters indicate significant differences between vehicle and cortisol doses (one-way ANOVA, Tukey-Test, P < 0.05).

Although the concentration could only be assessed from a single fish, plasma cortisol levels were highest in Atlantic salmon treated with 200 μg cortisol g BM^{-1} (Fig. 3A). In the 40 μg cortisol g BM^{-1} group plasma cortisol levels was significantly higher than controls at 14 and 30 days, but were significantly lower at 30 than at 14 days.

The gill NKA activity in Atlantic salmon increased in a dose-dependent manner at 14 and 30 dpi (Fig. 4A), reaching a maximum 2.2-fold increase in the 200 μg cortisol g BM^{-1} group at 30 dpi.

Plasma GH levels in the 40 μg cortisol g BM^{-1} group were 5.1 fold higher than controls at 30 dpi (Fig. 5). No plasma GH levels were obtained in the 200 μg cortisol g BM^{-1} group due to insufficient plasma volumes. Plasma IGF-I levels were significantly lower in cortisol-treated Atlantic salmon compared to the control groups only in the 200 μg cortisol g BM^{-1} treatment (Fig. 5).

3.2. Experiment 1b: Effects of cortisol on food intake in Atlantic salmon

There were no mortalities over the 14-day study. The hepatosomatic index (HSI), and relative gut content (g food in gut per g body mass) in Atlantic salmon treated with 40 μg cortisol g BM^{-1} were significantly

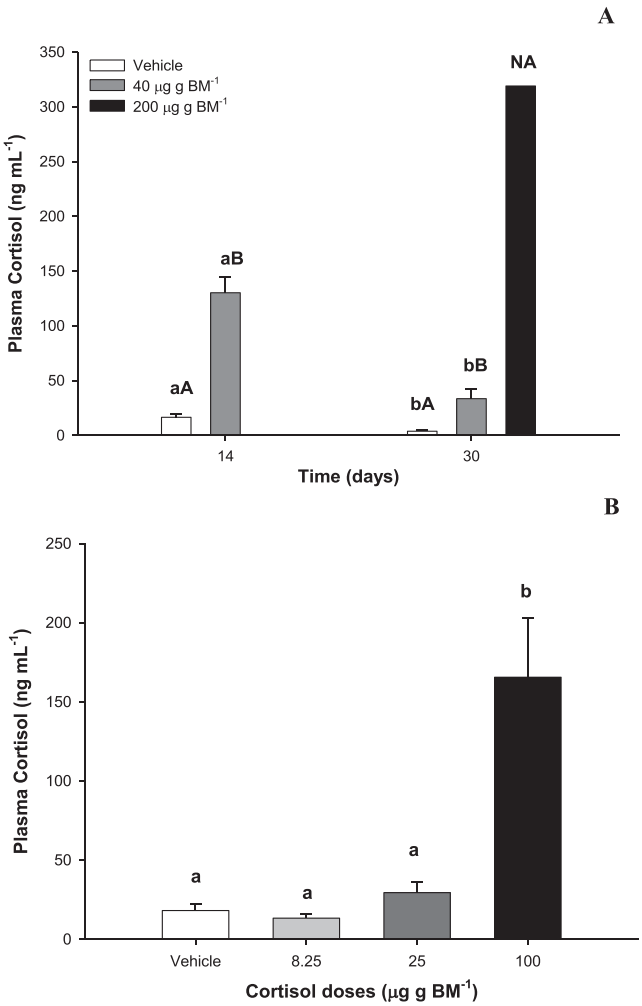

Fig. 3. Plasma cortisol levels in (A) *Salmo salar* treated with 0, 40 or 200 μg cortisol g BM^{-1} (n = 10 per treatment) for 14 or 30 d. Lowercase letters indicate significant differences between days for every cortisol dose. Upper case letters indicate significant differences between doses at the same days (Two-way ANOVA, Tukey-Test, $P < 0.05$). (B) *Salvelinus fontinalis* treated with 0, 8.25, 25 or 100 μg cortisol g BM^{-1} (n = 20 per treatment) for 30 d. Lowercase letters indicate significant differences between vehicle and cortisol doses (one-way ANOVA, Tukey-Test, $P < 0.05$). NA = not analysed because sample size was n = 1 (measurement of plasma GH and IGF-I was prioritized, and sufficient plasma volumes were only available for one fish in this group).

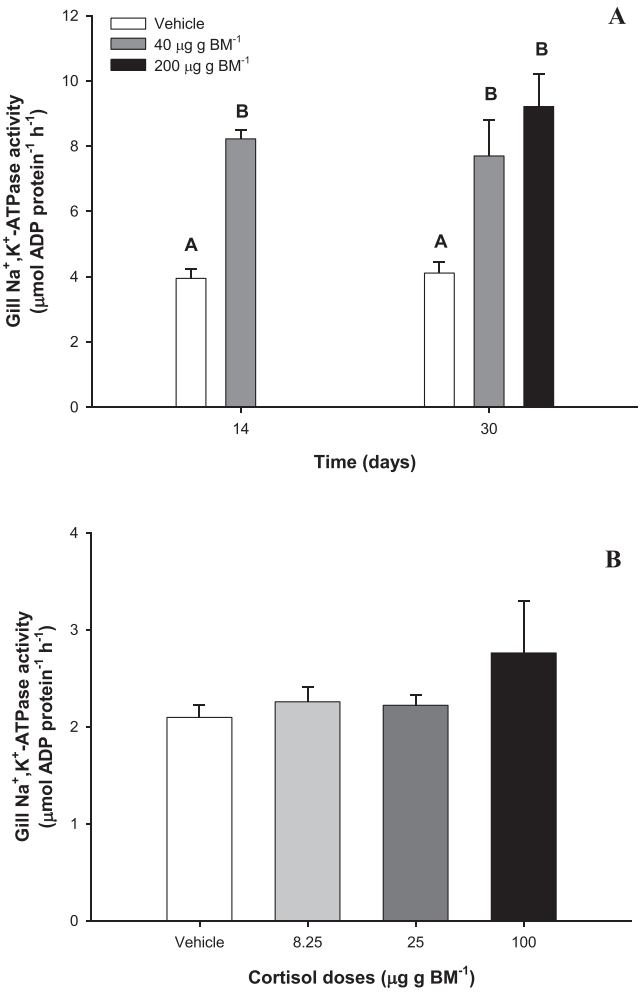

Fig. 4. Gill Na^+,K^+-ATPase activity in (A) *Salmo salar* treated with 0, 40 or 200 μg cortisol g BM^{-1} (n = 10 per treatment) for 30 d. No difference was observed between days for the vehicle and 40 μg cortisol g BM^{-1} treatments (Two-way ANOVA, Tukey-Test, $P > 0.05$). Upper case letters indicate significant differences between doses at the same days (one-way ANOVA, Tukey-Test, $P < 0.05$). (B) Gill Na^+,K^+-ATPase activity in *Salvelinus fontinalis* treated with 0, 8.25, 25 or 100 μg cortisol g BM^{-1} (n = 20 per treatment) for 30 d. No difference was observed between the vehicle and cortisol doses (one-way ANOVA, $P > 0.05$).

lower compared to the control group (Table 1). Plasma glucose was 23% higher in the cortisol treated group compared to controls, whereas plasma chloride levels did not significantly differ between the two groups (Table 1).

3.3. Experiment 2: Effects of cortisol on growth and osmoregulation in brook trout

No mortality or pathologies were observed in any brook trout during the experiment. The effects of cortisol implants on body mass of brook trout juveniles are shown in Fig. 1. In brook trout, the control group had significantly greater body mass at 20 and 30 days of treatment compared to initial mass. Body mass of the control group at day 30 was significantly greater than body mass of the fish in the two highest cortisol treatments (Fig. 1B). Condition factor in brook trout was significantly lower in all experimental groups compared to controls, with the control group having the highest K (1.22 ± 0.01) and the 100 μg cortisol g BM^{-1} group having the lowest K (0.98 ± 0.02) (Fig. 2A).

Growth rate in the brook trout control group was similar in magnitude to the Atlantic salmon control group (Fig. 2B). The lowest dose of cortisol resulted in a 75% decline in growth rate, and growth rate was negative (i.e. loss of mass) in the two highest doses (−0.20 ± 0.11 and −0.37 ± 0.08), having a 129% and 154% reduction in growth rate, respectively, relative to the control group (Fig. 2B).

Plasma glucose and chloride levels and hematocrit in brook trout at 30 dpi were not statistically different among cortisol treatments (Table 2). Plasma cortisol levels in brook trout increased significantly at 100 μg cortisol g BM^{-1} group, 9-fold more than the control group (Fig. 3B). Although cortisol levels were elevated in the 25 μg cortisol g BM^{-1} group compared to controls, they were not statistically different. Gill NKA activity in brook trout was not significantly altered by cortisol treatment (Fig. 4B).

4. Discussion

We investigated the role of cortisol in growth and ion homeostasis in Atlantic salmon and brook trout, two members of the sub-family Salmoninae, by treating them with cortisol implants for 30 days. We demonstrate that cortisol reduces growth rate in both species, but that growth rate in brook trout is far more sensitive to the effects of

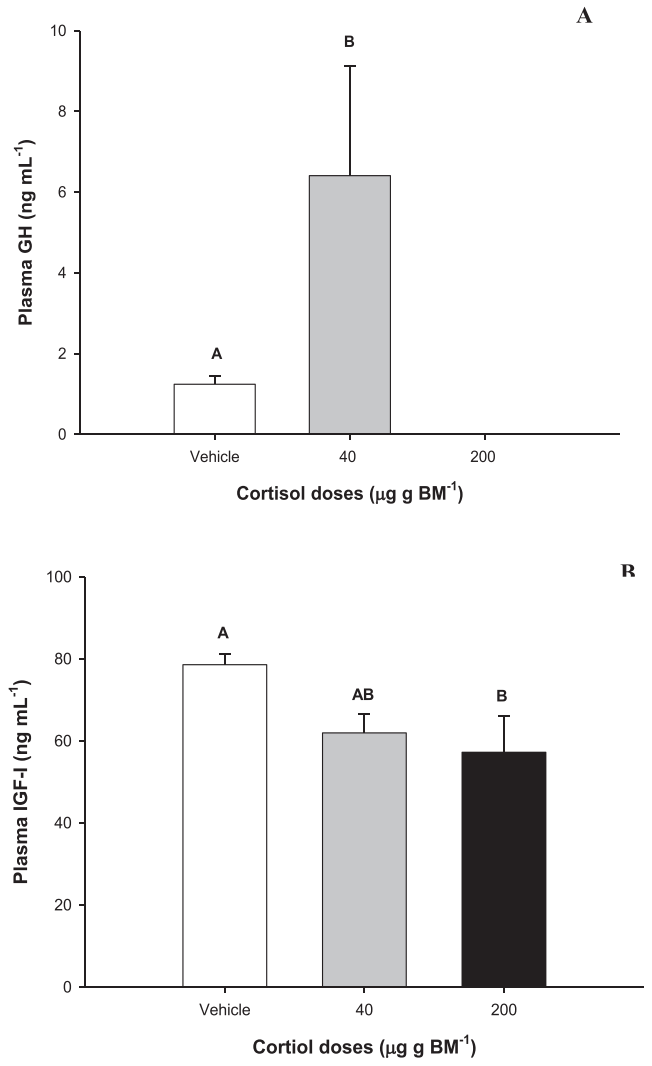

Fig. 5. Plasma growth hormone (GH; A) and plasma insulin-like growth factor I (IGF-I; B (histogram) in *Salmo salar* treated with 0, 40 or 200 µg cortisol g BM^{-1} (n = 10 per treatment) for 30 d. Lowercase letters indicate significant differences between vehicle and cortisol doses (one-way ANOVA, Tukey-Test, $P < 0.05$) for plasma GH and upper case letters indicate significant differences among cortisol doses for plasma IGF-I (one-way ANOVA, Tukey-Test, $P < 0.05$).

Table 1
Specific growth rate, hepatosomatic index (HSI), and relative gut content mass in Atlantic salmon treated with 0 (vehicle) and 40 µg cortisol g BM^{-1}. Animals were sampled at 14 days post-implantation (n = 16 per treatment). Values are mean + SEM. Asterisks (*) indicate significant differences between vehicle and cortisol dose (one-way ANOVA, $P < 0.05$).

	Control (vehicle)	40 µg cortisol g BM^{-1}
Growth rate (% BM d^{-1})	1.65 ± 0.32	0.58* ± 0.31
HSI (%)	1.56 ± 0.05	1.23* ± 0.06
Gut content (%)	1.11 ± 0.10	0.57* ± 0.10
Plasma glucose	5.57 ± 0.25	6.83* ± 0.35
Plasma chloride	128.9 ± 0.48	128.0 ± 0.76

exogenous cortisol than are Atlantic salmon. Conversely, cortisol treatment in brook trout does not upregulate gill NKA activity (a key component of salinity tolerance) to the same level as in Atlantic salmon. These results indicate that growth and osmoregulation are differentially responsive to cortisol in the two species, and in opposite directions.

The use of slow release of cortisol implants has been used in many previous studies (Laiz-Carrión et al., 2002; Mancera and McCormick, 1999; Specker et al., 1994) and in the present study resulted in elevated circulating cortisol in both species. The current results on Atlantic salmon at 40 µg cortisol g BM^{-1} were similar with other reports and resulted in circulating cortisol levels that were in the physiological range for this species when stressed (Carey and McCormick, 1998), while a cortisol dose of 200 µg cortisol g BM^{-1} induced 100-fold increases in plasma cortisol levels that are beyond the normal physiological range for this species. In brook trout, significant increases in plasma cortisol after 30 days were seen only at the highest dose of 100 µg cortisol g BM^{-1} (Fig. 3B). It is likely that plasma cortisol levels were elevated in the lower cortisol doses of 8.25 and 25 µg cortisol g BM^{-1} prior to the end of the experiment at 30 days, as was seen for Atlantic salmon at 40 µg cortisol g BM^{-1} at 14 days (Fig. 3A). In the present study, slightly different semi-solid cortisol-implant vehicles were used for the Atlantic salmon (vegetable shortening and oil) and the brook trout (coconut oil) experiments, which could potentially complicate direct comparison. Birnie-Gauvin et al. (2018) found that in brown trout (*Salmo trutta*), vegetable shortening implants containing cortisol (100 µg cortisol g BM^{-1}) resulted in elevated plasma cortisol for 9 days, whereas cortisol in cocoa butter at the same dose resulted in elevations of plasma cortisol that lasted for 3 days but not 6 days. The difference in plasma cortisol levels found in these studies may have limited application to the present study, however, as the same dose of cortisol in cocoa butter was used for brook trout, and we observed elevated plasma cortisol levels after 30 days. In addition, we used a vehicle of 1:1 of vegetable oil:shortening which should be more similar to cocoa butter than shortening alone, as the melting points are similar. The post-implant plasma cortisol profiles (Figs. 3 and 4), with a time-dependent decrease in plasma cortisol, which have been seen in previous studies on salmonids using similar implant methods (Specker et al., 1994), support the validity of the species comparison. In addition, the levels of plasma cortisol following cortisol treatment observed in the present study are similar to those seen previously in Atlantic salmon (McCormick et al., 2008; Specker et al., 1994) and brook trout (Shaughnessy and McCormick, 2018; Vijayan et al., 1991).

Table 2
Hematocrit, plasma glucose and chloride levels in brook trout (*Salvelinus fontinalis*) treated with 0, 8.25, 25 or 100 µg cortisol g BM^{-1}. Animals were sampled at 30 days post-implantation (n = 20 per treatment). Values are mean ± SEM.

Cortisol treatment	Hematocrit %	Glucose (mM)	Chloride (mM)
Vehicle	35 ± 0.9	5.1 ± 0.28	131.7 ± 0.86
8.25 µg cortisol g BM^{-1}	35 ± 0.9	6.9 ± 0.74	129.0 ± 1.85
25 µg cortisol g BM^{-1}	36 ± 0.9	5.7 ± 0.31	130.4 ± 1.07
100 µg cortisol g BM^{-1}	37 ± 1.3	6.0 ± 0.64	130.1 ± 2.19

The long-term elevation of plasma cortisol resulted in growth-suppressing effects in both salmonid species. Growth inhibition in response to cortisol treatment is consistent with previous data on goldfish (*Carassius auratus*), channel catfish (*Ictalurus punctatus*), rainbow trout (*Oncorhynchus mykiss*), largemouth bass (*Micropterus salmoides*) and sea bass (*Dicentrarchus labrax*) (Bernier et al., 2004; Leal et al., 2011; Madison et al., 2015; O'Connor et al., 2011; Peterson and Small, 2005; Small, 2004). However, the current study reveals that the cortisol-induced growth inhibition is much greater in brook trout than in Atlantic salmon for any given dose (Fig. 3). We found clear evidence that cortisol reduces food intake in Atlantic salmon, similar to data on cortisol-treated rainbow trout (Gregory and Wood, 1999; Madison et al., 2015), supporting the notion that decreased food intake is an important mechanism behind the cortisol-induced growth inhibition in salmonids and other fish species. Bernier et al. (2004) found that low doses of cortisol increased food intake in goldfish whereas high doses inhibited food intake, suggesting the impact of cortisol may be both species and dose dependent.

Appetite is regulated by the hypothalamus and peripheral signals via a range of neuropeptides that stimulate (orexigenic) or inhibit

(anorexigenic) food intake (Arora and Anubhuti, 2006; Kalra et al., 1999; Rønnestad et al., 2017; Volkoff et al., 2005). The orexigenic neuropeptides released within the hypothalamus include neuropeptide Y (NPY), α-, β-, y-melanocyte-stimulating hormones (MSH), while ghrelin is released from the gastrointestinal (GI) tract. Important anorexigenic peptides include corticotropin-releasing factor (CRF) from hypothalamus and leptin released from liver (Johansson and Björnsson, 2015; Schjolden et al., 2009). In goldfish, cortisol implants have been found to increase NPY mRNA levels and decrease CRF mRNA levels in the telencephalon-preoptic brain region, concomitant with lower food intake and growth rate (Bernier et al., 2004). In rainbow trout, cortisol treatment results in increased liver leptin (*lep-a1*) and brain preoptic area *crf* mRNA levels, along with reduced food intake and growth (Madison et al., 2015). Although appetite-regulating peptides were not measured in the present study, the decreased growth of cortisol-treated Atlantic salmon concomitant with decreased GI food content, makes it likely that cortisol is inhibiting appetite.

A key finding of the present study is the differentiated response in growth rate to cortisol between brook trout and Atlantic salmon. There are at least two, non-exclusive explanations for this. One could be a varying sensitivity of appetite-regulating hormones to cortisol between the species, and another could be linked to a species-specific effect of cortisol on metabolic rate and resource allocation. Cortisol treatment or high cortisol levels due to stress has been found to increase the resting metabolic rate of fish including Atlantic salmon, rainbow trout, Atlantic cod (*Gadus morhua*) and Nile tilapia (*Oreochromis niloticus*) (Abdel-Tawwab et al., 2014; Hvas and Oppedal, 2019; Li et al., 2018; Motyka et al., 2017; Thorarensen et al., 2017). Greater metaboic rate would lower conversion efficiency and decrease growth, which is consistent with studies showing that cortisol reduces conversion efficiency and lowers growth rate in goldfish (Bernier et al., 2004). The cortisol-induced decrease in HSI is likely the result of mobilization of triglyceride reserves and/or glycogen, allowing the production of substrates that enter glycolytic routes forming pyruvate and enabling amino acid and glucose production which can be used as fuel for metabolic demands (Mommsen et al., 1999). The stimulation of proteolytic and lipolytic pathways by cortisol (Valenzuela et al., 2017; Wendelaar-Bonga, 1997) would further indicate metabolic reallocation and explain the reduced condition factor that was observed in both salmonid species in the present study.

Plasma glucose levels were not affected by cortisol in the present study, which may relate to the relatively long-term treatment period used. Lack of elevated plasma glucose was found by Fast et al. (2008) in Atlantic salmon during 4 weeks of stress exposure, although in the first several hours, plasma glucose increased relative to control. Plasma glucose also did not differ in in Senegalese sole (*Solea senegalensis*) after 2 weeks of cortisol treatment (Arjona et al., 2011). These results are likely to be related to tertiary stress effects, at which stage glucose levels tend to reach basal levels, though the precise mechanisms behind this are not fully understood (Barton, 2002; Polakof et al., 2012; Wendelaar-Bonga, 1997).

The GH/IGF-I system is a key regulator of growth in fish as in other vertebrates (Björnsson et al., 2018). Our results indicate that cortisol's action to inhibit growth may act in part by impacting these hormones, as plasma GH levels in Atlantic salmon were significantly elevated 14 days after cortisol implant, while plasma IGF-I levels were suppressed. These results are consistent with previous studies on catfish, fine flounder and salmonids that have shown reduced liver IGF-I mRNA or plasma levels following cortisol or stress treatment that also resulted in reduced growth rate (Björnsson et al., 2018; Madison et al., 2015; Peterson and Small, 2005; Small, 2004; Valenzuela et al., 2017). Cortisol has been shown to affect the GH/IGF-I system by down-regulating GH receptor mRNA levels (Small et al., 2006) and/or activating intracellular pathways that attenuate GH receptor signal transduction (Philip and Vijayan, 2015). This 'uncoupling' of GH and IGF-I is also seen during catabolic stress and starvation and may facilitate shifting limited metabolic capacity away from growth and toward more immediate metabolic demands (Björnsson, 1997; Björnsson et al., 2018). Cortisol has also been shown to control specific IGF-I binding proteins (IGFBP) in salmonids (Shimizu and Dickhoff, 2017; Breves et al., 2020), which may provide further fine-tuning of the GH-IGF-I system during exposure to stressors. Similarly, cortisol treatment increased plasma levels of a 20 kD IGFBP in catfish (Peterson and Small, 2005), and 4 different plasma IGFBPs in tilapia (Kajimura et al., 2003), which are associated with reduced growth in response to cortisol. Overall, these results indicate a strong effect of cortisol on the GH-IGF-I system in fishes which may explain many of its negative impacts of cortisol on growth.

Many studies in salmonids and other teleost species have addressed the effects of cortisol on NKA activity in gills, indicating that this ion transport protein is directly regulated by cortisol (Arjona et al., 2011; Laiz-Carrión et al., 2002; Mancera and McCormick, 1999; McCormick, 1995; McCormick et al., 2013). Cortisol promotes seawater acclimation of teleosts by stimulating the development of seawater-type gill ionocytes, gill NKA activity, and other ion transporters involved in salt secretion, resulting in increased salinity tolerance (Madsen, 1990; Madsen et al., 1995; McCormick, 1995, 1996; McCormick et al., 2013; Shaughnessy and McCormick, 2018). In the present study, gill NKA activity of Atlantic salmon was elevated by cortisol treatment after 14 and 30 days of treatment, consistent with previous studies noted above. In contrast, gill NKA activity in the brook trout was not affected by cortisol at any of the doses used. These results contrast with a previous study indicating that brook trout treated for 12 day with 25 μg cortisol g BM^{-1} exhibited a 50% increase in gill NKA activity. As with plasma glucose, this difference may lie in the long-term cortisol treatment protocol used in the present study, as the sensitivity of gill NKA activity to cortisol is related to branchial levels of cortisol receptors (Shrimpton and McCormick, 1999). In addition, cortisol receptors in the gills of salmonids are downregulated after exogenous cortisol treatments (Shrimpton and Randall, 1994). Thus, the species-specific cortisol sensitivity seen in the present study may relate to both initial abundance of branchial cortisol receptors and their regulation in response to cortisol.

The lower sensitivity of gill NKA activity to plasma cortisol in the brook trout, in comparison with the Atlantic salmon, may ultimately relate to the greater degree of anadromy of the latter (Hoar, 1976). Most populations of Atlantic salmon are anadromous, and juveniles of this species undergo a parr-smolt transformation which prepares them for downstream migration and ocean entry (McCormick, 2013). Prominent among these developmental changes is an increase in salinity tolerance and gill NKA activity which is driven in part by increased plasma cortisol. In contrast, brook trout are primarily a freshwater species, with anadromous populations occurring relatively rarely and little evidence exists for preparatory changes for seawater entry (McCormick and Naiman, 1984a, 1984b). Selection may have favored a more robust response of gill NKA to cortisol in Atlantic salmon due to the more frequent and predictable movements into seawater that are part of their anadromous life history.

5. Conclusions

This study demonstrates that cortisol treatment of Atlantic salmon and brook trout in freshwater results in decreased growth rate, but with greater sensitivity in brook trout. Decreased growth rate is likely due, in part, to the observed reduction in food intake that was seen in Atlantic salmon. Lower levels of plasma IGF-I indicate that the GH-IGF-I system is impacted by cortisol, though it is currently unclear if this is a direct effect or indirectly driven by reduced food intake. More strongly elevated gill NKA activity levels in Atlantic salmon suggest that cortisol is more important for regulating salinity tolerance in this highly anadromous species compared to the more facultatively anadromous brook trout. Further work is needed to examine the mechanistic basis of these findings, including examining the relative abundance of branchial

cortisol receptors and the regulation of appetite-regulating neuropeptides and hormones by cortisol among these and other salmonids with varying sensitivity to cortisol.

Acknowledgements

We thank Ian Kinahan and Meghan Taylor for their help in rearing and sampling of fish used in this study. We thank Fondap-IDEAL grants 15150003. We thank Jason Breves and two anonymous reviewers for their comments on an early version of the manuscript. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US Government.

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