Am J Physiol Regul Integr Comp Physiol 318: R410–R417, 2020.
First published November 20, 2019; doi:10.1152/ajpregu.00033.2019.

RESEARCH ARTICLE | *Fluid and Electrolyte Homeostasis*

Osmoregulatory role of the intestine in the sea lamprey (*Petromyzon marinus*)

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[1]*Department of Biology, Faculty of Marine and Environmental Sciences, Campus de Excelencia Internacional del Mar, University of Cádiz, Cádiz, Spain;* [2]*Centre of Marine Sciences, University of Algarve, Gambelas, Faro, Portugal;* [3]*United States Geological Survey, Leetown Science Center, S.O. Conte Anadromous Fish Research Laboratory, Turners Falls, Massachusetts; and* [4]*Graduate Program in Organismic and Evolutionary Biology, University of Massachusetts, Amherst, Massachusetts*

Submitted 28 January 2019; accepted in final form 18 December 2019

Barany A, Shaughnessy CA, Fuentes J, Mancera JM, McCormick SD. Osmoregulatory role of the intestine in the sea lamprey (*Petromyzon marinus*). *Am J Physiol Regul Integr Comp Physiol* 318: R410–R417, 2020. First published November 20, 2019; doi:10.1152/ajpregu.00033.2019.—Lampreys are the most basal vertebrates with an osmoregulatory strategy. Previous research has established that the salinity tolerance of sea lamprey increases dramatically during metamorphosis, but underlying changes in the gut have not been examined. In the present work, we examined changes in intestinal function during metamorphosis and seawater exposure of sea lamprey (*Petromyzon marinus*). Fully metamorphosed juvenile sea lamprey had 100% survival after direct exposure to 35 parts per thousand seawater (SW) and only slight elevations in plasma chloride (Cl^-) levels. Drinking rates of sea lamprey juveniles in seawater were 26-fold higher than juveniles in freshwater (FW). Na^+-K^+-ATPase (NKA) activity in the anterior and posterior intestine increased 12- and 3-fold, respectively, during metamorphosis, whereas esophageal NKA activity was lower than in the intestine and did not change with development. Acclimation to SW significantly enhanced NKA activity in the posterior intestine but did not significantly change NKA activity in the anterior intestine, which remained higher than that in the posterior intestine. Intestinal Cl^- and water uptake, which were observed in ex vivo preparations of anterior and posterior intestine under both symmetric and asymmetric conditions, were higher in juveniles than in larvae and were similar in magnitude of those of teleost fish. Inhibition of NKA by ouabain in ex vivo preparations inhibited intestinal water absorption by 64%. Our results indicate drinking and intestinal ion and water absorption are important to osmoregulation in SW and that preparatory increases in intestinal NKA activity are important to the development of salinity tolerance that occurs during sea lamprey metamorphosis.

development; drinking rate; intestine Na^+-K^+-ATPase; ion transport; water absorption

INTRODUCTION

Lamprey and hagfish are the most basal vertebrates and the only extant members of the vertebrate superclass *Agnatha*. Unlike hagfish, which are marine osmoconformers, lamprey have an osmoregulatory strategy in which blood ion levels are maintained relatively constant in both freshwater (FW) and seawater (SW). Larvae (also called "ammocoetes") of the anadromous sea lamprey (*Petromyzon marinus* L.) spend 4–6 yr in FW before metamorphosing and migrating to the sea. Metamorphosis takes 4–5 mo and involves the radical transformation from a substrate-dwelling, filter-feeding ammocoete into a free-swimming, parasitic juvenile. Juveniles spend another 2–3 yr in the sea before returning to FW to spawn and die. The capacity for osmoregulation in SW is very low in larval sea lamprey but increases dramatically during metamorphosis (39).

Lampreys and teleosts maintain an internal osmotic pressure at approximately one-third that of SW, with plasma osmolality values of ~300 mosmol/kgH_2O. Fishes living in FW must counteract the passive loss of ions and gain of water by a process of active, ATP-dependent uptake of ions (primarily Na^+ and Cl^-) across the gill epithelium and removal of excess water via the production of dilute urine. Fishes living in hyperosmotic environments such as SW (~1,050 mosmol/kgH_2O) must counteract the passive gain of ions and loss of water. To do this, fish in SW increase drinking and absorb water in the gut while excreting excess divalent ions (primarily Ca^{2+} and Mg^{2+}) via the gut and kidney and monovalent ions via the gills (14).

For most teleost fishes ingested SW is consecutively processed by the different intestinal regions including the esophagus, pyloric cecas, stomach, anterior intestine, middle-posterior intestine, and rectum. Together, these regions desalinate ingested SW until it is close to iso-osmotic with respect to the blood and facilitate net water absorption, which occurs primarily in the intestine (23, 29, 44). As ingested SW moves through the gut, the osmolality of the lumen compartment is substantially reduced, allowing for simultaneous net water absorption (44) via two possible paths: transcellularly, in which aquaporins are involved (8, 50, 51), and paracellularly (37). Both paths for water absorption from the lumen are driven by the osmotic gradient, indicating Na^+ and Cl^- absorption as major contributors to reduction of gut osmolality that then allows water uptake (24, 33). In teleosts it has been established that luminal Na^+ and Cl^- uptake occurs through the apical Na^+-K^+-$2Cl^-$ cotransporter (NKCC2) (11, 12, 20, 42). Transcellular movement of Na^+ is driven by basolateral Na^+-K^+-ATPase (NKA). NKA also exchanges three K^+ for two Na^+, which provides an electrical gradient that favors movement of Cl^- into the blood. The excess of monovalent ions taken up by the gut to desalinize the imbibed SW is secreted by the gills (21, 25, 31). For most teleosts, SW acclimation results in elevated levels of NKA activity in the gut and gill, which produce a

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favorable electrochemical gradients for the passive movement of ions via cotransporters and ion channels (21, 24, 47).

The importance of the intestine to osmoregulation has been clearly established in teleost fish; however, there is little understanding of the role of the intestine in osmoregulation of lamprey. Although there is some information on morphological and molecular changes that occur in the intestine related to changes in external salinity and metamorphosis (15, 26), there is very limited direct physiological evidence for the role of the gut in ion uptake of juvenile marine lamprey. Drinking rate and intestinal ion uptake have been examined in migrating river lamprey (38), but these studies were limited to use of 50% SW due to the limited salinity tolerance of upstream-migrating adults.

The present study focused on the ionoregulatory changes in the sea lamprey intestine during metamorphosis and after salinity acclimation as juveniles. Our aims were to characterize changes in the osmoregulation of the gut during metamorphosis, the developmental period when there are large increases in the salinity tolerance of sea lamprey. Specifically, we examined developmental changes in NKA activity and examined drinking rates, ion uptake, and water absorption during SW acclimation of anadromous sea lamprey.

MATERIALS AND METHODS

Animals and experimental designs. Sea lamprey were caught in the Sawmill River, a tributary of the Connecticut River (Massachusetts), by electrofishing or Fyke net capture from July to November in 2016. For the metamorphic series, lamprey were sampled in the field immediately upon capture. The metamorphic stage of individuals was determined according to the descriptions presented by Youson and Potter (54). Salinity acclimation experiments were carried out under laboratory conditions at the United States Geological Survey (USGS) Conte Anadromous Fish Research Center (Turners Falls, MA) with larvae and postmetamorphic juveniles captured from the Sawmill River. Lamprey were placed into 1-m diameter tanks with flow-through Connecticut River water at ambient temperatures. Juveniles were then directly transferred to SW [35 parts per thousand (ppt)] or FW; only juveniles were exposed to SW because larvae cannot survive above 8 ppt (39). Experimental SW was prepared by mixing artificial sea salt (Crystal Sea Salt, Baltimore, MD) and dechlorinated municipal fresh water. Lamprey were kept under natural photoperiod conditions and at a constant temperature of 15°C in 60-liter recirculating glass aquaria equipped with mechanical, chemical, and biological filtration. The animals were not offered food because anadromous sea lamprey naturally stop feeding during metamorphosis and do not resume feeding until they begin parasitic feeding once they reach the ocean. Water quality and mortalities were monitored daily. All experiments were carried out in accordance with USGS guidelines and approved by the USGS Institutional Animcal Care and Use Committee (Protocol No. LB00A3O-117).

Sampling protocol and blood analysis. Lamprey were euthanized using a lethal dose of 160 mL MS-222/L water (400 mg/L buffered with $NaHCO_3$, pH 7.0; Argent Chemical Laboratories, Redmond, WA) and then measured for length and body mass and sampled for blood, muscle, and intestine. Blood was collected from caudal vessels into 0.5-mm heparinized capillary tubes. Hematocrit (measured as percentage of total blood volume) and plasma were obtained via centrifugation (5,000 *g* for 5 min). The anterior intestine was ligated at either end, removed, and emptied into 1.5-mL vials. Anterior intestinal fluid was obtained via centrifugation (13,000 *g* for 5 min) from SW-acclimated juveniles to determine osmolality. Muscle (~0.2 g) was collected from the region posterior to the anal vent, blotted dry, and placed in a 60°C drying oven for determination of muscle water content, which was used as an index of tissue hydration state. Intestinal tissues (esophagus, anterior intestine, and posterior intestine) were collected for determination of NKA activity in 1.5-mL microcentrifuge tubes containing homogenization buffer (SEI in mM: 150 sucrose, 10 EDTA, and 50 imidazole, pH 7.3). All plasma and intestinal tissue samples were immediately frozen and stored at −80°C. Plasma Cl^- concentration was determined using a digital chloridometer (Haake Buchler Instruments, Saddlebrook, NJ). Plasma osmolality was measured with a vapor pressure osmometer (model 550, Wescor 5500, Logan, UT).

Determination of drinking rate. Drinking rates were determined by following a modified protocol of Petzel (34). Lamprey were exposed to 0.04 g/L phenol red (114529, Sigma) in aerated glass aquaria for 2.5 h (in FW) or 0.5 h (in SW) to avoid imbibed phenol-water leaking through the rectum. During sampling, intestinal fluid and the remaining digestive tract were collected in 1.5-mL microcentrifuge tubes, and 500 μL of deionized water were added to each tube. The tubes containing the samples, as well as a water blank and a exposure water control (containing phenol red), were rotated for 1 h at 4°C to ensure the complete release of phenol red from between microvilli into solution. The samples were then centrifuged at 3,000 *g* for 10 min, and 250 μL of the supernatant were added to 125 μL 10% trichloro-acetic acid, then vortexed and centrifuged again at 13,000 *g* for 5 min. After centrifugation, 250 μL of the supernatant were added to 125 μL 2 N NaOH and then vortexed and centrifuged at 13,000 *g* for 5 min. Finally, 200 μL of the final supernatant were measured at 560 nm (THERMOmax microplate reader using SOFTmax software, Molecular Devices, Menlo Park, CA). Absorbance of a water blank was subtracted from the absorbance reading from the final supernatant. Drinking rate was calculated using the following formula (expressed in $mL \cdot h^{-1} \cdot kg^{-1}$): volume of water ingested ($mL \cdot h^{-1} \cdot kg^{-1}$) = $[(A_g \times V \times D/A_w)/T]/W$, where A_g is absorbance in the gut sample, A_w is absorbance in the exposure water (containing phenol red), V is mL (deionized water added to the sample), D is 2.25 (dilution factor = $V_{final}/V_{initial}$), T is exposure time, and W is weight.

Determination of intestinal water absorption and net chloride (Cl^-) flux. Water absorption and net Cl^- fluxes in whole intestine and isolated intestinal regions (anterior and posterior) were determined following previous methods (20, 25, 28). The intestinal sections were isolated, flushed and then incubated for 30 min in serosal saline bubbled with a physiological gas mixture (99.5% O_2-0.5% CO_2). The lumen was then filled via a syringe with either *1*) a serosa-like saline ("iso-osmotic"; "symmetric model") that was specifically formulated to recreate lamprey plasma (in mM: 128.0 NaCl, 1.3 NaH_2PO_4, 5.0 $NaHCO_3$, 4.0 KCl, 2.4 $CaCl_2$, 0.9 $MgSO_4$, 0.9 $MgCl_2$, and 5.5 glucose; 270 mosmol/kgH_2O and pH 7.8); *2*) a 50% SW solution (530 mosmol/kgH_2O; 50% dilution of 100% SW solution; "asymmetric model"); or *3*) a 100% SW solution (1,072 mosmol/kgH_2O; "asymmetric model") made from the commercial sea salt mix described above (in mM: 524.0 Cl^-, 452.0 Na^+, 53.0 Mg^{2+}, 10.2 Ca^{2+}, and 9.7 K^+). Saline was maintained at 15°C and pregassed for at least 30 min before experimentation. The ends of the intestinal samples were sealed at each end using dental line and placed in a beaker containing serosal saline supplied with the gas mixture. The intestinal preparations were weighed every 15 min for 1 h. Samples from the luminal fluid for Cl^- analysis were taken at the beginning and end of the 1-h experiment. At the end of the experiment, each intestinal section was opened, placed on graph paper, and then photographed. Surface area of the intestinal segment within the tied region was determined using ImageJ software (NIH, Bethesda, MD). Change in mass over time was used to determine a slope, converted to volume, and then normalized to the intestinal surface to calculate a rate of water absorption ($\mu L \cdot cm^{-2} \cdot h^{-1}$). To determine net Cl^- flux, luminal Cl^- concentrations (t_1-t_0) were normalized to their respective intestinal surfaces just as in calculation of water absorption rates ($\mu Eq \cdot cm^{-2} \cdot h^{-1}$). A negative rate indicated Cl^- secretion (from sero-

sal to luminal), and a positive rate indicated Cl^- absorption (from luminal to serosal).

Na^+-K^+-ATPase activity. Intestinal NKA activity was analyzed using an NADH-linked kinetic assay in a 96-well microplate run at 25°C for 10 min, as described by McCormick (30). Frozen intestinal regions were homogenized on ice in either 150 μL (larvae to stage 5) or 600 μL (stage 6 to juvenile) of SEID (0.1% sodium deoxycholate in SEI buffer, pH 7.3) and centrifuged at 3,200 *g* for 5 min at 4°C. The supernatant was assayed in duplicate for ATPase activity in the presence and absence of the NKA-specific inhibitor ouabain (0.5 mM). NADH was determined spectrophotometrically at 340 nm, and the difference in NADH reduction rates between the inhibited and uninhibited solutions was used to calculate NKA-specific (ouabain-sensitive) activity (expressed as μmol·ADP mg protein^{-1}·h^{-1}. Total protein was measured using the bicinchoninic acid protein assay with a bovine serum albumin standard (Thermo Scientific, Rockford, IL). Both assays were run on a THERMOmax microplate reader using SOFTmax software (Molecular Devices, Menlo Park, CA).

Calculations and statistical analysis. Fulton's condition factor (*K*) was calculated with the formula 1,000 × [wet weight (g) × length^{-3} (cm)] (41). Percent water content was calculated as: [(wet mass − dry mass) × 100]/wet mass. All data are represented as the means ± SE. Detection of significant differences were carried out by using unpaired Student's *t* test, one-way ANOVA, or two-way ANOVA, followed by a Tukey's or Sidak's post hoc analysis. All statistical analyses were performed with GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA). Significance for all tests was set at $P < 0.05$.

RESULTS

There were no mortalities of fully metamorphosed juvenile sea lamprey transferred directly from FW to SW over the 3-wk exposure period. SW juveniles had significantly lower condition factor compared with FW larvae and FW juveniles (Table 1). Plasma Cl^- was significantly (37%) higher in FW juveniles than in FW larvae, and exposure of juveniles to SW resulted in a slight increase in plasma Cl^- (12%). Muscle water content did not significantly differ between larvae and juveniles in FW but was slightly higher (2.7%) in SW juveniles (Table 1). Hematocrit values were significantly lower in SW juveniles compared with FW larvae and FW juveniles (Table 1). Osmolality of anterior intestinal fluid of SW juveniles was 575 ± 79 mosmol/kgH_2O (~50% SW).

Drinking rates. Drinking rates in FW larvae and FW juveniles were relatively low (Fig. 1). Drinking rates in juveniles increased with increasing salinity and were over 4-fold higher in 10 ppt and 26-fold higher in SW juveniles compared with FW juveniles.

Whole intestinal water absorption. Whole intestine water absorption under iso-osmotic conditions were over twofold higher in FW juveniles compared with FW larvae (Fig. 2*A*). Acclimation of juveniles to SW resulted in a 25% increase in water absorption that was significantly different from FW juveniles. Intestinal water absorption in SW-acclimated juveniles was 64% lower with serosal addition of ouabain (500 μM) compared with the control (Fig. 2*B*).

Table 1. *Biometric data, muscle water content, hematocrit, and plasma Cl^- in larvae and FW- and SW-acclimated juvenile sea lamprey*

	FW Larvae	FW Juvenile	SW Juvenile
Length, cm	13.4 ± 0.3	14.8 ± 0.3	15.2 ± 0.3
Mass, g	3.0 ± 0.2	4.3 ± 0.2	4.1 ± 0.3
Condition factor, *K*	1.24 ± 0.03^{a,b}	1.34 ± 0.02^{a}	1.17 ± 0.06^{b}
Muscle water content, %	72.0 ± 1.0^{a}	74.1 ± 0.4^{a}	76.8 ± 0.8^{b}
Plasma chloride, mM	85 ± 1.3^{a}	117 ± 1.1^{b}	131 ± 1.4^{c}
Hematocrit, %	27 ± 1.0^{a}	29 ± 1.0^{a}	19 ± 0.9^{b}

Values represent means ± SE ($n = 10$). FW, freshwater; SW, seawater. Different lowercase letters indicate significant differences ($P < 0.05$, one-way ANOVA, Tukey's post hoc).

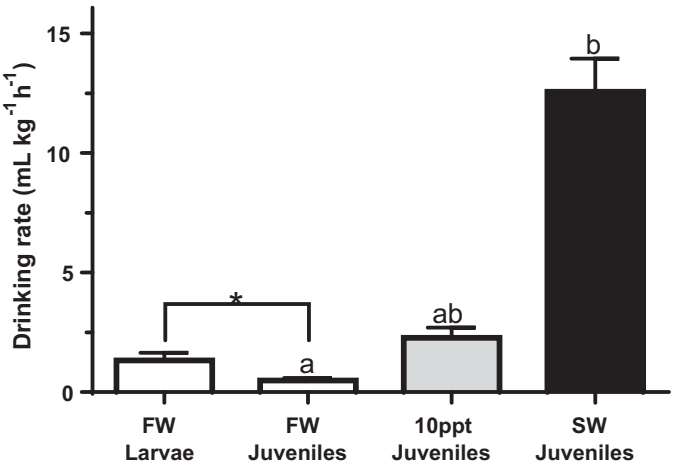

Fig. 1. Drinking rate for larvae and freshwater (FW)-, 10 ppt-, and seawater (SW; 35 ppt) -acclimated juvenile sea lamprey. Values represent means ± SE ($n = 4$–9). *Significant difference ($P < 0.05$, unpaired *t* test analysis). Different letters indicate significant differences among salinities in juveniles ($P < 0.05$, one-way ANOVA, Tukey's post hoc).

Water absorption and net Cl^- flux by intestinal region. Water absorption rates of the anterior intestine did not differ between symmetrical (iso-osmotic) and asymmetrical (50 and 100% SW) conditions (Fig. 3*A*). In the posterior intestine, the highest water absorption rate was observed in 50% SW (Fig. 3*A*). Significantly higher water absorption rates (~2-fold) were observed in anterior compared with posterior intestine for iso-osmotic and 100% SW conditions but were similar for 50% SW (Fig. 3*A*). Net Cl^- absorptive flux in the anterior intestine increased with increasing osmotic concentration of the luminal solution (iso-osmotic < 50% SW < 100% SW; Fig. 3*B*). For posterior intestine a significant enhancement of Cl^- uptake was observed for luminal 50% SW but was lower in iso-osmotic and 50% SW (Fig. 3*B*). Interestingly net Cl^- flux showed small secretory rates (from serosa to lumen) under iso-osmotic conditions across the membranes (Fig. 3*B*). There were no significant differences in water absorption or net Cl^- flux between FW- and SW-acclimated juveniles in either anterior or posterior intestine (data not shown).

Na^+-K^+-ATPase activity. NKA activity levels in the anterior and posterior intestine of wild-caught sea lamprey were low in larvae and stage 2–3 individuals and increased progressively through the later stages of metamorphosis (Fig. 4, *A* and *B*, respectively). In the anterior and posterior intestine, NKA activity increased 18- and 3-fold, respectively, between stage 2 and 3 and downstream migrating juveniles (Fig. 4, *A* and *B*). Esophageal NKA activity remained low throughout metamorphosis (Fig. 4*C*). The highest NKA activity was observed in the anterior intestine, followed by the posterior intestine, and then the esophagus (Fig. 4, *A*–*C*). Biometric data for the samples in the field are shown in Table 2. Laboratory-reared juveniles had significantly higher NKA activity in the anterior and posterior intestinal regions compared with larvae (over 36- and 4-fold,

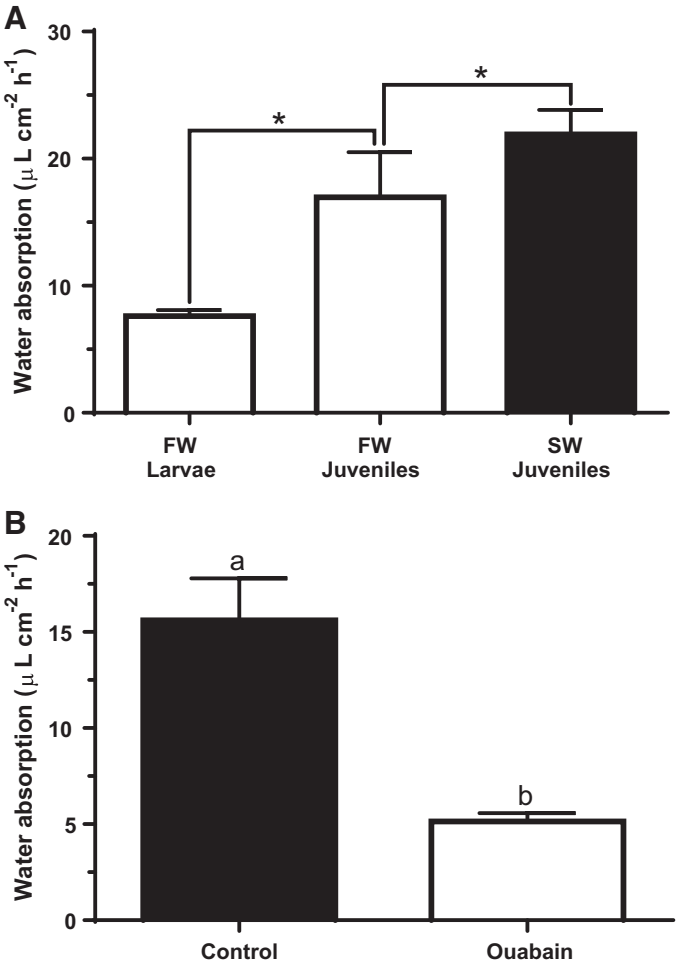

Fig. 2. Whole intestinal water absorption for larvae and freshwater (FW)- and seawater (SW)-acclimated juvenile sea lamprey (*A*). Values represent means ± SE (n = 6–10). *Significant differences ($P < 0.05$, unpaired Student's t test). Effect of ouabain on whole intestinal water absorption in SW-acclimated juvenile sea lamprey (*B*). In *B*, ouabain was added to the serosal side (500 μM) and intestinal sacs were incubated for 30 min with the test agent before the effect was evaluated every 15 min for 1 h. Values represent means ± SE (n = 4). Different letters indicate significant differences ($P < 0.05$, unpaired Student's t test).

respectively, Fig. 5). In the anterior intestine, NKA activity increased slightly after SW-acclimation but was not significantly different between FW- and SW-acclimated juveniles. However, in the posterior intestine, SW-acclimated juveniles had fourfold higher NKA activity than FW-acclimated juveniles (Fig. 5).

DISCUSSION

In the present study we have found that NKA activity in anterior and posterior intestine increases dramatically during metamorphosis in association with high levels of salinity tolerance. Drinking rates of fully transformed juveniles in SW are more than 20-fold higher than juveniles in FW. Both the anterior and posterior intestine can take up Cl^- and water. Water absorption is substantially inhibited by ouabain, a specific inhibitor of NKA. These are the first studies to examine intestinal function in sea lamprey in FW and SW, and these data provide direct evidence that intestinal ion and water uptake may be driven by NKA and critical to osmoregulation in SW.

We found that fully metamorphosed juvenile sea lamprey survived for 3 wk after direct exposure from FW to SW, consistent with previous research demonstrating that larval sea lamprey have poor salinity tolerance, which increases dramatically during metamorphosis (3, 4, 39). Other studies in our laboratory indicate that they will survive under these SW conditions for many months (C. A. Shaughnessy and S. D. McCormick, unpublished results). Lamprey acclimated to SW experienced only moderate (14 mM) increases in plasma Cl^-, and their absolute levels of plasma Cl^- (131 mM) are similar to those seen in fully acclimated teleosts in SW (14). It has been shown that adult sea lamprey experiencing poor survival after reexposure to brackish water (25 ppt) have much higher levels of plasma Cl^- (276 mM) (15) and even that plasma Cl^- of surviving adults in brackish water is higher (149 mM) than the SW-acclimated juveniles in the present study. In addition, muscle water content of SW-acclimated juveniles was actually higher than that of FW-acclimated juveniles, indicating that they were not experiencing tissue dehydration that would occur

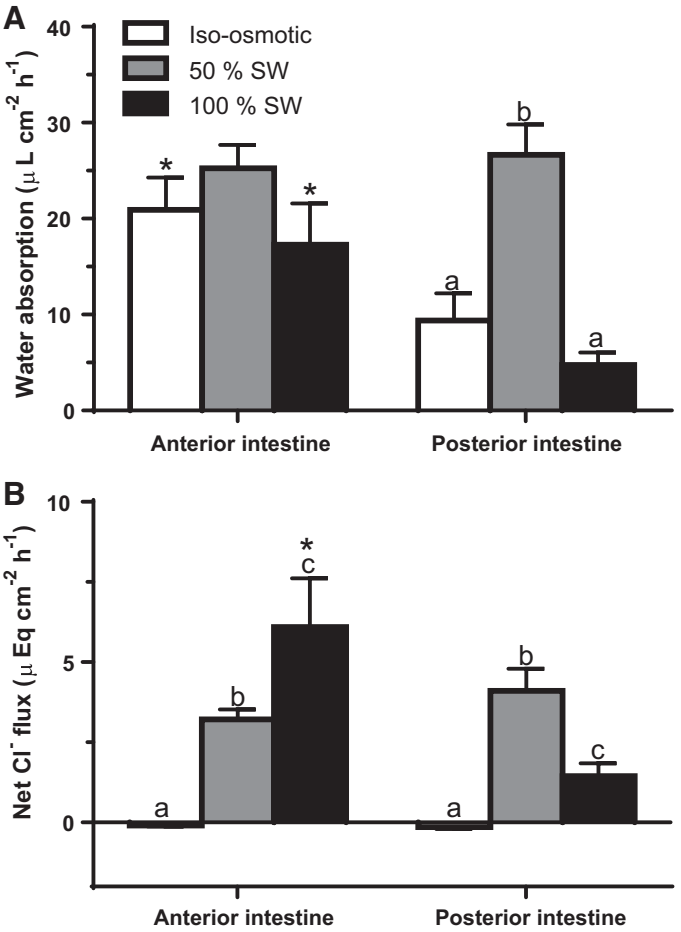

Fig. 3. Intestinal water absorption (*A*) and net chloride (Cl^-) flux (*B*) in the anterior and posterior intestinal regions under different luminal solutions [iso-osmotic saline, 50% seawater (SW), or 100% SW] in juvenile sea lamprey acclimated to SW. Values represent means ± SE (n = 5–8). Different letters indicate significant differences among luminal solutions within the same intestinal region. *Significant differences between regions under the same luminal solution ($P < 0.05$, two-way ANOVA, Sidak's post hoc).

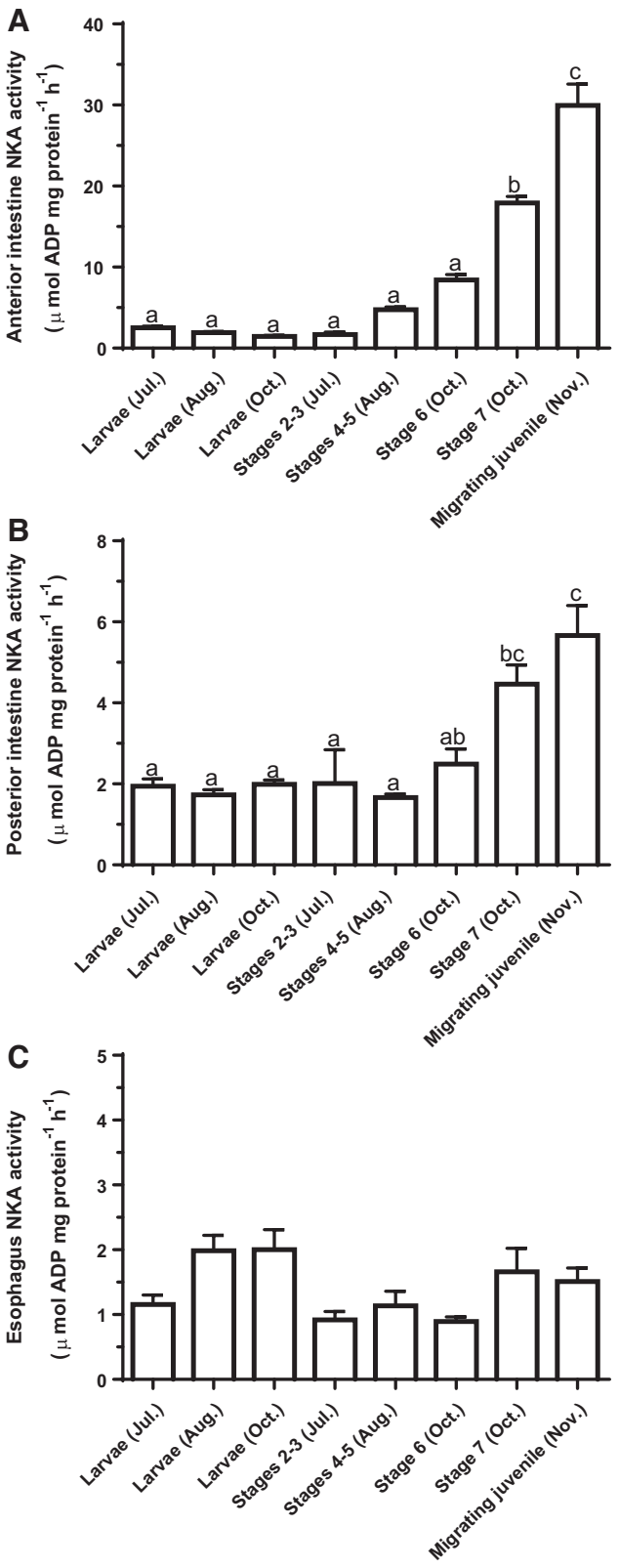

Fig. 4. Na^+-K^+-ATPase (NKA) activity in the anterior (*A*) and posterior intestine (*B*) intestine and esophagus (C), during metamorphosis of sea lamprey from larvae to downstream migrating juveniles. Values represent means ± SE (n = 2–19). Different letters indicate significant differences ($P < 0.05$, one-way ANOVA, Tukey's post hoc).

Table 2. *Biometric data from various developmental stages and downstream migrating juvenile sea lamprey collected and sampled in the field*

	Length, cm	Mass, g	Condition Factor, *K*
Larvae (Jul.)	12.2 ± 0.6[a]	2.8 ± 0.4[a]	1.39 ± 0.06
Larvae (Aug.)	14.4 ± 0.4[b]	4.3 ± 0.3[a,b]	1.39 ± 0.05
Larvae (Oct.)	14.7 ± 0.3[b]	4.6 ± 0.3[b]	1.41 ± 0.06
Stages 2–3	16.0 ± 0.8[b]	4.8 ± 0.2[a,b]	1.16 ± 0.11
Stages 4–5	15.2 ± 0.2[b]	5.3 ± 0.5[b]	1.49 ± 0.09
Stage 6	14.8 ± 0.5[b]	4.9 ± 0.6[a,b]	1.48 ± 0.10
Stage 7	15.3 ± 0.5[b]	5.8 ± 0.3[b]	1.50 ± 0.03
Migrating juvenile	15.9 ± 0.3[b]	5.3 ± 0.3[b]	1.29 ± 0.02

Values represent means ± SE (n = 2–19). Different lowercase letters indicate significant differences ($P < 0.05$, one-way ANOVA, Tukey's post hoc).

in fish that are unable to osmoregulate in SW. These higher levels of muscle hydration may be related to the initial energetic demands of osmoregulation in SW, resulting in lower lipid and higher muscle water, a relationship seen in most teleost fish (6). In the case of a teleost fish, Atlantic salmon (*Salmo salar*) acclimated to SW experience a moderate (3%) decrease in muscle water content showing a similar total muscle water content (76%) (5) to that seen in SW-acclimated sea lamprey juveniles. We found a 10% decrease in sea lamprey exposed to SW, similar in direction but lower in magnitude than the 1–4% decrease in hematocrit of Atlantic salmon exposed to SW (5). Although the lower levels of hematocrit seen in SW juveniles may be due lower red blood cell volume, this seems unlikely because plasma ion levels in FW and SW were similar. Alternatively, lower hematocrit in SW may be the result of higher plasma volume in SW or simply reduced concentration of red blood cells.

Drinking rates in SW-acclimated juvenile lamprey were 26-fold higher than juveniles in FW, which agrees with previous studies in teleosts in which drinking rates are substantially higher in fish in SW compared with FW (17). Drinking rates in the sea lamprey reported here are twofold higher than those reported in the migrating European river lamprey (*Lampetra fluviatilis*) acclimated to 50% SW (6 $mL \cdot h^{-1} \cdot kg^{-1}$) and the

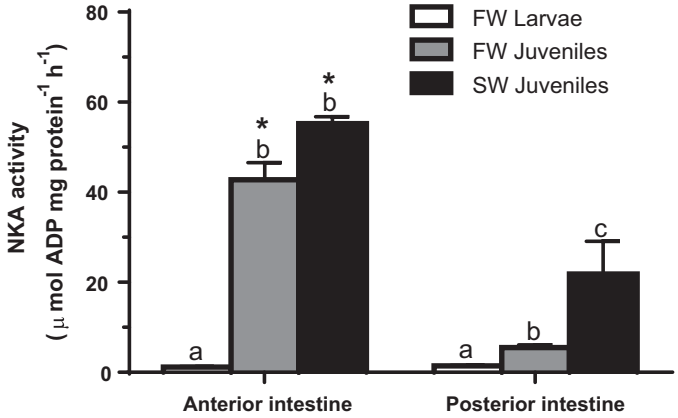

Fig. 5. Intestinal Na^+-K^+-ATPase (NKA) activity in laboratory-reared larvae and freshwater (FW)- and seawater (SW)-acclimated juvenile sea lamprey. Values represent means ± SE (n = 9–10). Different letters indicate significant differences within an intestinal region. *Significant differences between intestinal regions of the same group ($P < 0.05$, two-way ANOVA, Sidak's post hoc).

same for FW acclimated river lamprey (0.4 $mL \cdot h^{-1} \cdot kg^{-1}$) (37). When these are compared with values for teleost fishes, the observed drinking rates in SW-acclimated sea lamprey in the present study were similar to that reported for Red River pupfish (*Cyprinodon rubrofluviatilis*), ~14 $mL \cdot h^{-1} \cdot kg^{-1}$ (40) and higher than those reported for other teleost species, which ranged from 2 to 6 $mL \cdot h^{-1} \cdot kg^{-1}$ (16, 17, 34). These higher drinking rates in sea lamprey may relate in part to the relatively small size of the animals. Irrespective of these differences, our overall finding of higher drinking rate in SW compared with FW in sea lamprey is consistent with a role of the gut in recovering water that is passively lost while in a hyperosmotic SW environment.

In a series of ex vivo experiments, we characterized the magnitude and nature of water transport across the intestinal epithelium in juvenile sea lamprey and sought to further examine the possible regionalization of water absorption in the sea lamprey intestine. Water absorption rates using the whole intestine under symmetrical conditions showed higher rates of water absorption in FW- and SW-acclimated juveniles compared with FW larvae. These results indicate that the intrinsic capacity for intestinal water uptake (and likely also ion uptake) in metamorphosed sea lamprey is greater than that for larval sea lamprey and that this may be related to the observed increases in whole animal osmoregulatory ability of juveniles. These findings agree with previous literature indicating that that water absorption rates increase during smolt development of salmonids when SW tolerance increases (46). In addition, the higher levels of intestinal water absorption observed after SW acclimation in the present study is consistent with findings in coho (*Oncorhynchus kisutch*) and Atlantic salmon (9, 48).

Our results indicate that, as in teleost fishes, water absorption in sea lamprey is strongly dependent on NKA activity and active uptake of ions. Serosal addition of ouabain resulted in 64% reduction in intestinal water absorption under symmetrical conditions. Because net Cl^- fluxes under symmetrical conditions were so low, we were unable to demonstrate an effect of ouabain on Cl^- fluxes. In teleost fishes an effect of ouabain on intestinal water uptake has been shown in coho and Atlantic salmon (9, 28, 48). Previous studies demonstrated that ouabain significantly reduce Na^+ influx in isolated anterior intestine of adult sea lamprey acclimated to 50% seawater (35).

In the present study, the vast majority of total intestinal ATPase activity was ouabain sensitive. NKA activity was greater in the anterior intestine compared with the posterior intestine, similar to what has been shown in migrating river lamprey (35). Intestinal NKA activity increases dramatically during metamorphosis, especially in the anterior intestine. These changes correspond to what has been observed in the gill, where NKA activity and ionocyte abundance increased during metamorphosis and were associated with increased salinity tolerance (39). Increases in intestinal NKA activity have also been observed in salmonids during smolt development (46, 49). Our results indicate that intestinal changes during metamorphosis is critical to the development of salinity tolerance that occurs before downstream migration of sea lamprey juveniles.

We cannot rule out the possibility that some of the changes in intestinal NKA activity are due to the demands of a new feeding regime in parasitic sea lamprey following metamorphosis. Nutrient absorption processes are likely to change during parasitic feeding and so could be linked with changes in NKA activity. Most nutrient absorption processes, such as movement of carbohydrates (52, 53), amino acids (27, 32), and some vitamins and minerals (27) across the apical membrane of intestinal enterocytes are Na^+-dependent, and these nutrients have to move across highly polarized epithelial cell layers. NKA might also be involved in regulating the intracellular Na^+ levels that are critical for nutrient transport, in addition to its role in osmoregulation. Further research is needed to understand the relationship between nutrient absorption mechanisms and osmoregulation in the intestine of anadromous lamprey.

To better understand how water movement is related to macro-scale solute gradients, water absorption preparations for anterior and posterior intestinal regions were isolated and luminally exposed to iso-osmotic, 50% SW and 100% SW solutions. Anterior intestine exhibited no change in water absorption rates across the three luminal solutions tested, but the water absorption rate in posterior intestine increased significantly under 50% SW, reaching the same range as anterior intestine. This is in accordance with previous studies on European eel (*Anguilla anguilla*) that showed increasing intestinal water absorption in preparations with a slightly higher osmotic solution in the lumen with respect to plasma (44). The rate of water absorption in preparations of whole intestine that we observed in the present study (20 $\mu L \cdot cm^{-2} \cdot h^{-1}$; ~250 $mL \cdot kg^{-1} \cdot day^{-1}$ after unit conversion) is higher than rates previously reported in adult lampreys (~20 $mL \cdot kg^{-1} \cdot day^{-1}$) (36) and similar to rates reported in marine teleosts (14–20 $\mu L \cdot cm^{-2} \cdot h^{-1}$) (7, 28, 40).

Interestingly, the intestine of the sea lamprey was able to transport water from lumen to serosa even when the osmolality of the luminal fluid was markedly higher than plasma. This phenomenon of apparent nonosmotic water uptake has been reported before in chicken cloaca (43), European eel intestine (44), and European river lamprey (36), although these previous reports did not test for or observe intestinal water uptake under as large as a gradient difference as we have tested for and observed in this study. This apparent nonosmotic water flow is assumed to be caused by osmotic flow into the hyperosmotic lateral interspaces between enterocytes (10, 13, 22), which agrees with the hypothesis that water movement against a gradient is caused by local osmosis due to the salt-linked water flow (45). Water uptake may also be facilitated by the complex morphology of the gut which can allow for osmotic concentrations in the interstitial spaces to be much lower than those of the bulk flow and would be further facilitated by the high levels of Cl^- influx.

In the sea lamprey, increasing luminal osmolality in the anterior intestinal region (under asymmetric conditions) increased net Cl^- absorptive net fluxes from lumen to serosa; however, very small net Cl^- secretory net fluxes were observed for both intestinal regions under symmetric conditions. An explanation for the apparent Cl^- secretory net fluxes we observed could either be *1*) they are the result of a transepithelial pathway for Cl^- secretion present in the sea lamprey intestine, or *2*) they are an artifact of the observed net water absorption from the lumen to serosa, which may be due to solute-linked water flow occurring in the hyperosmotic lateral interspaces between enterocytes. Anecdotally, during our sampling of SW-acclimated juveniles, we observed aggregate pre-

cipitates in both intestinal regions (anterior and posterior), but did not observe any precipitate formation in FW-acclimated larvae or FW juveniles, indicating a similar role of carbonate precipitation as seen in teleost fishes (7, 20).

We observed greater NKA activity and greater maximum capacity for ion uptake in the anterior intestine compared with the posterior intestine. The two regions had similar levels of water uptake capacity when the luminal solution was 50% SW, but the anterior intestine had higher levels of water uptake under isosmotic and 100% SW. Similar differences in between anterior and posterior intestine in NKA activity, ion uptake, and water absorption highlighted in this study are similar to what has been reported for other fishes, including mummichog (*Fundulus heteroclitus*) (19), *Sparus aurata* (20); *Solea senegalensis* (2), *Anguilla spp* (1, 25), rainbow trout (*Oncorhynchus mykiss*) (18), and *Lampetra fluviatilis* (35).

The findings of the present study support the hypotheses *1*) that processes of ion and water absorption are coupled, and *2*) that major driving forces water absorption across the intestinal epithelium are NKA activity and Cl^- uptake (3, 24, 47). We propose the mechanism responsible for this absorptive net Cl^- flux is the immediate action of the passive apical cotransporters (possibly NKCC2) and/or Na^+/Cl^- cotransporter (NCC) in response to the polarization of membranes due to the forced gradient across the epithelia generated by NKA activity. We propose that the anterior intestine is the primary site for active ion uptake, which likely occurs via a mechanism of removing excess of monovalent ions from the imbibed SW, as indicated by this region having the highest NKA activity and the largest increases in NKA activity during metamorphosis.

Perspectives and Significance

The observation that basal vertebrates such as sea lamprey exhibit proximal to distal intestinal regionalization of ion- and water-absorptive processes indicates that development and environmental salinity have been major factors driving intestinal differentiation from early on in the vertebrate lineage. The observations of increased drinking in SW and active uptake of ions and absorption of water by the intestine provide strong parallels between the osmoregulatory strategy of lamprey and teleost fishes, indicating that this is an ancient and conserved strategy among ion- and osmo- regulating fishes in SW. We provide evidence of substantial increases in NKA activity in the intestine during lamprey metamorphosis, indicating that preparatory changes in the intestine and gill are similar to those in smolting salmonids and critical to the development of salinity tolerance that allows for rapid movement from FW to SW. Further studies on the specific pathways for the movement of monovalent ions and water are necessary to fully understand the likely complex role of the intestine in osmoregulation in *Agnathans* and to establish an evolutionary relationship with teleost fishes. The establishment of juvenile sea lamprey as a viable model for examining osmoregulatory physiology in these basal vertebrates could open up new areas of investigation, including examination of the specific transport pathways used for ion, nutrients, and water movement in the intestine, as well as the hormonal control of drinking rate and osmoregulatory function of the gut.

ACKNOWLEDGMENTS

We thank Amy Regish, Jessica Norstog, Daniel Hall, Andrew Weinstock, Diogo Martins, Aaron Hendry, and Yoko Yamaguchi for help in collecting and sampling wild juvenile sea lamprey. We also thank Diogo Martins for reviewing an early version of the manuscript. CCMar is supported by national funds from the Portuguese Foundation for Science and Technology (FCT) through project UID/Multi/04326/2019. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the United States Government.

GRANTS

This research was funded by an International Mobility Program Fellowship from the University of Cadiz (to A. Barany) and National Science Foundation Grant IOS-1558025 (to S. D. McCormick).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

A.B., C.A.S., and S.D.M. conceived and designed research; A.B. and C.A.S. performed experiments; A.B. analyzed data; A.B. interpreted results of experiments; A.B. prepared figures; A.B. drafted manuscript; A.B., C.A.S., J.F., J.M.M., and S.D.M. edited and revised manuscript; A.B., C.A.S., and S.D.M. approved final version of manuscript.

REFERENCES

1. **Aoki M, Kaneko T, Katoh F, Hasegawa S, Tsutsui N, Aida K.** Intestinal water absorption through aquaporin 1 expressed in the apical membrane of mucosal epithelial cells in seawater-adapted Japanese eel. *J Exp Biol* 206: 3495–3505, 2003. doi:10.1242/jeb.00579.
2. **Ruiz-Jarabo I, Barany A, Jerez-Cepa I, Mancera JM, Fuentes J.** Intestinal response to salinity challenge in the Senegalese sole (*Solea senegalensis*). *Comp Biochem Physiol A Mol Integr Physiol* 204: 57–64, 2017. doi:10.1016/j.cbpa.2016.11.009.
3. **Beamish FWH.** Osmoregulation in juvenile and adult lampreys. *Can J Fish Aquat Sci* 37: 1739–1750, 1980b. doi:10.1139/f80-219.
4. **Beamish FWH, Strachan PD, Thomas E.** Osmotic and ionic performance of the anadromous sea lamprey, *Petromyzon marinus*. *Comp Biochem Physiol* 60: 435–443, 1978. doi:10.1016/0300-9629(78)90013-0.
5. **Brauner CJ, Seidelin M, Madsen SS, Jensen FB.** Effects of freshwater hyperoxia and hypercapnia and their influences on subsequent seawater transfer in Atlantic salmon (*Salmo salar*) smolts. *Can J Fish Aquat Sci* 57: 2054–2064, 2000. doi:10.1139/f00-161.
6. **Brett JR, Groves TDD.** Physiological energetics. In: *Fish Physiology*, edited by Hoar WS, Randall DJ, Brett JR. New York: Academic, 1979, vol. 8, p. 279–352.
7. **Carvalho ESM, Gregório SF, Power DM, Canário AVM, Fuentes J.** Water absorption and bicarbonate secretion in the intestine of the sea bream are regulated by transmembrane and soluble adenylyl cyclase stimulation. *J Comp Physiol B* 182: 1069–1080, 2012. doi:10.1007/s00360-012-0685-4.
8. **Cerdà J, Finn RN.** Piscine aquaporins: an overview of recent advances. *J Exp Zool A Ecol Genet Physiol* 313: 623–650, 2010. doi:10.1002/jez.634.
9. **Collie NL, Bern HA.** Changes in intestinal fluid transport associated with smoltification and sea-water adaptation in coho salmon, *Oncorhynchus kisutch* (Wal-baum). *J Fish Biol* 21: 337–348, 1982. doi:10.1111/j.1095-8649.1982.tb02839.x.
10. **Curran PF.** Na, Cl, and water transport by rat ileum in vitro. *J Gen Physiol* 43: 1137–1148, 1960. doi:10.1085/jgp.43.6.1137.
11. **Cutler CP, Cramb G.** Molecular physiology of osmoregulation in eels and other teleosts: the role of transporter isoforms and gene duplication. *Comp Biochem Physiol A Mol Integr Physiol* 130: 551–564, 2001. doi:10.1016/S1095-6433(01)00435-4.
12. **Cutler CP, Sanders IL, Luke G, Hazon N, Cramb G.** Ion transport in teleosts: identification and expression of ion transporting proteins in branchial and intestinal epithelia of the European eel (*Anguilla anguilla*). In: *Seminar Series. Society For Experimental Biology*, edited by Ennion SJ, Goldspink G. Cambridge, UK: Cambridge University Press, 1996, p. 43–74.

13. **Diamond JM.** The mechanism of isotonic water transport. *J Gen Physiol* 48: 15–42, 1964. doi:10.1085/jgp.48.1.15.
14. **Evans DH.** Teleost fish osmoregulation: what have we learned since August Krogh, Homer Smith, and Ancel Keys. *Am J Physiol Regul Integr Comp Physiol* 295: R704–R713, 2008. doi:10.1152/ajpregu.90337.2008.
15. **Ferreira-Martins D, Coimbra J, Antunes C, Wilson JM.** Effects of salinity on upstream-migrating, spawning sea lamprey, *Petromyzon marinus*. *Conserv Physiol* 4: cov064, 2016. doi:10.1093/conphys/cov064.
16. **Fuentes J, Eddy FB.** Drinking in Atlantic salmon presmolts and smolts in response to growth hormone and salinity. *Comp Biochem Physiol A Physiol* 117: 487–491, 1997. doi:10.1016/S0300-9629(96)00397-0.
17. **Fuentes J, Eddy FB.** Effect of manipulation of the renin-angiotensin system in control of drinking in juvenile Atlantic salmon (*Salmo salar* L) in fresh water and after transfer to sea water. *J Comp Physiol B* 167: 438–443, 1997. doi:10.1007/s003600050094.
18. **Genz J, Esbaugh AJ, Grosell M.** Intestinal transport following transfer to increased salinity in an anadromous fish (*Oncorhynchus mykiss*). *Comp Biochem Physiol A Mol Integr Physiol* 159: 150–158, 2011. doi:10.1016/j.cbpa.2011.02.011.
19. **Genz J, Grosell M.** *Fundulus heteroclitus* acutely transferred from seawater to high salinity require few adjustments to intestinal transport associated with osmoregulation. *Comp Biochem Physiol A Mol Integr Physiol* 160: 156–165, 2011. doi:10.1016/j.cbpa.2011.05.027.
20. **Gregório SF, Carvalho ESM, Encarnação S, Wilson JM, Power DM, Canário AV, Fuentes J.** Adaptation to different salinities exposes functional specialization in the intestine of the sea bream (*Sparus aurata L.*). *J Exp Biol* 216: 470–479, 2013. doi:10.1242/jeb.073742.
21. **Grosell M.** Intestinal anion exchange in marine fish osmoregulation. *J Exp Biol* 209: 2813–2827, 2006. doi:10.1242/jeb.02345.
22. **Grosell M.** The role of the gastrointestinal tract in salt and water balance. In: *Fish Physiology: the multifunctional Gut of Fish*, edited by Grosell M, Farrel SD, Brauner CJ. Amsterdam: Academic, 2011, p. 135–164.
23. **Grosell M, Taylor JR.** Intestinal anion exchange in teleost water balance. *Comp Biochem Physiol A Mol Integr Physiol* 148: 14–22, 2007. doi:10.1016/j.cbpa.2006.10.017.
24. **Grosell M, Wood CM, Wilson RW, Bury NR, Hogstrand C, Rankin C, Jensen FB.** Bicarbonate secretion plays a role in chloride and water absorption of the European flounder intestine. *Am J Physiol Regul Integr Comp Physiol* 288: R936–R946, 2005. doi:10.1152/ajpregu.00684.2003.
25. **Hirano T, Mayer-Gostan N.** Eel esophagus as an osmoregulatory organ. *Proc Natl Acad Sci USA* 73: 1348–1350, 1976. doi:10.1073/pnas.73.4.1348.
26. **Hilliard RW, Bird DJ, Potter IC.** Metamorphic changes in the intestine of three species of lampreys. *J Morphol* 176: 181–196, 1983. doi:10.1002/jmor.1051760207.
27. **Kiela PR, Ghishan FK.** Physiology of intestinal absorption and secretion. *Best Pract Res Clin Gastroenterol* 30: 145–159, 2016. doi:10.1016/j.bpg.2016.02.007.
28. **Madsen SS, Olesen JH, Bedal K, Engelund MB, Velasco-Santamaría YM, Tipsmark CK.** Functional characterization of water transport and cellular localization of three aquaporin paralogs in the salmonid intestine. *Front Physiol* 2: 56, 2011. doi:10.3389/fphys.2011.00056.
29. **Marshall WS, Grosell M.** Ion osmoregulation, and acid-base balance. In: *The Physiolology of Fishes*, edited by Evans D, Claiborne JB. New York: CRC, 2006, p. 179–230.
30. **McCormick SD.** Methods for nonlethals gill biopsy and measurement of Na^+, K^+-ATPase activity. *Can J Aquat Sci* 50: 656–658, 1993. doi:10.1139/f93-075.
31. **McCormick SD, Regish AM, Christensen AK, Björnsson BT.** Differential regulation of sodium-potassium pump isoforms during smolt development and seawater exposure of Atlantic salmon. *J Exp Biol* 216: 1142–1151, 2013. doi:10.1242/jeb.080440.
32. **McGivan JD, Pastor-Anglada M.** Regulatory and molecular aspects of mammalian amino acid transport. *Biochem J* 299: 321–334, 1994. doi:10.1042/bj2990321.
33. **Musch MW, Orellana SA, Kimberg LS, Field M, Halm DR, Krasny EJ Jr, Frizzell RA.** Na^+-K^+-Cl^- co-transport in the intestine of a marine teleost. *Nature* 300: 351–353, 1982. doi:10.1038/300351a0.
34. **Petzel D.** Drinking in Antarctic fishes. *Polar Biol* 28: 763–768, 2005. doi:10.1007/s00300-005-0005-5.
35. **Pickering AD, Morris R.** Localization of ion-transport in the intestine of the migrating river lamprey, *Lampetra fluviatilis L. J Exp Biol* 58: 165–176, 1973.
36. **Pickering AD, Morris R.** Osmoregulation of *Lampetra fluviatilis L.* and *Petromyzon marinus* (Cyclostomata) in hyperosmotic solutions. *J Exp Biol* 53: 231–243, 1970.
37. **Preston GM, Carroll TP, Guggino WB, Agre P.** Appearance of water channels in *Xenopus* oocytes expressing red cell CHIP28 protein. *Science* 256: 385–387, 1992. doi:10.1126/science.256.5055.385.
38. **Rankin JC.** Drinking in hagfishes and lampreys. In: *Symposia of the Society for Experimental Biology*. 2002, p. 1–17.
39. **Reis-Santos P, McCormick SD, Wilson JM.** Ionoregulatory changes during metamorphosis and salinity exposure of juvenile sea lamprey (*Petromyzon marinus L.*). *J Exp Biol* 211: 978–988, 2008. doi:10.1242/jeb.014423.
40. **Renfro JL, Hill LG.** Sodium balance of the Red River pupfish *Cyprinodon rubrofluviatilis*. *Comp Biochem Physiol A Comp Physiol* 44: 1353–1367, 1973. doi:10.1016/0300-9629(73)90275-2.
41. **Ricker WE.** Computation and interpretation of biological statistics of fish populations. *Bull Fish Res Board Can* 191: 1–382, 1975.
42. **Ruhr IM, Bodinier C, Mager EM, Esbaugh AJ, Williams C, Takei Y, Grosell M.** Guanylin peptides regulate electrolyte and fluid transport in the Gulf toadfish (*Opsanus beta*) posterior intestine. *Am J Physiol Regul Integr Comp Physiol* 307: R1167–R1179, 2014. doi:10.1152/ajpregu.00188.2014.
43. **Skadhauge E.** *In vivo* perfusion studies of the cloacal water and electrolyte resorption in the fowl (*Gallus domesticus*). *Comp Biochem Physiol* 23: 483–501, 1967. doi:10.1016/0010-406X(67)90401-X.
44. **Skadhauge E.** The mechanism of salt and water absorption in the intestine of the eel (*Anguilla anguilla*) adapted to waters of various salinities. *J Physiol* 204: 135–158, 1969. doi:10.1113/jphysiol.1969.sp008904.
45. **Skadhauge E.** Coupling of transmural flows of NaCl and water in the intestine of the eel (*Anguilla anguilla*). *J Exp Biol* 60: 535–546, 1974.
46. **Sundell K, Jutfelt F, Agustsson T, Olsen RE, Sandblom E, Hansen T, Björnsson BT.** Intestinal transport mechanisms and plasma cortisol levels during normal and out-of-season parr- smolt transformation of Atlantic salmon, *Salmo salar*. *Aquacult* 222: 265–285, 2003. doi:10.1016/S0044-8486(03)00127-3.
47. **Tresguerres M, Levin LR, Buck J, Grosell M.** Modulation of NaCl absorption by $[HCO_3^-]$ in the marine teleost intestine is mediated by soluble adenylyl cyclasE HCO_3^-. *Am J Physiol Regul Integr Comp Physiol* 299: R62–R71, 2010. doi:10.1152/ajpregu.00761.2009.
48. **Veillette PA, White RJ, Specker JL.** Changes in intestinal fluid transport in Atlantic salmon (Salmo salar L) during parr-smolt transformation. *Fish Physiol Biochem* 12: 193–202, 1993. doi:10.1007/BF00004367.
49. **Veillette PA, Young G.** Tissue culture of sockeye salmon intestine: functional response of Na^+-K^+-ATPase to cortisol. *Am J Physiol Regul Integr Comp Physiol* 288: R1598–R1605, 2005. doi:10.1152/ajpregu.00741.2004.
50. **Whittamore JM.** Osmoregulation and epithelial water transport: lessons from the intestine of marine teleost fish. *J Comp Physiol B* 182: 1–39, 2012. doi:10.1007/s00360-011-0601-3.
51. **Wilson RW, Wilson JM, Grosell M.** Intestinal bicarbonate secretion by marine teleost fish–why and how? *Biochim Biophys Acta Biomembr* 1566: 182–193, 2002.
52. **Wood IS, Trayhurn P.** Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. *Br J Nutr* 89: 3–9, 2003. doi:10.1079/BJN2002763.
53. **Wright EM, Sala-Rabanal M, Loo DDF, Hirayama BA.** Sugar absorption. In: *Physiology of the Gastrointestinal Tract*, edited by Johnson LR. London: Academic, 2012. p. 1583–1593.
54. **Youson JH, Potter IC.** A description of the stages in the metamorphosis of the anadromous sea lamprey, *Petromyzon marinus L. Can J Zool* 57: 1808–1817, 1979. doi:10.1139/z79-235.