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Photoperiod and dietary treatment in freshwater modulate the short-term intestinal response to seawater in Atlantic salmon (*Salmo salar*)

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ABSTRACT

Stimulation and timing of smoltification are essential for successful Atlantic salmon (*Salmo salar)* aquaculture. This study investigated intestinal responses during dietary and photoperiod manipulation in freshwater (FW) and after a subsequent seven days residence in seawater (SW). "Small" and "large" Atlantic salmon parr (~40 g and ~ 130 g respectively) were treated in FW for 12 weeks and thereafter transferred to SW for seven days. During the FW phase, fish underwent two different light conditions, 24 L:0D – 24 L ("LL-LL" groups) and 7 L:17D – 24 L ("SP-LL" groups) or fed with either regular feed ("LL-LL C" and "SP-LL C" groups) or feed enriched with a salt mix plus free tryptophan ("LL-LL + diet" and "SP-LL + diet" groups). We analyzed Na^+/K^+-ATPase (NKA) activity, tissue bioelectrical properties in Ussing chambers, and intestinal fluid composition. The NKA activity showed minor variations in relation to fish size, treatments, or intestinal region (anterior or posterior). Photoperiod modulated epithelial bioelectrical properties (I_{sc} and R_t) of the anterior and posterior intestine, particularly transepithelial resistance (R_t). Pharmacological experiments, targeting apical $Na^+/K^+/2Cl^-$ (NKCC2) and Na^+/Cl^- (NCC) co-transporters revealed intestinal region- and water salinity-dependent effects. In addition, stimulation of the intracellular cAMP with forskolin and IBMX showed intestinal region-, water salinity, and treatment-dependence responses with clear functional specialization of the anterior and posterior intestine. The intestinal fluid composition reflected the ability to process ingested SW and showed little variation in large fish. In summary, our data suggest a better pre-adaptation of the intestine during light-stimulated smoltification (SP-LL groups), and the combination of light and diet might give, in an industrial aquaculture setting, an advantage to smaller, but not larger smolts. Intestinal fluid composition in small fish can be used as an index of intestinal function and may act as a long-term performance proxy in SW Atlantic salmon.

1. Introduction

In nature, Atlantic salmon (*Salmo salar*) juveniles spend a variable period in freshwater (FW) before undergoing several preparatory changes for seawater (SW) entry. The set of morphological, physiological, endocrine, and behavioral changes that transform FW parr into a SW-ready smolts are known as parr-smolt transformation (PST) or simply smoltification (Hoar, 1988; Stefansson et al., 2008). When fish have surpassed a minimum body size, increasing day length in spring activates the light-brain-pituitary axis to promote neuroendocrine mechanisms regulating the onset of smoltification (Ebbesson et al., 2003; McCormick et al., 1998). Other environmental factors, such as water temperature and flow, and endogenous mechanisms, also contribute to the stimulation and timing of PST (Eriksson and Lundqvist, 1982; McCormick, 2012).

In both FW and SW, the ionic and osmotic differences between smolts plasma and the surrounding medium cause an osmotic imbalance, opposite in the two environments. The gills, gastrointestinal tract, and

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kidney are the main organs that work in synergy to oppose the disequilibrium and maintain body homeostasis (Edwards and Marshall, 2012). A crucial change that allows smolts to survive and reach a new osmoregulatory steady state in SW is the development of hypo-osmoregulatory capacity while still in FW and the fast reorganization of the osmoregulatory mechanisms after SW transfer (McCormick, 2012).

Earlier studies on the development of the hypo-osmoregulatory mechanism during PST have focused mainly on the gill (McCormick, 2012). However, ion- and osmotic homeostasis in SW is a highly integrated process where the intestine plays a fundamental role. Thus, upon transfer to SW, the first osmoregulatory response required is the onset of drinking, which increases about 30-folds compared to FW salmon smolts (Fuentes and Eddy, 1997). This increase of drinking causes an increased absorption of ions and water in the gastrointestinal tract (Smith, 1930) and the subsequent active secretion of ions by the chloride cells in the gills (Evans et al., 2005).

Water ingested requires intestinal processing to become available to the plasma pool. This processing starts in the esophagus, where a relevant water-independent absorption of Na^+ and Cl^- take place (Hirano and Mayer Gostan, 1976; Parmelee and Renfro, 1983). The functional consequence is a substantial reduction of osmolality of the imbibed fluid which constitutes a first step to promote water absorption in the intestine (Marshall and Grosell, 2005).

The main driving force for water absorption is NaCl movement. Several mechanisms are believed to be involved in this process. Thus, NaCl is or might be absorbed by the apical Na^+/Cl^- co-transporter (NCC) (Frizzell et al., 1979; Watanabe et al., 2011) and, together with K^+, by the apical $Na^+/K^+/2Cl^-$ co-transporter (NKCC2) (Musch et al., 1982; Sundh et al., 2014; Trischitta et al., 1992). In addition, recent studies in SW species (Grosell et al., 2005; Grosell and Genz, 2006; Kurita et al., 2008) highlighted a putative important role for apical anion exchangers (SLC26 family, Ohana et al., 2009) in facilitating Cl^- absorption and subsequent water movements. Thus, Cl^- enters the enterocyte by apical Cl^-/HCO_3^- exchangers and generates apical HCO_3^- secretion. As a result, there is an alkalinization of the intestinal fluid that, combined with the high concentrations of Ca^{2+} and Mg^{2+} originated from the high drinking, creates the optimal chemical conditions for $CaCO_3$ and $MgCO_3$ precipitation in the form of aggregates (Walsh et al., 1991; Wilson and Grosell, 2003). The precipitation in the form of carbonates removes ions from the solution, reduces the intestinal fluid osmolality, and favors net water absorption. This processing and the putative involvement of intestinal precipitation generates a unique ion profile in the intestinal fluid of SW fish. Na^+ and Cl^- concentration become lower than plasma levels, while divalent ions Ca^{2+} and Mg^{2+}, are several folds higher in the fluid than in the plasma (Alves et al., 2019; Grosell et al., 2007). How this process takes place in Atlantic salmon smolts remains still unknown. The electrochemical potential required to energize ion movement through channels and transporters is generated by the basolateral Na^+/K^+-ATPase, whose activity in fish intestine is generally higher after SW adaptation (Barany et al., 2020; Skou and Esmann, 1992).

The intestine of fish is a heterogeneous tissue at both morphological and functional levels. Using functional approaches, several studies described a region-specific role of the different tracts of the intestine in osmoregulatory mechanisms (Alves et al., 2019; Gregório et al., 2013; Grosell et al., 2005; Ruhr et al., 2014; Ruiz-Jarabo et al., 2017b). In Atlantic salmon such studies are scarce but relevant. For instance, Sundell et al. (2003) described intestinal region-dependent tissue resistance measured in Ussing chambers in FW and SW anterior and posterior intestine. Which are also evident in rainbow trout in FW and SW (Sundell and Sundh, 2012). Usher et al. (1991a) showed changes in water absorption in vitro in the intestine of Atlantic salmon during the PST and after transfer to SW, and Veillette et al. (1993) show a functional intestinal regionalization of fluid transport during the PST.

In the industrial production of Atlantic salmon, several methods are used to stimulate smoltification, e.g., osmoregulatory maturation. A compressed natural photoperiod is commonly adopted, where the fish is exposed to a period of short-day length ("winter") followed by an increase in day length ("spring") during the last part of the FW phase (Duston and Saunders, 1990; Sigholt et al., 1995). This treatment is hereafter termed "light treatment". However, to enhance the growth rate in FW, many farmers skip the short-day treatment to achieve bigger smolt size and a shorter growth period in SW (Pino Martinez et al., 2021; Ytrestøyl et al., 2020). In the absence of light treatment, so-called "smoltification feeds" may be used to stimulate PST. Generally, these feeds are added a salt/ion mixture known to stimulate SW tolerance in juvenile salmonids (Basulto, 1976; Staurnes and Finstad, 2000). The feed used in the present experiment was in addition added free tryptophan, which, like ions, is known to activate G-protein coupled calcium-sensitive receptors (CaSR) to promote SW tolerance (Loretz, 2008). This method, hereafter termed a "dietary stimulation", is adopted in the weeks preceding SW transfer.

The transfer from FW to SW still represents a critical period with significant loss of animals in salmon farming due to suboptimal smolt quality and high level of infections (Aunsmo et al., 2008; Hjeltnes et al., 2019). Therefore, the aim of the present study was to understand if the intestinal function provides valuable information about the timing of SW transfer of Atlantic salmon undergoing different strategies of smolting stimulation. To achieve this objective, we investigated the intestinal responses in FW and after a short-term (seven days) SW challenge to 1) characterize the region-specific osmoregulatory mechanisms evoked during smoltification in the anterior and posterior intestine by mean of a functional approach in Ussing chamber; 2) focusing on the end result of the intestinal function (fluid composition), investigate if dietary stimulation, with a mix of salt and amino acid tryptophan, in combination with photoperiod manipulation, can improve the intestinal physiological machinery required for the FW-SW transition, in small (~40 g) and large (~130 g) Atlantic salmon pre-smolts.

2. Materials and methods

2.1. Chemicals

Forskolin (FK) and 3-isobutyl-1-methylxanthine (IBMX) were from Cayman Chemical (MI, USA). All other chemicals were from Sigma-Aldrich (Madrid, Spain). In pharmacology experiments, final concentrations of the chemicals in Ussing chambers were as follows: bumetanide (BUM) 200 μM, hydrochlorothiazide (HCTZ) 1000 μM, forskolin (FK) 10 μM and 3-isobutyl-1-methylxanthine (IBMX) 500 μM, prepared as concentrated stocks in DMSO. The amount of DMSO in the solution never exceeded 0.2% of the total volume of the Ussing chamber.

2.2. Animals and experimental design

The experimental design of the present study has been described in detail by Striberny et al. (2021). In brief, fertilized Atlantic salmon eggs (*Salmo salar*) were obtained from AquaGen strain (AquaGen, Trondheim, Norway) and hatched and raised to parr at the Aquaculture Research Station in Tromso (Norway). In mid-March 2017, at start-feeding, juveniles were divided into two groups. A group of fish was kept in FW at 4 °C till two weeks before the start of the experiment (6th February 2018). During the two weeks, the temperature was increased by 0.5 °C/day till 10 °C. This group at the beginning of the experiment had a body mass mean ~ 40 g and it is referred to them as "small" fish. The other group was kept in FW at 10 °C until September 2017, after which the temperature was decreased and kept on 4 °C till November 2017 and increased again to 10 °C until the start of the experiment (6th February 2018). At the beginning of the experiment, these fish had a body mass average ~ 130 g and termed "large" fish. Until this point, both size groups had been kept at continuous light (24 L:0D).

On 6th February 2018, 1400 small fish and 1000 large fish were

divided into eight circular tanks (300 L/tank) for each size group and kept in flow-through FW at 10 °C. Four tanks per size were subjected to six weeks of short photoperiod with 7 h of light, and 17 h of darkness (7 L:17D, "SP" groups) and the others remained under 24 h of light (24 L:0D, "LL" groups). At the end of the six weeks under short photoperiod for SP groups (21st March), all fish were brought back to continuous light (24 L:0D). At this time-point, the water temperature was increased to 12 °C, and two tanks, for each light condition and size, were fed with pellet supplemented with salt and the amino acid tryptophan ("LL- LL + diet" and "SP-LL + diet" groups). The rest of the tanks were fed with the usual commercial pellet ("LL-LL C" and "SP-LL C" control groups). The dietary treatment was carried out in the last six weeks in FW, before SW transfer (11th May). The composition of the two used feeds is shown in Table 1. On 11th May, 50 fish from each treatment and size were transfer to 33‰ SW at 8 °C in circular tanks (300 L/tank).

Fish were fed in excess and continuously by automatic systems with pellets suitable for the life stage (Skretting AS, Stavanger, Norway). During the short photoperiod for SP-LL groups, all groups were fed during the 7 h of light for SP-LLs. Feeding was withheld in the 24 h before each sampling. However, food was present in the intestine of FW fish. SW fish intestine were devoid of food.

The experimental design and the sampling time-points used in the present work are schematically represented in Fig. 1. For each time-point, size, and experimental condition, ten fish were sampled, five from each duplicate tank. Animals were anesthetized with an overdose of benzocaine (160 ppm), blood collected, and fish sacrificed by decapitation. The intestine was isolated, washed in salmon saline (see below), and samples of the anterior intestine (2–3 cm caudal of the point of insertion of the last pyloric caeca) and posterior intestine (2–3 cm posterior to the ileorectal sphincter) were collected for electrophysiological characterization and intestinal Na^+/K^+-ATPase activity measurements. Also, during the sampling of fish in SW at day seven after transfer, intestinal fluid from individual animals was collected.

The experiment was conducted following guidelines provided in Norwegian and European legislation related to animal research. Formal approval of the experimental protocol was given by the Norwegian Food Safety Authority, FOTS ID 13891.

2.3. Intestinal fluid

FW fish lacked intestinal fluid; therefore, the fluid collection took place only after seven days in SW. After anesthesia, the abdominal cavity was cut open, and the intestinal tract clamped with two hemostatic forceps (from the end of pyloric caeca to the anal sphincter), the contents were then emptied into 1.5 mL tubes, centrifuged (12,000 rpm, 6 min, RT) and fluid stored at −20 °C for later analysis. Osmolality was measured using a Vapor Pressure Osmometer (Model 5600 Wescor, Logan, UT, USA). Sodium ion concentration was determined using a flame photometer (BWB-XP, BWB Technologies, UK), and chloride ion was measured by coulometric titration with a Chloride Analyzer (Model 925 Corning, Medfield, MA, USA). The difference in osmolality (ΔOsm) and chloride ion (ΔCl^-) were calculated between plasma (PL) and intestinal fluid (IF) per individual fish (plasma values from Striberny et al. (2021) following the formula Δvalue = Plasma level-Intestinal fluid level; in mOsm.kg^{-1} for osmolality or mmol L^{-1} for chloride ion.

Table 1
Diet composition.

Diet composition	Control (%)	Salt (%)
Wheat	15.00	9.90
Wheat gluten	10.00	12.00
Sunflower meal	5.00	2.00
Soy protein concentrate	15.50	15.00
Fababean dehulled	4.80	2.00
Fish meal	31.30	32.30
Rapeseed oil	8.50	8.60
Fish oil	8.50	8.60
Water	0.30	1.00
Vitamin and mineral premixes	1.10	1.10
Sodium chloride	0.00	6.00
Calcium chloride	0.00	0.75
L-tryptophan	0.00	0.40
Magnesium chloride	0.00	0.25
Total	100.00	100.00
Moisture	8.30	8.30
Protein	43.55	43.24
Fat	21.99	21.99
Ash	6.98	13.36
Gross energy (MJ)	22.17	21.21

2.4. Intestinal Na^+/K^+-ATPase activity

Mucosal biopsies of the anterior and posterior intestine were placed in 200 μl of ice-cold SEI buffer (150 mM sucrose, 10 mM EDTA, 50 mM imidazole, pH 7.3), rested on ice for 2–3 min and immediately frozen at −80 °C to avoid tissue degradation by proteases. Intestinal Na^+/K^+-ATPase (NKA) activity was determined using a previously developed method (McCormick, 1993).

Samples were randomly analyzed and NKA activity was measured within 25 min of samples thawing. The specific activity (μmol ADP mg protein^{-1} h^{-1}) was determined by linking ATP hydrolysis to the oxidation of nicotinamide adenine dinucleotide (NADH) measured at 340 nm for 10 min at 25 °C in the presence or absence of 0.5 mM ouabain. Immediately after each NKA assay, protein content of each sample was adjusted to 1 mg mL^{-1}. Protein content was determined by the Bradford Protein Assay (BioRad Laboratories, Hercules, CA, USA) using bovine serum albumin as standard (BSA, BioRad Laboratories, Hercules, CA, USA).

2.5. Ussing chamber experiments

Intestinal sections from the anterior and posterior intestine were washed with chilled fresh saline to remove undigested food and mounted within five minutes of sacrifice on tissue holders of 0.25 cm^2 or 0.5 cm^2 (P2404 and P2305, Physiological Instruments, San Diego, CA, USA) between two half-Ussing chambers (P2400 or P2300, Physiological Instruments, San Diego, CA, USA) holding 2 mL of saline kept at 10 °C and gassed bilaterally with humidified air. All intestinal sections of a single tank (five fish, ten intestinal preparations, five anterior and five posterior intestines) were run simultaneously. Saline composition for FW experiments was: 7.5 mM $NaHCO_3$, 2.5 mM KCl, 130 mM NaCl, 1 mM NaH_2PO_4, 1 mM $MgSO_4$, 2.5 mM $CaCl_2$, 5 mM Na-HEPES. Apical saline received 10 mM mannitol to equilibrate 10 mM glucose in the basolateral saline. Saline pH was adjusted at 7.80 using 4-(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid (HEPES), and osmolality set to 320 mOsm kg^{-1} with mannitol (Vapor Pressure Osmometer Model 5600 Wescor, Logan, UT, USA). For SW experiments, saline received an additional 20 mM NaCl, pH was adjusted to 7.80, and osmolality to 350 mOsm kg^{-1} with mannitol.

In our experimental setup the transepithelial potential (TEP, mV) was referenced (grounded) to the apical side (mucosa). Short-circuit current (I_{sc}, μA cm^{-2}) was monitored by clamping the epithelia to 0 mV. For clarity, I_{sc} was expressed as negative for anion absorption, and positive for the secretion of anions. Transepithelial resistance (R_t, Ω cm^2) was calculated by Ohm's law using the current deflections induced by 2 mV bipolar pulses of 3 s every minute. When the tissue achieved steady state (usually 30–40 min after mounting), basal values were recorded.

The response to pharmacological treatments was tested in the last period in FW (FW-May) and after seven days in SW (SW 7d-May). Bumetanide (BUM, 200 μM, apical) or hydrochlorothiazide (HCTZ, 1000 μM, apical) were used to inhibit respectively $Na^+/K^+/2Cl^-$ (NKCC) and Na^+/Cl^- (NCC) co-transporters. A cocktail of forskolin and 3-isobutyl-1-methylxanthine (FK, 10 μM + IBMX, 500 μM) applied bilaterally

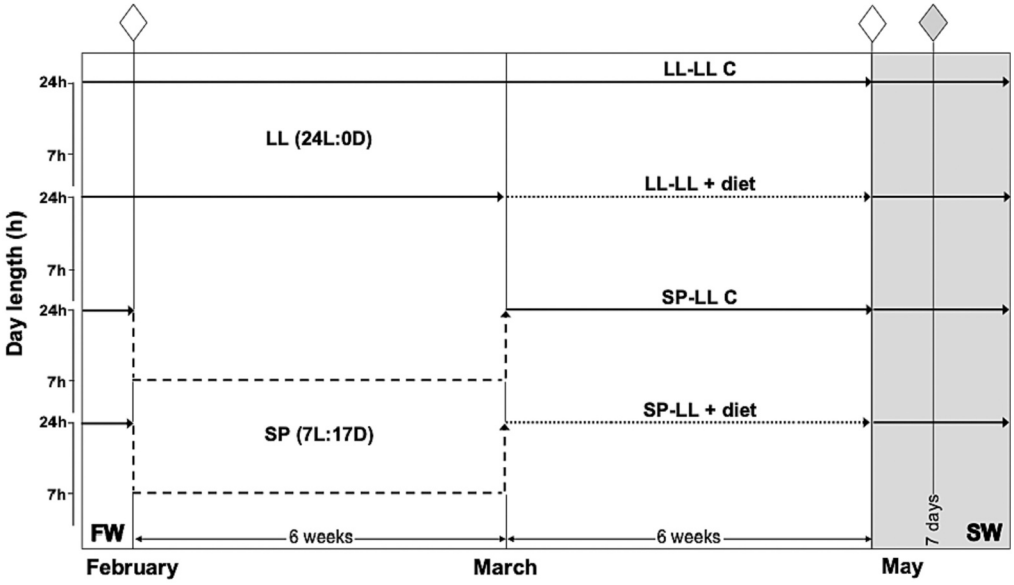

Fig. 1. Graphic representation of the experimental design showing the four experimental conditions to which small (~40 g) and large (~130 g) Atlantic salmon (*Salmo salar*) were subjected from February to May. The white background represents the freshwater phase (FW) while the grey the seawater phase (SW). Solid arrows indicate the use of continuous light (24 L:0D, LL), dash arrows the use of short photoperiod (7 L:17D, SP), and round dot arrows the use of salt and tryptophan diet. White diamonds represent the FW sampling-points while grey diamond the SW sampling-point.

was used to raise intracellular cAMP. After monitoring effects for 30 min, individual responses were recorded and shown as short-circuit current differences between the treatment values and the basal values of individual preparations (ΔI_{sc}, μA cm^{-2}).

Voltage clamping and current injections used VCC MC8 Multichannel voltage/current clamp or VCC600 amplifiers (with automatic fluid resistance compensation) (Physiological Instruments, San Diego, CA, USA), connected with Ag/AgCl electrodes (with tip asymmetry <1 mV) to either side of the Ussing chamber with 3-mm-bore agar bridges (1 M KCl in 3% agar). Bioelectrical parameters for each tissue were recorded onto a computer through Lab-Trax-4/16 acquisition systems (World Precision Instruments, Sarasota, FL, USA) using LabScribe3 (iWorx Systems Inc., CB Sciences, NH, USA).

2.6. Statistical analysis

All results are shown as mean ± SEM. Homogeneity of variances and normality was assessed before statistical analysis. When necessary, data were transformed to comply with ANOVA assumptions. In each sampling point and for each fish size and intestinal region, differences between experimental groups were assessed by two-way ANOVA, considering light (LL-LL and SP-LL) and diet (control and dietary treatment) as main factors. The ANOVA analysis were followed by the Bonferroni *post-hoc* test when significant interaction between the two factors was observed. In the specific case of pharmacological experiments with bumetanide, when an experimental group was missing one-way ANOVA was carried out, followed by the Bonferroni *post-hoc* test. All statistical analyses were performed using Prism 6.0 (GraphPad Software). Statistical significance was accepted at $p < 0.05$.

3. Results

3.1. Animals and mortality

At the beginning of the experiment small fish body mass averaged 42.2 ± 2.0 g while large fish 131.8 ± 5.9 g. From February to May small and large fish increased their body mass reaching respectively the weight of 108.4 ± 3.1 g and 296.6 ± 9.9 g at the time of SW transfer in May.

During the experimental period, low mortality was observed in the SW stage. For small fish, a total of 8 animals died out of 130. Two fish died in the LL-LL C group and 3 in the SP-LL C during the SW challenge test in May (Striberny et al., 2021), 1 fish died in the LL-LL + salt and 2 in the SP-LL C during the first seven days in SW.

3.2. Intestinal fluid analysis

3.2.1. Carbonate aggregates

Regardless of their treatment, carbonate aggregates were present in the intestinal fluid of all fish sacrificed after seven days in SW. However, due to the intensive sampling and ex-vivo experimentation, quantification was not possible.

3.2.2. Osmolality

Intestinal fluid osmolality ($Osm_{(IF)}$, mOsm Kg^{-1}) was consistently lower than plasma osmolality in individual fish. $Osm_{(IF)}$ in small fish averaged between 276 and 317 mOsm Kg^{-1} and was significantly affected by diet ($p = 0.0021$, two-way ANOVA, Fig. 2-A) with the lowest $Osm_{(IF)}$ shown by SP-LL + diet group (276 mOsm Kg^{-1}, Fig. 2-A). In small fish, ΔOsm between plasma and intestinal fluid was significantly affected by diet ($p = 0.0157$, two-way ANOVA, Fig. 2-B). The dietary treated groups presented an ΔOsm ~70% higher than control groups (Fig. 2-B). Large fish had $Osm_{(IF)}$ averages between 290 and 313 mOsm Kg^{-1}. There was a significant interaction between light and diet ($p = 0.0071$, two-way ANOVA, Fig. 2-C). The LL-LL + diet group had a significantly higher osmolality than SP-LL + diet (Bonferroni *post-hoc* test, Fig. 2-C). In large fish, no significant differences in ΔOsm between plasma and intestinal fluid existed in response to treatments at seven days in SW (two-way ANOVA, Fig. 2-D).

3.2.3. Chloride ion

Chloride ion concentration in the intestinal fluid ($Cl^-_{(IF)}$, mmol L^{-1}) was consistently lower than plasma concentration in individual fish. In small fish, $Cl^-_{(IF)}$ concentration was between 38 mmol L^{-1} and 55 mmol L^{-1} (Fig. 3-A). Diet significantly affected $Cl^-_{(IF)}$ levels ($p = 0.0020$), and there was a significant interaction between light and diet ($p = 0.0149$, two-way ANOVA, Fig. 3-A). In the group LL-LL + diet, $Cl^-_{(IF)}$ concentration was ~40% lower than the group LL-LL C (Fig. 3-A). In small fish, ΔCl^- between plasma and intestinal fluid did not differ significantly between treatments (two-way ANOVA, Fig. 3-B). However, LL-LL C presented the lowest ΔCl^- (98 mmol L^{-1}). In large fish, $Cl^-_{(IF)}$ ranged between 49 and 58 mmol L^{-1} and was significantly affected by light ($p = 0.0376$, two-way ANOVA, Fig. 3-C). ΔCl^- between plasma and intestinal fluid in large fish were significantly affected by light ($p =$

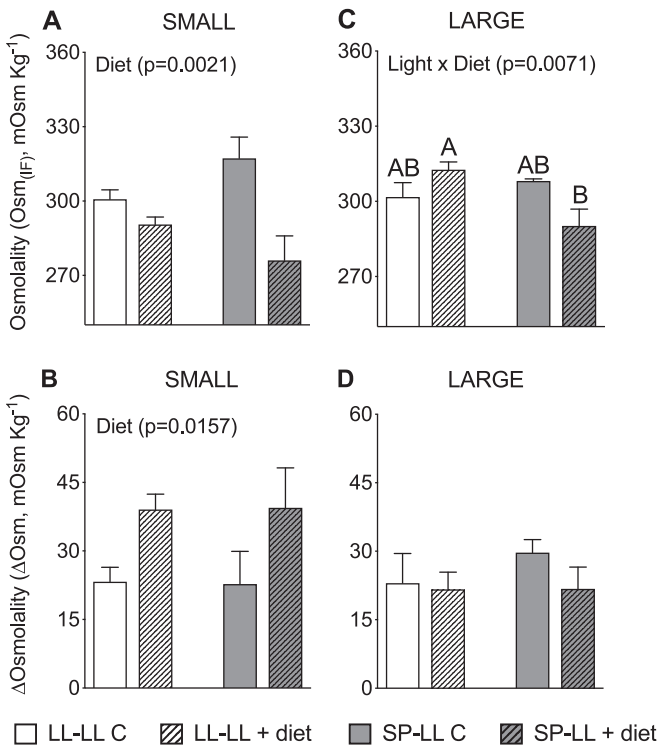

Fig. 2. Intestinal fluid osmolality ($Osm_{(IF)}$, mOsm Kg^{-1}) and difference in osmolality between plasma and intestinal fluid (ΔOsm, mOsm Kg^{-1}) in small (~40 g, A-B) and large (~130 g, C—D) Atlantic salmon (*Salmo salar*), after seven days in SW (SW 7d - May). Each bar represents the mean ± SEM (n = 8–11). In the analysis, light and dietary treatment are the two considered factors; significant p values are reported in the graph ($p < 0.05$, two-way ANOVA). When significant interaction between light and diet was observed, Bonferroni *post-hoc* test was carried out and significant differences among experimental groups indicated with different uppercase letters.

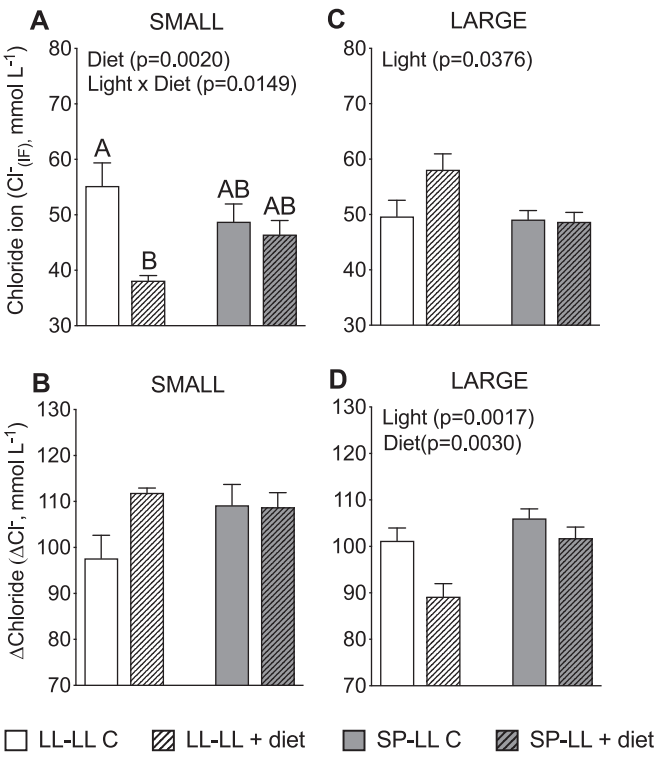

Fig. 3. Chloride ion concentrations in the intestinal fluid ($Cl^-_{(IF)}$, mmol L^{-1}) and difference in chloride ion concentrations between plasma and intestinal fluid (ΔCl^-, mmol L^{-1}) in small (~40 g, A-B) and large (~130 g, C—D) Atlantic salmon (*Salmo salar*), after seven days in SW (SW 7d - May). Each bar represents the mean ± SEM (n = 7–11). In the analysis, light and dietary treatment are the two considered factors; significant p values are reported in the graph ($p < 0.05$, two-way ANOVA). When significant interaction between light and diet was observed, Bonferroni *post-hoc* test was carried out and significant differences among experimental groups indicated with different uppercase letters.

0.0017) and diet ($p = 0.0030$, two-way ANOVA, Fig. 3-D).

3.2.4. Sodium ion

In small fish, sodium ion concentrations in the intestinal fluid ($Na^+_{(IF)}$, mmol L^{-1}) were affected by light ($p = 0.0345$) and diet ($p < 0.0001$) and showed a significant interaction of the two factors ($p = 0.0207$, two-way ANOVA, Fig. 4-A). $Na^+_{(IF)}$ ranged from 41 mmol L^{-1} to 59 mmol L^{-1} with significantly higher levels in LL-LL C group than the other groups (Bonferroni *post-hoc* test, Fig. 4-A). $Na^+_{(IF)}$ in large fish was significantly affected by light ($p = 0.0041$) and presented significant interaction of light and diet ($p = 0.0312$, two-way ANOVA, Fig. 4-B). Averages were between 45 and 69 mmol L^{-1}, and LL-LL groups had $Na^+_{(IF)}$ levels significantly higher (~65–69 mmol L^{-1}) than SP-LL + diet (45 mmol L^{-1}) (Bonferroni *post-hoc* test, Fig. 4-B).

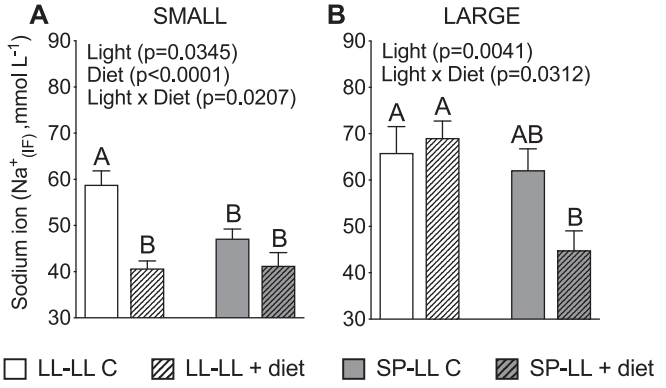

Fig. 4. Sodium ion concentrations in the intestinal fluid ($Na^+_{(IF)}$, mmol L^{-1}) in small (~40 g, A) and large (~130 g, B) Atlantic salmon (*Salmo salar*), after seven days in SW (SW 7d - May). Each bar represents the mean ± SEM (n = 8–11). In the analysis, light and dietary treatment are the two considered factors; significant p values are reported in the graph ($p < 0.05$, two-way ANOVA). When significant interaction between light and diet was observed, Bonferroni *post-hoc* test was carried out and significant differences among experimental groups indicated with different uppercase letters.

3.3. Intestinal Na^+/K^+-ATPase activity

This study has no data on gill ATPase activity; however, a cohort of a parallel experiment was shown to perform well long-term and grow in SW (Striberny et al., 2021). Due to the small size of the intestine at the beginning of the experiment in February, the Na^+/K^+-ATPase (NKA) activity assay could not be successfully performed. Therefore, the NKA activity results are reported only for FW and SW sampling points in May.

In small fish, NKA activities averaged between 3.2 and 4.6 µmol ADP mg $protein^{-1}$ h^{-1} in the anterior intestine. The activity did not change significantly in response to light or diet in either FW or SW (two-way ANOVA, Fig. 5-A). In contrast, the NKA activity in the posterior intestine was affected by the light in FW ($p = 0.0012$, two-way ANOVA, Fig. 5-B), presenting values between 2.5 and 4.8 µmol ADP mg $protein^{-1}$ h^{-1}. After seven days in SW, all groups had similar NKA activities (Fig. 5-B).

In large fish, NKA activity in the anterior intestine averaged between 2.8 and 4.9 µmol ADP mg $protein^{-1}$ h^{-1} (Fig. 5-C). NKA activity was affected by light in FW ($p = 0.0121$, two-way ANOVA, Fig. 5-C). Upon transfer to SW, there were no significant differences among treatments

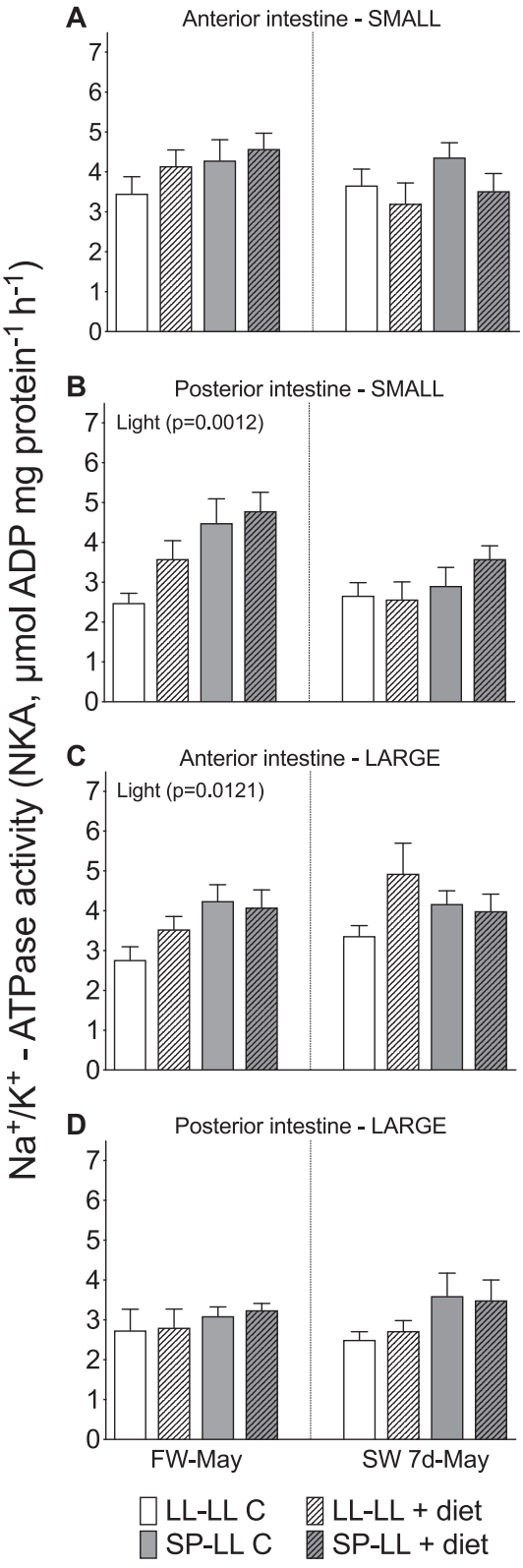

Fig. 5. Na^+ K^+-ATPase activity (NKA, µmol ADP mg protein^{-1} h^{-1}) of the anterior and posterior intestine in small (~40 g, A-B) and large (~130 g, C—D) Atlantic salmon (*Salmo salar*). NKA values were measured before SW transfer (FW - May) and after seven days in SW (SW 7d - May). Each bar represents the mean ± SEM (n = 7–10). In the analysis, light and dietary treatment are the two considered factors; significant p values are reported in the graph ($p < 0.05$, two-way ANOVA).

(two-way ANOVA, Fig. 5-C). NKA activity was stable in the posterior intestine in both FW and SW, averaging between 2.5 and 3.6 µmol ADP mg protein^{-1} h^{-1} (two-way ANOVA, Fig. 5-D).

3.4. Ussing chamber experiments: basal short-circuit current (I_{sc})

In the open circuit mode, intestinal preparations showed small but consistent transepithelial voltages, either positive or negative depending on the region, experimental group or salinity. The experiments were subsequently performed under voltage clamp and in our setup, negative currents indicate net absorption of anions, while positive values indicate secretory currents. Current values (I_{sc}) are shown as µA cm^{-2}.

The anterior intestine of small fish presented absorptive I_{sc} (negative current) in both FW and SW, except for the SP-LL C group that showed a positive/secretory I_{sc} at seven days in SW (Fig. 6-A). I_{sc} in the anterior intestine averaged between +2.15 and − 18.8 µA cm^{-2} (Fig. 6-A). After seven days in SW, there was a significant effect of light ($p = 0.0045$, two-way ANOVA, Fig. 6-A). The posterior intestine of small fish presented negative I_{sc} in FW and SW, with average values between −4.78 and − 11.5 µA cm^{-2} (Fig. 6-B). In FW in May, light significantly affected I_{sc} ($p = 0.0202$, two-way ANOVA, Fig. 6-B) with lower values for fish subjected to SP-LL than groups under LL-LL conditions. After seven days in SW, I_{sc} was not significantly affected by light or diet or by the combination of both (two-way ANOVA, Fig. 6-B).

In large fish, the anterior and the posterior intestine presented, in all the experimental groups, negative/absorptive I_{sc} (Fig. 6-C and 6-D). In the anterior intestine, I_{sc} averaged between −5.16 and − 12.32 µA cm^{-2} (Fig. 6-C), while in the posterior intestine, averages were between −3.78 and − 9.56 µA cm^{-2} (Fig. 6-D). For the two considered regions, I_{sc} was not significantly affected by light or diet or by the interaction of the two factors (two-way ANOVA, Fig. 6-C and 6-D).

3.5. Pharmacology treatments

In pharmacology experiments carried out in Ussing chamber in the last period in FW (May) and after seven days in SW, short-circuit current differences (ΔI_{sc}, µA cm^{-2}) with positive values indicate inhibition of the prevailing absorptive current (I_{sc}), and negative values show stimulation of absorption. Experiments with levels of DMSO equivalent to those used in the pharmacological characterizations were performed, and were without effect on tissue bioelectrical parameters, ruling out effects of the vehicle alone (Fig. 7-A).

3.5.1. Bumetanide effect on basal I_{sc}

In both fish sizes, the effect of apical bumetanide (BUM, 200 µM), a specific inhibitor of $Na^+/K^+/2Cl^-$ co-transporter (NKCC), showed positive ΔI_{sc} for all the considered groups in the anterior and posterior intestine (Fig. 7), therefore inhibition of the absorptive I_{sc} (Fig. 7-B). Due to technical problems during the experiments in the posterior intestine of the SP-LL C in FW in May and in the anterior and posterior intestine of large fish SP-LL + diet in SW, the bumetanide effect was not determined (ND, Fig. 7-E and 7- F).

Bumetanide effect in the anterior intestine of small fish had ΔI_{sc} between +1.80 and + 4.91 µA cm^{-2} (Fig. 7-C), while in the posterior intestine it had ΔI_{sc} between +0.30 and + 0.91 µA cm^{-2} (Fig. 7-D). The response to bumetanide was not significantly affected by light or diet in the two intestinal region (two-way ANOVA, Fig. 7-C and 7-D).

In the anterior intestine of large fish, the ΔI_{sc} response to apical bumetanide, ranged from +0.90 to +2.75 µA cm^{-2} (Fig. 7-E), while it ranged from +0.20 to +1.26 µA cm^{-2} in posterior intestine (Fig. 7-F).

3.5.2. Hydrochlorothiazide effect on basal I_{sc}

The response to the apical application of hydrochlorothiazide (HCTZ, 1000 µM), a specific inhibitor of Na^+/Cl^- co-transporter (NCC), showed positive ΔI_{sc} for all the experimental groups (Fig. 8), with inhibition of the absorptive I_{sc} (Fig. 8-A).

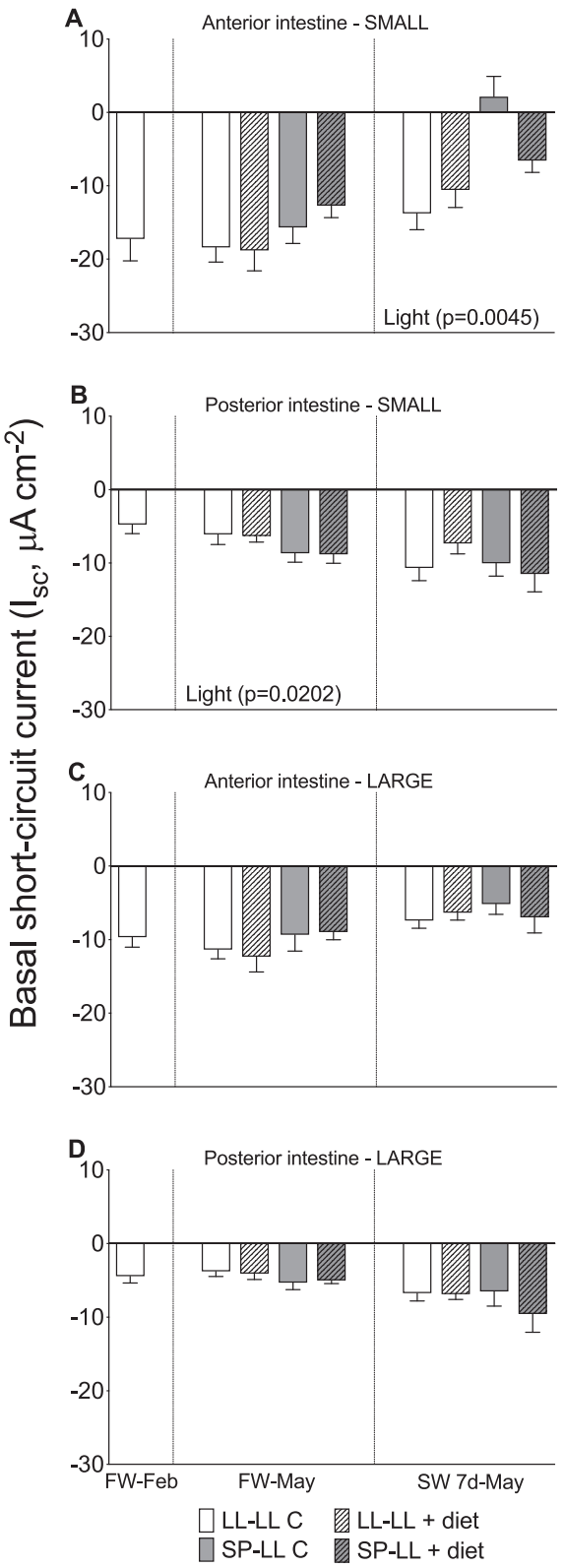

Fig. 6. Basal short circuit current (I_{sc}, μA cm^{-2}) of the anterior and posterior intestine in small (~40 g, A-B) and large (~130 g, C—D) Atlantic salmon (*Salmo salar*). I_{sc} values were measured in Ussing chamber using the voltage-clump technique, under symmetric osmotic conditions, at the beginning of the experiment (FW - Feb), before SW transfer (FW - May) and after seven days in SW (SW 7d - May). Each bar represents the mean ± SEM (n = 7–10). In the analysis, light and dietary treatment are the two considered factors; significant p values are reported in the graph ($p < 0.05$, two-way ANOVA).

The response to apical HCTZ was not significantly affected by light or diet in either intestinal region of small fish (two-way ANOVA, Fig. 8-B and 8-C). In the anterior intestine, average ΔI_{sc} ranged from +1.68 to +8.8 μA cm^{-2}, while in the posterior intestine, ΔI_{sc} ranged from +0.56 to +2.8 μA cm^{-2}.

In the anterior intestine of large fish, the response to apical HCTZ (Fig. 8-D) was not significantly affected by light or diet (two-way ANOVA) and ΔI_{sc} ranged from +0.27 to +3.20 μA cm^{-2}. The effect of apical HCTZ in the posterior intestine of large fish had ΔI_{sc} between 0 and + 1.80 μA cm^{-2} (Fig. 8-E). In FW, the posterior intestine ΔI_{sc} was significantly affected by light ($p = 0.0055$) and presented a significant interaction between light and diet ($p = 0.0472$, two-way ANOVA, Fig. 8-E). Fish from the SP-LL + diet group had significantly higher ΔI_{sc} than those from the LL-LL + diet treatment (Bonferroni *post-hoc* test, Fig. 8-E).

3.5.3. *Forskolin and 3-isobutyl-1-methylxanthine effect on basal I_{sc}*

The response to bilateral forskolin (FK, 10 μM) + 3-isobutyl-1-methylxanthine (IBMX, 500 μM), used to raise intracellular cAMP, resulted in higher (Fig. 9-A), less (Fig. 9-B) or unchanged absorptive I_{sc} in both intestinal regions of small and large fish.

In the anterior intestine of small fish, ΔI_{sc} ranged between −4.50 and + 2.00 μA cm^{-2} (Fig. 9-C). In both FW and SW, LL-LL groups and SP-LL groups presented opposite responses, with positive ΔI_{sc} for LL-LLs and negative ΔI_{sc} in the case of SP-LLs. After seven days in SW, light significantly affected the response to FK + IBMX ($p < 0.0001$, two-way ANOVA, Fig. 9-C). The posterior intestine of small fish presented values between 0 and + 0.75 μA cm^{-2} and light significantly affected ΔI_{sc} in FW ($p = 0.0016$, two-way ANOVA, Fig. 9-D).

ΔI_{sc} in the anterior intestine of large fish, in response to bilateral FK + IBMX, averaged between −0.12 and + 1.54 μA cm^{-2} with a decrease of absorptive I_{sc} for all the experimental groups except SP-LL + diet after seven days in SW (Fig. 9-E). The response to bilateral FK + IBMX in the anterior intestine was not affected by light or diet, neither in FW nor in SW (two-way ANOVA, Fig. 9-E). The posterior intestine of large fish showed ΔI_{sc} between −1.6 and 0.36 μA cm^{-2} (Fig. 9-F). In FW, all the experimental groups responded to bilateral FK + IBMX with a higher absorptive I_{sc}. ΔI_{sc} was significantly affected by light ($p = 0.0007$) and presented a significant interaction between light and diet ($p = 0.0469$, two-way ANOVA, Fig. 9-F). After seven days in SW, dietary treatment significantly affected ΔI_{sc} ($p = 0.0001$, two-way ANOVA, Fig. 9-F); while control groups showed an increase in the absorptive I_{sc} after bilateral FK + IBMX, fish treated with the enriched diet, showed reduction of it.

3.6. *Tissue resistance (R_t)*

In small fish, the anterior intestine had a tissue resistance (R_t, Ω cm^2) between 45 and 85 Ω cm^2 (Fig. 10-A) and it was significantly affected by light in both FW and SW in May ($p = 0.0394$ and $p = 0.0006$ respectively, two-way ANOVA, Fig. 10-A). In FW, the posterior intestine R_t averaged between 147 and 209 Ω cm^2 and, in May, R_t was significantly affected by the light ($p = 0.0204$, two-way ANOVA, Fig. 10-B). After seven days in SW, R_t averaged between 75 and 188 Ω cm^2 and it was significantly affected by both light and diet ($p = 0.0003$ and $p = 0.0278$ respectively, two-way ANOVA, Fig. 10-B).

In large fish, the R_t of the anterior intestine was between 89 and 138 Ω cm^2, without significant effects of treatments not in FW nor in SW (two-way ANOVA, Fig. 10-C). In the posterior intestine of FW animals, R_t averages were between 152 and 293 Ω cm^2. In FW (May), light significant affected R_t of the posterior intestine ($p = 0.0040$) and it was observed, also, a significant interaction between the two experimental factors ($p = 0.0218$, two-way ANOVA, Fig. 10-D). SP-LL C group had the lowest R_t, significantly different from the LL-LL groups (Bonferroni *post-hoc* test, Fig. 10-D). After seven days in SW, R_t averages in the posterior intestine were between 128 and 227 Ω cm^2 (Fig. 10-D). A significant effect of light was observed ($p = 0.0012$, two-way ANOVA, Fig. 10-D) with LL-LL groups showing higher R_t than SP-LL groups.

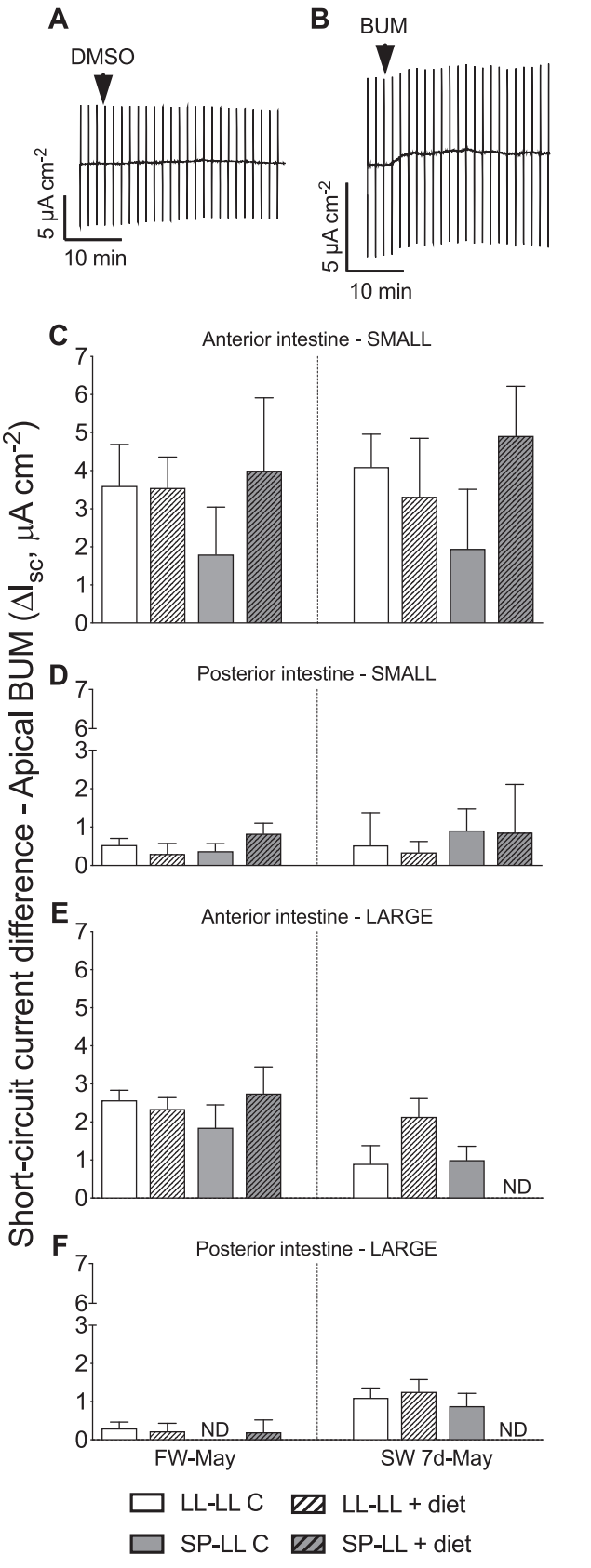

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Fig. 7. Response to DMSO (0.2%, original trace, A) and difference in short-circuit current (ΔI_{sc}, µA cm^{-2}) in response to apical bumetanide (200 µM, original trace, B) in the anterior and posterior intestine of small (~40 g, C—D) and large (~130 g, *E*-F) Atlantic salmon (*Salmo salar*). Pharmacological treatments were carried out in Ussing chamber, using the voltage-clump technique, under symmetric osmotic conditions, before SW transfer (FW - May) and after seven days in SW (SW 7d - May). Each bar represents the mean ± SEM (n = 6–8). In the analysis, light and dietary treatment are the two considered factors; significant differences were not detected among treatments ($p < 0.05$, two-way ANOVA).

4. Discussion

The present study functionally characterized the effects of light vs. dietary stimulated PST on intestinal osmoregulatory responses before and after SW transfer in Atlantic salmon of two different size classes, representative of 0+ and 1+ smolts in the farming industry. All experimental conditions allowed small and large fish to develop some intestinal hypo-osmoregulatory competence required for SW acclimation, albeit with an improved ability found in fish subjected to short photoperiods and/or feeding with salt and tryptophan enriched feed compared to untreated fish.

Previous studies in Atlantic salmon demonstrate the capacity of smolts to completely re-equilibrate their osmoregulation after 4–8 days in SW (McCormick et al., 1989; Prunet and Boeuf, 1985). Therefore, we focused on the intestinal physiological changes in FW and after the first seven days in SW.

Atlantic salmon smolts need to increase drinking to compensate for the dehydrating effect of SW (Fuentes and Eddy, 1997). However, an efficient processing of the ingested water is required to achieve an optimal osmoregulatory balance. Intestinal fluid processing in marine and euryhaline teleosts causes a decrease in osmolality from ~1100 mOsm (of the ingested SW) to values close to plasma osmolality (Alves et al., 2019; Ruiz-Jarabo et al., 2017a). In such conditions, NaCl absorption across the enterocytes generates an osmotic gradient between plasma and intestinal fluid to favor water absorption (Veillette et al., 1993). Here, the osmotic gradient (ΔOsm, Fig. 2-B and 2-D) was positive in all experimental groups with lower osmolality in the intestinal fluid than the plasma. This observation probably reflects differences in drinking and intestinal processing of ingested SW, or likely a combination of both. However, the rationale applied to this combined processing should be as follows: the higher the difference between plasma and intestinal fluid osmolality, the better the processing of ingested SW. Therefore, ΔOsm could be considered a suitable index of the fish capacity to process the ingested SW and their consequent ability to regulate water absorption. In this study, dietary treated small fish had a ~ 70% higher ΔOsm (Fig. 2-B) than the control groups indicating positive effects of diet on intestinal osmoregulatory mechanisms. Interestingly, ΔOsm in large fish was unaffected by dietary or light treatment (Fig. 2-D), thus arguing that the benefits of dietary and photoperiod manipulation are better suited for smaller smolts to enhance performance upon SW challenge.

In the intestine, water movement follows Cl^- and Na^+ absorption (Marshall and Grosell, 2005); therefore, the concentration of Cl^- and Na^+ in the intestinal fluid are also important indicators of how fish cope with osmotic stress in SW. Similarly to the results on osmolality, lower Cl^- and Na^+ concentration in the intestinal fluid in the groups treated with the combination of short photoperiod and dietary salt indicate beneficial effects of light and dietary stimulated PST, particularly in small fish (Fig. 3-A and Fig. 4-A).

The presence of carbonate precipitates in the intestine of all fish in SW, and the results of the intestinal fluid analysis confirm the acquired capacity of SW processing, regardless of fish size and experimental treatment. However, the results suggest that for small fish, but not for large fish, the combination of light and dietary treatments allows better SW processing in the intestine and better performance at the

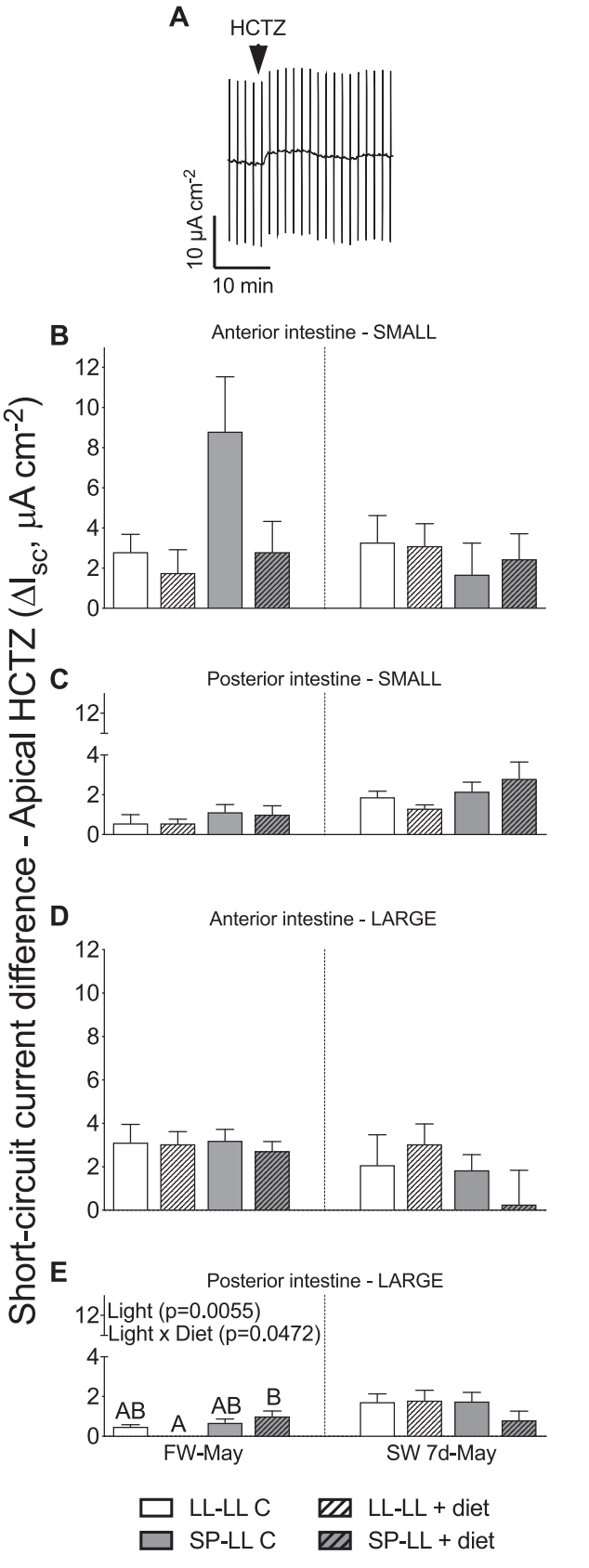

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Fig. 8. Difference in short-circuit current (ΔI_{sc}, µA cm^{-2}) in response to apical hydrochlorothiazide (HCTZ, 1000 µM, original trace, A) in the anterior and posterior intestine of small (~40 g, B—C) and large (~130 g, D-E) Atlantic salmon (*Salmo salar*). Pharmacological treatment was carried out in Ussing chamber, using the voltage-clump technique, under symmetric osmotic conditions, before SW transfer (FW - May) and after seven days in SW (SW 7d - May). Each bar represents the mean ± SEM (n = 3–8). In the analysis, light and dietary treatment are the two considered factors; significant p values are reported in the graph ($p < 0.05$, two-way ANOVA). When significant interaction between light and diet was observed, Bonferroni *post-hoc* test was carried out and significant differences among experimental groups indicated with different uppercase letters.

osmoregulatory level.

The intestine of fish has a dual function; it is involved in nutrient absorption and osmoregulatory mechanisms, being particularly challenging in SW teleost (Collie and Ferraris, 1995). Nutrient uptake occurs predominantly in the anterior intestine, while osmoregulatory processes are present along the whole intestine (Loretz, 1995). In our experimental conditions, comparisons between FW and SW animals must be interpreted with caution, considering the different feeding statuses of the fish in the two environmental salinities. One of the objectives of the present study was to recreate the conditions usually adopted in the aquaculture systems where fish receive daily meals. Still, feeding is withheld in the initial period in SW, due to the lack of appetite in the fish after the transfer (Usher et al., 1991b). In FW fish, undigested food was still present in the intestine on sampling days, while it was absent in SW fish. Therefore, the results may be due to a cumulative effect of feeding mechanisms and pre-adaptative changes in FW animals. At the same time, they are exclusively due to osmoregulation processes in SW. Despite this subtle dissimilarity in conditions, the present study results allow us to describe changes in the intestinal response in FW and SW that occur differently in relation to the group considered.

Ion movement across the epithelium and the intestinal fluid absorption relies on the activity of the basolateral Na^+/K^+-ATPase (NKA). After SW adaptation, salmonids species generally show a higher intestinal NKA activity in SW than in FW. However, in the present study, NKA activity in the intestine of small and large fish did not significantly differ between the last period in FW and the initial SW phase (Fig. 5). The dissimilarity in results could be due to the different residence times in SW, lower in this study than the previous, and experimental conditions (Colin et al., 1985; Fuentes et al., 1997). A further deviation is represented by the similar NKA values observed between anterior and posterior intestine, for most of the groups investigated (Fig. 5), unlike previous studies in trout (Colin et al., 1985; Nielsen et al., 1999). This may be associated with the increase in the posterior intestine functionality after smoltification, with levels of activity similar to those measured in the anterior intestine. The activity of NKA was affected by the light treatment. In the last period in FW, in fact, the posterior intestine of small fish and the anterior intestine of large fish (Fig. 5-B and 5-C) showed higher NKA activity in groups that went through short photoperiod, indicating a potentially higher readiness of these groups for SW entry.

Active absorptive processes in the intestine of Atlantic salmon were marginally affected by the experimental conditions used in the present study (Fig. 6), however, the transepithelial resistance (R_t) showed photoperiod dependence with lower values for the short-photoperiod groups (SP-LLs) (Fig. 10). Short-day experience could represent a signal for the increased need of intestinal absorption by the reduction of selectivity in the intestine.

The functional differences between the anterior and posterior intestine were reflected in the basal bioelectrical properties of the two regions. Under symmetric conditions in the Ussing chamber, the small and large fish showed prevailing absorbing processes, both in FW and SW, but with different magnitude for the anterior and posterior intestine (Fig. 6). Also, the selectivity of the two regions showed differences as

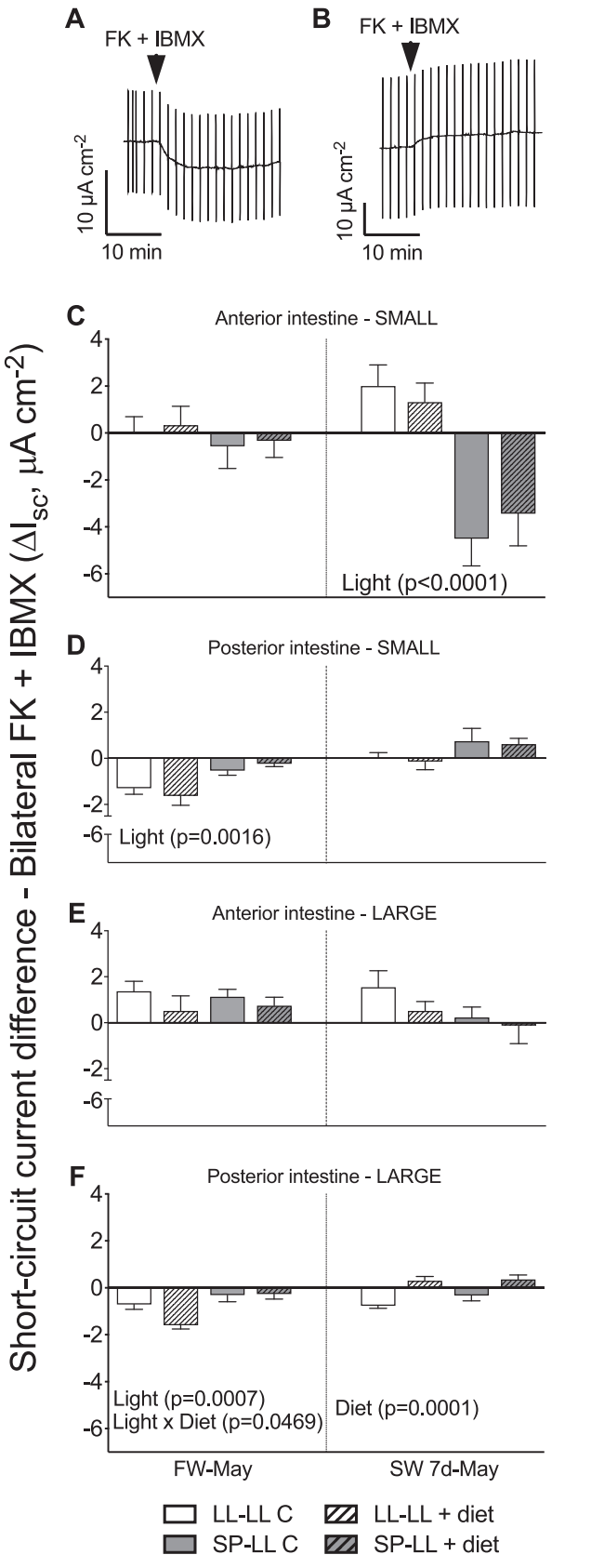

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Fig. 9. Difference in short-circuit current (ΔI_{sc}, µA cm^{-2}) in response to bilateral forskolin (FK, 10 µM) + 3-isobutyl-1-methylxanthine (IBMX, 500 µM) (original traces, A-B) in the anterior and posterior intestine of small (~40 g, C—D) and large (~130 g, E-F) Atlantic salmon (*Salmo salar*). Pharmacological treatment was carried out in Ussing chamber using the voltage-clump technique, under symmetric osmotic conditions, before SW transfer (FW-May) and after seven days in SW (SW 7d - May). Each bar represents the mean ± SEM (n = 5–10). In the analysis, light and dietary treatment are the two considered factors; significant p values are reported in the graph ($p < 0.05$, two-way ANOVA). When significant interaction between light and diet was observed, Bonferroni *post-hoc* test was carried.

previously described (Loretz, 1995). The anterior intestine (Fig. 10-A and Fig. 10-C) did not show differences in the resistance between FW and SW as observed in eel (Ando et al., 1975), while the resistance in the posterior intestine (Fig. 10-B and Fig. 10-D) tended to be lower after seven days in SW than before SW exposure. The reduction of tissue resistance after transfer to SW was previously observed in Coho salmon (Collie, 1985). However, later studies in Atlantic salmon and rainbow trout showed an increase in the transepithelial resistance in SW fish (Sundell et al., 2003; Sundell and Sundh, 2012). Differences between our data and these studies are likely related to fish size and time in SW.

The putative effects of light and dietary treatments in intestinal ion transport were tested using pharmacological experiments in the Ussing chamber, focusing on Cl^- and Na^+ movements. The coupled flow of both ions in the enterocytes was analyzed using bumetanide as the specific inhibitor of the $Na^+/K^+/2Cl^-$ co-transporter (NKCC) (Fig. 7), of which NKCC2 represents the apical isoform (Gamba, 2005), and HCTZ, a specific inhibitor of the apical Na^+/Cl^- co-transporter (NCC) (Fig. 8) (Bazzini et al., 2005). Our results confirm the presence of functional NKCC2 and NCC in the apical side of the enterocytes of the Atlantic salmon intestine. However, the inhibitory effect of bumetanide and HCTZ on the absorptive current was poorly affected by the light and diet, showing an independent functioning of NKCC2 and NCC pathways from the treatments used. The pharmacological experiment with bumetanide (Fig. 7) also showed a regional distribution of NKCC2 with more functional protein (larger bumetanide-sensitive effects) in the anterior intestine, particularly in FW animals, and in line with observations in other marine teleosts (Ando et al., 2014; Fuentes et al., 2018).

Even if the immediate need for SW teleosts is Cl^- absorption to take up water from the ingested SW, Cl^- efflux and fluid secretion can be observed in teleost as demonstrated in killifish (Marshall et al., 2002), Japanese eel (Ando et al., 2014) and toadfish (Ruhr et al., 2014). The capacity to move Cl^- in both directions could be related to a different localization of the cystic fibrosis transmembrane conductance regulator channels (CFTR) present in the apical and/or basolateral membrane of the enterocytes or in cytosolic vesicles established in several species (Bodinier et al., 2009; Gregório et al., 2013; Marshall et al., 2002; Marshall and Singer, 2002; Wong et al., 2016). Studies in killifish and seabream highlight the importance of CFTR during SW acclimation (Gregório et al., 2013; Marshall and Singer, 2002) while gene expression studies in eel, showed higher expression of CFTR in FW animals suggesting an important role also in FW, to maintain hydration, decreasing fluid viscosity, and avoiding obstruction during the passage of the intestinal content (Mekuchi et al., 2013; Wong et al., 2016). In Atlantic salmon gills, two different isoforms of CFTR were identified for the first time in an organism, CFTR I and CFTR II (Chen et al., 2001), and both isoforms are also expressed in the intestine (Sundh et al., 2014). In gills, Singer et al. (2002) suggested a temporal response of the two isoforms during SW acclimation of Atlantic salmon, with CFTR II involved in the early response to SW and CFTR I in more extended periods. In the present study, the pharmacological manipulation with FK + IBMX was carried out to increase intracellular cAMP, thus stimulating CFTR activity by the cAMP-PKA signaling pathway (Moon et al., 2015). Suprigsingly, the responses to cAMP stimulation were heterogeneus, i.e. in some groups I_{sc} increased to show secretory currents, and in other

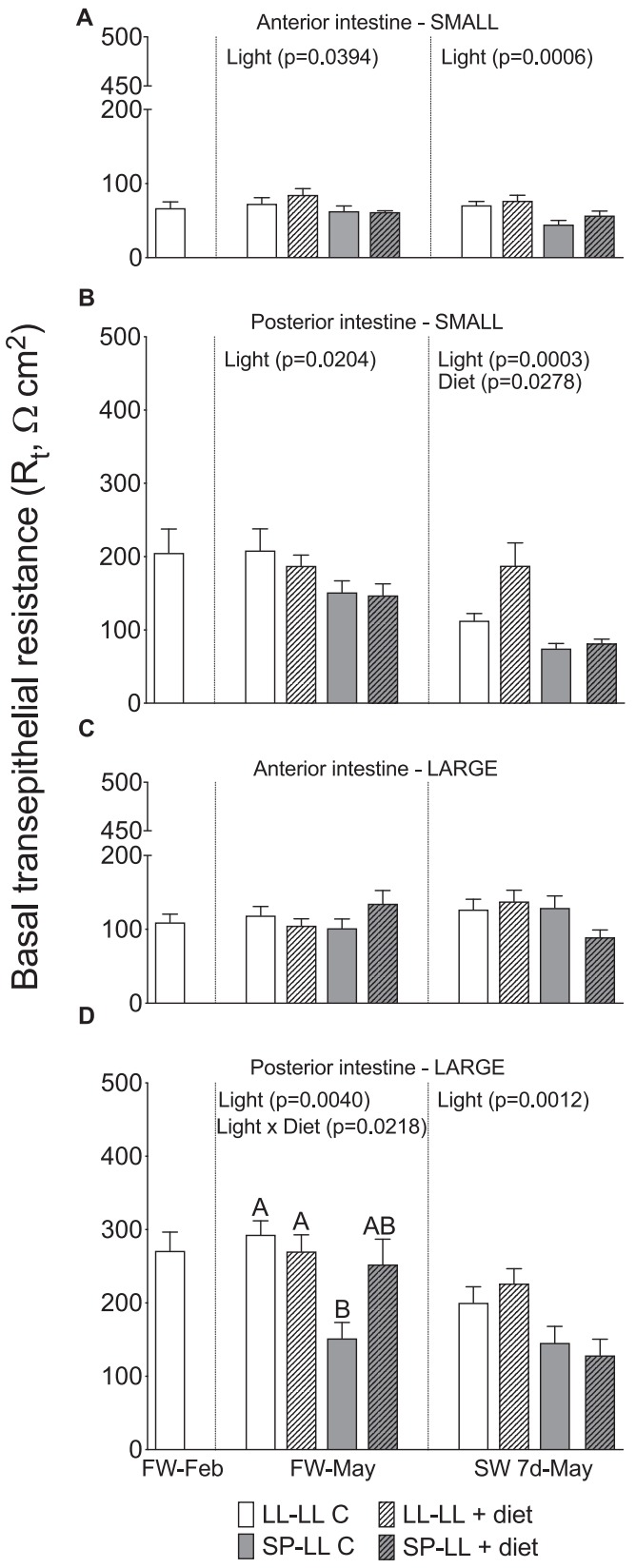

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Fig. 10. Basal transepithelial resistance (R_t, Ω cm^2) of the anterior and posterior intestine in small (~40 g, A-B) and large (~130 g, C—D) Atlantic salmon (*Salmo salar*). R_t was manually calculated (Ohm's law), during short-circuit current experiments, using current deflections induced by a 2 mV bipolar pulse, at the beginning of the experiment (FW - Feb), before SW transfer (FW-May) and after seven days in SW (SW 7d-May). Each bar represents the mean ± SEM (n = 7–10). In the analysis, light and dietary treatment are the two considered factors; significant p values are reported in the graph ($p < 0.05$, two-way ANOVA). When significant interaction between light and diet was observed, Bonferroni *post-hoc* test was carried out and significant differences among experimental groups indicated with different uppercase letters.

groups current levels were more absoprtive after cAMP stimulation (Fig. 9). The only likely explanation for these observations is the distribution of the CFTR protein to the apical or basolateral membrane of the enterocytes, to drive the chloride current to secretion or absorption respectively. The basolateral located channels could facilitate the mobilization of Cl^- into the bloodstream for subsequent elimination at the gill level (Evans et al., 2005), while the apical CFTR could be involved in Cl^- recycling to ensure the functioning of the Cl^-/HCO_3^- exchangers for carbonate aggregate formation(Bodinier et al., 2009; Grosell et al., 2005). In our study, stimulation of the cAMP showed differences in response to the treatments received and the environmental salinity, suggesting an essential function of CFTR in the modulation of intestinal osmoregulatory processes in Atlantic salmon. Immunohistochemistry studies could clarify CFTR distribution in the enterocytes and help to better understand the functional role of this channel during SW acclimation in Atlantic salmon, but despite several attempts, also by us, a specific antibody for CFTR in Atlantic salmon has not yet been optimized.

In conclusion, the present study showed a functional specialization of the anterior and posterior intestine in FW and upon transfer to SW in Atlantic salmon. While the anterior intestine is strongly involved in absorptive processes already in FW, the posterior intestine undergoes physiological maturation to optimize its function when in SW. The intestinal fluid composition upon transfer to SW may become an advantageous index to predict SW performance in juvenile Atlantic salmon. Low osmolality, combined with low levels of chloride and sodium ions (much lower than plasma levels), indicate, in fact, proper processing of the ingested SW, as expected in well-acclimated SW fish.

Experimental manipulation of parr with photoperiod and diet does not have a significant impact in larger Atlantic salmon parr. However, smaller fish respond to the combination of both experimental factors with a better performance at the intestinal level after SW transfer, possibly contributing to improved growth (Striberny et al., 2021). Thus, our data indicate that, for smaller smolts, the combination of light "as winter signal" and dietary treatment with salt mix plus free tryptophan can enhance adaptation and performance in SW of Atlantic salmon in the aquaculture system.

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Declaration of Competing Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Data availability

No data was used for the research described in the article.

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