Journal of Comparative Physiology B (2022) 192:577–592
https://doi.org/10.1007/s00360-022-01443-8

ORIGINAL PAPER

Na^+/HCO_3^- cotransporter 1 (*nbce1*) isoform gene expression during smoltification and seawater acclimation of Atlantic salmon

Jason P. Breves[1] · Ian S. McKay[1] · Victor Koltenyuk[1] · Nastasia N. Nelson[1] · Sean C. Lema[2] · Stephen D. McCormick[3]

Received: 19 January 2022 / Revised: 4 May 2022 / Accepted: 20 May 2022 / Published online: 17 June 2022

Abstract

The life history of Atlantic salmon (*Salmo salar*) includes an initial freshwater phase (parr) that precedes a springtime migration to marine environments as smolts. The development of osmoregulatory systems that will ultimately support the survival of juveniles upon entry into marine habitats is a key aspect of smoltification. While the acquisition of seawater tolerance in all euryhaline species demands the concerted activity of specific ion pumps, transporters, and channels, the contributions of Na^+/HCO_3^- cotransporter 1 (Nbce1) to salinity acclimation remain unresolved. Here, we investigated the branchial and intestinal expression of three *Na^+/HCO_3^- cotransporter 1* isoforms, denoted *nbce1.1*, *-1.2a*, and *-1.2b*. Given the proposed role of Nbce1 in supporting the absorption of environmental Na^+ by ionocytes, we first hypothesized that expression of a branchial *nbce1* transcript (*nbce1.2a*) would be attenuated in salmon undergoing smoltification and following seawater exposure. In two separate years, we observed spring increases in branchial Na^+/K^+-ATPase activity, *$Na^+/K^+/2Cl^-$ cotransporter 1*, and *cystic fibrosis transmembrane regulator 1* expression characteristic of smoltification, whereas there were no attendant changes in *nbce1.2a* expression. Nonetheless, branchial *nbce1.2a* levels were reduced in parr and smolts within 2 days of seawater exposure. In the intestine, gene transcript abundance for *nbce1.1* increased from spring to summer in the anterior intestine, but not in the posterior intestine or pyloric caeca, and *nbce1.1* and *-1.2b* expression in the intestine showed season-dependent transcriptional regulation by seawater exposure. Collectively, our data indicate that tissue-specific modulation of all three *nbce1* isoforms underlies adaptive responses to seawater.

Keywords Gill · Intestine · Ionocyte · Parr · Pyloric caeca · Smolts

Introduction

Approximately 5% of teleost species are considered euryhaline, and can maintain osmotic and ionic homeostasis when exposed to salinities ranging from fresh water (FW) to full-strength seawater (SW) (Schultz and McCormick 2013). Through the physiological adaptations that confer their tolerance to a broad range of salinities, euryhaline species are equipped to inhabit environments befitting particular life-history stages. Atlantic salmon (*Salmo salar*) undergo parr-smolt transformation (smoltification), a springtime transformation that includes the development of physiological, morphological, and behavioral traits that prepare them to migrate from FW to marine environments at 1–4 years of age (Hoar 1988; Boeuf 1993; McCormick et al. 1998). Like all teleosts residing in FW, salmon in the FW phase are at risk for both excessive hydration and the diffusive loss of ions across body surfaces. Conversely, salmon in the marine phase of their life cycle face the passive gain of ions and dehydration (Evans et al. 2005). Prior to entering the ocean, smolts acquire SW tolerance, in part, through synchronized changes that occur within the gill and gastrointestinal tract (Veillette et al. 1993; Sundell et al. 2003; McCormick et al.

Communicated by B. Pelster.

✉ Jason P. Breves
jbreves@skidmore.edu

[1] Department of Biology, Skidmore College, 815 N. Broadway, Saratoga Springs, NY 12866, USA

[2] Biological Sciences Department, Center for Coastal Marine Sciences, California Polytechnic State University, San Luis Obispo, CA 93407, USA

[3] U.S. Geological Survey, Eastern Ecological Science Center, Conte Anadromous Fish Research Laboratory, One Migratory Way, Turners Falls, MA 01376, USA

2013). While these developmental changes pre-adapt smolts to maintain hydromineral balance upon entering SW, smolts must also possess the ability to rapidly attenuate the ion-absorptive processes that were necessary in FW (McCormick et al. 2013).

While the gut and kidney certainly contribute to maintaining ionic homeostasis in teleosts, the branchial epithelium is the primary site of Na^+ and Cl^- transport via specialized ionocytes (Marshall and Grosell 2006). In marine environments, ion secretion by 'SW-type' ionocytes entails the operation of Na^+/K^+-ATPase and $Na^+/K^+/2Cl^-$ cotransporter 1 (Nkcc1) in the basolateral membrane and cystic fibrosis transmembrane conductance regulator 1 (Cftr1) in the apical membrane (Marshall and Grosell 2006; Hiroi and McCormick 2012). With respect to 'FW-type' ionocytes, several models have been presented describing how they absorb ions from dilute environments. These varying models reflect, in part, the evolution of different strategies for ion uptake across the teleost lineage (Dymowska et al. 2012; Hiroi and McCormick 2012; Guh et al. 2015). For a sub-population of rainbow trout (*Oncorhyncus mykiss*) ionocytes, termed peanut lectin agglutinin negative (PNA^-) cells, electrogenic Na^+/HCO_3^- cotransporter 1 (Nbce1) was proposed to mediate the basolateral exit of Na^+ following its entry through an apical Na^+ channel (Parks et al. 2007; Leguen et al. 2015). This model therefore proposes that Nbce1 augments the basolateral movement of Na^+ sustained by Na^+/K^+-ATPase. Furthermore, Nbce1 may equip PNA^- cells to support systemic acid–base balance (via net acid secretion) by providing a route for basolateral HCO_3^- efflux (Perry and Gilmour 2006). More recently, Tse et al. (2011) and Lema et al. (2018) proposed that Nbce1 supports Na^+ uptake in the gill of Japanese eel (*Anguilla japonica*) and desert pupfish (*Cyprinodon nevadensis amargosae*) by working in parallel with an apical Na^+/H^+ exchanger. To our knowledge, however, there is no information on the transcriptional regulation of three Atlantic salmon *nbce1* isoforms, denoted *nbce1.1*, *-1.2a*, and *-1.2b* (Lema et al. 2018), and more specifically, on whether their levels are modulated in the gill during smoltification or salinity acclimation.

Seawater-acclimated teleosts, including Atlantic salmon in their oceanic phase, combat dehydration by drinking ambient SW (Fuentes and Eddy 1997). Imbibed SW is desalinated by the esophagus via the active and passive transport of Na^+ and Cl^- to produce a fluid that is closer to the osmolality of plasma (Hirano and Mayer-Gostan 1976; Takei et al. 2017). Upon passing through the stomach and entering the intestine, monovalent ions are further decreased allowing water to be absorbed from the luminal fluid through transcellular and paracellular routes (Sundell and Sundh 2012; Madsen et al. 2015). Na^+ and Cl^- enter into enterocytes across the apical surface through Nkcc2 prior to their basolateral exit via Na^+/K^+-ATPases and ClC-family Cl^- channels, respectively (Takei 2021). Cftr2, on the other hand, is elevated in FW and seemingly supports intestinal Cl^- transport in FW-acclimated fishes (Marshall et al. 2002; Sundh et al. 2014; Wong et al. 2016). To promote solute-linked water absorption, enterocytes secrete HCO_3^- to form luminal Ca^{2+} and Mg^{2+} precipitates. The formation of these precipitates enhances water absorption by lowering the osmolality of the luminal fluid (Grosell 2014). In a subset of studied species, enterocytes move HCO_3^- from blood plasma into the intestinal lumen via basolaterally located Nbce1 and apically expressed Cl^-/HCO_3^- exchangers (Grosell et al. 2007; Kurita et al. 2008; Takei 2021). As in the gill, there is currently no information on whether the expression of *nbce1* isoforms is associated with the capacity of salmon intestine (including pyloric caeca) to absorb ions and water (Veillette et al. 1993, 2005; Sundell et al. 2003).

In the present study, we investigated the dynamics of *nbce1.1*, *-1.2a*, and *-1.2b* expression in Atlantic salmon during the parr-smolt transformation and following the exposure of parr and smolts to SW. Given the proposed roles of Nbce1 in the absorption of environmental Na^+ by branchial ionocytes in other teleosts, we hypothesized that *nbce1* transcripts with robust branchial expression would be downregulated in salmon undergoing smoltification and following SW exposure. On the other hand, we hypothesized that *nbce1* transcripts expressed in pyloric caeca, anterior intestine, and posterior intestine would be stimulated during smoltification and following SW transfer to promote fluid absorptive processes.

Materials and methods

Animals

Prior to initiation of the experiments described below, Atlantic salmon (*Salmo salar*) parr were obtained from the Kensington National Fish Hatchery (Kensington, CT, USA) and held at the U.S. Geological Survey, Eastern Ecological Science Center, Conte Anadromous Fish Research Laboratory (Turners Falls, MA, USA). 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, Longview, WA, USA). All experiments were conducted in accordance with U.S. Geological Survey institutional guidelines and an approved IACUC review (LSC-9070).

Experiment 1: tissue distribution of *nbce1.1*, *-1.2a*, and *-1.2b*

A series of tissues were collected in July of 2018 from post-smolts (mixed sex) maintained in FW ($n = 5–7$). Fish weighed 39.7 ± 2.4 g (mean ± S.E.M.) at the time of sampling. Fish were anesthetized with buffered MS-222

(100 mg/l; pH 7.0; Sigma, St. Louis, MO, USA) and the following tissues were collected: whole brain, gill filaments, heart, liver, esophagus, stomach, pyloric caeca, anterior intestine, posterior intestine, body kidney, urinary bladder, muscle, fat, and whole blood. Anterior (proximal) and posterior (distal) intestine samples were collected in relation to the ileorectal sphincter following Sundh et al. (2014). At the time of collection, all tissue samples were immediately frozen on dry ice prior to storage at − 80 °C.

Experiment 2: effects of season and salinity on plasma osmolality, branchial Na^+/K^+-ATPase activity, and *nbce1.2a* expression in parr and smolts

In the first seasonal profile/SW-challenge experiment with juvenile salmon, our primary objective was to describe branchial *nbce1.2a* expression patterns. At the start of the experiment (January 2019), fish were separated by size into parr and presmolt groups based on a previously established winter threshold for smolt development (McCormick et al. 2007). Each group was maintained under natural photoperiod in duplicate tanks throughout the experiment. Parr and smolts ($n = 11$–12; mixed sex) maintained in dechlorinated tap water (FW) were sampled on February 18, April 1, May 6, and July 15. On May 6, 35 parr and 36 smolts were transferred to separate recirculating tanks containing SW (35 ppt) with particle and charcoal filtration and continuous aeration. Parr and smolts ($n = 11$–13) were sampled after 1, 4, and 10 days in SW. Fish sampled on May 6 represented FW controls (time zero controls). Fish in SW were fed ad libitum once daily. Food was withheld for 24 h prior to all samplings that occurred between 09:00 and 11:00 Eastern Standard Time. Parr and smolts weighed 14.0 ± 1.9 g and 44.8 ± 3.0 g at the time of sampling, respectively.

Experiment 3: steady-state branchial *nbce1.2a* expression in parr and smolts

We assessed branchial *nbce1.2a* expression in parr (6.0 ± 1.0 g) and smolts (64.9 ± 3.5 g) fully acclimated to either FW or SW (30 ppt). In May of 2017, we sampled gill filaments from parr and smolts ($n = 6$–10; mixed sex) transferred to 30 ppt after 2 and 2.5 weeks, respectively.

Experiment 4: effects of season and salinity on plasma Cl^-, branchial Na^+/K^+-ATPase activity, and *nbce1* expression in smolts

To resolve intestinal patterns of *nbce1* expression, we analyzed an additional seasonal profile/SW-challenge experiment. Fish ($n = 8$; mixed sex) were sampled from a cohort that was expected to smolt in the spring of 2014 on the basis of their size in early February. Fish were sampled on March 3, April 8, May 1, and July 10. Food was withheld for 24 h prior to sampling that occurred between 09:00 and 11:00 h Eastern Standard Time. SW challenges were conducted on March 3 and May 1. 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 09:00 at 24 and 48 h after transfer to SW. Fish weighed 45.7 ± 1.7 g at the time of sampling. Branchial Na^+/K^+-ATPase, *nkcc1*, and *cftr1* data from this experiment were reported in a previous study (Breves et al. 2017).

Sampling

At the time of all samplings, fish were netted and anesthetized in buffered MS-222 as 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 from the initial netting. Blood was separated by centrifugation at 4 °C and plasma stored at − 80 °C until subsequent analyses. Depending on the experiment, gill filaments, pyloric caeca, anterior intestine, and posterior intestine were collected and immediately frozen on dry ice and stored at − 80 °C. Four to six 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.

Plasma parameters and branchial Na^+/K^+-ATPase activity

Plasma osmolality was measured using a vapor pressure osmometer (Wescor 5100C, Logan, UT, USA). Plasma Cl^- was analyzed by the silver titration method using a Buchler-Cotlove digital chloridometer (Labconco, Kansas City, MO, USA) and external standards. Ouabain-sensitive branchial Na^+/K^+-ATPase 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, Winooski, VT, USA). Protein concentration of the homogenate was determined using a BCA protein assay (Thermo Fisher Scientific, Rockford, IL, USA).

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

Total RNA was extracted from tissue by the TRI Reagent procedure (MRC, Cincinnati, OH, USA) according to the manufacturer's protocols. RNA concentration and purity were assessed by spectrophotometric absorbance (Nanodrop 1000, Thermo Fisher Scientific). First-strand cDNA was

synthesized with a High-Capacity cDNA Reverse Transcription Kit that included random primers (Life Technologies, Carlsbad, CA, USA). Relative mRNA levels were determined by qRT-PCR using the StepOnePlus real-time PCR system (Life Technologies). We employed previously described primer sets for all target and normalization genes aside from *nbce1.1* (XM_014172772), *-1.2a* (XM_014140945), and *-1.2b* (XM_014128056) (Supplementary Table 1). We follow the nomenclature for Atlantic salmon *nbce1s* described by Lema et al. (2018). Primers for *nbce1.1*, *-1.2a*, and *-1.2b* were designed using OligoAnalyzer tool software (Integrated DNA Technologies, Inc.) to span predicted exon–exon junctions and to amplify products of 132, 123, and 88 base pairs, respectively. Non-specific product amplification and primer-dimer formation were assessed by melt curve analyses. qRT-PCR reactions were set up in a 15 µl final reaction volume with 400 nM of each primer, 1 µl cDNA, and 7.5 µl of 2×SYBR Green PCR Master Mix (Life Technologies). 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 gill, pyloric caeca, anterior intestine, and posterior intestine cDNA and included on each plate to calculate the PCR efficiencies for target and normalization genes (Supplementary Table 1). Relative mRNA levels are reported as a fold-change from a given tissue or treatment group as specified in the figure legends.

Statistical analyses

Multiple group comparisons for the tissue expression profiles were performed by one-way ANOVA followed by Tukey's HSD test. For the two seasonal/SW-challenge experiments, one-way ANOVAs followed by Tukey's HSD tests were performed separately for parr and smolts. Significance was set at $P < 0.05$. For a single comparison, a Student's *t* test was employed and significant differences are indicated in figures. All statistical analyses were performed using GraphPad Prism 6 (San Diego, CA, USA). Significance for all tests was set at $P < 0.05$.

Results

Experiment 1: tissue distribution of *nbce1.1*, *-1.2a*, and *-1.2b*

In FW-acclimated post-smolts, *nbce1.1* mRNA levels were higher in anterior intestine with markedly lower levels in all other examined tissues (Fig. 1a). By contrast, relative *nbce1.2a* mRNA expression was highest in gill and muscle (Fig. 1b). The highest *nbce1.2b* mRNA expression was detected in pyloric caeca followed by anterior and posterior intestine (Fig. 1c).

Experiment 2: effects of season and salinity on plasma osmolality, branchial Na^+/K^+-ATPase activity, and *nbce1.2a* expression in parr and smolts

Aside from an increase between April and May, there was no clear seasonal effect on plasma osmolality in smolts; plasma osmolality remained constant in parr from February to July (Fig. 2a). Exposure to SW in May caused a significant increase in plasma osmolality in parr at 1 and 4 days after transfer; SW exposure did not affect plasma osmolality in smolts (Fig. 2b, c). Branchial Na^+/K^+-ATPase activity in smolts increased progressively from February to May prior to declining to low levels in July. In parr, Na^+/K^+-ATPase activity was stable across the sampled period and did not exhibit an appreciable increase between February and May (Fig. 2d). There were no changes in Na^+/K^+-ATPase activity in smolts transferred to SW (Fig. 2f). Parr, on the other hand, showed increases in Na^+/K^+-ATPase activity at 4 and 10 days after transfer to SW (Fig. 2e).

In smolts, branchial *nkcc1* and *cftr1* gene transcript abundance peaked in April prior to declining thereafter; there were no seasonal effects on *nkcc1* and *cftr1* mRNA levels in parr (Fig. 3a, d). There were no clear changes in *nkcc1* and *cftr1* mRNA levels in both parr and smolts following SW exposures (Figs. 3b, c, e, f). For smolts, there was a significant increase in branchial *nbce1.2a* expression between February and April prior to a return to low levels in May and July; a similar, albeit non-significant trend toward an increase in *nbce1.2a* mRNA levels occurred between February and April in parr, as well (Fig. 3g). SW exposures reduced *nbce1.2a* mRNA levels in parr and smolts at 1 and 10 days after transfer to SW, respectively (Fig. 3h, i).

Experiment 3: steady-state branchial *nbce1.2a* expression in parr and smolts

Branchial *nbce1.2a* mRNA levels were markedly lower in parr and smolts maintained in 30 ppt SW for 2 and 2.5 weeks, respectively, when compared with animals held in FW (Fig. 4).

Experiment 4: effects of season and salinity on plasma Cl^-, branchial Na^+/K^+-ATPase activity, and *nbce1* expression in smolts

Plasma Cl^- was significantly lower in April compared with all other sampled time points (Fig. 5a). SW challenges in

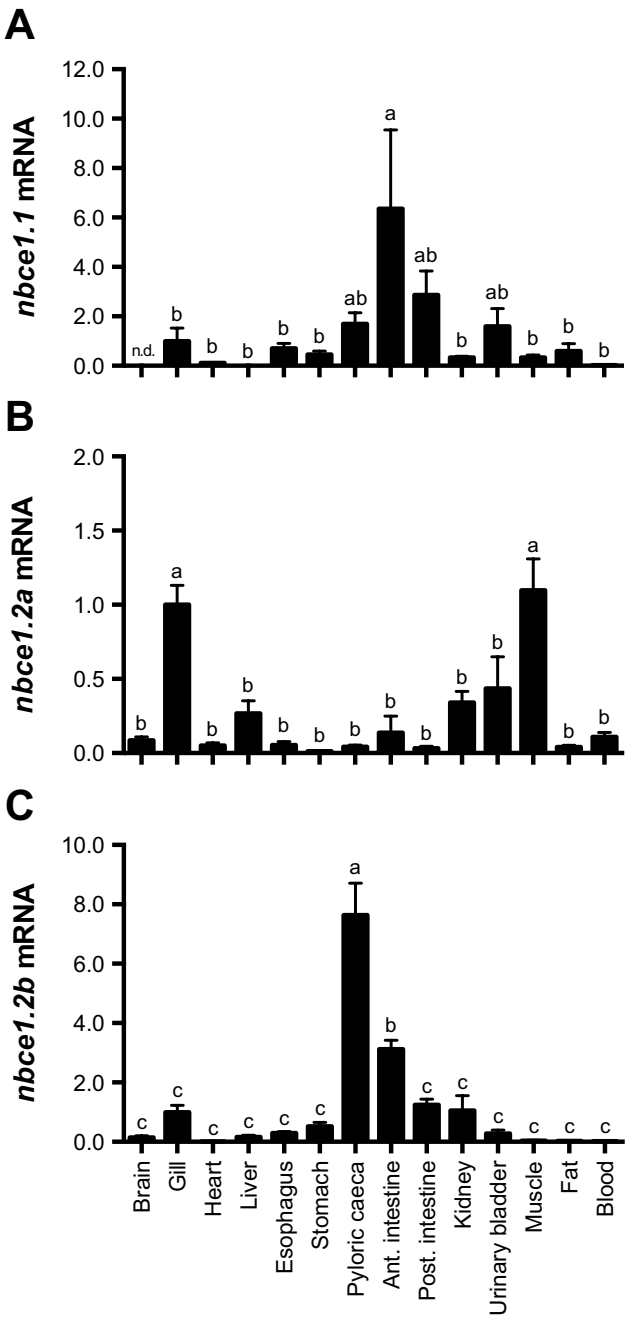


Fig. 1 Tissue expression of *nbce1.1* (**a**), *nbce1.2a* (**b**), and *nbce1.2b* (**c**) in Atlantic salmon post-smolts maintained in fresh water. Data were normalized to *ef1α* as a reference gene and are presented relative to branchial expression levels. Means ± SEM ($n = 5$–7). Means not sharing the same letter are significantly different (one-way ANOVA, Tukey's HSD test, $P < 0.05$). *n.d.* no detection

both March and May elicited elevations in plasma Cl^-, with increases in plasma Cl^- more pronounced in March (Fig. 5b, c). Branchial Na^+/K^+-ATPase activity was elevated in May compared with pre-smolts (March 3 and April 8) and post-smolts (July 10) (Fig. 5d). SW exposures did not elicit changes in Na^+/K^+-ATPase activity (Fig. 5e, f). Branchial *nkcc1* and *cftr1* mRNA expression was higher in May compared with all other time points (Fig. 6a, d). While *nkcc1* levels were not impacted by SW challenges (Figs. 6b, c), *cftr1* expression was elevated following SW exposure in March but not May (Fig. 6e, f). There were no clear seasonal changes in branchial *nbce1.2a* (Fig. 6g), but in a fashion consistent with Experiment #2, SW exposures resulted in marked decreases in *nbce1.2a* expression (Fig. 6h, i).

There was a significant effect of season on both *nkcc2* and *cftr2* transcript abundances in pyloric caeca; both transcripts showed their highest expression in April and were significantly elevated from March (Fig. 7a, d). In May, *nkcc2* levels were elevated 1 day after exposure to SW, while an effect of SW was not detected in March (Fig. 7b, c). There was no clear effect of SW transfer on *cftr2* levels (Fig. 7e, f). There were no effects of season or SW exposure on either *nbce1.1* or *nbce1.2b* in pyloric caeca (Fig. 7g–l).

In anterior intestine, there was a seasonal effect on *nkcc2* expression; *nkcc2* was elevated in April compared with March and July (Fig. 8a). In March and May, *nkcc2* expression was elevated at 1 and 2 days following SW exposure, respectively (Fig. 8b, c). While there was no seasonal effect on *cftr2* levels (Fig. 8d), *cftr2* in anterior intestine was significantly reduced 2 days after transfer to SW in March and May (Fig. 8e, f). *nbce1.1* expression steadily rose throughout the sampling period (Fig. 8g); SW exposure resulted in elevated *nbce1.1* in March but not May (Fig. 8h, i). There were no effects of season or SW exposure on *nbce1.2b* in anterior intestine (Fig. 8j–l).

In posterior intestine, *nkcc*2 mRNA levels did not change across season, but were elevated following SW exposure in May (Fig. 9a–c). *cftr2* levels, on the other hand, were decreased following an SW exposure in March (Fig. 9e). In May, *cftr2* levels were transiently elevated 1 day after SW exposure (Fig. 9f). While there were no seasonal effects on *nbce1.1* or *-1.2b* (Fig. 9g, j), SW exposure stimulated the expression of each transcript in March and May, respectively (Fig. 9h–i, k–l).

Discussion

Phylogenetic analyses indicated that two Nbce1s, denoted Nbce1.1 and -1.2, evolved within teleosts (Lee et al. 2011; Chang et al. 2012; Lema et al. 2018). A subsequent duplication of Nbce1.2, into Nbce1.2a and -1.2b, occurred within the salmonid lineage (Lema et al. 2018). Therefore, our first objective was to characterize the distributions of *nbce1.1*, *-1.2a*, and *-1.2b* mRNAs to target subsequent analyses on the appropriate isoform(s) when assessing branchial and intestinal *nbce1* expression patterns. The *nbce1.1* and *-1.2b* isoforms were expressed within the segments of the intestine

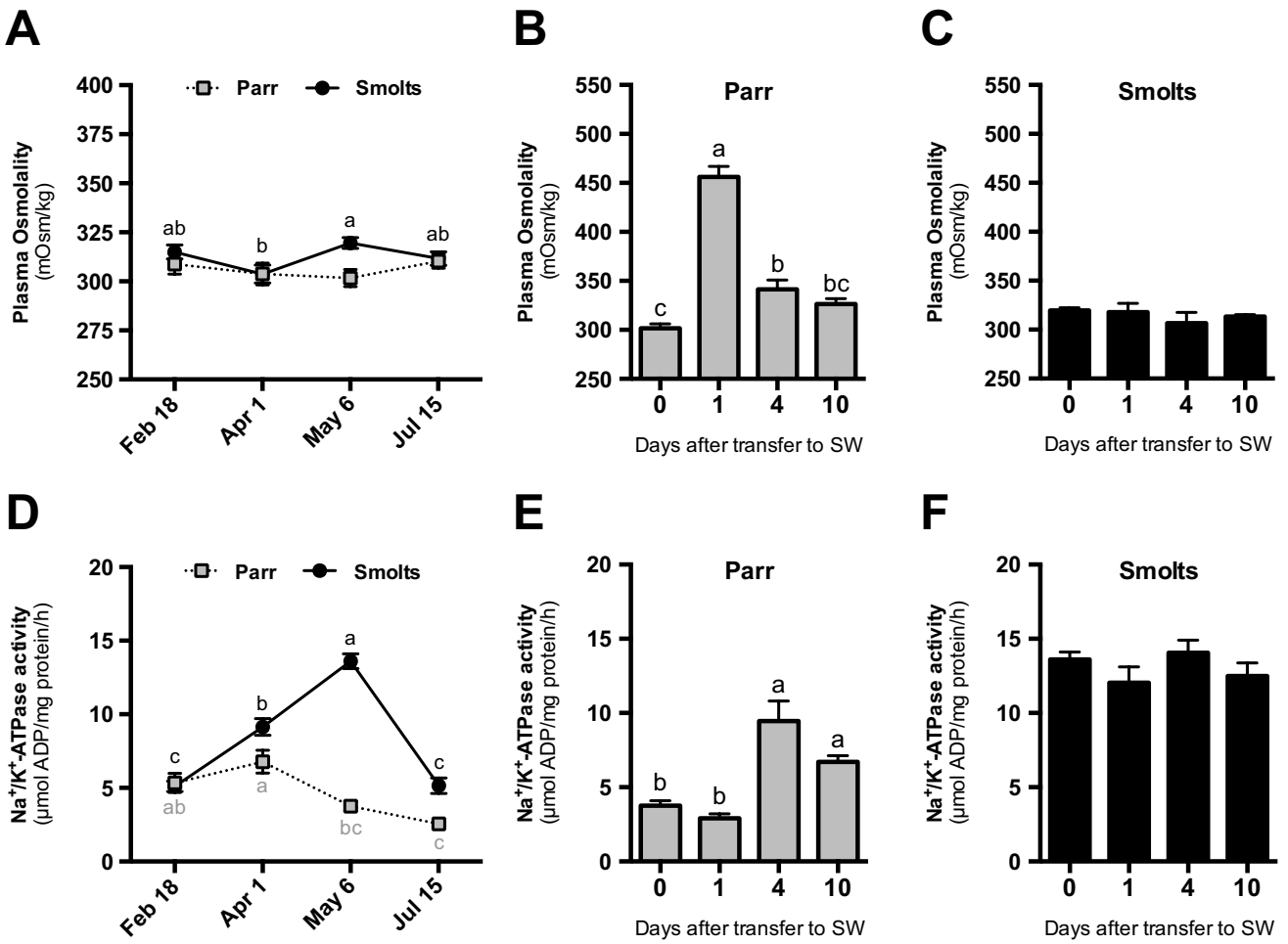


Fig. 2 Plasma osmolality (**a**) and branchial Na^+/K^+-ATPase activity (**d**) in Atlantic salmon parr (shaded squares; dotted line) and smolts (filled circles; solid line) maintained in fresh water from February 18 through July 15. Means ± S.E.M. ($n = 11–12$). Within a life stage, denoted by gray (parr) or black (smolts) letters, means not sharing the same letter are significantly different (one-way ANOVA, Tukey's HSD test, $P < 0.05$). Plasma osmolality (**b**, **c**) and branchial Na^+/K^+-ATPase activity (**e**, **f**) in parr (shaded bars) and smolts (solid bars) subjected to 1-, 4-, and 10-day seawater (SW) exposures in May. Means ± S.E.M. ($n = 11–13$). Means not sharing the same letter are significantly different (one-way ANOVA, Tukey's HSD test, $P < 0.05$)

(including pyloric caeca), whereas *nbce1.2a* showed low intestinal expression but robust expression in the gill. In rainbow trout, *nbce1* gene transcript levels (corresponding to *nbce1.2* based on Lema et al. 2018) were similar in the pyloric caeca and anterior intestine (Grosell et al. 2007), a pattern that aligns with high rates of gastrointestinal HCO_3^- secretion in these tissues (Grosell et al. 2009). Comparable to the expression patterns of *nbce1.1* and *-1.2b* in the current study, *nbce1* expression in toadfish (*Opsanus beta*) was greater in the anterior/middle intestine compared to the posterior intestine/rectum (Taylor et al. 2010). With the distribution of salmon *nbce1* isoforms more clearly resolved, we then characterized their dynamics in the gill and intestine during SW acclimation along with other mediators of ionoregulation.

In two separate years, salmon sampled from late-February/early March to July showed springtime elevations in branchial Na^+/K^+-ATPase activity characteristic of smoltification (Tipsmark et al. 2002; McCormick et al. 2007; Nilsen et al. 2007). Furthermore, smolts displayed springtime increases in the expression of genes indicative of SW-type ionocyte recruitment, such as *nkcc1* and *cftr1* (Tipsmark et al. 2002; Kiilerich et al. 2007; Mackie et al. 2007; Nilsen et al. 2007), which allow them to maintain hydromineral balance upon transfer to SW. The observation that smolts in both years did not show marked changes in Na^+/K^+-ATPase activity or *nkcc1* and *cftr1* transcript abundance in response to SW suggested that preparatory increases in SW-type ionocytes and their associated ion transporters enabled them to maintain

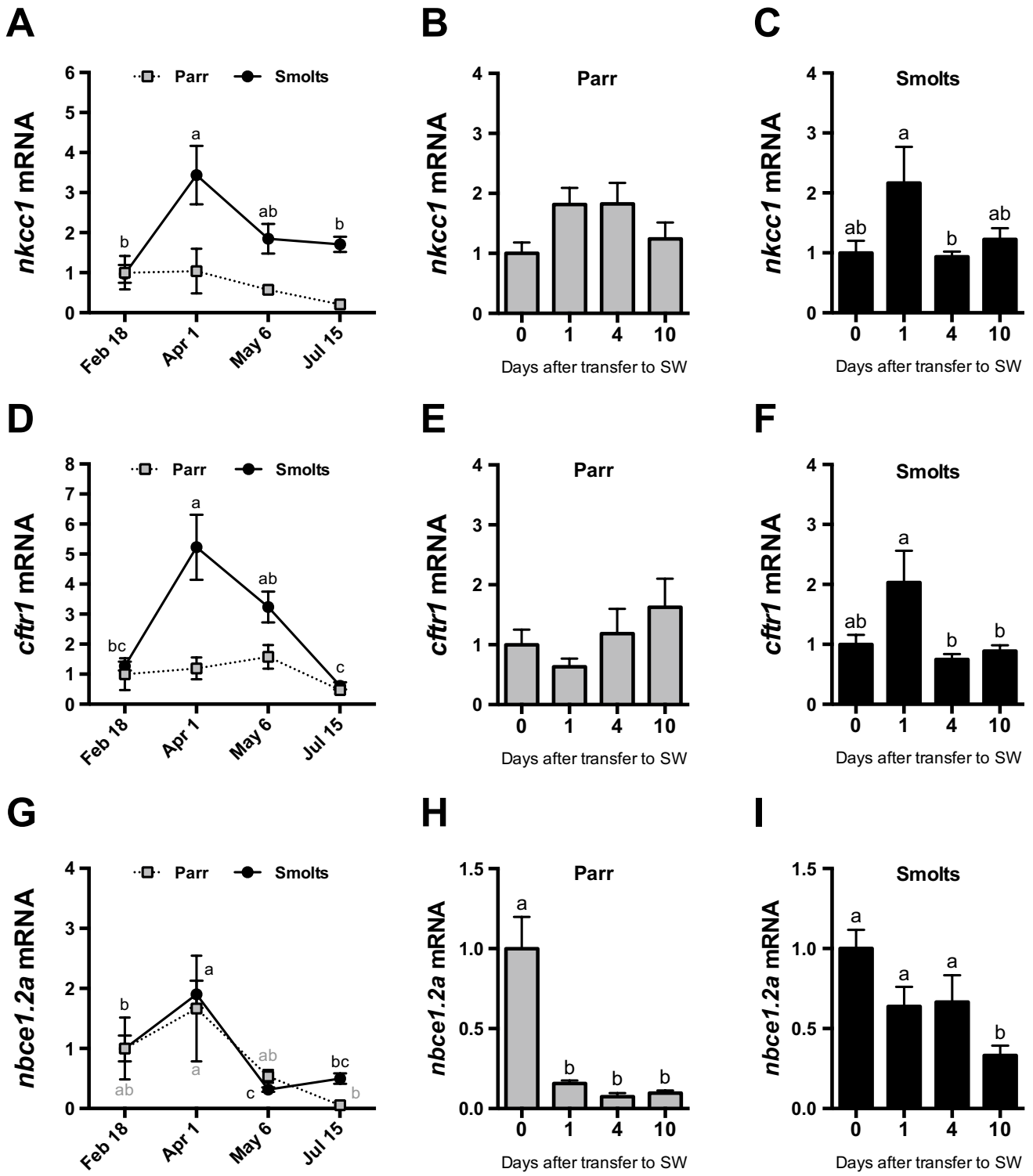


Fig. 3 Branchial *nkcc1* (**a**), *cftr1* (**d**), and *nbce1.2a* (**g**) gene expression in Atlantic salmon parr (shaded squares; dotted line) and smolts (filled circles; solid line) maintained in fresh water from February 18 through July 15. Gene expression is presented as a fold-change from the February 18 parr group. Means ± S.E.M. ($n = 11–12$). Within a life stage, denoted by gray (parr) or black (smolts) letters, means not sharing the same letter are significantly different (one-way ANOVA, Tukey's HSD test, $P < 0.05$). Branchial *nkcc1* (**b**, **c**), *cftr1* (**e**, **f**), and *nbce1.2a* (**h**, **i**) gene expression in parr (shaded bars) and smolts (solid bars) subjected to 1-, 4-, and 10-day seawater (SW) exposures in May. Gene expression is presented as a fold-change from the 0-day groups. Means ± S.E.M. ($n = 11–13$). Means not sharing the same letter are significantly different (one-way ANOVA, Tukey's HSD test, $P < 0.05$)

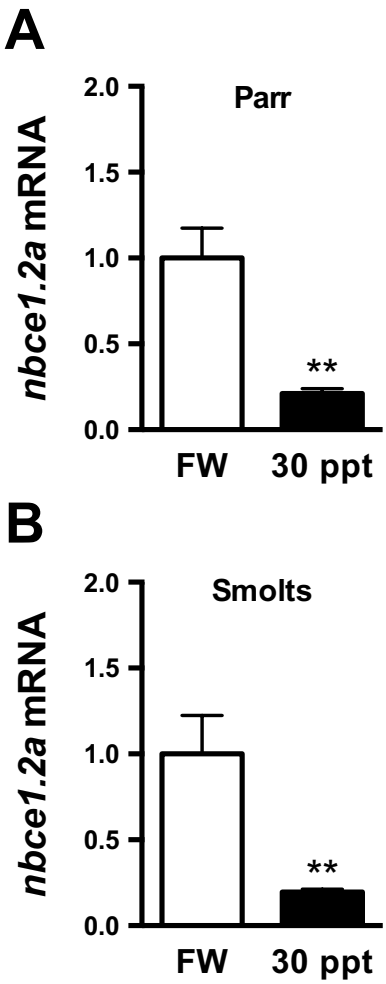


Fig. 4 Branchial *nbce1.2a* gene expression in parr (**a**) and smolts (**b**) acclimated to 30 ppt for 2 and 2.5 weeks, respectively. Means ± SEM ($n = 6$–10). Gene expression in 30 ppt (solid bars) is presented as a fold-change from the freshwater (FW)-acclimated group (open bars). Asterisks indicate significant difference between groups by Student's t test (**$P < 0.01$)

hydromineral balance in SW without recruiting more transporters (McCormick et al. 2013). We hypothesized that *nbce1.2a* would be downregulated in salmon undergoing smoltification, but unlike *nkcc1* and *cftr1*, there were no seasonal changes in *nbce1.2a* in smolts. Nonetheless, there were sustained drops in *nbce1.2a* expression within 2 days after SW exposure. An inverse relationship between environmental salinity and branchial *nbce1.2* expression was similarly observed in euryhaline pupfish transferred from brackish water (7.5 ppt) to FW (Lema et al. 2018). We therefore propose that Nbce1.2a is less abundant, and presumably less important, in SW than in FW. In particular, the acute transcriptional downregulation of *nbce1.2a* within the first day of SW acclimation may reflect the attenuation of Na^+ transport across the basolateral membrane of FW-type ionocytes. Under this scenario, the movement of Na^+ from FW-type ionocytes into the plasma via Nbce1.2a assumes that intracellular HCO_3^- is high enough to overcome the inward Na^+ gradient or that multiple HCO_3^- molecules bind to Nbc1, allowing it to respond to electrical gradients driven by Na^+/K^+-ATPase (Parks et al. 2007).

To our knowledge, there is no description of a FW-type ionocyte in Atlantic salmon that incorporates the function of Nbce1. In another salmonid, the rainbow trout, Nbce1 supports the absorption of environmental Na^+ by PNA^- cells by co-transporting Na^+ and HCO_3^- across the basolateral membrane (Parks et al. 2007). The initial entry of Na^+ into PNA^- cells through the apical Na^+ channel, acid-sensing ion channel 4 (Asic4), is electrochemically linked to an apical V-type H^+-ATPase (Dymowska et al. 2014). Intracellular HCO_3^- is produced by carbonic anhydrase (Parks et al. 2007). In Mozambique tilapia (*Oreochromis mossambicus*) and zebrafish (*Danio rerio*), Nbce1 is located in the basolateral membrane of a sub-population of FW-type ionocytes that employ Na^+/Cl^- cotransporter 2 (Ncc2) (Furukawa et al. 2011; Guh et al. 2015), whereas in eel and pupfish, Nbce1 may work in tandem with an apical Na^+/H^+ exchanger (Tse et al. 2011; Lema et al. 2018). Given the transcriptional regulation of *nbce1.2a* described in the current study, an important next step will be to localize its encoded product within the branchial epithelium of FW-acclimated salmon. Because salmonids do not express Ncc2 in the gill (Hiroi and McCormick 2012), and there is no information on Na^+/H^+ exchangers or Asic4 in Atlantic salmon, it should first be determined whether Nbce1.2a is found within ionocytes containing the FW-type isoform of the Na^+/K^+-ATPase alpha subunit (McCormick et al. 2009). The sustained expression of branchial *nbce1.2a* levels during smoltification suggests that its translated product mediates essential ionoregulatory functions that cannot cease until smolts leave FW. In a similar fashion, Na^+/K^+-ATPase alpha 1a protein abundance is not greatly altered until their entry into SW, when

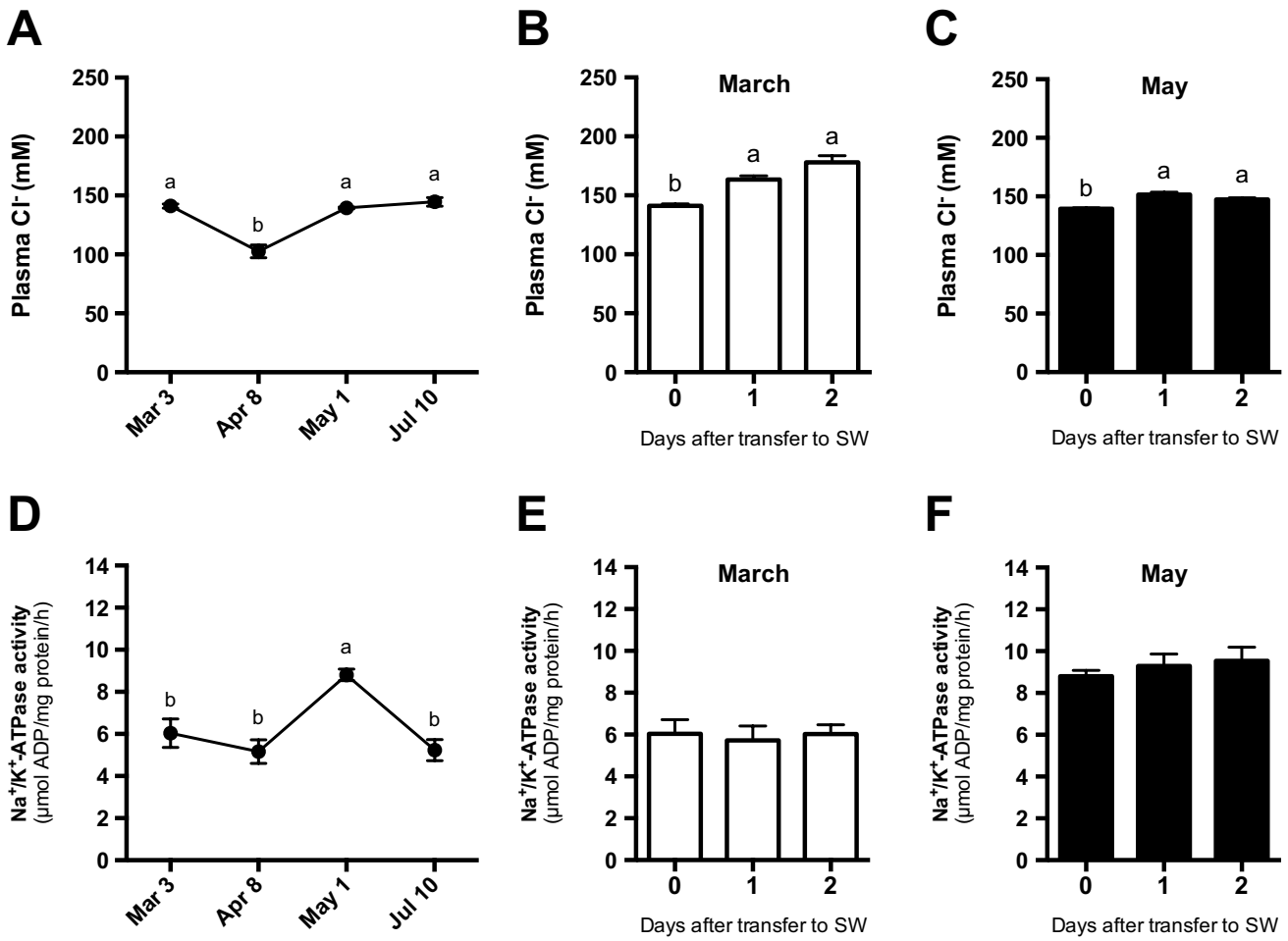


Fig. 5 Plasma Cl^- (**a**) and branchial Na^+/K^+-ATPase activity (**d**) in Atlantic salmon smolts maintained in fresh water from March 3 through July 10. Plasma Cl^- (**b**, **c**) and branchial Na^+/K^+-ATPase activity (**e**, **f**) in smolts subjected to 1- and 2-day seawater (SW) exposures in March (open bars) and May (solid bars). Means ± S.E.M. ($n = 8$). Means not sharing the same letter are significantly different (one-way ANOVA, Tukey's HSD test, $P < 0.05$)

both transcription and protein abundance decrease (Nilsen et al. 2007; McCormick et al. 2013). The common drop in gill Na^+/K^+-ATPase alpha 1a (McCormick et al. 2013) and *nbce1.2a* (present study) transcription after SW exposure provides some support for the idea that Nbce1.2a will be present in the FW-type, Na^+/K^+-ATPase alpha 1a-rich ionocyte.

The intestinal epithelium of salmonids undergoes structural and functional changes during smoltification in preparation for encountering a dehydrating marine environment (Sundell and Sundh 2012). For instance, smoltification entails an enhanced capacity for solute-linked fluid absorption (Veillette et al. 1993; Nielsen et al. 1999). While intestinal Na^+/K^+-ATPase activity is known to increase during springtime in Atlantic salmon (Sundell et al. 2003), a complete picture of the molecular underpinnings of enhanced fluid absorptive capacity, and in particular with regards to *nbce1* expression, has not been fully developed. First, we observed springtime increases in *nkcc2* in the pyloric caeca and anterior intestine prior to further increases in *nkcc2* levels following the transfer of smolts to SW, a pattern previously reported by Sundh et al. (2014). In this respect, Atlantic salmon are consistent with the general pattern of how euryhaline species utilize Nkcc2 to

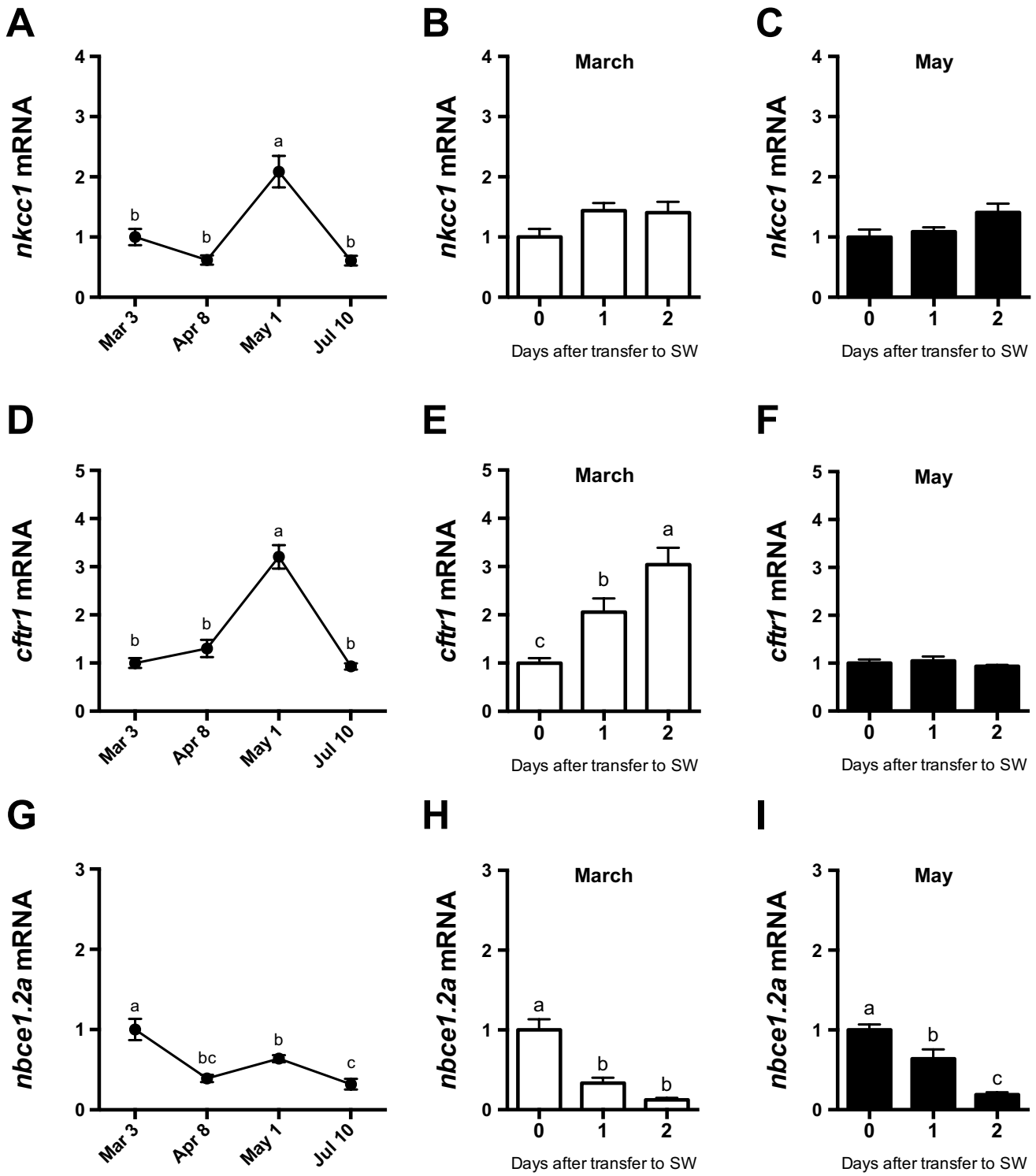


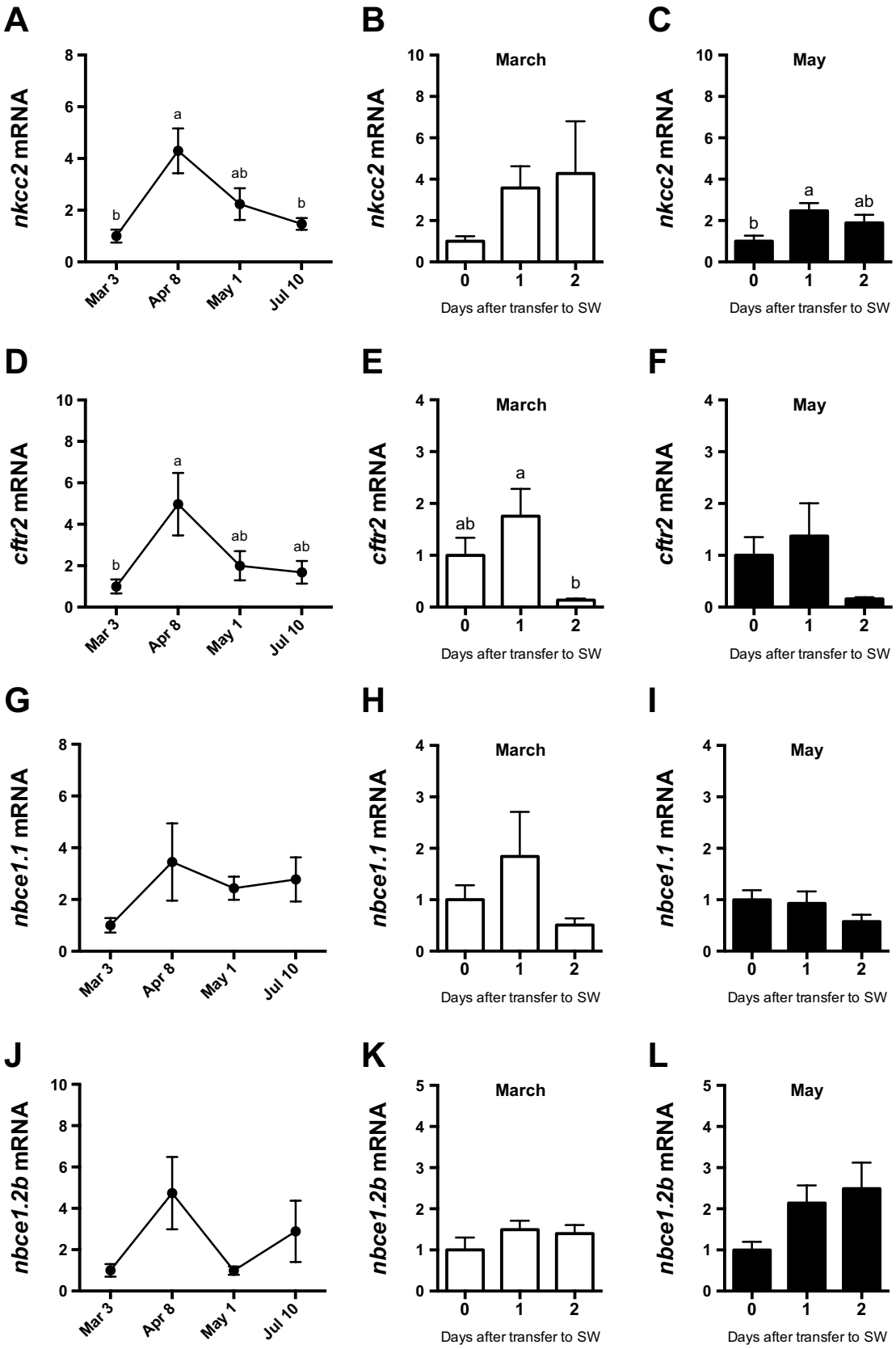
Fig. 6 Branchial *nkcc1* (**a**), *cftr1* (**d**), and *nbce1.2a* (**g**) gene expression in Atlantic salmon smolts maintained in fresh water from March 3 through July 10. Gene expression is presented as a fold-change from the March 3 group. Branchial *nkcc1* (**b**, **c**), *cftr1* (**e**, **f**), and *nbce1.2a* (**h**, **i**) gene expression in smolts subjected to 1- and 2-day seawater (SW) exposures in March (open bars) and May (solid bars). Gene expression is presented as a fold-change from the 0-day groups. Means ± S.E.M. ($n = 8$). Means not sharing the same letter are significantly different (one-way ANOVA, Tukey's HSD test, $P < 0.05$)

support solute-linked fluid absorption (Watanabe et al. 2011; Gregório et al. 2013; Li et al. 2014; Esbaugh and Cutler 2016; Ruhr et al. 2016; Zhang et al. 2019). The abundance of gene transcripts encoding *cftr2*, on the other hand, was diminished in the anterior intestine of smolts transferred to SW in both March and May, providing further support that Cftr2 function is counter-productive to SW acclimation in euryhaline species (Gregório et al. 2013; Sundh et al. 2014; Wong et al. 2016). However, functional studies are still required to deduce the role(s) for Cftr2 in FW-acclimation given its apical localization within enterocytes (Marshall et al. 2002).

Concomitant with the regulation of *nkcc2* and *cftr2* following exposure to SW, *nbce1.1* levels increased in the anterior and posterior intestine in March and *nbce1.2b* levels increased in the posterior intestine in May. Because Nbce1 facilitates the basolateral acquisition of HCO_3^- by enterocytes, these responses ostensibly support the enhanced secretion of HCO_3^- across the apical surface of enterocytes via Cl^- exchange (Kurita et al. 2008; Grosell 2011). This action will lower intestinal osmolality in two ways: by reducing luminal Cl^- and alkalinizing the gut leading to formation of divalent salt precipitates, which in turn promotes water uptake. The intestinal expression of Cl^-/HCO_3^- exchangers in Atlantic salmon, therefore, warrants future characterization. While there is considerable evidence indicating the anterior intestine of SW-acclimated fishes secretes HCO_3^- at a substantial rate (Grosell 2006), direct assessments of HCO_3^- secretion by different regions of the intestine of Atlantic salmon are lacking, but will be necessary to integrate *nbce1* isoform expression patterns with actual capacities for HCO_3^- secretion. This is important to resolve, because, alternatively, the hydration of metabolic CO_2 by carbonic anhydrase may serve as a source of HCO_3^- for apical secretion by teleost enterocytes (Grosell 2006, 2011). Moreover, different cellular mechanisms to acquire HCO_3^- as a substrate for Cl^- exchange dominate along the intestine in region-specific fashions (e.g., anterior vs. posterior intestine) (Sattin et al. 2010; Grosell 2011). Given that intestinal *nbce1* expression patterns have only been reported for a few species (Grosell et al. 2007; Taylor et al. 2010), our observation that Atlantic salmon express multiple *nbce1* isoforms in the intestine provides a new perspective on how fishes regulate HCO_3^- transport in response to hyperosmotic environments.

It is well established that a broad suite of hormones promotes hydromineral balance in teleosts by orchestrating effectors of solute transport in the gill and intestine (Takei et al. 2014). The pituitary hormone prolactin promotes the expression of ion transporters/channels within ionocytes that enable teleosts residing in FW environments to actively absorb ambient Na^+ and Cl^- (Breves et al. 2014; Shaughnessy and Breves 2021). Salmon Nbce1.2a emerges from our study as a candidate for regulation by prolactin given its enhanced expression under FW conditions when prolactin signaling is activated (Hirano et al. 1985). Prolactin also dampens processes associated with SW acclimation, and notably, intestinal HCO_3^- secretion by inhibiting the basolateral acquisition of HCO_3^- via Nbce1 (Ferlazzo et al. 2012). Given their expression patterns in salmon, Nbce1.1 and -1.2b should now be examined as potential intermediaries between systemic prolactin and the function of teleost enterocytes. Cortisol, on the other hand, promotes branchial and intestinal phenotypes associated with SW acclimation (Hirano and Utida 1968; Utida et al. 1972; Pelis and McCormick 2001; Tipsmark et al. 2002; Nilsen et al. 2007). For instance, Atlantic salmon smolts increase their capacity for intestinal fluid absorption in response to plasma cortisol (Cornell et al. 1994; Veillette et al. 1995). Our current findings poise us to determine whether changes in plasma cortisol (Nichols and Weisbart 1985) are linked with the transcriptional control of intestinal *nbce1.1* and *-1.2b* during SW acclimation.

In conclusion, our collective findings provide the first evidence that multiple Nbce1 isoforms underlie salinity acclimation in Atlantic salmon. The first key finding of this study was that *nbce1.2a* expression is salinity-dependent in the gill, and thus, we propose that FW-type ionocytes in Atlantic salmon employ Nbce1.2a for the uptake of environmental Na^+. This certainly does not exclude the possibility that Nbce1.2a also contributes to acid–base balance in a fashion similar to how it operates within rainbow trout ionocytes (Perry and Gilmour 2006). Second, we found that intestinal *nbce1.1* and *-1.2b* levels increased upon exposure to SW in parallel with *nkcc2*. The development of paralog-specific Nbce1 antibodies is now warranted to localize Nbce1s in key ion-transporting cells, particularly ionocytes and enterocytes, and to resolve their sub-cellular localization patterns. Future work is certainly needed to also begin addressing the functional/adaptive significance


A
B
C
D
E
F
G
H
I
J
K
L
nkcc2 mRNA
cftr2 mRNA
nbce1.1 mRNA
nbce1.2b mRNA
Mar 3
Apr 8
May 1
Jul 10
March
May
Days after transfer to SW
a
ab
b

◂**Fig. 7** *nkcc2* (**a**), *cftr2* (**d**), *nbce1.1* (**g**), and *nbce1.2b* (**j**) gene expression in pyloric caeca of Atlantic salmon smolts maintained in fresh water from March 3 through July 10. Gene expression is presented as a fold-change from the March 3 group. *nkcc2* (**b**, **c**), *cftr2* (**e**, **f**), *nbce1.1* (**h**, **i**), and *nbce1.2b* (**k**, **l**) gene expression in pyloric caeca of smolts subjected to 1- and 2-day seawater (SW) exposures in March (open bars) and May (solid bars). Gene expression is presented as a fold-change from the 0-day groups. Means ± S.E.M. ($n = 8$). Means not sharing the same letter are significantly different (one-way ANOVA, Tukey's HSD test, $P < 0.05$)

of Atlantic salmon (and other salmonids) expressing three Nbce1 isoforms in tissue-specific fashions. Analyses of this nature promise to shed further light on how Nbce1s support the broad salinity tolerance of euryhaline fishes and may pave the way for their identification as targets of endocrine factors.

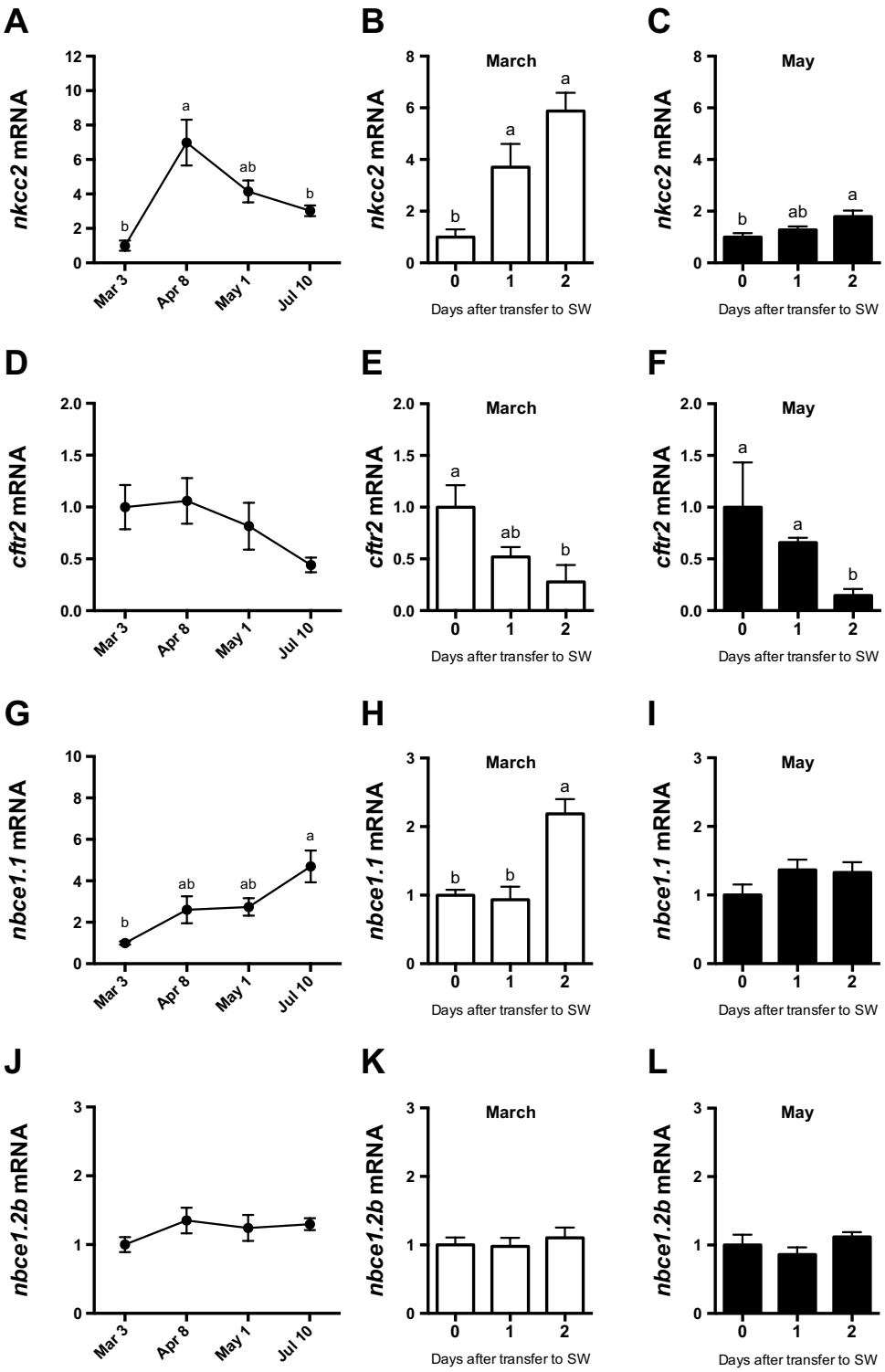


Fig. 8 *nkcc2* (**a**), *cftr2* (**d**), *nbce1.1* (**g**), and *nbce1.2b* (**j**) gene expression in anterior intestine of Atlantic salmon smolts maintained in fresh water from March 3 through July 10. Gene expression is presented as a fold-change from the March 3 group. *nkcc2* (**b**, **c**), *cftr2* (**e**, **f**), *nbce1.1* (**h**, **i**), and *nbce1.2b* (k, l) gene expression in anterior intestine of smolts subjected to 1- and 2-day seawater (SW) exposures in March (open bars) and May (solid bars). Gene expression is presented as a fold-change from the 0-day groups. Means ± S.E.M. ($n = 8$). Means not sharing the same letter are significantly different (one-way ANOVA, Tukey's HSD test, $P < 0.05$)

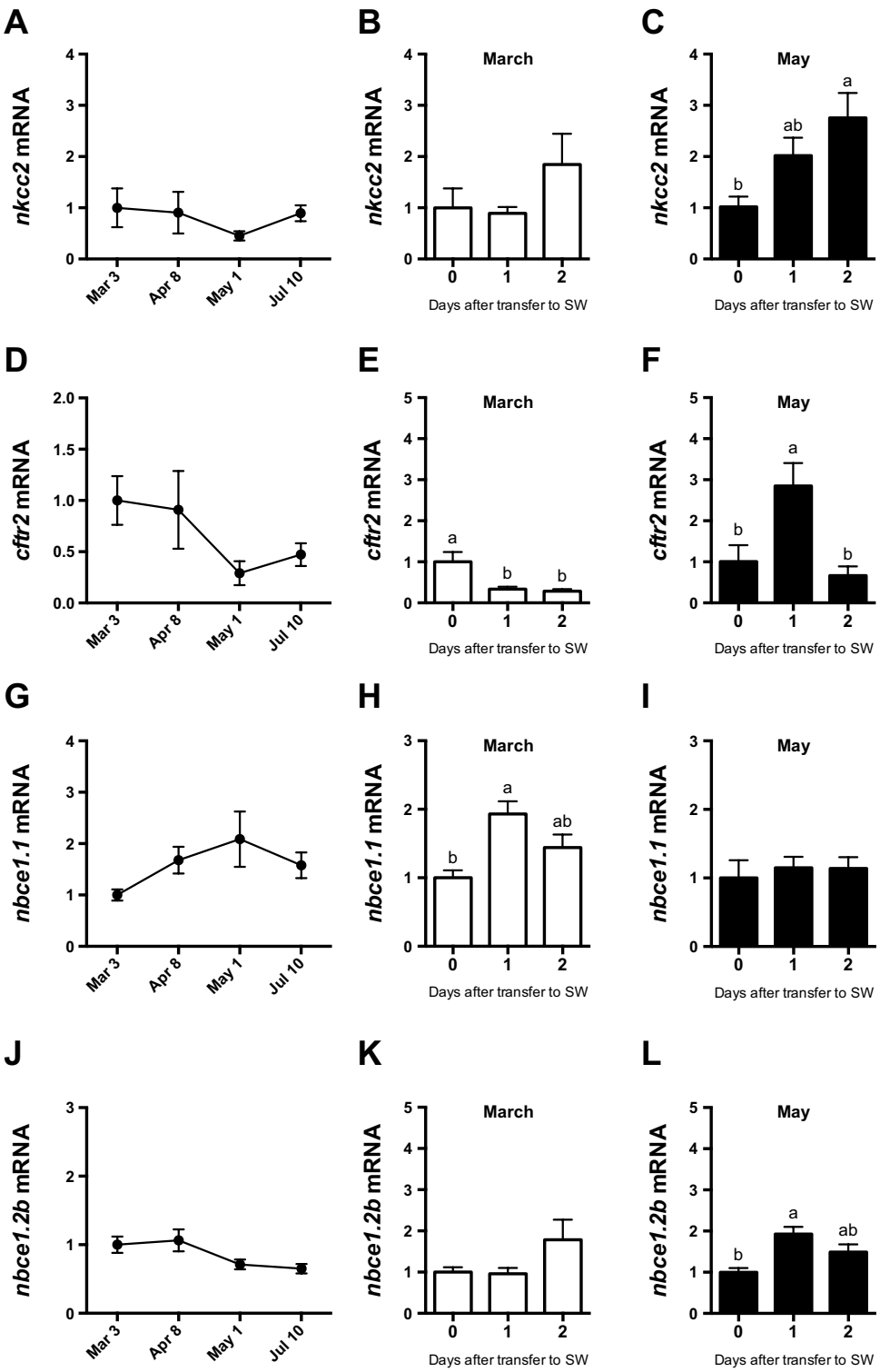


Fig. 9 *nkcc2* (**a**), *cftr2* (**d**), *nbce1.1* (**g**), and *nbce1.2b* (**j**) gene expression in posterior intestine of Atlantic salmon smolts maintained in fresh water from March 3 through July 10. Gene expression is presented as a fold-change from the March 3 group. *nkcc2* (**b**, **c**), *cftr2* (**e**, **f**), *nbce1.1* (**h**, **i**), and *nbce1.2b* (**k**, **l**) gene expression in posterior intestine of smolts subjected to 1- and 2-day seawater (SW) exposures in March (open bars) and May (solid bars). Gene expression is presented as a fold-change from the 0-day groups. Means ± S.E.M. ($n = 8$). Means not sharing the same letter are significantly different (one-way ANOVA, Tukey's HSD test, $P < 0.05$)

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00360-022-01443-8.

Acknowledgements We appreciate the excellent laboratory assistance and fish care provided by Amy Regish and Dan Hall during the course of this study.

Funding This work was supported by the National Science Foundation (IOS-1755131 to JPB). Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the US Government.

Declarations

Conflict of interest The authors have no competing interests to declare that are relevant to the content of this article.

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

References

Boeuf G (1993) Salmonid smolting: a pre-adaptation to the oceanic environment. In: Rankin JC, Jensen FB (eds) Fish ecophysiology. Chapman and Hall, London, pp 105–135

Bower NI, Li X, Taylor R, Johnston IA (2008) Switching to fast growth: the insulin-like growth factor (IGF) system in skeletal muscle of Atlantic salmon. J Exp Biol 211:3859–3870

Breves JP, McCormick SD, Karlstrom RO (2014) Prolactin and teleost ionocytes: new insights into cellular and molecular targets of prolactin in vertebrate epithelia. Gen Comp Endocrinol 203:21–28

Breves JP, Fujimoto CK, Phipps-Costin SK, Einarsdottir IE, Björnsson BT, McCormick SD (2017) Variation in branchial expression among *insulin-like growth-factor binding proteins* (*igfbps*) during Atlantic salmon smoltification and seawater exposure. BMC Physiol 17(1):2

Chang MH, Plata C, Kurita Y, Kato A, Hirose S, Romero MF (2012) Euryhaline pufferfish NBCe1 differs from nonmarine species NBCe1 physiology. Am J Physiol Cell 302(8):C1083-1095

Cornell SC, Portesi DM, Veillette PA, Sundell K, Specker JL (1994) Cortisol stimulates intestinal fluid uptake in Atlantic salmon (*Salmo salar*) in the post-smolt stage. Fish Physiol Biochem 13:183–190

Dymowska AK, Hwang PP, Goss GG (2012) Structure and function of ionocytes in the freshwater fish gill. Respir Physiol Neurobiol 18:282–292

Dymowska AK, Schultz AG, Blair SD, Chamot D, Goss GG (2014) Acid-sensing ion channels are involved in epithelial Na^+ uptake in the rainbow trout *Oncorhynchus mykiss*. Am J Physiol Cell 307(3):C255–C265

Esbaugh AJ, Cutler B (2016) Intestinal Na^+, K^+, $2Cl^-$ cotransporter 2 plays a crucial role in hyperosmotic transitions of a euryhaline teleost. Physiol Rep 4(22):e13028–e13028

Evans DH, Piermarini PM, Choe KP (2005) The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol Rev 85:97–177

Ferlazzo A, Carvalho ESM, Gregorio SF, Power DM, Canario AVM, Trischittla F, Fuentes J (2012) Prolactin regulates luminal bicarbonate secretion in the intestine of the sea bream (*Sparus aurata* L.). J Exp Biol 215:3836–3844

Fuentes J, Eddy FB (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. J Comp Phys B 167(6):438–443

Furukawa F, Watanabe S, Inokuchi M, Kaneko T (2011) Responses of gill mitochondria-rich cells in *Mozambique tilapia* exposed to acidic environments (pH 4.0) in combination with different salinities. Comp Biochem Physiol A 158(4):468–476

Gregório SF, Carvalho ESM, Encarnação S, Wilson JM, Power DM, Canário AVM, Fuentes J (2013) Adaptation to different salinities exposes functional specialization in the intestine of the sea bream (*Sparus aurata* L.). J Exp Biol 216:470–479

Grosell M (2006) Intestinal anion exchange in marine fish osmoregulation. J Exp Biol 209:2813–2827

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

Grosell M (2014) Intestinal transport. In: Evans DH, Claiborne JB, Currie S (eds) The physiology of fishes. CRC Press, Boca Raton, pp 175–203

Grosell M, Gilmour KM, Perry SF (2007) Intestinal carbonic anhydrase, bicarbonate, and proton carriers play a role in the acclimation of rainbow trout to seawater. Am J Physiol Regul Integr Comp Physiol 293(5):R2099-2111

Grosell M, Genz J, Taylor JR, Perry SF, Gilmour KM (2009) The involvement of H^+-ATPase and carbonic anhydrase in intestinal HCO_3^- secretion in seawater-acclimated rainbow trout. J Exp Biol 212:1940–1948

Guh Y, Lin C, Hwang PP (2015) Osmoregulation in zebrafish: ion transport mechanisms and functional regulation. EXCLI J 14:627–659

Hirano T, Mayer-Gostan N (1976) Eel esophagus as an osmoregulatory organ. Proc Nat Acad Sci USA 73:1348–1350

Hirano T, Utida S (1968) Effects of ACTH and cortisol on water movement in isolated intestine of the eel, *Anguilla Japonica*. Gen Comp Endocrinol 11(2):373–380

Hirano T, Prunet P, Kawauchi H, Takahashi A, Ogasawara T, Kubota J, Nishioka RS, Bern HA, Takada K, Ishii S (1985) Development and validation of a salmon prolactin radioimmunoassay. Gen Comp Endocrinol 59(2):266–276

Hiroi J, McCormick SD (2012) New insights into gill ionocyte and ion transporter function in euryhaline and diadromous fish. Resp Physiol Neurobiol 184:257–268

Hoar WS (1988) The physiology of smolting salmonids. In: Hoar WS, Randall DJ (eds) Fish physiology, vol XIB. Academic Press, New York, pp 275–343

Kiilerich P, Kristiansen K, Madsen SS (2007) Cortisol regulation of ion transporter mRNA in Atlantic salmon gill and the effect of salinity on the signaling pathway. J Endocrinol 194:417–427

Kurita Y, Nakada T, Kato A, Doi H, Mistry AC, Chang MH, Romero MF, Hirose S (2008) Identification of intestinal bicarbonate transporters involved in formation of carbonate precipitates to stimulate water absorption in marine teleost fish. Am J Physiol Regul Integr Comp Physiol 294(4):R1402–R1412

Lee YC, Yan JJ, Cruz SA, Horng JL, Hwang PP (2011) Anion exchanger 1b, but not sodium-bicarbonate cotransporter 1b, plays a role in transport functions of zebrafish H^+-ATPase-rich cells. Am J Physiol Cell 300(2):C295-307

Leguen I, Le Cam A, Montfort J, Peron S, Fautrel A (2015) Transcriptomic analysis of trout gill ionocytes in fresh water and sea water using laser capture microdissection combined with microarray analysis. PLoS ONE 10(10):e0139938

Lema SC, Carvalho PG, Egelston JN, Kelly JT, McCormick SD (2018) Dynamics of gene expression responses for ion transport proteins and aquaporins in the gill of a euryhaline pupfish during freshwater and high-salinity acclimation. Physiol Biochem Zool 91(6):1148–1171

Li Z, Lui EY, Wilson JM, Ip YK, Lin Q, Lam TJ, Lam SH (2014) Expression of key ion transporters in the gill and esophageal-gastrointestinal tract of euryhaline Mozambique tilapia *Oreochromis mossambicus* acclimated to fresh water, seawater and hypersaline water. PLoS ONE 9(1):e87591

Mackie PM, Gharbi K, Ballantyne JS, McCormick SD, Wright PA (2007) $Na^+/K^+/2Cl^-$ cotransporter and CFTR gill expression after seawater transfer in smolts (0+) of different Atlantic salmon (*Salmo salar*) families. Aquaculture 272:625–635

Madsen SS, Engelund MB, Cutler CP (2015) Water transport and functional dynamics of aquaporins in osmoregulatory organs of fishes. Biol Bull 229(1):70–92

Marshall WS, Grosell M (2006) Ion transport, osmoregulation and acid-base balance. In: Evans DH, Claiborne JB (eds) The physiology of fishes. CRC Press, Boca Raton, pp 177–230

Marshall WS, Howard JA, Cozzi RR, Lynch EM (2002) NaCl and fluid secretion by the intestine of the teleost *Fundulus heteroclitus*: involvement of CFTR. J Exp Biol 205:745–758

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

McCormick SD, Shrimpton JM, Carey JB, O'Dea MF, Sloan KE, Moriyama S, Björnsson BT (1998) Repeated acute stress reduces growth rate of Atlantic salmon parr and alters plasma growth hormone, insulin-like growth factor I and cortisol. Aquaculture 168:221–235

McCormick SD, Shrimpton JM, Moriyama S, Björnsson BT (2007) Differential hormonal responses of Atlantic salmon parr and smolt to increased daylength: a possible developmental basis for smolting. Aquaculture 273:337–344

McCormick SD, Regish AM, Christensen AK (2009) Distinct freshwater and seawater isoforms of Na^+/K^+-ATPase in gill chloride cells of Atlantic salmon. J Exp Biol 212:3994–4001

McCormick SD, Regish AM, Christensen AK, Björnsson BT (2013) Differential regulation of sodium-potassium pump isoforms during smolt development and seawater exposure of Atlantic salmon. J Exp Biol 216:1142–1151

Nichols DJ, Weisbart M (1985) Cortisol dynamics during seawater adaptation of Atlantic salmon *Salmo salar*. Am J Physiol 248(6 Pt 2):R651-659

Nielsen C, Madsen SS, Björnsson BT (1999) Changes in branchial and intestinal osmoregulatory mechanisms and growth hormone levels during smolting in hatchery-reared and wild brown trout. J Fish Biol 54:799–818

Nilsen TO, Ebbesson LOE, Madsen SS, McCormick SD, Andersson E, Björnsson BT, Prunet P, Stefansson SO (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*. J Exp Biol 210:2885–2896

Parks SK, Tresguerres M, Goss GG (2007) Interactions between Na^+ channels and Na^+-HCO_3^- cotransporters in the freshwater fish gill MR cell: a model for transepithelial Na^+ uptake. Am J Physiol Cell 292(2):C935-944

Pelis RM, McCormick SD (2001) Effects of growth hormone and cortisol on Na^+-K^+-$2Cl^-$ cotransporter localization and abundance in the gills of Atlantic salmon. Gen Comp Endocrinol 124:134–143

Perry SF, Gilmour KM (2006) Acid-base and CO_2 excretion in fish: unanswered questions and emerging models. Resp Physiol Neurobiol 154:199–215

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucl Acids Res 29(9):e45

Ruhr IM, Takei Y, Grosell M (2016) The role of the rectum in osmoregulation and the potential effect of renoguanylin on SLC26a6 transport activity in the Gulf toadfish (*Opsanus beta*). Am J Physiol Regul Integr Comp Physiol 311:R179-191

Sattin G, Mager EM, Beltramini M, Grosell M (2010) Cytosolic carbonic anhydrase in the Gulf toadfish is important for tolerance to hypersalinity. Comp Biochem Physiol A 156(2):169–175

Schultz ET, McCormick SD (2013) Euryhalinity in an evolutionary context. In: McCormick SD, Farrell AP, Brauner CJ (eds) Euryhaline fishes. Elsevier, New York, pp 477–529

Shaughnessy CA, Breves JP (2021) Molecular mechanisms of Cl^- transport in fishes: new insights and their evolutionary context. J Exp Zool A 335(2):207–216

Sundell K, Sundh H (2012) Intestinal fluid absorption in anadromous salmonids: importance of tight junctions and aquaporins. Front Physiol 3:338

Sundell K, Jutfelt E, Agustsson T, Olsen RE, Sandblom E, Hansen 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

Sundh H, Nilsen TO, Lindström J, Hasselberg-Frank L, Stefansson SO, McCormick SD, Sundell K (2014) Development of intestinal ion-transporting mechanisms during smoltification and seawater acclimation in Atlantic salmon *Salmo salar*. J Fish Biol 85:1227–1252

Takei Y (2021) The digestive tract as an essential organ for water acquisition in marine teleosts: lessons from euryhaline eels. Zool Lett 7(1):10

Takei Y, Hiroi J, Takahashi H, Sakamoto T (2014) Diverse mechanisms for body fluid regulation in teleost fishes. Am J Physiol Regul Integr Comp Physiol 307:R778-792

Takei Y, Wong MK, 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. Am J Physiol Regul Integr Comp Physiol 312(2):R231-244

Taylor JR, Mager EM, Grosell M (2010) Basolateral NBCe1 plays a rate-limiting role in transepithelial intestinal HCO_3^- secretion, contributing to marine fish osmoregulation. J Exp Biol 213(3):459–468

Tipsmark CK, Madsen SS, Seidelin M, Christensen AS, Cutler CP, 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*). J Exp Zool 293:106–118

Tse WK, Chow SC, Lai KP, Au DW, Wong CK (2011) Modulation of ion transporter expression in gill mitochondrion-rich cells of eels acclimated to low-Na^+ or -Cl^- freshwater. J Exp Zool A 315(7):385–393

Utida S, Hirano T, Oide H, Ando M, Johnson DW, Bern HA (1972) Hormonal control of the intestine and urinary bladder in teleost osmoregulation. Gen Comp Endocrinol 3(Suppl):317–327

Veillette PA, White RJ, Specker JL (1993) Changes in intestinal fluid transport in Atlantic salmon (*Salmo salar* L.) during parr-smolt transformation. Fish Physiol Biochem 12(3):193–202

Veillette PA, Sundell K, Specker JL (1995) Cortisol mediates the increase in intestinal fluid absorption in Atlantic salmon during parr-smolt transformation. Gen Comp Endocrinol 97:250–258

Veillette PA, White RJ, Specker JL, Young G (2005) Osmoregulatory physiology of pyloric ceca: regulated and adaptive changes in chinook salmon. J Exp Zool A 303(7):608–613

Watanabe S, Mekuchi M, Ideuchi H, Kim YK, Kaneko T (2011) Electroneutral cation-Cl^- cotransporters NKCC2β and NCCβ expressed in the intestinal tract of Japanese eel *Anguilla japonica*. Comp Biochem Physiol A 159:427–435

Wong MK, Pipil S, Kato A, Takei Y (2016) Duplicated CFTR isoforms in eels diverged in regulatory structures and osmoregulatory functions. Comp Biochem Physiol A 199:130–141

Zhang K, Zhang X, Wen H, Qi X, Fan H, Tian Y, Liu Y, Li Y (2019) Spotted sea bass (*Lateolabrax maculatus*) *cftr*, *nkcc1a*, *nkcc1b*, and *nkcc2*: genome-wide identification, characterization and expression analysis under salinity stress. J Ocean Univ China 18:1470–1480

Supplementary Table 1. Specific primer sequences for quantitative real-time PCR.

Gene	Primer Sequence (5′-3′)	Efficiency (%)	Reference/Acc. No.
cftr1	F: CCTTCTCCAATATGGTTGAAGAGGCAAG R: GAGGCACTTGGATGAGTCAGCAG	103	Nilsen et al. 2007
cftr2	F: GCCTTATTTCTTCTATTTGTATGCACT R: GCCACCATGAAAAACTAAAGAGTACCT	108	Nilsen et al. 2007
ef1α	F: GAATCGGCTATGCCTGGTGAC R: GGATGATGACCTGAGCGGTG	99	Bower et al. 2008
nbce1.1	F: GACAATATGCAGGCAGGGTG R: AGCCTCTCGAAGACCAGAAC	100	XM_014172772
nbce1.2a	F: GTCAAGGAGGAGGAGGG R: TCGTAGAAATCACTGGCGAAG	98	XM_014140945
nbce1.2b	F: TCAGGGAGGAGGCGGAC R: CCGCTTGATGTCCAGAATGAG	96	XM_014128056
nkcc1	F: GATGATCTGCGGCCATGTTC R: AGACCAGTAACCTGTCGAGAAAC	95	Nilsen et al. 2007
nkcc2	F: CCGCGTGCCCAACATC R: GCACGGTTACCGCTCACACT	103	Sundh et al. 2014