Received: 31 July 2023 | Revised: 5 November 2023 | Accepted: 8 November 2023

DOI: 10.1002/jez.2766

RESEARCH ARTICLE

JEZ-A ECOLOGICAL AND INTEGRATIVE PHYSIOLOGY WILEY

Transcriptional regulation of esophageal, intestinal, and branchial solute transporters by salinity, growth hormone, and cortisol in Atlantic salmon

Jason P. Breves[1] | Ellie R. Runiewicz[1] | Sierra G. Richardson[1] | Serena E. Bradley[1] | Daniel J. Hall[2] | Stephen D. McCormick[2]

[1]Department of Biology, Skidmore College, Saratoga Springs, New York, USA

[2]Department of Biology, University of Massachusetts, Amherst, Massachusetts, USA

Correspondence
Jason P. Breves, Department of Biology, Skidmore College, 815 N. Broadway, Saratoga Springs, NY 12866, USA.
Email: jbreves@skidmore.edu

Funding information
National Science Foundation

Abstract

In marine habitats, Atlantic salmon (*Salmo salar*) imbibe seawater (SW) to replace body water that is passively lost to the ambient environment. By desalinating consumed SW, the esophagus enables solute-linked water absorption across the intestinal epithelium. The processes underlying esophageal desalination in salmon and their hormonal regulation during smoltification and following SW exposure are unresolved. To address this, we considered whether two Na^+/H^+ exchangers (Nhe2 and −3) expressed in the esophagus contribute to the uptake of Na^+ from lumenal SW. There were no seasonal changes in esophageal *nhe2* or *−3* expression during smoltification; however, *nhe3* increased following 48 h of SW exposure in May. Esophageal *nhe2*, *−3*, and *growth hormone receptor b1* were elevated in smolts acclimated to SW for 2.5 weeks. Treatment with cortisol stimulated branchial Na^+/K^+-ATPase (Nka) activity, and *$Na^+/K^+/2Cl^-$ cotransporter 1* (*nkcc1*), *cystic fibrosis transmembrane regulator 1 (cftr1)*, and *nka-α1b* expression. Esophageal *nhe2*, but not *nhe3* expression, was stimulated by cortisol. In anterior intestine, cortisol stimulated *nkcc2*, *cftr2*, and *nka-α1b*. Our findings indicate that salinity stimulates esophageal *nhe2* and *−3*, and that cortisol coordinates the expression of esophageal, intestinal, and branchial solute transporters to support the SW adaptability of Atlantic salmon.

KEYWORDS
cortisol, esophagus, gill, growth hormone, intestine, receptor, smoltification

1 | INTRODUCTION

Most teleost fishes exhibit osmoregulatory strategies that maintain extracellular fluids between 270 and 400 mOsm/kg with the major dissolved ions, Na^+ and Cl^-, maintained between 130–180 and 100–150 mmol/L, respectively (Marshall & Grosell, 2006). Given the composition of seawater (SW), teleosts residing in marine habitats must mitigate the diffusive entry of Na^+ and Cl^- and the osmotic loss of body water. Alternatively, threats to hydromineral balance for teleosts residing in fresh water (FW) stem from the passive loss of ions and the osmotic gain of water. Thus, to successfully maintain hydromineral balance at the systemic level, the ion- and water-transporting capacities of multiple osmoregulatory organs including the gill, gastrointestinal tract, kidney, and urinary bladder must operate in concert (Takei et al., 2014).

Marine/SW-acclimated fish drink ambient SW to support water balance. Accordingly, drinking rates are higher in fish (including salmon) residing in SW versus FW conditions (Fuentes & Eddy, 1997; Takei, 2021). Consumed SW is desalinated within the esophagus to yield a fluid that is closer to the osmolality of plasma (Hirano & Mayer-Gostan, 1976). It is currently thought that Na^+ and Cl^- are removed from the lumenal fluid and transported into the blood via

Na^+/K^+-ATPase (Nka), Na^+/H^+ exchangers (Nhes), Cl^-/HCO_3^- exchangers, and ClC-family Cl^- channels (Esbaugh & Grosell, 2014; Takei, 2021; Takei et al., 2017), though this is based on limited study of only a few euryhaline species. The absorbed Na^+ and Cl^- is subsequently secreted by branchial ionocytes (Kaneko et al., 2008). The desalinated SW then moves through the stomach before entering the intestine at ~400 mOsm/kg (Grosell, 2014). Upon entering the intestine, monovalent ions and water are absorbed from the lumenal fluid through both transcellular and paracellular routes (Sundell & Sundh, 2012). The major transporter for the apical entry of Na^+ and Cl^- into enterocytes is $Na^+/K^+/2Cl^-$ cotransporter 2 (Nkcc2). The basolateral exit of Na^+ and Cl^- occurs via Nka and ClC-family Cl^- channels, respectively (Takei, 2021). The dominant Nka isoforms expressed within Atlantic salmon enterocytes are *nka-α1b* and *-α1c* (Sundh et al., 2014; Tipsmark et al., 2010). Enterocytes additionally support the formation of Ca^{2+} and Mg^{2+} carbonate aggregates by moving HCO_3^- from the blood into the intestinal lumen via basolaterally located Na^+/HCO_3^- cotransporter 1 (Nbce1) and an apically located Cl^-/HCO_3^- exchanger (Grosell et al., 2007; Takei, 2021). The formation of these aggregates enhances water absorption by lowering the osmolality of the lumenal fluid (Grosell, 2014). Within the branchial epithelium, ionocytes operate as the conduit for the active secretion of excess Na^+ and Cl^- into marine environments. "SW-type" ionocytes rely upon the functions of Nka and Nkcc1 in the basolateral membrane and cystic fibrosis transmembrane conductance regulator 1 (Cftr1) in the apical membrane (Kaneko et al., 2008). Collectively, the branchial and gastrointestinal processes engaged under marine conditions are contrasted with the ion-conserving processes that operate in FW-acclimated fish (Marshall & Grosell, 2006; Shaughnessy & Breves, 2021).

Endocrine control of the molecular and cellular effectors of solute and water transport is an essential aspect of how fishes maintain hydromineral balance. Among the hormones that regulate the expression and localization of solute transporters, growth hormone (Gh), and cortisol are regarded as "SW-adapting hormones" because they promote the survival of fish in hyperosmotic environments (Takei et al., 2014). Gh and cortisol do not operate in isolation, as there are important interactions between these axes in the regulation of hormone levels and their effects on target tissues (Pelis & McCormick, 2001; Tipsmark & Madsen, 2009). While the widespread expression of Gh receptors (Ghrs) suggests that Gh is highly pleiotropic, exactly how Gh regulates the molecular underpinnings of solute and water transport remains unclear (Reindl & Sheridan, 2012). Reflecting its mineralocorticoid activities, cortisol directly stimulates the expression of Nka and ion transporters/channels (e.g., Nkcc1 and Cftr1) that support branchial ion extrusion (Breves et al., 2020; Kiilerich et al., 2007; Pelis & McCormick, 2001; Tipsmark et al., 2002). On the other hand, regulatory links between cortisol and Gh/insulin-like growth factor signaling and gastrointestinal functions befitting SW acclimation remain largely unknown (Veillette et al., 1995).

For juvenile Atlantic salmon (*Salmo salar*) between 1 and 4 years of age, springtime elevations in circulating Gh and cortisol underlie the timing of "parr-smolt transformation" or "smoltification" (Björnsson, 1997; McCormick et al., 2002; Sundell et al., 2003). This life-stage transition entails the orchestrated development of physiological, morphological, and behavioral traits that support the survival of smolts in the ocean (Boeuf, 1993; Hoar, 1988). Springtime elevations in plasma Gh and cortisol increase the SW tolerance of smolts before, and during, their downstream migration (Hoar, 1988). Atlantic salmon parr and smolts therefore provide fitting models from which to resolve how Gh and cortisol coordinate branchial, esophageal, and intestinal functions within the context of anadromous life-history strategies. As noted above, our understanding of esophageal function in teleosts is based on evidence from only a few species, and there is currently no information on the hormonal regulation of esophageal ion transporters. Given the paucity of information on esophageal processes in salmon, we first assessed *nhe2* and *−3* expression patterns in tissues of FW postsmolts. We then assessed *nhe2* and *−3* during smoltification and following SW exposures to consider how their encoded proteins support the desalination of SW. Next, we compared the esophageal expression of *nhe2* and *−3* along with multiple *ghr*, *glucocorticoid receptor* (*gr*), and *mineralocorticoid receptor* (*mr*) genes between salmon fully acclimated to either FW or SW. In our final experiment, we determined how a suite of gene transcripts that encode effectors of branchial, esophageal, and intestinal ion transport was impacted by the administration of Gh and cortisol in FW parr.

2 | MATERIALS AND METHODS

2.1 | Animals

Before initiation of the experiments described below, Atlantic salmon parr were obtained from the Kensington State Fish Hatchery (Kensington, CT) and held at the US Geological Survey, Eastern Ecological Science Center, Conte Anadromous Fish Research Laboratory (Turners Falls, MA). Fish were held in 1.5-m diameter fiberglass tanks supplied with dechlorinated tap water under natural photoperiod. Water temperature was maintained at 8–11°C. Fish were fed to satiation twice daily with commercial feed (Bio-Oregon). All experiments were conducted in accordance with US Geological Survey institutional guidelines and an approved IACUC review (LSC-9070).

2.2 | Tissue distribution of nhe2 and −3

The following tissues were collected in July of 2018 from post-smolts (n = 5–7; mixed sex) maintained in FW: whole brain, gill filaments, heart, liver, esophagus, stomach, pyloric caeca, anterior intestine, posterior intestine, body kidney, urinary bladder, muscle, fat, and

whole blood. At the time of collection, all tissue samples were immediately frozen on dry ice before storage at −80°C.

2.3 | Effects of season and salinity on esophageal nhe2 and −3 expression

To further investigate esophageal *nhe2 and −3* expression patterns, we analyzed fish from a cohort that underwent smoltification in the spring of 2014 (Breves et al., 2022). Fish were sampled on March 3, April 8, May 1, and July 10 ($n = 8$; mixed sex). At the time of sampling, fish weighed 45.7 ± 1.7 g (mean ± SEM). Food was withheld for 24 h before sampling. SW challenges were conducted on March 3 and May 1, the latter representing the time of peak smoltification. Sixteen smolts were transferred to a tank with recirculating SW (35 ppt) with particle and charcoal filtration and continuous aeration. Food was withheld for the duration of the SW challenge. Fish were sampled ($n = 8$) at 24 and 48 h after transfer to SW.

2.4 | Steady-state esophageal gene expression in smolts

In May of 2017, we assessed esophageal expression of *nhe2*, *−3*, *ghrb1*, *−2*, *gr1*, *−2*, and *mr* in smolts (64.9 ± 3.5 g; $n = 9–10$; mixed sex) fully acclimated to either FW or SW (30 ppt). Smolts were held in SW for 2.5 weeks before sampling. Food was withheld from FW and SW smolts for 24 h before sampling.

2.5 | Effects of growth hormone and cortisol in parr

In February of 2020, parr (10.3 ± 1.1 g; $n = 47$; mixed sex) were randomly distributed into a 190-L tank maintained at 12°C with particle and charcoal filtration, continuous aeration, and supplied with dechlorinated tap water at 2 L/h. Fish were acclimated to the experimental tanks for 1 week before the beginning of the experiment. Following the acclimation period, fish were anesthetized with buffered MS-222 (100 mg/L; pH 7.0; Sigma) and randomly assigned to one of four groups: vehicle, Gh (5 μg/g body mass), cortisol (10 μg/g body mass; hydrocortisone; Pfaltz and Bauer), and Gh+cortisol. Ovine Gh (Lot No. AFP9220A) was obtained from NIDDK's National Hormone and Pituitary Program. Hormones were suspended in a 1:1 mixture of vegetable oil and shortening (Crisco, B&G Foods) and then intraperitoneally injected into parr at a volume of 10 μL/g body mass. The control group received the vehicle only (1:1 mixture). Fish were given an identifying color paint mark between the anal fin rays to identify their group. All fish were returned to the original 190-L tank after a 0.5 h recovery period. Food was withheld for the remainder of the experiment and sampling occurred 5 days after injection.

The cortisol concentration was selected based on a previous study where parr treated with 10 μg cortisol/g body mass exhibited plasma cortisol concentrations of ~100 ng/mL (Breves et al., 2020). Plasma cortisol increased to about ~130 ng/mL when Atlantic salmon were transferred directly from FW to SW (Nichols & Weisbart, 1985). The oGh concentration was chosen based upon a previous study utilizing Gh-treated parr (Pelis & McCormick, 2001).

2.6 | Sampling

Fish were netted and anesthetized in buffered MS-222 at the time of the samplings described above. Blood was collected from the caudal vasculature by a needle and syringe treated with ammonium heparin. Blood samples were collected within 5 min of the initial netting. Blood was separated by centrifugation at 4°C and plasma stored at −80°C until subsequent analyses. Depending on the experiment, esophagus, gill filaments, and anterior intestine were collected and immediately frozen on dry ice and stored at −80°C. Anterior (proximal) intestine was collected in relation to the ileorectal sphincter following Sundh et al. (2014). For the hormone injection experiment, 4–6 additional gill filaments were placed in ice-cold SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3) and stored at −80°C.

2.7 | Plasma chloride and branchial Nka activity

Plasma chloride was analyzed by the silver titration method using a Buchler-Cotlove digital chloridometer (Labconco) and external standards. Ouabain-sensitive branchial Nka activity was measured as described by McCormick (1993). This assay couples the production of ADP to NADH using lactate dehydrogenase and pyruvate kinase in the presence and absence of 0.5 mmol/L ouabain. Samples (10 μL) were run in duplicate in 96-well microplates at 25°C and read at a wavelength of 340 nm for 10 min on a BioTek Synergy 2 spectrophotometer (BioTek). Protein concentration of the homogenate was determined using a BCA protein assay (Thermo Fisher Scientific).

2.8 | RNA extraction, cDNA synthesis, and quantitative real-time PCR (qRT-PCR)

Total RNA was extracted from tissue by the TRI Reagent procedure according to the manufacturer's protocols (Molecular Research Center). RNA concentration and purity were assessed by spectrophotometric absorbance (Nanodrop 1000, Thermo Fisher Scientific). First strand cDNA was synthesized by reverse transcribing 100 ng total RNA with a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Relative mRNA levels were determined by qRT-PCR using the StepOnePlus real-time PCR system (Applied Biosystems). We employed previously described primer sets for all target and normalization genes aside from *ghrb1* (NM_001123576),

ghrb2 (NM_001123594), *nhe2* (XM_014174391), and *nhe3* (XM_014166803) (Table 1). We follow the nomenclature for Atlantic salmon Gh receptors (Ghrbs) presented by Ocampo Daza and Larhammar (2018). Primers for *ghrb1*, *ghrb2*, *nhe2*, and *nhe3* were designed using NCBI Primer-BLAST to span predicted exon-exon junctions and to amplify products of 80, 123, 74, and 150 base pairs, respectively. Nonspecific product amplification and primer-dimer formation were assessed by melt curve analyses. qRT-PCR reactions

TABLE 1 Specific primer sequences for quantitative real-time PCR.

Gene	Primer sequence (5′-3′)	Efficiency (%)	Reference
cftr1	F: CCTTCTCCAATATGGTTGAAGAGGCAAG	91	Nilsen et al. (2007)
	R: GAGGCACTTGGATGAGTCAGCAG		
cftr2	F: GCCTTATTTCTTCTATTTGTATGCACT	108	Nilsen et al. (2007)
	R: GCCACCATGAAAAACTAAAGAGTACCT		
ef1α	F: GAATCGGCTATGCCTGGTGAC	95	Bower et al. (2008)
	R: GGATGATGACCTGAGCGGTG		
ghrb1	F: CCCACCTCTAAACAAGCCCTC	106	NM_001123576
	R: CAGCGGAACGTGTTCATGTC		
ghrb2	F: TGCCTGCCCCACCTCTTATC	105	NM_001123594
	R: GGCACATCAGTTCAGACCTTG		
gr1	F: ACGACGATGGAGCCGAAC	96	Kiilerich et al. (2007)
	R: ATGGCTTTGAGCAGGGATAG		
gr2	F: TGGTGGGCTGCTGGATTTCTGC	92	Kiilerich et al. (2011)
	R: CTCCCTGTCTCCCTCTGTCA		
mr	F: TCGTCCACAGCCAAAGTGTG	94	Madaro et al. (2015)
	R: TTCTTCCGGCACACAGGTAG		
nbce1.1	F: GACAATATGCAGGCAGGGTG	100	Breves et al. (2022)
	R: AGCCTCTCGAAGACCAGAAC		
nbce1.2b	F: TCAGGGAGGAGGCGGAC	96	Breves et al. (2022)
	R: CCGCTTGATGTCCAGAATGAG		
nhe2	F: GCCCGTATACAGGTGCCATT	97	XM_014174391
	R: ACATGGAAACCAATCTTGGCG		
nhe3	F: GGGCCTATAGAGTAGAAGCTGG	97	XM_014166803
	R: CCTGGACCCAGGATTAGGGG		
nka-α1a	F: CCAGGATCACTCAATGTCACTCT	89	Nilsen et al. (2007)
	R: GCTATCAAAGGCAAATGAGTTTAATATCATTGTAAAA		
nka-α1b	F: GCTACATCTCAACCAACAACATTACAC	102	Nilsen et al. (2007)
	R: TGCAGCTGAGTGCACCAT		
nka-α1c	F: AGGGAGACGTACTACTAGAAAGCAT	96	Nilsen et al. (2007)
	R: CAGAACTTAAAATTCCGAGCAGCAA		
nka-α3	F: GGAGACCAGCAGAGGAACAG	102	Nilsen et al. (2007)
	R: CCCTACCAGCCCTCTGAGT		
nkcc1	F: GATGATCTGCGGCCATGTTC	97	Nilsen et al. (2007)
	R: AGACCAGTAACCTGTCGAGAAAC		
nkcc2	F: CCGCGTGCCCAACATC	103	Sundh et al. (2014)
	R: GCACGGTTACCGCTCACACT		

were setup in a 15 µL final reaction volume with 400 nM of each primer, 1 µL cDNA, and 7.5 µL of 2x SYBR Green PCR Master Mix (Applied Biosystems). The following cycling parameters were employed: 10 min at 95°C followed by 40 cycles at 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s. After verification that levels did not vary across treatments, *elongation factor 1α* (*ef1α*) levels were used to normalize target genes (Bower et al., 2008). Reference and target gene levels were calculated by the relative quantification method with PCR efficiency correction (Pfaffl, 2001). Standard curves were prepared from serial dilutions of esophagus, anterior intestine, or gill cDNA and included on each plate to calculate the PCR efficiencies for target and normalization genes (Table 1). Relative mRNA levels are reported as a fold-change from a given tissue or treatment group as specified in the figure legends.

2.9 | Statistical analyses

Multiple group comparisons of *nhe2* and *−3* expression between tissues were conducted by Kruskal–Wallis test, followed by Dunn's test (Figure 1). For the seasonal/SW exposure experiment (Figure 2), multiple group comparisons were performed by one-way ANOVA, followed by Tukey's HSD test. Student's *t* tests were employed for single comparisons (Figure 3). The Gh and cortisol injection experiment (Figures 4–6) was analyzed by two-way ANOVA with Gh and cortisol as factors. When there was a significant interaction, post hoc testing was performed by Tukey's HSD test. Significant differences are indicated in figures. All statistical analyses were performed using GraphPad Prism 9. Significance for all tests was set at $p < 0.05$.

3 | RESULTS

3.1 | Tissue distribution of nhe2 and −3

In FW-acclimated postsmolts, *nhe2* showed robust expression in the gill, esophagus, stomach, urinary bladder, and blood (Figure 1a). While relative *nhe3* expression was high in the esophagus, it was only significantly different from the gill; *nhe3* was undetectable in multiple tissues (Figure 1b). The mean Ct values for *nhe2* and *−3* in esophagus were 21.1 and 23.4, respectively.

3.2 | Effects of season and salinity on esophageal nhe2 and −3 expression

There were no clear seasonal changes in esophageal *nhe2* expression (albeit there was a transient increase in April); there was no effect of SW exposure on *nhe2* levels (Figure 2a,b). *nhe3* expression did not change seasonally but was elevated by ~2-fold after 48 h of SW exposure in May (Figure 2c,d).

3.3 | Steady-state esophageal gene expression in smolts

Esophageal *nhe2* and *−3* expression was elevated by ~1.5- and ~2.5-fold, respectively, in smolts maintained in SW (30 ppt) for 2.5 weeks when compared with animals held in FW (Figure 3a,b). Esophageal *ghrb1* expression was enhanced by ~2-fold in SW-acclimated smolts whereas *ghrb2*, *gr1*, and *−2* were unaffected by salinity (Figure 3c–f). Esophageal *mr* expression was not impacted by salinity (data not shown).

3.4 | Effects of growth hormone and cortisol in parr

Cortisol stimulated branchial Nka activity relative to vehicle controls (Figure 4a). Plasma chloride in FW and branchial *nka-α1a* expression were not affected by Gh or cortisol (Figure 4b,e). Treatment with cortisol elevated branchial *nkcc1* and *cftr1* expression by ~4-fold compared to control levels; there was also a significant effect of cortisol on *nka-α1b* expression (Figure 4c,d,f). In the esophagus,

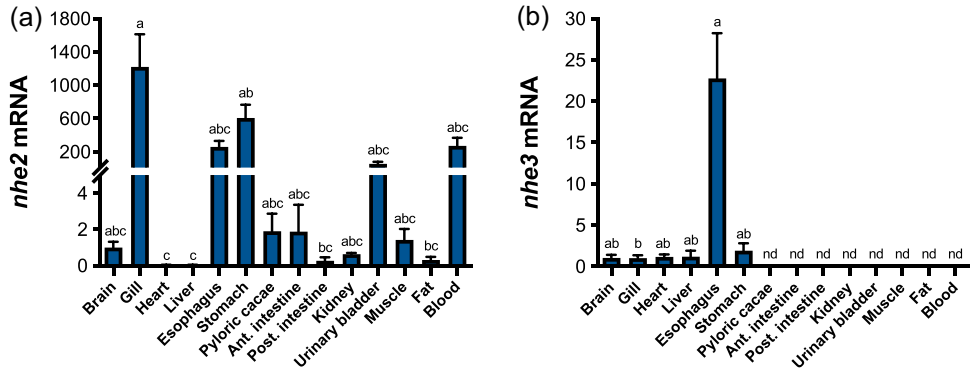

FIGURE 1 Tissue gene expression of *nhe2* (a) and *nhe3* (b) in Atlantic salmon postsmolts maintained in fresh water. Data were normalized to *ef1α* as a reference gene and are presented relative to brain expression levels. Means ± SEM ($n = 5–7$). Means not sharing the same letter are significantly different (Kruskal–Wallis, Dunn's test, $p < 0.05$). nd, no detection.

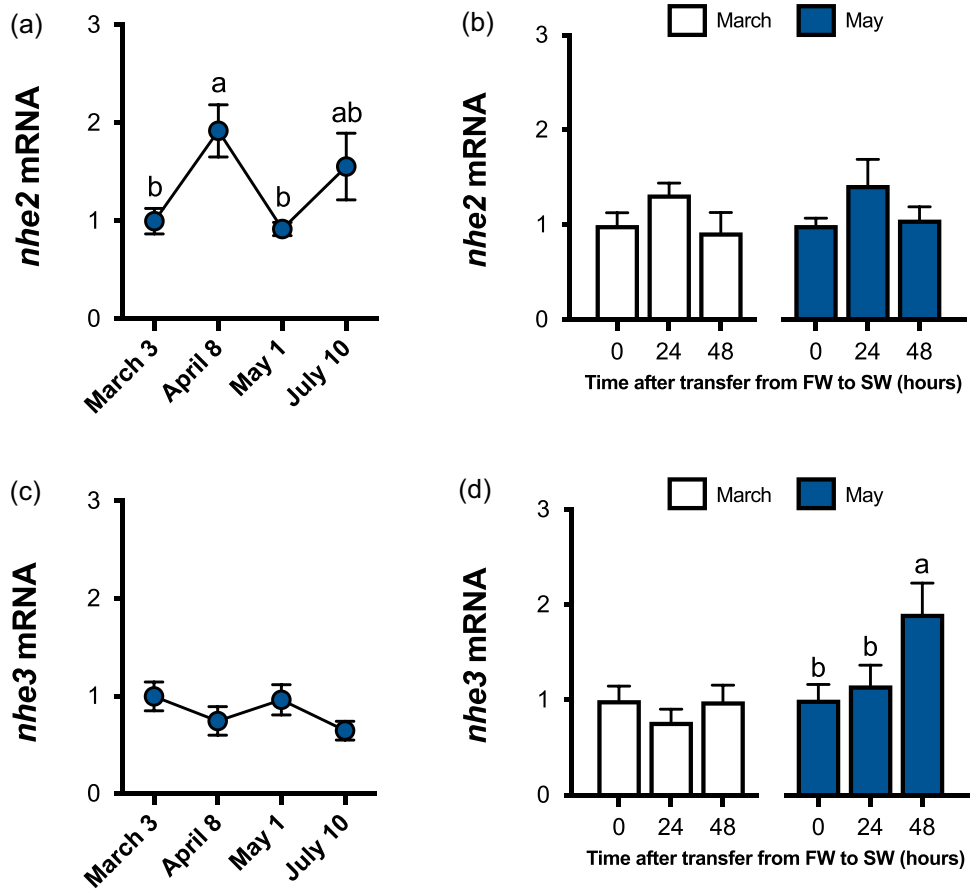

FIGURE 2 Esophageal *nhe2* (a) and *nhe3* (c) gene expression in Atlantic salmon smolts maintained in fresh water (FW) from March 3 through July 10. Esophageal *nhe2* (b) and *nhe3* (d) gene expression in smolts subjected to 24- and 48-h seawater (SW) exposures in March (open bars) and May (solid bars). Means ± SEM ($n = 8$). Within a given experiment, means not sharing the same letter are significantly different (one-way ANOVA, Tukey's HSD, $p < 0.05$).

cortisol treatment resulted in a ~1.5-fold elevation in *nhe2* expression relative to vehicle controls whereas *nhe3* was not affected by Gh or cortisol (Figure 5). In the anterior intestine, cortisol stimulated *nkcc2* expression by ~7-fold compared to control levels (Figure 6a). There was a significant effect of cortisol, and an interaction with Gh on *cftr2* expression (Figure 6b). Only the cortisol group was significantly elevated from vehicle controls. Resembling patterns in the gill, treatment with cortisol stimulated intestinal *nka-α1b* expression, but in this case, by ~13-fold relative to control levels (Figure 6c). Gh and cortisol did not impact intestinal expression of *nka-α1c*, *-α3*, *nbce1.1*, or *−1.2b* (Figure 6d–g).

4 | DISCUSSION

To resolve how Gh and cortisol control gastrointestinal solute transporters, we first characterized the distribution of *nhe* transcripts to assess whether their encoded products might support esophageal desalination. Both *nhe2* and *−3* were detected at high levels in the esophagus (Figure 1), a pattern that resembles their expression in Japanese eel (*Anguilla japonica*) (Takei et al., 2017). Interestingly, esophageal expression of both *nhe2* and *−3* in salmon and eel contrasts with the scenario in gulf toadfish (*Opsanus beta*) where *nhe2* is high in the esophagus but *nhe3* levels are low throughout the gastrointestinal tract (Esbaugh & Grosell, 2014), indicating that only Nhe2 is involved in esophageal desalination in this species.

Our tissue expression analysis also revealed *nhe2* expression in the gill, which is noteworthy, because to our knowledge, there is no description of a FW-type ionocyte or other cell type in the gill of Atlantic salmon that incorporates the function of Nhe2. In rainbow trout (*Oncorhyncus mykiss*), Nhe2 and −3 are expressed within a subtype of ionocytes termed peanut lectin agglutinin positive (PNA^+) ionocytes that support Na^+ absorption (Hiroi & McCormick, 2012; Ivanis et al., 2008). Note that *nhe3* showed either negligible or undetectable expression outside of the esophagus, and previously we did not detect branchial immunoreactivity when using an anti-trout Nhe3 antibody (Christensen & McCormick, unpublished). These observations indicate that Na^+ absorption may not occur via Nhe3 in FW-type Atlantic salmon ionocytes. In any case, because we detected *nhe2* and *−3* transcripts in the esophagus, we next considered their expression patterns during smoltification and SW acclimation.

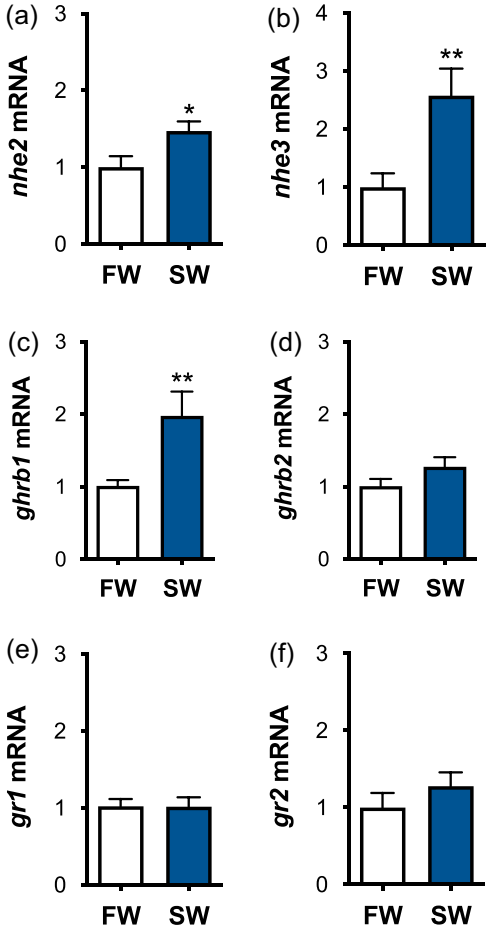

FIGURE 3 Esophageal *nhe2* (a), *nhe3* (b), *ghrb1* (c), *ghrb2* (d), *gr1* (e), and *gr2* (f) gene expression in Atlantic salmon smolts acclimated to seawater (SW; 30 ppt) for 2.5 weeks. Means ± SEM (n = 9–10). Gene expression in SW (solid bars) is presented as a fold-change from the freshwater (FW)-acclimated group (open bars). Asterisks indicate significant difference between groups by Student's t test (*p < 0.05 and **p < 0.01).

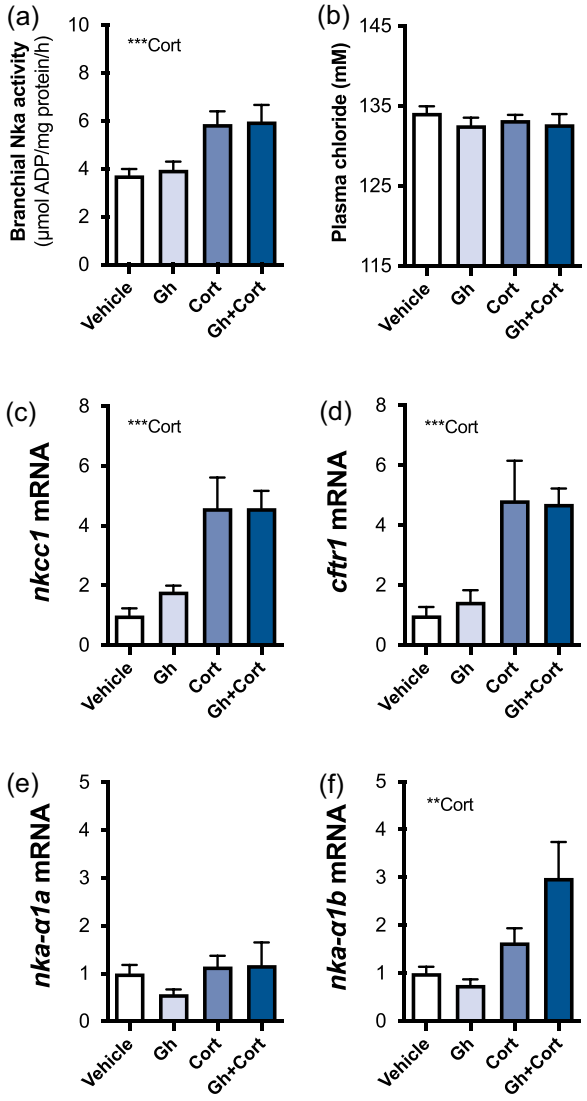

FIGURE 4 Effects of ovine growth hormone (Gh; 5 µg/g body mass), cortisol (Cort; 10 µg/g body mass), and the combination of both hormones (Gh+Cort) on branchial Nka activity (a), plasma chloride (b), and branchial *nkcc1* (c), *cftr1* (d), *nka-α1a* (e), and *nka-α1b* (f) gene expression in Atlantic salmon parr maintained in fresh water. Means ± SEM (n = 11–12). mRNA levels are presented as a fold-change from the vehicle group. Significant main effects are indicated within panels (two-way ANOVA, **p < 0.01 and ***p < 0.001).

Among the myriad physiological changes that smolts undergo before entering marine habitats (Boeuf, 1993; Hoar, 1988), a suite of solute- and water-transporting pathways are activated in the intestine to enhance the capacity for fluid absorption (Breves et al., 2020; Sundh et al., 2014; Tipsmark et al., 2010; Veillette et al., 1993). We predicted that *nhe2* and *−3* would exhibit seasonal changes and responses to SW reflecting the associated demand for desalination by the esophagus (Esbaugh & Grosell, 2014; Takei et al., 2017). Juvenile salmon sampled from March to July underwent smoltification as indicated by a springtime peak in branchial Nka activity (as described in our previous report) (Breves et al., 2022). Moreover, smolts in May were better able to maintain systemic chloride balance when challenged with SW than presmolts in March (Breves et al., 2022). Despite observing these hallmarks of enhanced SW tolerance, we found no seasonal changes in esophageal *nhe2* or *−3* expression (Figure 2a,c). However, *nhe3* expression doubled 48 h after SW exposure (Figure 2d) and was similarly elevated under chronic SW conditions (Figure 3b). Esophageal *nhe2* expression was also elevated under chronic SW conditions (Figure 3a), indicating that regulated increases in *nhe2* and *−3* expression accompany essential morpho-functional changes that occur in the esophagus with salinity acclimation (Yamamoto & Hirano, 1978). Our results with Atlantic salmon contrast with Japanese eel where *nhe2* is constitutively expressed in the esophagus and is not influenced by exposure to SW (Takei et al., 2017). As in other surveyed teleosts (Carrick & Balment, 1983; Hirano, 1974), drinking rates are greater in Atlantic salmon residing in SW versus FW (Fuentes & Eddy, 1997). Thus, given the lack of preparatory/seasonal increases in *nhe2* or *−3*, the introduction of SW into the esophagus may be a key activator of

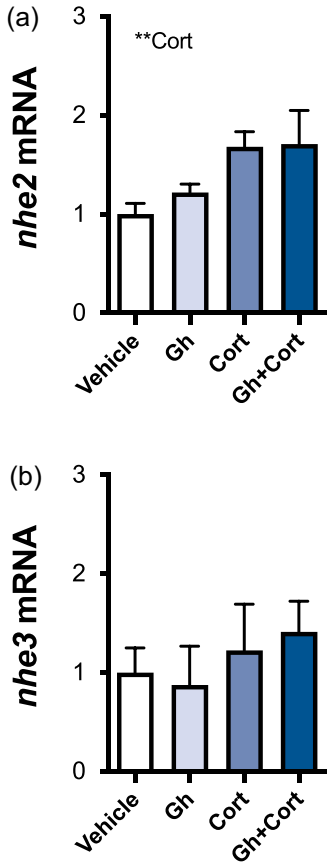

FIGURE 5 Effects of ovine growth hormone (Gh; 5 μg/g body mass), cortisol (Cort; 10 μg/g body mass), and the combination of both hormones (Gh+Cort) on esophageal *nhe2* (a) and *nhe3* (b) gene expression in Atlantic salmon parr maintained in fresh water. Means ± SEM (n = 11–12). mRNA levels are presented as a fold-change from the vehicle group. Significant main effects are indicated within panels (two-way ANOVA, $^{**}p < 0.01$).

nhe2 and/or *-3* expression to recruit more transporters when smolts imbibe SW. Indeed, drinking in teleosts starts immediately after SW transfer with the detection of elevated chloride (Hirano, 1974).

In addition to the direct actions of environmental salinity on teleost osmoregulatory organs (Kültz, 2012), hormones released during challenges to hydromineral balance regulate effectors of solute transport (Takei et al., 2014). In salmon smolts, Gh, and cortisol levels increase both before, and following, SW exposure (Breves et al., 2017; Nichols & Weisbart, 1985; Nilsen et al., 2008). Atlantic salmon express duplicate Ghr-encoding genes, designated *ghrb1* and *−2* (Ocampo Daza & Larhammar, 2018). While both transcripts are expressed in the esophagus, only *ghrb1* showed enhanced expression under SW conditions (Figure 3c,d). Similarly, pre-smolts exhibited an increase in branchial *ghr* (*ghrb1*) expression following SW exposure, a pattern ascribed to an increased requirement for Gh signaling (Kiilerich et al., 2007). With the expression of *gr1*, *−2*, and *mr* genes (Figure 3e,f), numerous receptors for Gh and cortisol are poised to provide connectivity between their ligands and key esophageal activities.

We utilized parr to test whether *nhe2* and *−3* are targets of hormonal signaling because they reliably activate hyposmoregulatory activities in response to exogenous Gh and cortisol (Bisbal & Specker, 1991; Breves et al., 2020; Pelis & McCormick, 2001; Specker et al., 1994; Veillette et al., 1995). The hormone implants administered in the current study were clearly effective in stimulating adaptive responses to SW such as branchial Nka activity and *nkcc1*, *cftr1*, and *nka-α1b* expression (Figure 4a,c,d,f) (McCormick et al., 2008, 2013; Nilsen et al., 2007; Pelis & McCormick, 2001; Tipsmark et al., 2002). However, we did not observe the previously identified synergistic effects of cortisol and Gh on *nkcc1* and *nka-α1b* expression (Tipsmark & Madsen, 2009). *nka-α1a* expression, which decreases during SW acclimation (McCormick et al., 2013), was not impacted by Gh or cortisol (Figure 4e) in contrast to previous studies where *nka-α1a* was stimulated by cortisol (McCormick et al., 2008; Tipsmark & Madsen, 2009). Collectively, these branchial responses are in strong agreement with how heightened corticosteroid signaling induces smoltification-related changes in branchial ion-secretion capacity (McCormick et al., 2013).

The most significant finding of this study is that cortisol orchestrates the expression of solute transporters across multiple segments of the gastrointestinal tract to presumably promote intestinal water absorption. Within the esophagus, *nhe2*, but not *nhe3*, was stimulated by cortisol. Considering that both *nhe2* and *−3* responded to SW transfer (Figures 2d and 3a,b), elevated cortisol after SW exposure (Nichols & Weisbart, 1985) may be sufficient to induce *nhe2* whereas SW itself is necessary for the induction of *nhe3*. To our knowledge, these findings present the first evidence that cortisol regulates a Na^+-transporting pathway linked to desalination in the esophagus (Takei et al., 2017). Nonetheless, we anticipate that with a more nuanced understanding of the molecular underpinnings of ion transport in salmon esophagus, informed by recent progress in Japanese eel (Takei, 2021; Takei et al., 2017), the identification of additional cortisol targets will follow (e.g., Nka isoforms, Cl^-/HCO_3^- exchangers, and ClC-family Cl^- channels). It is important to recognize that in addition to regulating *nhe2* expression, cortisol also initiates adaptive cellular changes. For instance, apoptosis underlies the transformation of the esophageal epithelium from a stratified to columnar state during SW acclimation, and accordingly, cortisol increased the presence of TUNEL-positive nuclei in medaka (*Oryzias latipes*) esophagus (Takagi et al., 2011).

Atlantic salmon smolts develop an increased capacity for intestinal fluid absorption in response to springtime elevations in plasma cortisol (Specker, 1982; Sundell et al., 2003; Veillette et al., 1993). Cortisol promotes fluid absorptive capacity, at least in part, by stimulating Nka activity (Cornell et al., 1994; Veillette et al., 1995, 2005), a pattern consistent with the enhanced *nka-α1b* expression we observed in response to cortisol (Figure 6c). Surprisingly, *nkaα1c*, the other dominant Nka transcript within enterocytes, was not sensitive to cortisol (Figure 6d). We also found that cortisol promotes *nkcc2* expression (Figure 6a), which through its translated product, enhances solute-linked fluid absorption by facilitating the entry of Na^+ and Cl^- into enterocytes for subsequent exit via basolateral pathways (Takei, 2021). In parallel with intestinal Nka activity, Nkcc2 expression increases during Atlantic salmon smoltification and following transfer to SW (Duarte et al., 2023), the latter

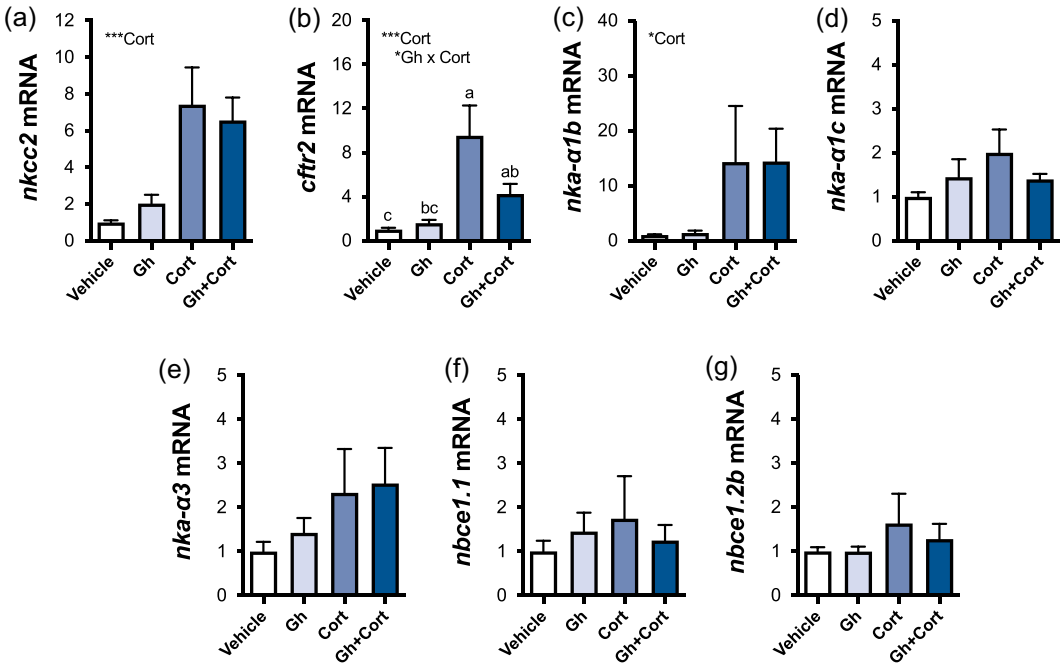

FIGURE 6 Effects of ovine growth hormone (Gh; 5 μg/g body mass), cortisol (Cort; 10 μg/g body mass), and the combination of both hormones (Gh+Cort) on intestinal *nkcc2* (a), *cftr2* (b), *nka-α1b* (c), *nka-α1c* (d), *nka-α3* (e), *nbce1.1* (f), and *nbce1.2b* (g) gene expression in Atlantic salmon parr maintained in fresh water. Means ± SEM (n = 11–12). mRNA levels are presented as a fold-change from the vehicle group. Significant main or interaction effects are indicated within panels (two-way ANOVA, $^{*}p < 0.05$ and $^{***}p < 0.001$). When there is a significant interaction effect, means not sharing the same letter are significantly different (Tukey's HSD, $p < 0.05$).

comparable to how other euryhaline species in SW upregulate Nkcc2 (Esbaugh & Cutler, 2016; Gregório et al., 2013). It will be interesting to learn whether a cortisol-Nkcc2 link operates in nonsalmonid models to activate intestinal fluid absorption in SW. Cortisol's stimulatory effect on *cftr2* expression (Figure 6b) was unexpected, since *cftr2* is elevated in FW- versus SW-acclimated salmon (Breves et al., 2022; Sundh et al., 2014) and is seemingly deleterious to euryhaline species inhabiting SW (Gregório et al., 2013; Marshall et al., 2002; Wong et al., 2016). Finally, knowing that intestinal *nbce1.1* expression increases concomitantly with *nkcc2* during SW-acclimation (Breves et al., 2022), we hypothesized that cortisol and/or Gh also facilitates the basolateral acquisition of HCO_3^- via Nbce1 to support the enhanced secretion of HCO_3^- across the apical surface of enterocytes. However, we did not detect any responses by *nbce1.1* (or *−1.2b*) to Gh or cortisol (Figure 6f,g). Alternatively, the hydration of metabolic CO_2 by carbonic anhydrase may serve as the source of HCO_3^- for apical secretion by enterocytes (Grosell, 2011). This warrants future consideration given that carbonic anhydrase activity is regulated by cortisol in trout gill (Gilmour et al., 2011).

5 | CONCLUSION AND PERSPECTIVES

Our results indicate that *nhe2* and *−3* are abundant in the esophagus and upregulated by exposure to SW. In addition to its well-documented actions on branchial ion-secretion, cortisol plays a key role in orchestrating solute-transporting activities within various segments of the gastrointestinal tract in fashions that facilitate fluid absorption. More specifically, our study identified genes encoding Nhe2 and Nkcc2 as targets of cortisol regulation within the esophagus and intestine, respectively. For effective desalination to occur in the esophagus, both transcellular and paracellular water permeability must be minimized upon SW entry (Takei, 2021). It stands entirely unresolved, however, whether aquaporin- and claudin-encoding genes are regulated in salmon esophagus during SW acclimation. In addition to solute-transporters such as Cl^-/HCO_3^- exchangers and ClC-family Cl^- channels, future studies are now warranted to determine whether molecular determinants of osmotic permeability are transcriptional targets of cortisol.

ACKNOWLEDGMENTS

This work was supported by the National Science Foundation (IOS-1755131 to J. P. B.). We appreciate the excellent laboratory assistance provided by Amy Regish during this study.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

All experiments were conducted in accordance with U.S. Geological Survey institutional guidelines and an approved IACUC protocol (LSC-9070).

ORCID

Jason P. Breves https://orcid.org/0000-0003-1193-4389

Stephen D. McCormick http://orcid.org/0000-0003-0621-6200

REFERENCES

Bisbal, G. A., & Specker, J. L. (1991). Cortisol stimulates hypo-osmoregulatory ability in Atlantic salmon, *Salmo salar* L. *Journal of Fish Biology*, *39*, 421–432.

Björnsson, B. T. (1997). The biology of salmon growth hormone: From daylight to dominance. *Fish Physiology and Biochemistry*, *17*, 9–24.

Boeuf, G. (1993). Fish ecophysiology. In J. C. Rankin, & F. B. Jensen (Eds.), *Salmonid smolting: A pre-adaptation to the oceanic environment* (pp. 105–135). Chapman and Hall.

Bower, N. I., Li, X., Taylor, R., & Johnston, I. A. (2008). Switching to fast growth: The insulin-like growth factor (IGF) system in skeletal muscle of Atlantic salmon. *Journal of Experimental Biology*, *211*, 3859–3870.

Breves, J. P., Fujimoto, C. K., Phipps-Costin, S. K., Einarsdottir, I. E., Björnsson, B. T., & McCormick, S. D. (2017). Variation in branchial expression among *insulin-like growth-factor binding proteins* (*igfbps*) during Atlantic salmon smoltification and seawater exposure. *BMC Physiology*, *17*(1), 2.

Breves, J. P., McKay, I. S., Koltenyuk, V., Nelson, N. N., Lema, S. C., & McCormick, S. D. (2022). Na^+/HCO_3^- cotransporter 1 (*nbce1*) isoform gene expression during smoltification and seawater acclimation of Atlantic salmon. *Journal of Comparative Physiology B*, *192*, 577–592.

Breves, J. P., Springer-Miller, R. H., Chenoweth, D. A., Paskavitz, A. L., Chang, A. Y. H., Regish, A. M., Einarsdottir, I. E., Björnsson, B. T., & McCormick, S. D. (2020). Cortisol regulates *insulin-like growth-factor binding protein* (*igfbp*) gene expression in Atlantic salmon parr. *Molecular and Cellular Endocrinology*, *518*, 110989.

Carrick, S., & Balment, R. J. (1983). The renin-angiotensin system and drinking in the euryhaline flounder, *Platichthys flesus*. *General and Comparative Endocrinology*, *51*, 423–433.

Cornell, S. C., Portesi, D. M., Veillette, P. A., Sundell, K., & Specker, J. L. (1994). Cortisol stimulates intestinal fluid uptake in Atlantic salmon (*Salmo salar*) in the post-smolt stage. *Fish Physiology and Biochemistry*, *13*, 183–190.

Duarte, V., Gaetano, P., Striberny, A., Hazlerigg, D., Jørgensen, E. H., Fuentes, J., & Campinho, M. A. (2023). Modulation of intestinal growth and differentiation by photoperiod and dietary treatment during smoltification in Atlantic salmon (*Salmo salar*, L.). *Aquaculture*, *566*, 739164.

Esbaugh, A. J., & Cutler, B. (2016). Intestinal Na^+, K^+, $2Cl^-$ cotransporter 2 plays a crucial role in hyperosmotic transitions of a euryhaline teleost. *Physiological Reports*, *4*(22):e13028.

Esbaugh, A. J., & Grosell, M. (2014). Esophageal desalination is mediated by Na^+, H^+ exchanger-2 in the gulf toadfish (*Opsanus beta*). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *171*, 57–63.

Fuentes, J., & Eddy, F. B. (1997). Effect of manipulation of the renin-angiotensin system in control of drinking in juvenile Atlantic salmon (*Salmo salar* L) in fresh water and after transfer to sea water. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*, *167*(6), 438–443.

Gilmour, K. M., Collier, C. L., Dey, C. J., & Perry, S. F. (2011). Roles of cortisol and carbonic anhydrase in acid-base compensation in rainbow trout, *Oncorhynchus mykiss*. *Journal of Comparative Physiology. B, Biochemical, Systemic, and Environmental Physiology*, *181*(4), 501–515.

Gregório, S. F., Carvalho, E. S., Encarnação, S., Wilson, J. M., Power, D. M., Canário, A. V., & Fuentes, J. (2013). Adaptation to different salinities exposes functional specialization in the intestine of the sea bream (*Sparus aurata* L.). *The Journal of Experimental Biology*, *216*, 470–479.

Grosell, M. (2011). Intestinal anion exchange in marine teleosts is involved in osmoregulation and contributes to the oceanic inorganic carbon cycle. *Acta Physiologica*, *202*, 421–434.

Grosell, M. (2014). The physiology of fishes. In D. H. Evans, J. B. Claiborne, & S. Currie (Eds.), *Intestinal transport* (4th ed., pp. 175–203). CRC Press.

Grosell, M., Gilmour, K. M., & Perry, S. F. (2007). Intestinal carbonic anhydrase, bicarbonate, and proton carriers play a role in the acclimation of rainbow trout to seawater. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *293*(5), R2099–R2111.

Hirano, T. (1974). Some factors regulating water intake by the eel, *Anguilla japonica*. *Journal of Experimental Biology*, *61*(3), 737–747.

Hirano, T., & Mayer-Gostan, N. (1976). Eel esophagus as an osmoregulatory organ. *Proceedings of the National Academy of Sciences*, *73*, 1348–1350.

Hiroi, J., & McCormick, S. D. (2012). New insights into gill ionocyte and ion transporter function in euryhaline and diadromous fish. *Respiratory Physiology & Neurobiology*, *184*, 257–268.

Hoar, W. S. (1988). Fish physiology, vol. XIB. In W. S. Hoar, & D. J. Randall (Eds.), *The physiology of smolting salmonids* (pp. 275–343). Academic Press.

Ivanis, G., Esbaugh, A. J., & Perry, S. F. (2008). Branchial expression and localization of SLC9A2 and SLC9A3 sodium/hydrogen exchangers and their possible role in acid-base regulation in freshwater rainbow trout (*Oncorhynchus mykiss*). *Journal of Experimental Biology*, *211*, 2467–2477.

Kaneko, T., Watanabe, S., & Lee, K. M. (2008). Functional morphology of mitochondrion-rich cells in euryhaline and stenohaline teleosts. *Aqua-BioScience Monographs*, *1*, 1–62.

Kiilerich, P., Kristiansen, K., & Madsen, S. S. (2007). Cortisol regulation of ion transporter mRNA in Atlantic salmon gill and the effect of salinity on the signaling pathway. *Journal of Endocrinology*, *194*, 417–427.

Kiilerich, P., Pedersen, S. H., Kristiansen, K., & Madsen, S. S. (2011). Corticosteroid regulation of Na^+,K^+-ATPase α1-isoform expression in Atlantic salmon gill during smolt development. *General and Comparative Endocrinology*, *170*, 283–289.

Kültz, D. (2012). The combinatorial nature of osmosensing in fishes. *Physiology*, *27*, 259–275.

Madaro, A., Olsen, R. E., Kristiansen, T. S., Ebbesson, L. O. E., Nilsen, T. O., Flik, G., & Gorissen, M. (2015). Stress in Atlantic salmon: Response to unpredictable chronic stress. *Journal of Experimental Biology*, *218*, 2538–2550.

Marshall, W. S., & Grosell, M. (2006). Ion transport, osmoregulation and acid-base balance. In D. H. Evans, & J. B. Claiborne (Eds.), *The physiology of fishes* (pp. 177–230). CRC Press.

Marshall, W. S., Howard, J. A., Cozzi, R. R. F., & Lynch, E. M. (2002). NaCl and fluid secretion by the intestine of the teleost *Fundulus heteroclitus*: Involvement of CFTR. *Journal of Experimental Biology*, *205*, 745–758.

McCormick, S. D. (1993). Methods for nonlethal gill biopsy and measurement of Na^+, K^+,-ATPase activity. *Canadian Journal of Fisheries and Aquatic Sciences*, *50*, 656–658.

McCormick, S. D., Regish, A., O'Dea, M. F., & Shrimpton, J. M. (2008). Are we missing a mineralocorticoid in teleost fish? Effects of cortisol, deoxycorticosterone and aldosterone on osmoregulation, gill Na^+,K^+-ATPase activity and isoform mRNA levels in Atlantic salmon. *General and Comparative Endocrinology*, *157*, 35–40.

McCormick, S. D., Regish, A. M., Christensen, A. K., & Björnsson, B. T. (2013). Differential regulation of sodium-potassium pump isoforms during smolt development and seawater exposure of Atlantic salmon. *Journal of Experimental Biology*, *216*, 1142–1151.

McCormick, S. D., Shrimpton, J. M., Moriyama, S., & Björnsson, T. (2002). Effects of an advanced temperature cycle on smolt development and endocrinology indicate that temperature is not a zeitgeber for smolting in Atlantic salmon. *Journal of Experimental Biology*, *205*, 3553–3560.

Nichols, D. J., & Weisbart, M. (1985). Cortisol dynamics during seawater adaptation of Atlantic salmon *Salmo salar*. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *248*, R651–R659.

Nilsen, T. O., Ebbesson, L. O. E., Kiilerich, P., Björnsson, B. T., Madsen, S. S., McCormick, S. D., & Stefansson, S. O. (2008). Endocrine systems in juvenile anadromous and landlocked Atlantic salmon (*Salmo salar*): Seasonal development and seawater acclimation. *General and Comparative Endocrinology*, *155*, 762–772.

Nilsen, T. O., Ebbesson, L. O. E., Madsen, S. S., McCormick, S. D., Andersson, E., Björnsson, T., Prunet, P., & Stefansson, S. O. (2007). Differential expression of gill Na^+/K^+-ATPase α- and β-subunits, $Na^+/K^+/2Cl^-$ cotransporter and CFTR anion channel in juvenile anadromous and landlocked Atlantic salmon *Salmo salar*. *Journal of Experimental Biology*, *210*, 2885–2896.

Ocampo Daza, D., & Larhammar, D. (2018). Evolution of the receptors for growth hormone, prolactin, erythropoietin and thrombopoietin in relation to the vertebrate tetraploidizations. *General and Comparative Endocrinology*, *257*, 143–160.

Pelis, R. M., & McCormick, S. D. (2001). Effects of growth hormone and cortisol on Na^+-K^+-$2Cl^-$ cotransporter localization and abundance in the gills of Atlantic salmon. *General and Comparative Endocrinology*, *124*, 134–143.

Pfaffl, M. W. (2001). A new mathematical model for relative quantification in real$^-$time RT-PCR. *Nucleic Acids Research*, *29*(9):45e.

Reindl, K. M., & Sheridan, M. A. (2012). Peripheral regulation of the growth hormone-insulin-like growth factor system in fish and other vertebrates. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *163*, 231–245.

Shaughnessy, C. A., & Breves, J. P. (2021). Molecular mechanisms of Cl^- transport in fishes: new insights and their evolutionary context. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *335*, 207–216.

Specker, J. L. (1982). Interrenal function and smoltification. *Aquaculture*, *28*, 59–66.

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

Sundell, K., Jutfelt, F., Ágústsson, T., Olsen, R. E., Sandblom, E., Hansen, T., & Björnsson, B. T. (2003). Intestinal transport mechanisms and plasma cortisol levels during normal and out-of-season parr-smolt transformation of Atlantic salmon, *Salmo salar*. *Aquaculture*, *222*, 265–285.

Sundell, K. S., & Sundh, H. (2012). Intestinal fluid absorption in anadromous salmonids: Importance of tight junctions and aquaporins. *Frontiers in Physiology*, *3*, 338.

Sundh, H., Nilsen, T. O., Lindström, J., Hasselberg-Frank, L., Stefansson, S. O., McCormick, S. D., & Sundell, K. (2014). Development of intestinal ion-transporting mechanisms during smoltification and seawater acclimation in Atlantic salmon *Salmo salar*. *Journal of Fish Biology*, *85*, 1227–1252.

Takagi, C., Takahashi, H., Kudose, H., Kato, K., & Sakamoto, T. (2011). Dual in vitro effects of cortisol on cell turnover in the medaka esophagus via the glucocorticoid receptor. *Life Sciences*, *88*, 239–245.

Takei, Y. (2021). The digestive tract as an essential organ for water acquisition in marine teleosts: Lessons from euryhaline eels. *Zoological Letters*, *7*(1), 10.

Takei, Y., Hiroi, J., Takahashi, H., & Sakamoto, T. (2014). Diverse mechanisms for body fluid regulation in teleost fishes. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *307*, R778–R792.

Takei, Y., Wong, M. K. S., Pipil, S., Ozaki, H., Suzuki, Y., Iwasaki, W., & Kusakabe, M. (2017). Molecular mechanisms underlying active desalination and low water permeability in the esophagus of eels acclimated to seawater. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *312*(2), R231–R244.

Tipsmark, C., Madsen, S., Seidelin, M., Christensen, A. S., Cutler, C. P., & Cramb, G. (2002). Dynamics of Na^+, K^+, $2Cl^-$ cotransporter and Na^+, K^+-ATPase expression in the branchial epithelium of brown trout (*Salmo trutta*) and Atlantic salmon (*Salmo salar*). *Journal of Experimental Zoology*, *293*, 106–118.

Tipsmark, C. K., & Madsen, S. S. (2009). Distinct hormonal regulation of *Na^+, K^+-atpase* genes in the gill of Atlantic salmon (*Salmo salar* L.). *Journal of Endocrinology*, *203*, 301–310.

Tipsmark, C. K., Sørensen, K. J., & Madsen, S. S. (2010). Aquaporin expression dynamics in osmoregulatory tissues of Atlantic salmon during smoltification and seawater acclimation. *Journal of Experimental Biology*, *213*(3), 368–379.

Veillette, P. A., Sundell, K., & Specker, J. L. (1995). Cortisol mediates the increase in intestinal fluid absorption in Atlantic salmon during parr-smolt transformation. *General and Comparative Endocrinology*, *97*, 250–258.

Veillette, P. A., White, R. J., & Specker, J. L. (1993). Changes in intestinal fluid transport in Atlantic salmon (*Salmo salar* L) during parr-smolt transformation. *Fish Physiology and Biochemistry*, *12*(3), 193–202.

Veillette, P. A., White, R. J., Specker, J. L., & Young, G. (2005). Osmoregulatory physiology of pyloric ceca: Regulated and adaptive changes in chinook salmon. *Journal of Experimental Zoology Part A: Comparative Experimental Biology*, *303*(7), 608–613.

Wong, M. K. S., Pipil, S., Kato, A., & Takei, Y. (2016). Duplicated CFTR isoforms in eels diverged in regulatory structures and osmoregulatory functions. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *199*, 130–141.

Yamamoto, M., & Hirano, T. (1978). Morphological changes in the esophageal epithelium of the eel, *Anguilla japonica*, during adaptation to seawater. *Cell and Tissue Research*, *192*, 25–38.

How to cite this article: Breves, J. P., Runiewicz, E. R., Richardson, S. G., Bradley, S. E., Hall, D. J., & McCormick, S. D. (2024). Transcriptional regulation of esophageal, intestinal, and branchial solute transporters by salinity, growth hormone, and cortisol in Atlantic salmon. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *341*, 107–117. https://doi.org/10.1002/jez.2766