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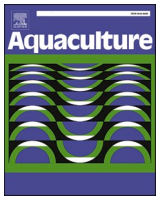

Dietary electrolyte imbalance alters drinking rate and gastrointestinal tract water fluxes of Atlantic salmon (*Salmo salar*) smolt in seawater

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ABSTRACT

It was hypothesized that dietary electrolyte balance (dEB) would influence the dynamics of water, ions, and nutrient fluxes in the gastrointestinal tract (GIT) of Atlantic salmon (*Salmo salar*) smolts differently depending on water salinity. To date, a comparative study on how dEB alters these dynamics in freshwater- and seawater-adapted fish is lacking. The test diets were low versus high dEB (−100 versus 500 mEq kg^{-1} DM^{-1}) and the test water salinities were 0 versus 30 ppt. Furthermore, the effect of the interaction between dEB and salinity on blood pH and osmolality were investigated. The experiment lasted for 6.5 weeks. Chyme was collected from four GIT segments (stomach, proximal, middle, and distal intestine) and analysed for dry matter (DM), pH, osmolality, crude protein, and ion (Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}) content. Water, ion, and nutrient fluxes were measured using yttrium oxide (Y_2O_3) as an inert marker. It was found that there was a diet effect on chyme pH in the stomach, being lower in fish fed the low dEB diet than the high dEB diet. Furthermore, the diet altered ion and nutrient fluxes in the stomach. Water salinity had the largest effect on chyme pH in all the GIT segments and on chyme osmolality in the stomach, which significantly increased in seawater conditions. The interaction between dEB and salinity had an effect on chyme DM, water and ion fluxes in the stomach, proximal and middle intestine. Our results showed that, depending on water salinity, dEB altered water fluxes differently. In freshwater-adapted fish, water influx to the stomach was higher in fish fed the high dEB diet than the low dEB diet, but the difference was neglectable. In contrast, in seawater-adapted fish, water influx into the stomach and proximal intestine was higher in fish fed the low dEB than the high dEB diet, and the amplitude was much larger. Additionally, in seawater conditions, drinking rate was 50% higher in fish fed the low dEB diet (3.07 ml kg^{-1} h^{-1}) than the high dEB diet (2.04 ml kg^{-1} h^{-1}). As a result, it was concluded that, in seawater conditions, a diet with a low dEB has a higher flux of water in the stomach and proximal intestine of Atlantic salmon smolts as well as enhanced drinking rate.

1. Introduction

Dietary minerals can influence the ability of anadromous fish to adapt to seawater conditions (Zaugg, 1982). Salmonids benefit from a mineral-rich diet, resulting in increased tolerance and survival when transitioning to seawater (Basulto, 1976; Pellertier and Besner, 1992; Zaugg et al., 1983). This change is attributed to salt-induced gill remodeling at both the structural and molecular levels, resulting in a partial seawater adaptation characterized by increased expression of ion transporters (Basulto, 1976; Eroldoğan et al., 2005; Harpaz et al., 2005; Pellertier and Besner, 1992; Perry et al., 2006; Zaugg et al., 1983). Furthermore, the role of the intestine in salt and water balance, particularly in saltwater fish, has been recognized (Grosell, 2010; Whittamore, 2012; Wilson et al., 2002). Inadequate preparation of the intestine for the transition from freshwater to seawater, which coincides with changes in osmotic conditions, can have a negative impact on the performance of Atlantic salmon in seawater (Vargas-Lagos et al., 2018). Over the years, aquaculture feed formulation has evolved towards the inclusion of ingredients alternative to fish meal and oil, primarily of plant origin. Next to impacts on macronutrient composition and digestibility the altered formulation can also change the dietary mineral or electrolyte composition and availability (Francis et al., 2001). In this

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regard, our understanding of how dietary electrolytes (e.g., the dietary electrolyte balance, dEB) influences the regulation of osmoregulatory responses in the gastrointestinal tract (GIT) is still limited.

Dietary electrolyte balance (dEB, mEq kg^{-1} DM), often represented by the difference between monovalent cations (K^+ and Na^+) and anion (Cl^-) in the feed, influences the acid-base balance of monogastric animals (Sauvant et al., 2004). Dietary electrolyte balance depends on the mineral profile of the diet, which can be changed by mineral supplementation and by using different types of ingredients. Large variability in cation and anion contents between ingredients results in considerable variability in dEB between commercial feed formulations (Saravanan et al., 2013). Historically, dEB in commercial fish feeds in Europe ranged between 200 and 400 mEq kg^{-1} DM (Tacon and De Silva, 1983). Recently, more data has been made available on the dEB of commercial salmon feeds in Norway and it ranges from −9 to 455 mEq kg^{-1} feed based on life stage or feed category (Philip et al., 2022; Sele et al., 2023).

The literature on the impact of dEB in fish is limited, but it has been shown to affect acid-base balance, amino acid metabolism, feed intake, growth, energy utilization, chyme characteristics, water, ion, and nutrient kinetics in freshwater fish (Ciavoni et al., 2023; Magnoni et al., 2018; Philip et al., 2022; Saravanan et al., 2013). Imbalance in dietary electrolyte levels in fish feeds are expected to disrupt acid-base secretion in the gut and subsequently result in post-prandial systemic metabolic alkalosis or acidosis (Bucking and Wood, 2008). These disturbances in acid-base balance may activate compensatory mechanisms which affected chyme characteristics (dry mater, pH, osmolality), water and ion fluxes, and digestion kinetics in various segments of the GIT (Ciavoni et al., 2023). Most of the above-mentioned studies, however, were done in freshwater environment. Philip et al. (2022) studied the effect of dEB on carbonate precipitation in the intestinal lumen in Atlantic salmon after transfer to seawater, an important osmoregulatory function of the intestine. A low dEB (−25 to −50 mEq kg^{-1} DM) diet increased carbonate precipitation 24 h post seawater transfer (Philip et al., 2022). Although the increased carbonate precipitate formation could be indicative of increased drinking in the low dEB diet, water and ion fluxes in the GIT were not studied by Philip et al. (2022).

Understanding the relationship between dietary composition and water salinity on osmoregulation in the GIT is critical for a species with a life cycle that includes both freshwater and saltwater environments, such as Atlantic salmon. This is because osmoregulation in teleosts, which change depending on environmental conditions, may interact with dietary characteristics and affect adaptation mechanisms. It was hypothesized that effects of dEB on osmoregulation mechanisms in salmon differ between freshwater and seawater. Therefore, feed formulation should be addressed to accommodate for optimal adaptation during freshwater to seawater transfer. To study this, the effect of contrasting dEB (−100 versus 500 mEq kg^{-1} DM) and water salinity (0 versus 30 ppt) on water, ion, and nutrient fluxes in the gastrointestinal tract of Atlantic salmon (*Salmo salar*) smolts was investigated.

2. Material and methods

The feeding trial and sampling were conducted at Matre Research Station of Institute of Marine Research (IMR, Norway). All the sampling procedures were performed on euthanized fish. The study was evaluated by the animal experimentation administration of IMR (Forsksdyrforvaltningen) and approved as a non-invasive animal study conducted in accordance with Norwegian regulations on the use of animals in research in line with the EU directive 2010/63/EU. This trial was exempt from an animal ethics approval (FOTS application) to the Norwegian Food Safety Authority, according to the regulation "FOR-2015-06-18-761 Regulation concerning the use of animals for scientific purposes, § 6. Godkjenning av forsøk". The approval requirement does not apply to experiments involving only the killing of animals to use organs or tissues from them.

2.1. Experimental design, animal housing and feeding

This experiment had a 2 × 2 factorial design. The first factor was the dietary electrolyte balance (dEB) (−100 versus 500 mEq kg^{-1} DM^{-1}). The second factor was water salinity (0 versus 30 ppt). The contrast in dEB was created by adding $CaCO_3$ and Na_2CO_3 to the high dEB diet and $CaCl_2$ to the low dEB diet (Table 1). The experiment involved a cohort of Atlantic salmon (*Salmo salar*) smolts comprising both male and female individuals (n = 640) prepared for transfer to seawater. The fish were reared under standard condition for production of yearling smolts (Fjelldal et al., 2006). In brief, fish were reared under continuous light from the first feeding and then switched to a photoperiod 12:12, light: dark cycle (L:D), for 6 weeks before the experiment began, followed by a photoperiod 24:0 (L:D) for 4 weeks to induce the parr-smolt transformation. The fish were then allocated randomly to 16 tanks of 1 m^3 each, accommodating 40 fish per tank. These 16 tanks were randomly assigned to the four experimental groups (two dEB and salinity levels), with four replicates for each treatment. The tanks received a continuous flow of water at a rate of 8 l min^{-1}, with the salinity adjusted according to the specific treatment. The salinity adjustment was done at the start of the experiment. This meant that the freshwater treatment groups were well acclimated to the conditions prior to the start of the experiment. The seawater groups however were subjected to elevated salinity during the experimental period. The photoperiod was set at 12:12 (L:D), water temperature at 12 °C, and the outlet's oxygen saturation was maintained above 80%. At the beginning of the experiment, the average weight of the fish was recorded (337 ± 5.5 g, mean ± SD). The experimental diets were 3.0 mm extruded sinking pellets and were produced by the Aquafeed technology center (Nofima AS, Bergen, Norway). The ingredient composition and analysed nutrient content of the diets are given in Table 1. Diets were formulated to meet the nutrient requirements for

Table 1
Ingredients and analysed nutrient composition of the experimental diets.

Test ingredients (%)	Low dEB	High dEB
Na_2CO_3	–	1.5
$CaCl_2$	2.4	–
$CaCO_3$	–	1.7
Basal ingredients (%)		
Wheat	21.2	20.4
Soya protein concentrate	20.0	20.0
Pea protein concentrate	20.0	20.0
Fishmeal LT (CP > 68%)	18.0	18.0
Fish oil	15.0	15.0
DL-Methionine	0.4	0.4
Histidine	0.5	0.5
Monocalciumphospate	1.5	1.5
Vitamin mineral premix[1]	1.0	1.0
Yttrium oxide	0.02	0.02
Sum	**100**	**100**
Proximate composition (%)		
Dry matter	94.0	90.0
Protein	45.7	47.8
Fat	19.4	19.6
Calcium	1.8	1.7
Sodium	0.5	1.2
Potassium	0.8	0.8
Magnesium	0.2	0.2
Phosphorus	1.4	1.4
dEB (mEq kg^{-1} DM)	−99	499

Basal ingredients suppliers: fish meal (Pelagia); soy protein concentrate (Selecta); pea protein concentrate (Tulip Stars, Pinnlee Europe B.V.); Wheat (Norgesmøllene); fish oil (Pelagia); vitamin mineral premix (Vilomix); monocalciumphosphate (Vilomix); yttium oxide (VWR).

[1] Vitamin premix (giving the following concentrations per kg diet): vitamin A: 3000 IU; vitamin D3: 3800 IU; vitamin E: 300 mg; vitamin K3: 30 mg; vitamin B1: 30 mg; vitamin B2: 45 mg; vitamin B6: 38 mg; vitamin B12: 0.08 mg; niacin: 300 mg; Ca-D-pantonat: 90 mg; biotin: 1.5 mg; folic acid: 15 mg; vitamin C: 300 mg. Mineral premix (giving the following concentrations per kg diet): Mg, 0.1 g; Fe: 100 mg, Mn: 30 mg, Zn:130 mg, Cu: 6 mg, I: 5 mg, Co: 0.05 mg, Se: 0.3 mg.

Atlantic salmon smolts (NRC, 2011). Yttrium oxide (Y_2O_3) served as an inert marker in the feed to assess digestion kinetics and water/ ion fluxes. The experiment lasted for 6.5 weeks, with 4 weeks on experimental diets to acclimate the fish to different salinities and stabilize their feed intake. The fish were fed twice daily, with each feeding lasting 2 h. Automatic feeders were used to ensure consistent feeding until apparent satiation, and feed intake was carefully monitored by collecting and weighing any spilled feed pellets 15 min after each meal. Feed intake estimation followed the method proposed by Helland et al. (1996).

2.2. Sampling

The final sampling was carried out over four days (days 43–46), with four tanks sampled each day. During this last feeding all the groups were fed 200 g feed tank^{-1}. Fish were sampled at fixed timepoint after the last meal, 6 h postprandial, to standardize the measurements of chyme content. Feed intake during the last meal was measured by recording feed refusal as described above. All fish were euthanized in tricaine methanesulfonate (Finquel, MS-222, 0.5 g l^{-1}), counted and batch weighed to measure the final biomass. Subsequently, four fish in each tank were sampled for blood from the caudal vein using 2 ml heparinized syringes (24G, 0.8 × 40 mm needle). Blood was then collected in 2 ml Eppendorf tubes, and pH was measured immediately after blood collection using a pH-meter (Seven2Go S2-Basic). Following the measurement of blood pH, the Eppendorf tubes were centrifuged (10,000 RPM, for 5 min). In fresh plasma, osmolality (Micro-Osmometer, Fiske, Model 210) and ion (Ca^{2+}, Na^+, K^+ and Cl^-) concentrations were measured (Radiometer, ABL90 FLEX plus). All fish per tank (n = 40) were then dissected for collection of chyme samples from the GIT. Chyme was quantitatively collected from four segments of the GIT: stomach, proximal, middle, and distal intestine. Chyme was analysed for pH, osmolality, dry matter, crude protein, mineral and yttrium content as described in detail by Ciavoni et al. (2023). In brief, the collected samples were pooled per tank, weighted, and stored in 150 ml plastic containers for each GIT segment. From these pooled chyme samples, a subsample of 2 ml was taken in an Eppendorf tube, centrifuged at 10,000 RPM, for 5 min to separate the fluid and solid phase of chyme for the analysis of osmolality and ions in the liquid phase. One more subsample (~ 3 g) of the chyme from each GIT segment was collected and diluted with cold distilled water ratio of 1:1 (w/v) to allow homogenization (Homogenizer, POLYTRON® PT 2100, Kinematica). After homogenization, the mixture was centrifuged at 3220 RPM for 30 min at 4 °C and the supernatant (enzyme extract) were stored at −80 °C for further measurement of digestive enzyme activity (method modified from Yasumaru and Lemos, 2014). The remaining pooled chyme samples were then freeze-dried for 72 h, homogenized by pestle and mortar into a fine powder, and stored at 4 °C until analysis to determine chyme nitrogen, mineral and yttrium content.

2.3. Analyses and calculations

The diets were homogenized and analysed for dry matter, ash, fat, and protein following standard procedures. Briefly, dry matter was measured after drying at 105 °C for 24 h; ash content determined by combustion in a muffle furnace at 550 °C for 16–18 h (NMKL, 1991). Total lipid was determined by ethyl-acetate extraction of tissue and acid-extraction in feeds (NS 9402, 1994). Total nitrogen was measured with a nitrogen analyser (Vario Macro Cube, Elementar Analysensysteme GmbH, Germany), according to AOAC official methods of analysis and crude protein calculated as N x 6.25 (AOAC, 1995). The concentration of minerals and yttrium in diets and chyme were analysed using a microwave assisted digestion and an inductively coupled plasma mass spectrometry (ICP-MS) as described elsewhere (Philip et al., 2022; Silva et al., 2019).

The feed intake per fish (FI, g fish^{-1}) was calculated as FI = (total offered feed – uneaten feed)/ (number of fish) (on DM basis). To determine the weight gain (Wg, g fish^{-1}), the difference between the average individual final (Wf) and initial (Wi) body weight per fish was calculated.

Water and mineral fluxes in the GIT were determined by using yttrium oxide (Y_2O_3) as a marker. As described by Harter et al. (2013) water and ion fluxes of Ca^{2+}, Mg^{2+}, Na^+, and K^+ were calculated in the stomach, proximal, middle, and distal intestine of the gastrointestinal tract. The water flux (ml g^{-1} of ingested DM feed) and ion fluxes (mg g^{-1} of ingested DM feed) in the stomach were calculated by subtracting the relative water or ion content in the stomach chyme from that in the diet and dividing by the relative amount of ingested feed dry matter. In the proximal, middle, and distal intestine, water and ion fluxes were calculated by subtracting the relative water or ion content in the chyme of the intestinal segment from that in the chyme of the previous segment and dividing the result by the relative amount of ingested feed dry matter. The relative amount of ingested feed dry matter (g DM mg^{-1} yttrium) was calculated by dividing the ingested dry matter on the sampling day by the yttrium content of the ingested feed.

For each diet group (low or high dEB), the drinking rate of Atlantic salmon in seawater (30 ppt) was calculated as a relative measure to those reared in freshwater, and hence referred to as relative drinking rate. The net flux of Mg^{2+} in the GIT was used as a proxy for ingested SW.

$$\text{Net GIT } Mg^{2+} \text{ flux } \left(\text{mg g}^{-1} \text{ of ingested DM feed}\right) = \sum Mg^{2+} \text{ flux}_{(\text{stomach+PI+MI+DI})}$$

Once the net GIT Mg^{2+} flux was obtained, the difference (delta, Δ) between the net GIT Mg flux of the SW group (30 ppt) for which the drinking rate is to be measured and the freshwater group (0 ppt) was calculated.

$$\Delta_{(30 \text{ ppt–0 ppt})}\ Mg^{2+} \text{ flux} = \text{Net GIT } Mg^{2+} \text{ flux}_{(30 \text{ ppt})} – \text{Net GIT } Mg^{2+} \text{ flux}_{(0 \text{ ppt})}$$

Then, to obtain the volume of seawater drunk per g of DM ingested, the $\Delta_{(30 \text{ ppt} - 0 \text{ ppt})}\ Mg^{2+}$ flux was divided by the Mg^{2+} concentration in the water (mg/ l) of the respective salinity (30 ppt). This provided the volume of SW drank by the fish (ml g^{-1} DM fed), which was further converted to per unit weight per hour.

$$\text{Relative drinking rate } \left(\text{ml}^{-1}\text{kg}^{-1}\text{h}^{-1}\text{g}^{-1} \text{ DM fed}\right) = \left(\left[\left(\Delta\ Mg^{2+} \text{ flux}\right)/\left(Mg^{2+} \text{ conc.SW}\right)\right]/\text{t}\right)^{*} (1000/\text{BW})$$

Where, t, is the post-prandial time point of sampling (6 h in this study) and BW, is the body weight of the fish.

Spectrophotometric (colorimetric) assays were performed for enzyme activity using enzyme-specific substrates. Pepsin activity (U ml^{-1}) in the stomach chyme was measured using hemoglobin as substrate (Anson and Mirsky, 1932). Pepsin activity was defined as the amount of enzyme that produces an increase in absorbance (at 280 nm) of 0.001 per minute at a temperature of 37 °C and pH of 2–3 (Andreeva and Rumsh, 2001). Alkaline protease activity (U ml^{-1}) of intestinal chyme was measured using casein as substrate, according to Walter (1984). One unit of protease activity was defined as 1 mg tyrosine released in 1 min using the extinction coefficient for tyrosine at 280 nm of 0.005 ml mg^{-1} cm^{-1} (Alarcón et al., 2002). Pepsin and alkaline protease activity in the chyme (U mg^{-1} ww) of each GIT segment were calculated by dividing the enzyme activity (U ml^{-1}) by the chyme wet weight (ww, mg).

2.4. Statistical analyses and graphical presentations

Fish tanks ($n = 16$) were used as experimental units for all analysed parameters and data are expressed as the mean ± SE per treatment of four replicates. All statistical analyses were carried out using the IBM Statistical Package for the Social Sciences (SPSS) program (version 27.0.1; New York, NY, USA). A multivariate analysis was performed to test the effect of dietary electrolyte balance, water salinity and their interaction on all analysed parameters. When an interaction effect was significant ($p < 0.05$), a Tukey HSD (honest significant difference), with multiple comparison and 95% level of significance was used to compare treatment means. Figures were made using GraphPad Prism version 8.

3. Results

Feed intake (g $fish^{-1}$) and survival (%) were lower ($p < 0.001$) in SW fish compared to FW fish (Supplementary table S1). Feed intake was also affected by dietary electrolyte balance (dEB), being lower in fish fed the low dEB diet (avg. over salinities, 60.5 g $fish^{-1}$) compared to high dEB diet (avg. over salinities, 84.3 g $fish^{-1}$). Fish weight gain (g $fish^{-1}$) and SGR (% d^{-1}) were only affected by the diet, being lower ($p < 0.001$) in fish fed the low dEB diet compared to the high dEB diet. FCR was affected by dEB and the interaction between diet and salinity and it was lower ($p < 0.001$) in fish fed the high dEB (avg. over salinities, 1.54) diet compared to the low dEB diet (avg. over salinities, 3.12). Averaged over all treatments, fish final weight was 382 ± 22 g $fish^{-1}$ and fish survival were 98.6%.

3.1. Chyme dry matter and relative water fluxes

There was an interaction effect ($p < 0.001$) of dEB and water salinity on chyme dry matter (DM) and relative water fluxes (RWF) in the stomach, proximal and middle intestine (Fig. 1; Supplementary table S2).

When fed the high dEB diet, chyme DM was lower in in the GIT of freshwater-adapted fish and higher in seawater-adapted fish compared to low dEB diet (interaction effect, $p < 0.001$). In FW conditions, the largest difference in chyme DM was found in the stomach, where it was 30% and 27.4% in the low and high dEB fed fish, respectively. In SW conditions, the largest difference was found in the proximal intestine, where chyme DM was 16.1% and 12.6% in the high and low dEB fed fish, respectively. When fed the high dEB diet, water influx was higher in the stomach of freshwater-adapted fish and lower in seawater-adapted fish (interaction effect, $p < 0.01$). In the proximal and middle intestine, the largest differences in RWF caused by the diet were present in seawater-adapted fish. In the proximal intestine of SW-adapted fish, water influx was almost three times higher ($p < 0.001$) in fish fed the low dEB diet (24.0 ml g^{-1} ingested DM) compared to the high dEB diet (8.9 ml g^{-1} ingested DM). In the middle intestine of SW-adapted fish, water efflux was more doubled ($p < 0.001$) in fish fed the low dEB diet (−26.7 ml g^{-1} ingested DM) compared to the high dEB diet (−11.2 ml g^{-1} ingested DM).

3.2. Chyme pH and osmolality

There was no interaction effect between water salinity and diet on chyme pH and chyme osmolality (Osm) in all the GIT tract segments (Supplementary table S2). Therefore, only the main effect of diet and salinity are addressed in Fig. 2.

Water salinity affected chyme pH in all segments of the GIT, being higher ($p < 0.001$) in seawater-adapted fish (Fig. 2 A). Chyme pH was

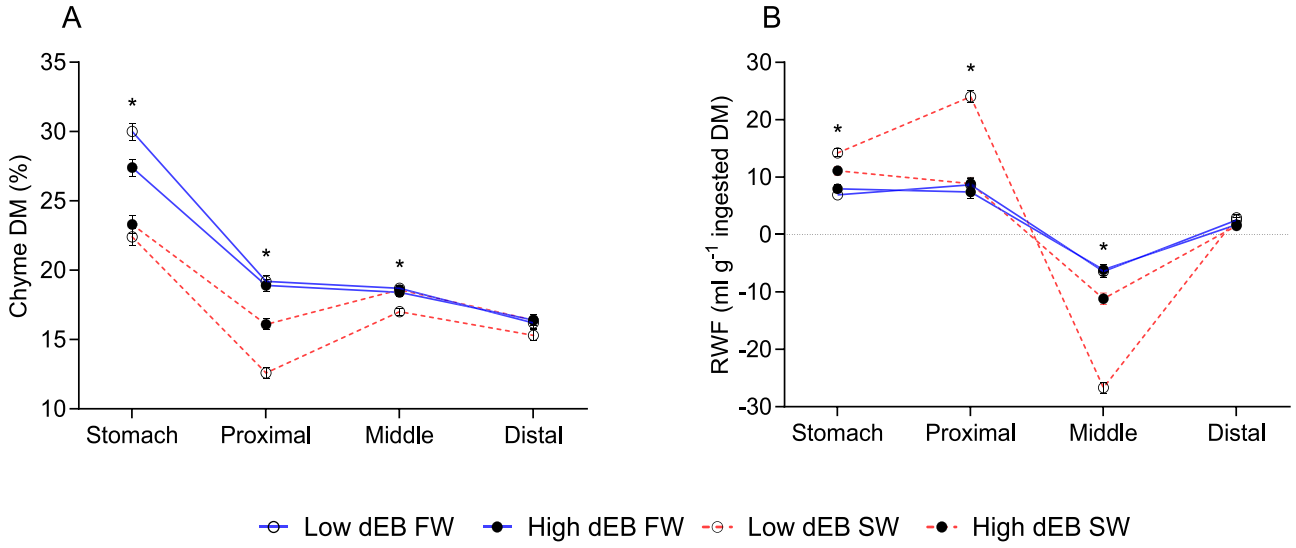

Fig. 1. (A) Chyme dry matter, DM and (B) relative water fluxes, RWF, as affected by increasing water salinity (0 and 35 ppt) and contrasting (low versus high) electrolyte balance (dEB) in the stomach, proximal, middle, and distal intestine of Atlantic salmon. The blue lines indicate freshwater (FW) condition and the red lines seawater (SW) condition. The empty dots (○) and dotted line represent the low dEB diet and the full dots (●) and continuous line represent the high dEB diets. The asterisk (*) indicates the presence of an interaction effect between water salinity and dietary treatment. Values are expressed as the mean per treatment ($n = 4$) and standard error of the means (SEM). The single effect of each treatment is presented in the Supplementary table S2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

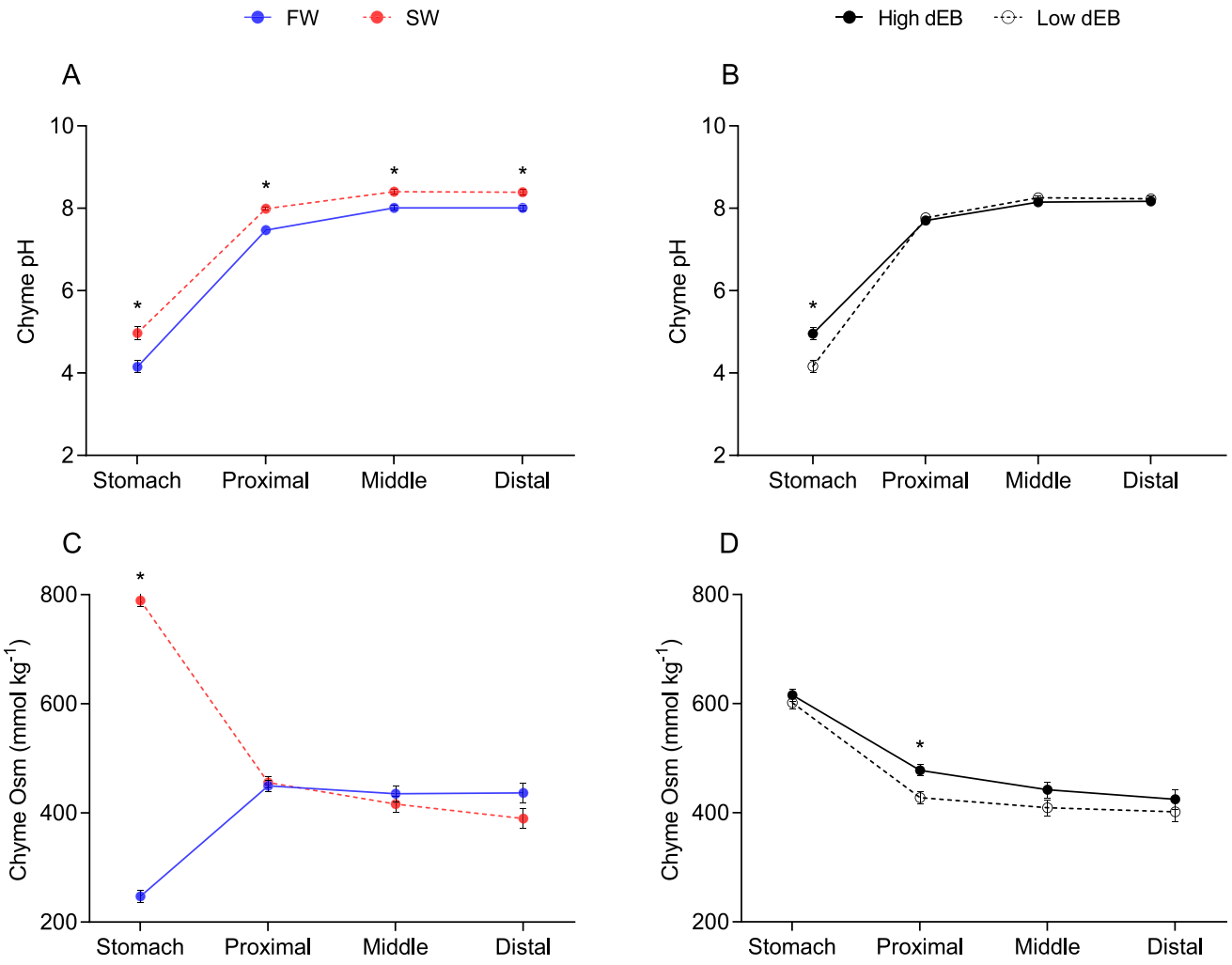

Fig. 2. (A, B) Chyme pH and (C, D) osmolality (Osm), as affected by increasing water salinity (0 and 35 ppt) and contrasting (low versus high) electrolyte balance (dEB) in the stomach, proximal, middle, and distal intestine of Atlantic salmon. In the left panels, the blue dots and continuous line represent freshwater (FW) condition and the red dots and dotted line represent seawater (SW) condition. In the right panels, the empty dots (○) and dotted line represent the low dEB diet and the full dots (●) and continuous line represent the high dEB diets. The asterisk (*) indicates the presence of an interaction effect between water salinity and dietary treatment. Values are expressed as the mean per treatment ($n = 4$) and standard error of the means (SEM). The interaction effects are presented in the Supplementary table S2.

affected by the diet only in the stomach ($p < 0.01$) (Fig. 2B). Averaged over water salinities, chyme pH in the stomach was 4.16 and 4.96 in the low dEB and high dEB group, respectively. In all the intestinal segments, chyme pH was similar between both diets. Water salinity affected ($p < 0.001$) chyme Osm only in the stomach (Fig. 2C). Averaged over diets, chyme Osm was 247 and 789 mmol kg^{-1} in the stomach of FW and SW fish, respectively. The diet had an effect ($p < 0.01$) on chyme Osm only in the proximal intestine. Averaged over salinities, chyme Osm was 428 and 478 mmol kg^{-1} in the low dEB and high dEB group, respectively.

3.3. Ion fluxes

The interaction effect of diet and water salinity had no effect on the majority of Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+} fluxes. As a result, the main effect is shown in Fig. 3, and significant interaction effects are mentioned in the text (Supplementary table S3). The main effect of the diet on relative Ca^{2+} flux (RCaF) was present in the stomach, proximal and distal intestine (Fig. 3B). In the stomach, RCaF was lower ($p < 0.001$) in fish fed the low dEB diet (−7.8 mg g^{-1} ingested DM) compared to the high dEB diet (0.2 mg g^{-1} ingested DM). In the proximal intestine, there was an influx of Ca^{2+} in fish fed both dietary treatments, being higher ($p < 0.001$) in fish fed the low dEB diet (8.5 mg g^{-1} ingested DM) compared to the high dEB diet (2.2 mg g^{-1} ingested DM). In the distal intestine, RCaF dropped being lower ($p < 0.05$) in fish fed the high dEB diet (−0.1 mg g^{-1} ingested DM) compared to the low dEB diet (2.1 mg g^{-1} ingested DM). Water salinity influenced RCaF only in the proximal intestine, where it was higher ($p < 0.05$) in SW (6.2 mg g^{-1} ingested DM) than in FW conditions (4.5 mg g^{-1} ingested DM) (Fig. 3A).

The main effects of the diet and water salinity on relative Mg^{2+} flux (RMgF) was present in the stomach (Fig. 3C, D). In the proximal and middle intestine, there was an effect of the interaction between diet and salinity, and the largest differences were present in seawater-adapted fish (Supplementary table S3). In the proximal intestine of SW-adapted fish, RMgF was almost twice higher ($p < 0.001$) in fish fed the low dEB diet (14.7 mg g^{-1} ingested DM) compared to the high dEB diet (8.8 mg g^{-1} ingested DM). In the middle intestine of SW-adapted fish, Mg^{2+} efflux was larger ($p < 0.001$) in fish fed the low dEB diet (−9.9 mg g^{-1} ingested DM) compared to the high dEB diet (−6.8 mg g^{-1} ingested DM).

The main effect of water salinity on relative Na^{+} flux (RNaF) was present in all GIT segments, whereas the main effect of the diet was present only in the stomach and middle intestine (Fig. 3E, F). Averaged over diets, Na^{+} influx was 16.0 and − 3.5 mg g^{-1} ingested DM in the stomach of seawater- and freshwater- adapted fish, respectively.

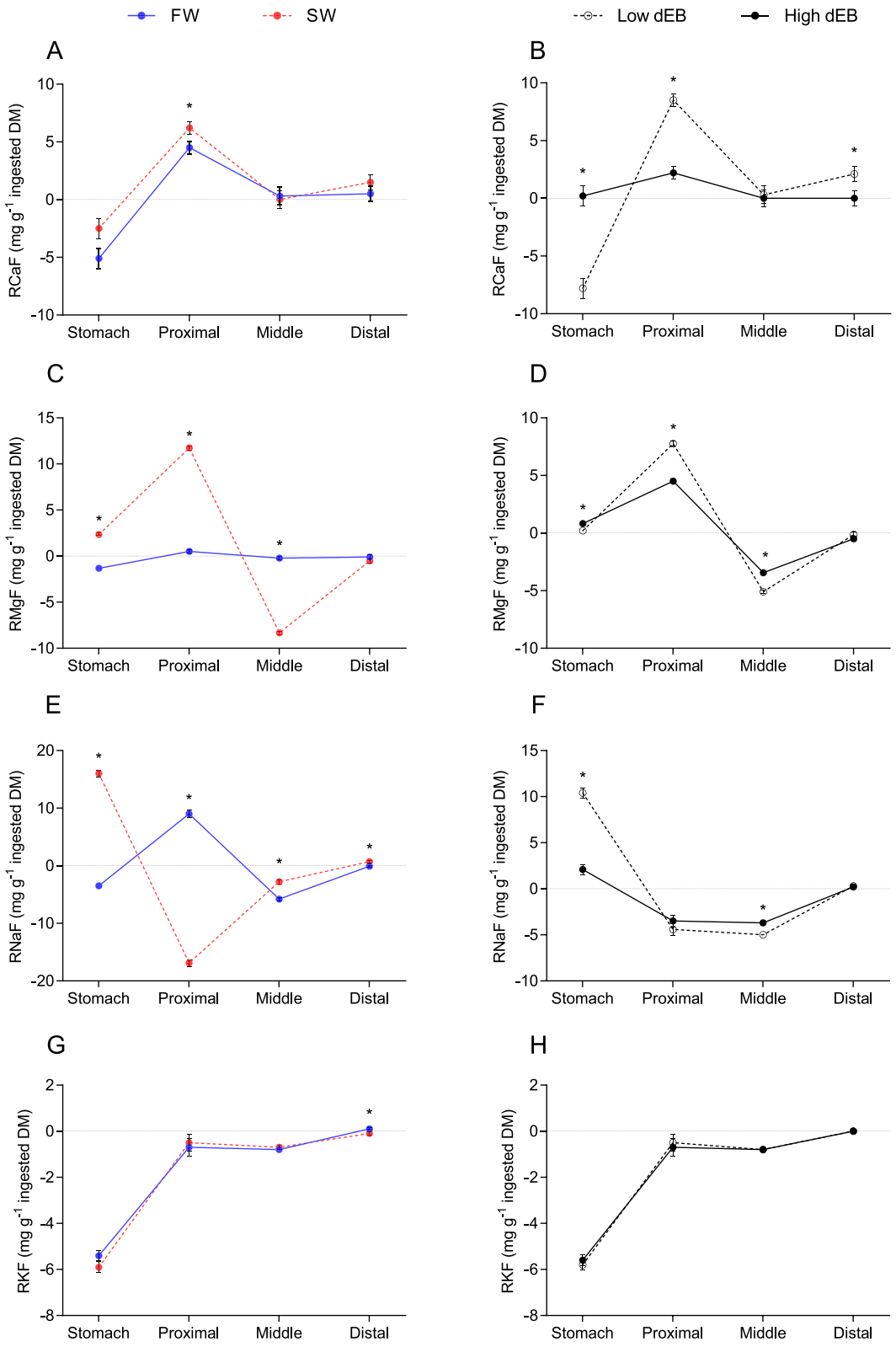

Fig. 3. Relative fluxes of (A, B) calcium, (RCaF); (C, D) magnesium, (RMgF); (E, F) sodium, (RNaF) and (G, H) potassium, (RKF) in the stomach, proximal, middle, and distal intestine of Atlantic salmon as affected by increasing water salinity and contrasting dietary electrolyte balance (dEB). In the left panels, the blue dots and continuous line represent freshwater (FW) condition and the red dots and dotted line represent seawater (SW) condition. In the right panels, the empty dots (○) and dotted line represent the low dEB diet and the full dots (●) and continuous line represent the high dEB diets. The asterisk (*) indicates the presence of an interaction effect between water salinity and dietary treatment. Values are expressed as the mean per treatment ($n = 4$) and standard error of the means (SEM). The interaction effects are presented in the Supplementary table S3. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

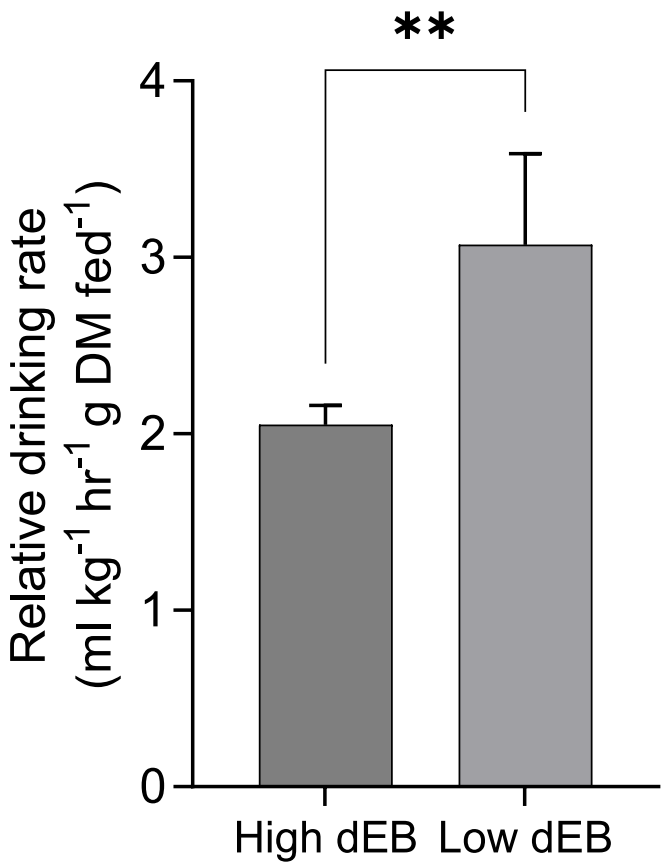

Fig. 4. Relative drinking rate of seawater-adapted Atlantic salmon smolts fed the low dEB diet (−100 mEq kg^{-1} DM) and the high dEB diet (500 mEq kg^{-1} DM). Data are presented as mean and standard deviation (SD), and the asterisks (** = $p < 0.01$) indicate the presence of a significant effect of diet on relative drinking rate.

Averaged over water salinities, the diet effect in the stomach caused a larger ($p < 0.001$) Na^+ influx in fish fed the low dEB diet (10.4 mg g^{-1} ingested DM) compared to the high dEB diet (2.1 mg g^{-1} ingested DM). In the proximal and distal intestine, Na^+ flux was only affected by water salinity ($p < 0.001$) and averaged over both diets, it was 9.0 and 16.9 mg g^{-1} ingested DM, in the proximal intestine and − 0.1 and 0.7 mg g^{-1} ingested DM in the distal intestine of freshwater- and seawater-adapted fish, respectively. In the middle intestine, there was an effect of the interaction between diet and salinity ($p < 0.05$) (Supplementary table S3).

Relative K^+ flux (RKF) was affected ($p < 0.05$) only by water salinity in the distal intestine, whereas no dietary effect was detected (Fig. 3G, H). Averaged over both diets and salinities, the relative K^+ flux was −5.7, −0.6, −0.8, and 0.03 mg g^{-1} ingested DM in the stomach, proximal, middle, and distal intestine, respectively (Supplementary table S3).

3.4. Relative drinking rate

Relative drinking rate (ml kg^{-1} h^{-1} g DM $ingested^{-1}$) of Atlantic salmon smolts adapted to seawater (30 ppt) fed diets contrasting in dietary electrolyte balance (dEB) is depicted in Fig. 4. Relative drinking rate increased by 50% from 2.05 to 3.07 ml kg^{-1} h^{-1} g DM $ingested^{-1}$ when fish were fed the high and the low dEB diet, respectively ($p < 0.001$).

3.5. Blood pH, plasma osmolality and ion content

The effect of diet, water salinity and the interaction between diet and salinity on caudal blood pH, plasma osmolality (Osm) and plasma ion concentration are presented in Table 2. Caudal blood pH was unaffected by diet, water salinity, and their interaction. Plasma osmolality was higher ($p < 0.001$) in seawater-adapted fish (avg. over diets, 333 mmol l^{-1}) than in freshwater-adapted fish (avg. over diets, 324 mmol l^{-1}). Plasma Na^+ and K^+ concentration was affected by the interaction between diet and salinity, whereas no interaction effect was present on plasma Ca^{2+} and Cl^- concentration.

3.6. Crude protein digestion kinetic

Kinetic of crude protein digestion (CP ADC) was not affected by the interaction between diet and water salinity in all the segments of the gastrointestinal tract. Therefore, the main effects of water salinity and diet are presented separately in Fig. 5. There was a main effect of the diet on CP ADC only in the stomach, where it was lower ($p < 0.05$) in fish fed the low dEB diet (avg. over water salinities, 14.5%) compared to the high dEB diet (avg. over water salinities, 23.3%).

3.7. Proteolytic enzyme activity in the chyme

The effect of diet and water salinity on pepsin activity in the stomach and protease activity in the proximal, middle, and distal intestine is presented in Table 3. There was no effect of the interaction between diet and salinity on enzyme activity in all the segments of the GIT. In the stomach and distal intestine, enzyme activity was affected only by water salinity. In the stomach, pepsin activity was lower ($p < 0.01$) in SW-adapted fish (avg. over diets, 57.7 U mg^{-1} chyme ww) than FW-adapted fish (avg. over diets, 93.5 U mg^{-1} chyme ww). In the distal intestine, protease activity was lower ($p < 0.05$) in SW-adapted fish (55.9 U mg^{-1} chyme ww) than FW-adapted fish (74.1 U mg^{-1} chyme ww). In the middle intestine, protease activity was affected only by the diet, being lower in fish fed the high dEB diet (avg. over water salinities, 92.0 U mg^{-1} chyme ww) compared to high dEB diet (avg. over water salinities, 150.3 U mg^{-1} chyme ww).

Table 2
Blood pH in the caudal vein, osmolality, and ion concentration (mmol l^{-1}) in plasma of Atlantic salmon smolt as affected by contrasting water salinity (FW versus SW) and dietary electrolyte balance (low versus high dEB).

	FW		SW			*p-values*		
	Low dEB	High dEB	Low dEB	High dEB	pSEM	Salinity	Diet	Salinity*Diet
Blood pH	7.17	7.15	6.69	7.02	0.19	ns	ns	ns
Plasma osmolality	323[a]	326[a]	333[b]	334[b]	1.24	***	ns	ns
Plasma Ca^{2+}	1.2	1.3	1.2	1.3	0.03	ns	ns	ns
Plasma Na^+	160[a]	162[a]	166[b]	165[b]	0.61	***	ns	*
Plasma K^+	3.9[a]	3.9[a]	3.8[a]	4.8[b]	0.17	*	**	**
Plasma Cl^-	134	133	135	135	0.63	ns	ns	ns

Ca^{2+}, calcium; Na^+, sodium, K^+, potassium, Cl^-, chloride. ns, not significant, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. Values are expressed as the mean per treatment (n = 4) and pooled standard errors of the mean (pSEM).

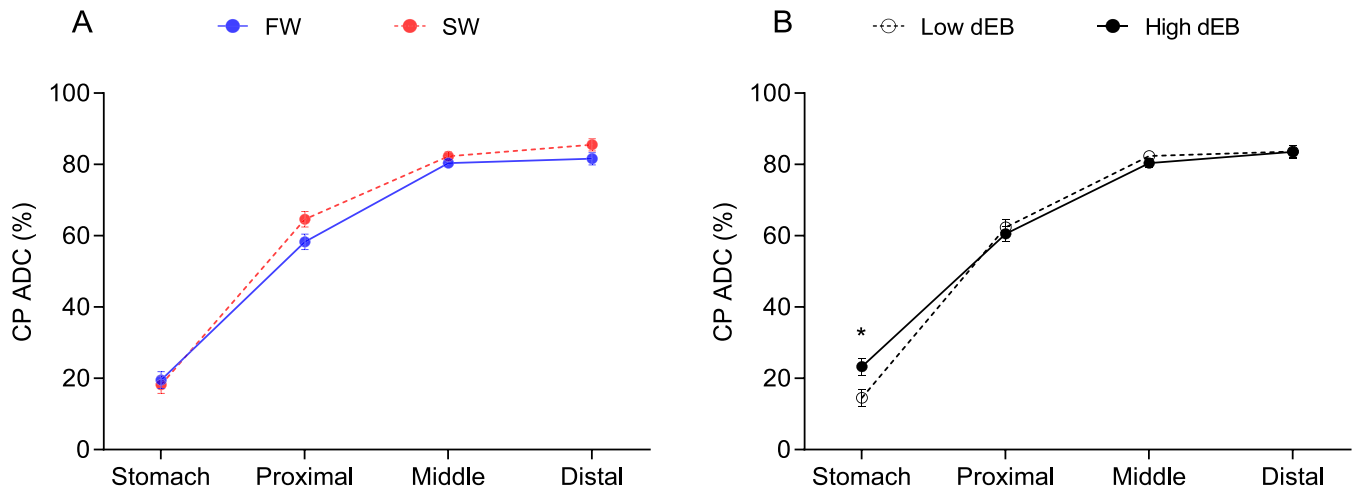

Fig. 5. Progression of digestion of crude protein (CP ADC) in the stomach, proximal, middle, and distal intestine of Atlantic salmon as affected by increasing water salinity and contrasting dietary electrolyte balance (dEB). In the left panels, the blue dots and continuous line represent freshwater (FW) condition and the red dots and dotted line represent seawater (SW) condition. In the right panels, the empty dots (○) and dotted line represent the low dEB diet and the full dots (●) and continuous line represent the high dEB diets. The asterisk (*) indicates the presence of an interaction effect between water salinity and dietary treatment. Values are expressed as the mean per treatment ($n = 4$) and standard error of the means (SEM). The single effect of each treatment is presented in the Supplementary table S5. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 3
Total putative protease activity (U mg^{-1} chyme ww) in the chyme of stomach (pepsin), proximal, middle, and distal intestine of Atlantic salmon smolt as affected by dietary electrolyte balance (dEB) and water salinity.

		FW		SW			*p-values*		
U mg^{-1} chyme ww		Low dEB	High dEB	Low dEB	High dEB	pSEM	Salinity	Diet	Salinity*Diet
Pepsin	**Stomach**	99.5^{c}	87.5^{bc}	62.7^{ab}	52.7^{a}	6.52	**	ns	ns
Protease	**Proximal**	116.1	85.6	86.4	77.9	18.7	ns	ns	ns
	Middle	169.2^{b}	88.1^{a}	131.4^{ab}	95.9^{ab}	18.6	ns	**	ns
	Distal	75.0	73.2	56.4	55.3	7.82	*	ns	ns

FW, 0 ppt salinity; SW, 30 ppt salinity; low dEB, −100 mEq kg^{-1} DM; high dEB, +500 mEq kg^{-1} DM.
ww, wet weight; ns, not significant, $p > 0.05$; *, $p < 0.05$; **, $p < 0.01$. Values are expressed as the mean per treatment ($n = 4$) and the pooled standard errors of the mean (pSEM).

4. Discussion

The current study investigated the role of dietary electrolyte balance (dEB) and water salinity on digestion and osmoregulation in the GIT of Atlantic salmon smolts. Increasing dEB led to higher chyme pH in the stomach of freshwater- and seawater-adapted salmon. Similarly, previous research has shown that, when fed high dEB diets, chyme pH increased in the stomach of freshwater Nile tilapia (*Oreochromis niloticus*) (Saravanan et al., 2013) and rainbow trout (*Oncorhynchus mykiss*) (Magnoni et al., 2018; Ciavoni et al., 2023). Furthermore, in the present study, a diet effect on ion fluxes was present in the stomach. Indeed, the low dEB diet promoted a significant efflux of Ca^{2+} and an influx of Na^{+} in the stomach of fish when compared to high dEB diet (Fig. 3B, F). The higher divalent ion efflux in low dEB fed fish may be a consequence of the lower chyme pH in the stomach, which may cause Ca^{2+} and Mg^{2+} to dissolve faster and move to the proximal intestine as suggested by Bucking and Wood (2007) and Elesho et al. (2022). Our results are consistent with previous studies and indicate that dEB mainly affects acid-base balance in the stomach of fish during digestion (Magnoni et al., 2018; Magnoni et al., 2018; Saravanan et al., 2013). Furthermore, the increase in divalent ions (Ca^{2+}, Mg^{2+}) influx in the proximal intestine was larger in the low dEB group than in the high dEB group. This implies that the increased acid secretion in the stomach drives more alkaline secretion in the proximal intestine. Additionally, chyme osmolality decreased in the proximal intestine of fish fed the low dEB diet, which could be attributed to the intestinal bicarbonate secretion to aid precipitation of calcium-magnesium carbonate for water absorption in the mid intestine (Philip et al., 2022; Wilson and Grosell, 2003). Elesho et al. (2022) proposed that when African catfish (*Clarias gariepinus*) were fed diets contrasting in starch versus fat, endogenous secretions in the stomach and proximal intestine were altered affecting Ca^{2+} and Mg^{2+} fluxes. This gives support to our observations that ion fluxes in the proximal intestine are regulated by changes in the stomach caused by diet. Furthermore, the calculated ADC of crude protein (CP) in the stomach suggests a quicker movement of solubilized proteins from the stomach to the proximal intestine in fish fed the low dEB diet than the high dEB diet, but no dietary effect was present in the intestine (Fig. 5B). Previous research has shown that high dEB diet may lead to higher hydration in the stomach of freshwater-adapted fish, hence facilitating digestive processes (Magnoni et al., 2018; Ciavoni et al., 2023). However, in the current study, water influx in the stomach was higher in fish fed the high dEB diet in freshwater conditions, but not in seawater conditions. This may be an indication that dEB alters water and nutrient fluxes in the stomach differently, depending on environmental salinity. In contrast to CP digestibility, enzyme activity in the stomach was not altered by dEB, but it was significantly higher in the middle intestine of fish fed the low dEB diet (Table 3). This could be a result of the more alkaline chyme. Magnoni et al. (2017) proposed that differences in dietary dEB may alter chyme pH in the gut of the euryhaline meagre fish (*Argyrosomus regius*) and affect enzyme activity. However, they did not find changes in trypsin and chymotrypsin activity in the intestine in fish fed contrasting dEB diets (200 versus 700 mEq kg^{-1} DM). Overall, when looking at chyme characteristics, the more acidic low dEB diet and the more alkaline high dEB diet only affected chyme

pH in the stomach. Similarly, ion and nutrient fluxes were differentially affected by dEB only in the stomach and proximal intestine.

Depending on environmental salinity, water fluxes in fish GIT change for osmotic purposes. Water fluxes include endogenous secretions produced during digestion or ingestion of water (drinking) from the environment. Drinking rate in unfed Atlantic salmon pre-smolt and smolt respectively increases from 1.9 or 2.4 ml kg^{-1} h^{-1} in FW to 7 or 6.5 ml kg^{-1} h^{-1} in SW (Smith et al., 1991; Usher et al., 1988). When fish are fed, their drinking rate increases further in both FW and SW environment (Bucking and Wood, 2006; Eddy, 2007; Wood and Bucking, 2010). Accordingly, water influx in the GIT was higher in SW-adapted fish than FW-adapted fish. The higher water influx in SW condition influenced chyme osmolality in the stomach (Fig. 2C), which was more than three times higher (789 mmol kg^{-1}) compared to FW condition (247 mmol kg^{-1}). However, chyme osmolality dropped from the proximal intestine to the distal intestine and the difference between FW- and SW-conditions were not significant, further confirming that the osmolality is only regulated in the intestinal segments but not in the stomach. Furthermore, chyme pH in the stomach and intestinal segments of Atlantic salmon increased along with water salinity (Fig. 2 A). In contrast, Usher et al. (1990) showed that chyme pH in the stomach did not change in Atlantic salmon smolts after transfer to seawater. Similarly, Ciavoni et al. (2024) proposed that, albeit drinking rate increased in seawater-adapted Atlantic salmon, water influx and chyme pH in the stomach did not differ between 0 and 35 ppt salinity when fed a commercial-like diet (ca. dEB of 260 mEq kg^{-1} DM). In both the studies, however, chyme pH was higher in the intestinal segments of seawater- than freshwater-adapted salmon.

Ciavoni et al. (2024) found that water influx increased more than three times between the stomach and the proximal intestine in seawater-adapted salmon. Therefore, it was proposed that, in seawater conditions, ingested water is shunted directly from the stomach into the proximal intestine, where it is primarily intended for osmoregulatory water uptake rather than chyme liquefaction in the stomach. In the present study, the salinity effect on water fluxes in the GIT changed significantly depending on the dEB of the feed (interaction effect) (Fig. 1A, B). Therefore, the water salinity effect on water and ion fluxes needs to be discussed considering its interaction with the diet effect.

The interaction between dEB and water salinity significantly altered chyme DM, water, and ion fluxes in the GIT of Atlantic salmon smolts. In the stomach, chyme DM was lower in freshwater-adapted fish fed the high dEB diet. Previous research suggested that, when freshwater fish were fed a high dEB diet, more acidic fluid secretion was needed to lower chyme pH into the stomach, decreasing chyme DM (Ciavoni et al., 2023; Magnoni et al., 2018; Saravanan et al., 2013). In the stomach, the effect of the interaction between diet and salinity on chyme DM was less pronounced, but it was stronger on water influx. Water influx in the stomach increased by 3.1 ml water g^{-1} ingested DM with the low dEB diet in seawater conditions. This could be due to a rise in endogenous secretions as well as water intake promoted by the diet. Indeed, in seawater conditions, relative drinking rate was higher in fish fed the low dEB diet (3.07 ml kg^{-1} h^{-1}) than the high dEB diet (2.04 ml kg^{-1} h^{-1}) (Fig. 4). Ingested seawater, therefore, appears to remain in the stomach after feeding the low dEB diet instead of quickly moving to the proximal intestine, as proposed by Ciavoni et al. (2024). As a result, our findings indicate that dEB may aid or inhibit drinking as well as endogenous secretions in the stomach in seawater conditions. This may be due to the intrinsic dietary characteristics which may demand more water addition into the stomach to facilitate nutrient hydrolysis. The measurement of ingested seawater in the current study was based on the net GIT flux of Mg^{2+} at 30 ppt salinity relative to the Mg^{2+} flux in freshwater and is thus referred to as the 'relative' drinking rate. Among the two divalent cations (Ca^{2+} and Mg^{2+}) that are ideal candidates for studying drinking in fish, magnesium was chosen because in our previous study (Ciavoni et al., 2024), an isosmotic point of 12 ppt was achieved based on Mg^{2+} flux, whereas it was unable to do so based on Ca^{2+} flux, as some Ca^{2+} uptake is known to occur in freshwater. Although it is possible that Mg^{2+} from ingested SW can also be absorbed in the GIT, Lin et al. (2013) demonstrated that Mg^{2+} from SW is absorbed only when the dietary Mg^{2+} supply is deficient, which was not the case in the present study. On the contrary, Mg^{2+} from ingested SW is concentrated (Shehadeh and Gordon, 1969; Wood et al., 2004) precipitated as calcium magnesium carbonate in the intestinal lumen of fish at higher water salinities (Philip et al., 2022; Walsh et al., 1991). Despite being fed the same dietry Mg^{2+} levels, differences in actual feed intake resulted in slightly different dietary Mg^{2+} intake levels across groups. However, when compared to the increase in Mg^{2+} concentrations in the chyme with increasing salinity, the dieatry differences were negligible. Furthermore, in seawater-adapted fish, water influx nearly doubled between the stomach and the proximal intestine when fed the low dEB diet, whereas it slightly decreased in fish fed the high dEB diet. This suggests that ingested water may transiently remain in the stomach and partially pass directly to the proximal intestine. Moreover, endogenous alkaline secretions may further contribute to increasing water influx in the proximal intestine (Grosell, 2010; Grosell, 2006). Accordingly, Ciavoni et al. (2024) proposed that chyme liquefaction in the stomach is endogenous, whereas in the intestine it is both endogenous and exogenous. However, whether of exogenous or endogenous origin, the current study found that the interaction between water salinity and dEB is critical in determining the magnitude of water fluxes along the GIT. Furthermore, when fed the low dEB diet, the influx of divalent ions (Ca^{2+}, Mg^{2+}) into the proximal intestine of seawater-adapted fish increased (Fig. 3B, D). Consequently, reabsorption of water and ions in the middle intestine of seawater-adapted fish was significantly higher in the low dEB group. The present results, further support the hypothesis that, depending on water salinity, dEB alters water and ion fluxes in the GIT differently.

In summary, dietary electrolyte balance alters drinking rate, water, ion, and nutrient fluxes in the GIT, but differently depending on environmental salinity. The influence of contrasting dEB on regulation of water dynamics in the GIT of Atlantic salmon was larger in seawater than in freshwater. As a result, it can be concluded that dEB plays a critical role in regulating water ingestion and fluxes in the GIT of Atlantic salmon smolts in seawater. Based on these findings, it is suggested that physiological constraints that may arise from the interaction between diet and environment warrant more consideration in formulation of smolt feeds. Within the context of smoltification, dietary formulation that promote water ingestion, such as a low dEB, may benefit fish by facilitating their physiological adaptation to seawater. However, the present study showed that a low dEB diet resulted in reduced feed intake and growth performance. These findings suggest that the optimal dEB formulation may vary depending on the specific objective, highlighting the need for careful consideration of potential trade-offs between physiological adaptation and growth performance.

CRediT authorship contribution statement

Elisa Ciavoni: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Johan W. Schrama:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Data curation, Conceptualization. **Øystein Sæle:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Antony J. Prabhu Philip:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2024.740685.

References

AOAC, 1995. Metode 992.15. AOAC Off. Methods Anal.

Alarcón, F.J., Moyano, F.J., Díaz, M., 2002. Evaluation of different protein sources for aquafeeds by an optimised pH-stat system. J. Sci. Food Agric. 82, 697–704. https://doi.org/10.1002/jsfa.1100.

Andreeva, N.S., Rumsh, L.D., 2001. Analysis of crystal structures of aspartic proteinases: on the role of amino acid residues adjacent to the catalytic site of pepsin-like enzymes. Protein Sci. 10, 2439–2450. https://doi.org/10.1101/ps.25801.

Anson, M.L., Mirsky, A.E., 1932. The estimation of pepsin with hemoglobin. J. Gen. Physiol. 16, 59. https://doi.org/10.1085/jgp.16.1.59.

Basulto, S., 1976. Induced saltwater tolerance in connection with inorganic salts in the feeding of Atlantic salmon (Salmo salar L.). Aquaculture 8, 45–55. https://doi.org/10.1016/0044-8486(76)90018-1.

Bucking, C., Wood, C.M., 2006. Water dynamics in the digestive tract of the freshwater rainbow trout during the processing of a single meal. J. Exp. Biol. 209, 1883–1893. https://doi.org/10.1242/jeb.02205.

Bucking, C., Wood, C.M., 2007. Gastrointestinal Transport of ca 2 + and mg 2 + during the Digestion of a Single Meal in the Freshwater Rainbow Trout, pp. 349–360. https://doi.org/10.1007/s00360-006-0134-3.

Bucking, C., Wood, C.M., 2008. The alkaline tide and ammonia excretion after voluntary feeding in freshwater rainbow trout. J. Exp. Biol. 211, 2533–2541. https://doi.org/10.1242/jeb.015610.

Ciavoni, E., Maas, R.M., Koppelaars, M., Sæle, Ø., Schrama, J.W., Philip, A.J.P., 2023. Effect of dietary electrolyte balance on the interplay between water fluxes and digestive functioning along the gastrointestinal tract of freshwater rainbow trout (Oncorhynchus mykiss). Aquaculture 563, 738928. https://doi.org/10.1016/j.aquaculture.2022.738928.

Ciavoni, E., Schrama, J.W., Radhakrishnan, G., Sæle, Ø., Prabhu Philip, A.J., 2024. Salinity induced changes in the progression of water, ion and nutrient fluxes along the gastrointestinal tract of Atlantic salmon smolt (Salmo salar). Aquaculture 580, 740331. https://doi.org/10.1016/j.aquaculture.2023.740331.

Eddy, F.B., 2007. Drinking in juvenile Atlantic salmon (Salmo salar) in response to feeding and activation of the endogenous renin-angiotensin system. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 148, 23–28. https://doi.org/10.1016/j.cbpa.2006.08.004.

Elesho, F.E., Sutter, D.A., Frenken, R., Verreth, J.A., Křockel, S., Schrama, J.W., 2022. Fishmeal hydrolysation and non-protein energy sources affect the kinetics of nutrient digestion in the gastrointestinal tract of African catfish (Clarias gariepinus). Aquaculture 547, 737425. https://doi.org/10.1016/j.aquaculture.2021.737425.

Eroldoğan, O.T., Kumlu, M., Kir, M., Kiris, G.A., 2005. Enhancement of growth and feed utilization of the European sea bass (Dicentrarchus labrax) fed supplementary dietary salt in freshwater. Aquac. Res. 36, 361–369. https://doi.org/10.1111/j.1365-2109.2004.01211.x.

Fjelldal, P.G., Lock, E.J., Grotmol, S., Totland, G.K., Nordgarden, U., Flik, G., Hansen, T., 2006. Impact of smolt production strategy on vertebral growth and mineralisation during smoltification and the early seawater phase in Atlantic salmon (Salmo salar, L.). Aquaculture 261, 715–728. https://doi.org/10.1016/j.aquaculture.2006.08.008.

Francis, G., Makkar, P.S., Becker, K., 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture 199, 197–227.

Grosell, M., 2006. Intestinal Anion Exchange in Marine Fish Osmoregulation, 1930, pp. 2813–2827. https://doi.org/10.1242/jeb.02345.

Grosell, M., 2010. The role of the gastrointestinal tract in salt and water balance. In: Fish Physiology. Academic Press. Elsevier Inc., pp. 135–164. https://doi.org/10.1016/S1546-5098(10)03004-9

Harpaz, S., Hakim, Y., Slosman, T., Eroldogan, O.T., 2005. Effects of adding salt to the diet of Asian sea bass Lates calcarifer reared in fresh or salt water recirculating tanks, on growth and brush border enzyme activity. Aquaculture 248, 315–324. https://doi.org/10.1016/j.aquaculture.2005.03.007.

Harter, T.S., Verreth, J.A.J., Heinsbroek, L.T.N., Schrama, J.W., 2013. Isoenergetic replacement of fat by starch in diets for African catfish (Clarias gariepinus): effect on water fluxes in the gastro intestinal tract. PLoS One 8, 1–9. https://doi.org/10.1371/journal.pone.0055245.

Helland, S.J., Grisdale-Helland, B., Nerland, S., 1996. A simple method for the measurement of daily feed intake of groups of fish in tanks. Aquaculture. https://doi.org/10.1016/0044-8486(95)01145-5.

Lin, Y.H., Ku, C.Y., Shiau, S.Y., 2013. Estimation of dietary magnesium requirements of juvenile tilapia, Oreochromis niloticus×Oreochromis aureus, reared in freshwater and seawater. Aquaculture 380–383, 47–51. https://doi.org/10.1016/j.aquaculture.2012.11.034.

Magnoni, L.J., Salas-Leiton, E., Peixoto, M.J., Pereira, L., Silva-Brito, F., Fontinha, F., Gonçalves, J.F.M., Wilson, J.M., Schrama, J.W., Ozório, R.O.A., 2017. Dietary electrolyte balance affects growth performance, amylase activity and metabolic response in the meagre (Argyrosomus regius). Comp. Biochem Physiol. Part - B Biochem. Mol. Biol. 211, 8–15. https://doi.org/10.1016/j.cbpb.2017.03.001.

Magnoni, L., Eding, E., Leguen, I., Prunet, P., Geurden, I., Ozório, R.O.A., Schrama, J.W., 2018. Hypoxia, But not an Electrolyte- Imbalanced Diet, Reduces Feed Intake, Growth and Oxygen Consumption in Rainbow Trout (*Oncorhynchus mykiss*), pp. 1–14. https://doi.org/10.1038/s41598-018-23352-z.

NMKL, 1991. Moisture and ash. In: Gravimetric Determination in Meat and Meat Products. UDC 637.5. NMKL Methods 23.

Pellertier, D., Besner, M., 1992. The effect of salty diets and gradual transfer to sea water on osmotic adaptation, gill Na+, K+-ATPase activation, and survival of brook charr, Salvelinus fontinalis. Mitchill. J. Fish Biol. 41, 791–803. https://doi.org/10.1111/j.1095-8649.1992.tb02707.x.

Perry, S.F., Rivero-Lopez, L., McNeill, B., Wilson, J., 2006. Fooling a freshwater fish: how dietary salt transforms the rainbow trout gill into a seawater gill phenotype. J. Exp. Biol. 209, 4591–4596. https://doi.org/10.1242/jeb.02558.

Philip, A.J.P., Fjelldal, P.G., Remø, S.C., Selvam, C., Hamre, K., Espe, M., Holen, E., Skjærven, K.H., Vikså, V., Subramanian, S., Schrama, J.W., Sissener, N.H., 2022. Dietary electrolyte balance of Atlantic salmon (Salmo salar) freshwater feeds: impact on osmoregulation, mineral metabolism and performance in seawater. Aquaculture 546. https://doi.org/10.1016/j.aquaculture.2021.737305.

Saravanan, S., Geurden, I., Orozco, Z.G.A., Kaushik, S.J., Verreth, J.A.J., Schrama, J.W., 2013. Dietary electrolyte balance affects the nutrient digestibility and maintenance energy expenditure of Nile tilapia. Br. J. Nutr. 110, 1948–1957. https://doi.org/10.1017/S0007114513001323.

Sauvant, D., Perez, J.M., Tran, G., 2004. Tables of Composition and Nutritional Value of Feed Materials: Pigs, Poultry, Cattle, Sheep, Goats, Rabbits, Horses and Fish. Wageningen Acad. Publ.

Sele, V., Silva, M., Philip, A., Hamre, K., Skjaerven, K., Espe, M., Liland, N., Lie, K.K., Berntssen, M., Lundebye, A.-K., 2023. Monitoring program for fish feed. Annual report for samples collected in 2022. Rapport fra havforskningen. Report nr. 2023-36 Nor. Fish Feed Rep. 35pp.

Shehadeh, Z.H., Gordon, M.S., 1969. The Role of the Intestine in Salinity Adaptation of the Rainbow Trout, p. 30.

Silva, M.S., Křockel, S., Jesu Prabhu, P.A., Koppe, W., Ørnsrud, R., Waagbø, R., Araujo, P., Amlund, H., 2019. Apparent availability of zinc, selenium and manganese as inorganic metal salts or organic forms in plant-based diets for Atlantic salmon (Salmo salar). Aquaculture 503, 562–570. https://doi.org/10.1016/j.

Smith, N.F., Eddy, F.B., Struthers, A.D., Talbot, C., 1991. Renin, atrial natriuretic peptide and blood plasma ions in parr and smolts of Atlantic salmon Salmo salar L. and rainbow trout Oncorhynchus mykiss (Walbaum) in fresh water and after short-term exposure to sea water. J. Exp. Biol. 157, 63–74. https://doi.org/10.1242/jeb.157.1.63.

Tacon, A.G.J., De Silva, S.S., 1983. Mineral composition of some commercial fish feeds available in Europe. Aquaculture 1, 11–20.

The National Academies Press, 2011. Nutrient Requirements of Fish and Shrimp. The National Academies Press.

Usher, M.L., Talbot, C., Eddy, F.B., 1988. Drinking in Atlantic salmon smolts transferred to seawater and the relationship between drinking and feeding. Aquaculture 73, 237–246. https://doi.org/10.1016/0044-8486(88)90058-0.

Usher, M.L., Talbott, C., Eddy, F.B., 1990. Effects of transfer to seawater on digestion and gut function in Atlantic salmon smolts (Salmo salar). Aquaculture 1, 85–96. https://doi.org/10.1016/0044-8486(90)90285-U.

Vargas-Lagos, C., Pontigo, J.P., Oyarzún, R., Soto-Dávila, M., Morera, F.J., Yáñez, A.J., Vargas-Chacoff, L., 2018. Intestinal incomplete process on the osmoregulation system during Salmo salar smoltification in a productive conditions. Aquaculture 491, 121–127. https://doi.org/10.1016/j.aquaculture.2018.03.022.

Walsh, P.J., Blackwelder, P., Gill, K.A., Danulat, E., Mommsen, T.P., 1991. Carbonate deposits in marine fish intestines: a new source of biomineralization. Limnol. Oceanogr. https://doi.org/10.4319/lo.1991.36.6.1227.

Walter, H.E., 1984. Proteases and their inhibitors. 2. 15. 2 method with haemoglobin, casein and azocoll as substrate. Meth. Enzym. Anal. 270–277.

Whittamore, J.M., 2012. Osmoregulation and epithelial water transport: lessons from the intestine of marine teleost fish. J. Comp. Physiol. B Biochem. Syst. Environ. Physiol. https://doi.org/10.1007/s00360-011-0601-3.

Wilson, R.W., Grosell, M., 2003. Intestinal Bicarbonate Secretion in Marine Teleost Fish — Source of Bicarbonate, pH Sensitivity, and Consequences for Whole Animal Acid – Base and Calcium Homeostasis, 1618, pp. 163–174. https://doi.org/10.1016/j.bbamem.2003.09.014.

Wilson, R.W., Wilson, J.M., Grosell, M., 2002. Intestinal bicarbonate secretion by marine teleost fish - why and how? Biochim. Biophys. Acta Biomembr. 1566, 182–193. https://doi.org/10.1016/S0005-2736(02)00600-4.

Wood, C.M., Bucking, C., 2010. The role of feeding in salt and water balance. In: Euryhaline Fishes, vol. 32. Fish Phsyology. Elsevier Inc., pp. 165–212. https://doi.org/10.1016/S1546-5098(10)03005-0

Wood, C.M., McDonald, M.D., Walker, P., Grosell, M., Barimo, J.F., Playle, R.C., Walsh, P.J., 2004. Bioavailability of silver and its relationship to ionoregulation and silver speciation across a range of salinities in the gulf toadfish (Opsanus beta). Aquat. Toxicol. 70, 137–157. https://doi.org/10.1016/j.aquatox.2004.08.002.

Yasumaru, F., Lemos, D., 2014. Species specific in vitro protein digestion (pH-stat) for fish: method development and application for juvenile rainbow trout (Oncorhynchus mykiss), cobia (Rachycentron canadum), and Nile tilapia (Oreochromis niloticus). Aquaculture 426, 74–84.

Zaugg, W.S., 1982. Some changes in smoltification and seawater adaptability of salmonids resulting from environmental and other factors. Aquaculture 28, 143–151. https://doi.org/10.1016/0044-8486(82)90017-5.

Zaugg, W.S., Roley, D.D., Prentice, E.F., Gores, K.X., Waknitz, F.W., 1983. Increased seawater survival and contribution to the fishery of Chinook salmon (Oncorhynchus tshawytscha) by supplemental dietary salt. Aquaculture 32, 183–188.