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Review

A review of osmoregulation in lamprey

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ABSTRACT

Lamprey are living representatives of the basal vertebrate agnathan lineage. Many lamprey species are anadromous with a complex life cycle that includes metamorphosis from a freshwater (FW) benthic filter-feeding larva into a parasitic juvenile which migrates to seawater (SW) or (in landlocked populations) large bodies of FW. After a juvenile/adult trophic period that can last up to two years, adults return to rivers and migrate upstream to spawn in FW. Therefore, the osmoregulatory challenges anadromous lamprey face during migrations are similar to those of derived diadromous jawed fishes because lamprey osmoregulate to maintain plasma osmolality at approximately one third SW as well. While in FW, lamprey gills actively take up ions and their kidneys excrete excess water to compensate for passive ion loss and water gain. When in SW, lamprey drink SW and their gills actively secrete excess ions (to compensate for salt loading and dehydration). Nevertheless, lampreys diverged from the rest of the vertebrate lineage more than 500 million years ago, which is reflected in similarities and differences in ionocyte (ion transport cell) ultrastructure and distribution as well as tight junctions in epithelia. The current review discusses recent advances in our understanding of ion transport mechanisms of lamprey with a focus on sea lamprey (*Petromyzon marinus*) due to the large literature on this species. We emphasize key molecular and cellular mechanisms in osmoregulatory organs (i.e., gill, kidney and gut) and provide insight relative to what is known in other fishes and identify areas where more research is needed.

Contents

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General introduction

Maintaining a relatively constant intracellular ionic environment is critical to the function of all animals (Bernard and Langley, 1878; Canon, 1929). In order to facilitate this, most vertebrates have adopted an osmoregulatory strategy in which plasma osmolality is maintained at approximately one-third the concentration of seawater (SW). The osmoregulatory strategy likely first evolved in lamprey, as their sister agnathan group the hagfishes are strictly marine and maintain plasma osmotic concentration similar to SW [although they have some ion regulatory capacity; Edwards and Marshall (2013)]. The evolutionary innovation of an osmoregulatory strategy may be one of several reasons for the success of subsequent vertebrate radiations (Betancur-R et al., 2012; Schultz and McCormick, 2013).

In fresh water (FW), osmoregulatory strategies require that fishes counteract the passive loss of ions and gain of water that occurs across body surfaces, especially the gills and skin (Fig. 1A). The basic mechanisms for counteracting these passive forces appear to be similar for lamprey and teleost fishes (Edwards and Marshall, 2013), although we will point out where there are gaps in our knowledge about lamprey osmoregulation. In FW, monovalent ions are acquired by the gills, i.e., actively taken up against a concentration gradient, as well as by the gut which can absorb ions from dietary sources. Meanwhile the kidney mitigates salt loss and excessive hydration by actively reabsorbing ions and producing a highly dilute urine.

In SW, most fishes must counteract the passive loss of water and gain of ions (Fig. 1B). In teleost fishes and lamprey, drinking rates are an order of magnitude greater in SW than in FW (Edwards and Marshall, 2013). Once water is taken in, the esophagus of fishes absorbs ions from the ingested water to reduce luminal osmolality so that it is nearly isosmotic with the plasma, which then allows for uptake of water by the intestine using solute linked water transport (Edwards and Marshall, 2013; Whittamore, 2012). In teleost fishes the intestine becomes highly alkaline through HCO_3^- secretion, resulting in precipitates of divalent ions which further reduces luminal osmolality (Whittamore, 2012). Excess of absorbed monovalent ions (Na^+ and Cl^-) are then actively excreted by the gill, and divalent ions are excreted by the kidney (Edwards and Marshall, 2013). Details of the mechanisms of passive and active transport by the gill, gut, kidney and skin in lamprey osmoregulation in FW and SW are given below.

To date, 41 species of lamprey have been documented worldwide; and, of these, 18 are parasitic at some point during their lifecycle. Only nine of these parasitic forms migrate between FW and SW while the other nine complete their lifecycle in FW (for a more extensive review refer to Potter et al., 2015). Due to the high variety of lamprey species and lack of common studies across multiple species, categorizing lamprey osmoregulatory mechanisms into a single model is challenging. Nevertheless, all species undergo a similar life cycle, and FW and SW osmoregulatory strategies appear

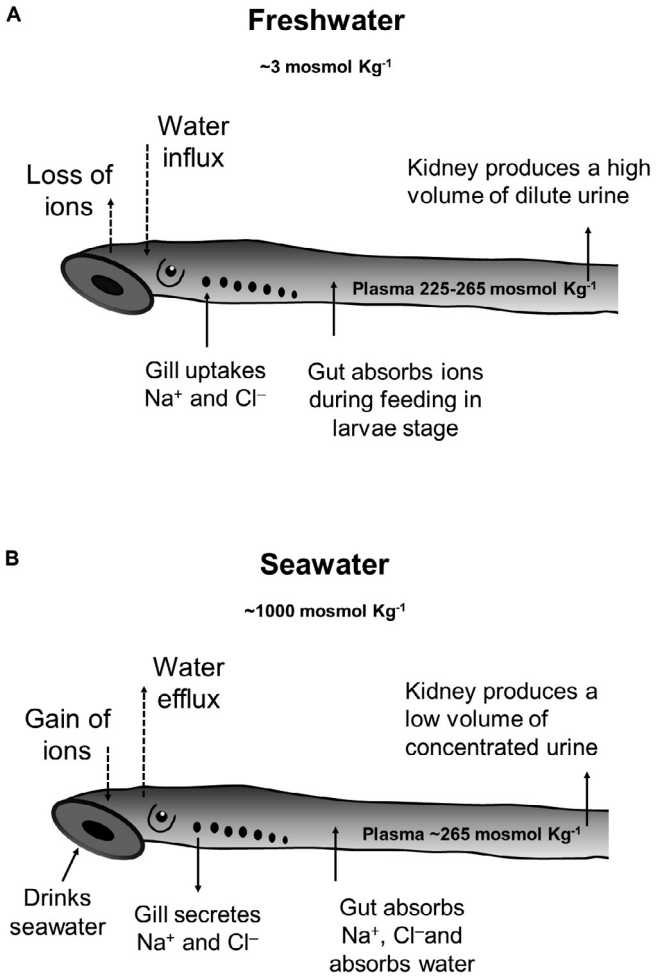

Fig. 1. Lamprey osmoregulatory mechanisms when in A) freshwater; and B) seawater. Dashed lines show passive water and ion fluxes, solid lines the active mechanisms for osmoregulation by the gill, gut and kidney.

to be similar in anadromous species. The current review focuses mainly on sea lamprey *Petromyzon marinus*, native to coastal regions of the northern Atlantic Ocean, which is the most extensively researched species of lamprey.

After a short prolarval phase, anadromous sea lamprey spend their first several years as filter-feeding larvae (also known as ammocoetes) in the sediment of FW streams and rivers. At the larval stage they maintain their internal milieu at $\geq$~225 mosmol kg^{-1} (Beamish et al., 1978; Morris, 1980; Reis-Santos et al., 2008), and they have low tolerance for elevated salinity and typically die if exposed to salinities >8 ppt (Reis-Santos et al., 2008, Shaughnessy and McCormick, 2020). Salinity tolerance increases during the latter stages of metamorphosis, and fully

transformed juveniles can survive direct transfer to SW (35 ppt) with only minor increases in plasma osmolality (Potter and Beamish, 1977; Reis-Santos et al., 2008). Interestingly, plasma osmolality of juveniles in FW is significantly higher than those of FW larvae, perhaps due to morphological changes and properties of tissues during metamorphosis to prepare the juveniles for the higher osmolality they will be experiencing in SW (Reis-Santos et al., 2008). After entering SW, juveniles maintain their internal milieu at ~260 mosmol kg^{-1} (Beamish et al., 1978). Upon return to FW as adults the capacity for osmoregulation in SW is greatly diminished (Ferreira-Martins et al., 2016a; Morris, 1958, 1956; Pickering and Morris, 1970).

Osmoregulation in lamprey was reviewed by Morris (1972), Beamish (1980) and Bartels and Potter (2004). In order to update and build on these earlier contributions, this review will focus on studies conducted in the past 15 years with an emphasis on the use of molecular approaches and complementary biochemical/-physiological studies that have further developed our understanding of ion transport mechanisms and epithelial function in sea lamprey. In this regard, we will consider where mechanisms are conserved, and diverge from those operating within teleost and elasmobranch fishes, as well as identify areas that are especially important and accessible for future research.

Osmoregulatory mechanisms of the gill

Alterations in ionocytes during sea lamprey lifecycle

Although teleost fishes and lampreys are not closely phylogenetically related (Janvier, 1999), the osmoregulatory mechanisms in sea lamprey appear to be analogous to those of teleost fishes, where the gill is a key osmoregulatory organ (Beamish, 1980; Hardisty et al., 1989; Morris, 1972). Even so, differences occur in ultrastructure and in the characteristics and distribution of lamprey epithelial cell types. The sea lamprey gill has several distinct cell types that are involved in ion regulation: larval FW ionocyte (previously designated by Bartels and Potter (2004) as ammocoete mitochondrion-rich cell, AMRC), FW-type ionocytes (also known as intercalated MRCs (IMRCs), SW-type ionocytes (also known as chloride cells) and pavement cells (PCs, which cover the surface of the gill). These cells are comparable to those of teleost fishes, and Bartels and Potter (2004) have suggested a resemblance to ion-transport epithelia of other vertebrates.

In the anadromous ecotype of sea lamprey, changes in the gill epithelia occur during metamorphosis in FW and during the time of migration from FW to SW and vice-versa. These changes include variations in cell type composition, cellular arrangement and the structure of the tight junctions (reviewed by Bartels and Potter, 2004). Bartels and co-workers (1998) identified two types of FW ionocytes in the larvae of sea lamprey: larval FW ionocytes and FW-type ionocytes. As lamprey undergo metamorphosis, the larval FW-type ionocyte disappears and the SW-type ionocytes develop (Peek and Youson, 1979a, 1979b). By the end of the metamorphic stage, the gill epithelia of the postmetamorphic downstream running sea lamprey contains SW-type ionocytes, PCs and FW-type ionocytes. It is not until entrance into SW and switching from hyper- to hypo-osmoregulatory mechanisms that the FW-type ionocytes disappear from the gill epithelia in the juveniles, which then return when the fully-grown adults re-enter FW for their spawning migration (Bartels et al., 1998; Ferreira-Martins et al., 2016a).

In terms of FW-type ionocytes in sea lamprey, the larval FW-type only occurs in the larvae stage, whilst the FW-type ionocyte is present in both FW phases (larval and upstream migrating adult) and is absent during the marine trophic phase. The presence of a FW-type ionocyte in two distinct life stages in FW suggests that it plays a crucial role in active ion uptake. Moreover, the absence of larval FW-type ionocytes in postmetamorphic juveniles in FW suggests that its role must be undertaken by other cell type(s) during the FW phases of post-larval life or has a function unique to the larval stage (Bartels et al., 2009).

As for the SW-type ionocytes in sea lamprey, these appear during metamorphosis and prior to entering SW (Reis-Santos et al., 2008). The presence of a SW-type ionocyte appears to contribute to postmetamorphic juveniles being fully tolerant of a direct transfer to SW (Reis-Santos et al., 2008). Upon completion of their marine trophic phase sea lamprey enter FW systems for spawning, and the SW-type ionocytes have been shown to vanish soon after exposure to FW, but these ionocytes can re-emerge with successful re-acclimation to brackish water (Ferreira-Martins et al., 2016a). Therefore, it is presumed that the SW-type ionocyte in the gill epithelia is involved in active ion secretion.

Finally, the only cell type that is present on the gill surface throughout the entire life cycle of the sea lamprey are the PCs. These cells are squamous which minimizes the diffusion distance for gas exchange (reviewed by Ferreira-Martins and Reis-Santos, 2018). It is hypothesized that PCs play a role in both FW and SW osmoregulatory mechanisms (Bartels and Potter, 2004), though there is currently no evidence for their active role in SW osmoregulation. The presence of PCs with a relatively impermeable apical membrane may help minimize passive osmotic and ionic fluxes across the gill (Bartels and Potter, 2004).

Transcellular ion transport and ion transport proteins in FW

When the sea lamprey is in FW, their internal milieu is hyperosmotic compared to the external environment resulting in a passive osmotic influx of water and an efflux of ions through the gills and skin. In order to maintain their blood osmolality constant, lamprey utilize active ion uptake through their gills (Fig. 2A) (Evans et al., 2005; Hwang et al., 2011; Takei et al., 2014). Although some studies have looked into the function and mechanisms of the sea lamprey gill in FW, most of the mechanisms have been assumed to be analogous to those of teleost fishes and have not yet been characterized and localized in the gill epithelia of lamprey.

Mechanisms for Na^+ uptake in FW fishes are a subject of controversy. Different theoretical and experimental studies with various species have led to various models of Na^+ uptake, which suggests that different species likely resort to different mechanisms (see Parks et al., 2008; Kumai and Perry, 2012 for detailed reviews). In order for Na^+ and Cl^- to be taken up by the gill, different active and passive ion transporters are located in FW-type ionocytes of fishes residing in FW. Na^+ uptake occurs through a mechanism of Na^+/H^+ exchange at the apical surface (reviewed by Wright and Wood, 2009) whereby the H^+ efflux drives Na^+ uptake against its concentration gradient. Two possible mechanisms have been demonstrated in teleost fishes: a Na^+/H^+ exchanger (NHE) protein driven by the H^+ gradient or a Na^+ channel indirectly coupled with a vacuolar-type H^+-ATPase (VHA) that creates the electrochemical gradient for Na^+ uptake (Fig. 2A). H^+-ATPase (e.g., B subunit) resides on the apical surface of sea lamprey larvae and postmetamorphic gill FW-ionocytes, and it has been demonstrated to decrease following exposure to increased environmental salinity indicating a role in branchial ion uptake (Reis-Santos et al., 2008; Sunga et al., 2020). A more recent study reported that VHA is located in the apical membrane region of gill ionocytes of upstream migrating adult lamprey and that its transcript (*atp6v1e*) abundance is greater in FW than in SW (Ferreira-Martins et al., 2016a).

In teleost fishes, there is evidence that acid-sensing channels (ASICs) are a Na^+ channel in FW-type ionocytes (Dymowska

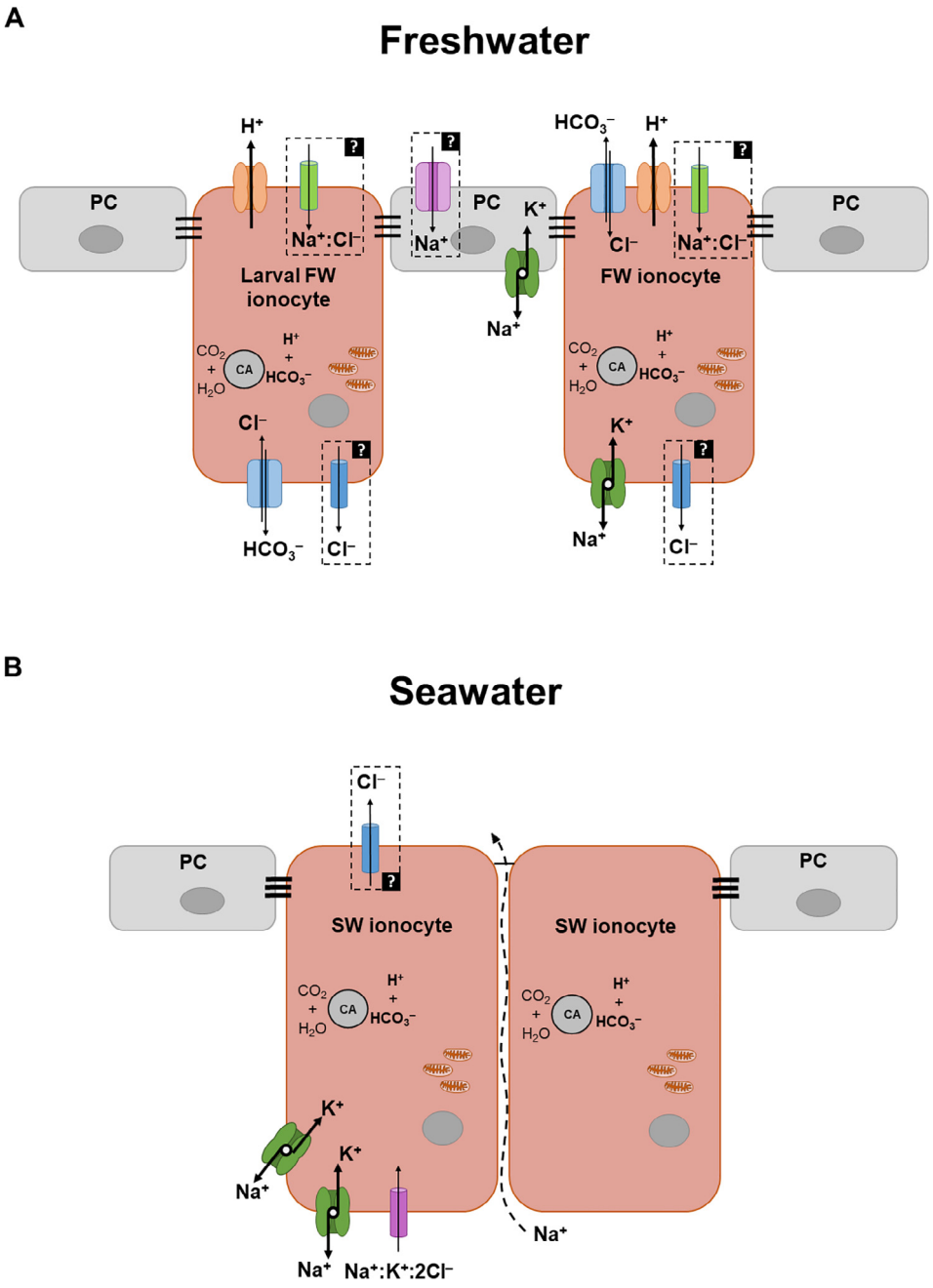

Fig. 2. Proposed branchial model for A) Na^+ and Cl^- uptake in freshwater (FW); and B) Na^+ and Cl^- secretion in seawater (SW). Thicker and slender arrows denote active and secondary transport, respectively. Dashed outline box with question mark "?" in ion transporters represent mechanisms without empirical evidence and hypothesized presence and function. FW larvae ionocyte occurs only in the larvae stage when in freshwater, while the FW ionocyte occurs in both larvae and adults when in freshwater.

et al., 2014). Although absent in ray finned fishes (Actinopterygii), lamprey possess an epithelial Na^+ channel (ENaC), homologous to that which is present in lobed finned fishes and tetrapods (Sarcopterygii) (Hanukoglu and Hanukoglu, 2016). Ferreira-Martins et al. (2016a) found that ENaCa/*scnn1a* mRNA levels decreased after exposure of adult sea lamprey to SW, indicating that ENaC may operate as an epithelial Na^+ channel. Nevertheless, the molecular characterization (gene expression, protein abundance and localization and biochemical properties) of ENaC in the context of the sea lamprey life cycle is still needed to better understand the role of this channel in ion uptake. A model for Na^+ and Cl^- uptake in adult lamprey (Bartels and Potter, 2004) suggests that ENaC is located in the PCs. However, this model is based on studies conducted in amphibians (Ehrenfeld and Klein, 1997; Harvey and Ehrendeld, 1986; Harvey et al., 1988; Nagel and Dörge, 1996) and to date no studies have localized this channel in the sea lamprey gill epithelium. In this same model, it is proposed that a Na^+/K^+-ATPase (NKA) is also required in the basolateral membrane of the PC that contain ENaC. Other studies conducted in sea lamprey demonstrated the presence of NKA in the basolateral membrane of FW ionocytes (Ferreira-Martins et al., 2016a, Reis-Santos et al., 2008). Thus, it is reasonable to hypothesize that Na^+ uptake can occur in this type of ion regulatory cell.

An electroneutral Na^+/H^+ exchanger (NHE) located in the apical region of FW-type ionocytes has been found and characterized in the gill epithelia of zebrafish (*Danio rerio*) (Yan et al., 2007),

Osorezan dace (*Tribolodon hakonensis*) (Hirata et al., 2003), rainbow trout (*Oncorhynchus mykiss*) (Ivanis et al., 2008; Edwards et al., 1999), and tilapia (*Oreochromis mossambicus*) (Wilson et al., 2000a). However, the function of NHEs in a whole cell model is controversial since it is thermodynamically irreconcilable for NHEs to work efficiently in environments where low ion concentrations and environmental pH (e.g., Na^+ <0.1 mM and pH <5, respectively) occur (Parks et al., 2008). More recently, the ammonia transporter rhesus (Rh) glycoprotein has been found in the apical membrane of FW-type ionocytes in teleosts (Nakada et al., 2007a, 2007b; Nawata et al., 2007) that forms a metabolon with NHE2 and 3 (Wright and Wood, 2009), thus offsetting and relieving the thermodynamic limits that a low pH environment implicates. Even so, this model does not resolve the challenges that a low-Na^+ environment poses (Dymowska et al., 2012) and thus more than one Na^+ uptake mechanism may be present in many fishes. To date, no studies have identified and characterized NHEs in lamprey, although Rh glycoproteins have been identified in Arctic lamprey (*Lethenteron camtschaticum*, Suzuki et al., 2014) and in sea lamprey skin (Blair et al., 2017) and gill (Sunga et al., 2020).

Cytosolic carbonic anhydrase (CAc) rapidly catalyzes both hydration and dehydration reactions of CO_2 providing an intracellular pool of H^+ and HCO_3^-, thus providing a multitude of functions in CO_2 transport, ionic and acid–base regulation (reviewed by Gilmour, 2012). Significantly, in the gills of fishes, CAc (*ca17a*) maintain an internal pool of H^+ for both the VHA and NHE (Gilmour, 2012). Two CAc isoforms (*ca18* and *ca19*) have been found and characterized in sea lamprey gill and red-blood cells (Esbaugh and Tufts, 2006; Ferreira-Martins et al., 2016b).

In some teleosts, Cl^- uptake from FW is achieved directly via an apical HCO_3^-/Cl^- exchanger (Bayaa et al., 2009; Perry et al., 2009). In zebrafish, a number of SLC26 anion exchange gene family members (*slc26a3, slc26a4*, and *slc26a6c*) have been implicated in Cl^- uptake (Bayaa et al., 2009; Perry et al., 2009). In FW-acclimated elasmobranchs, *slc26a4* (pendrin) has been identified as the likely anion exchanger (Piermarini et al., 2002). For the exchange to occur CAc plays a key role in providing an internal pool of HCO_3^-, and a Cl^- channel is located in the basolateral membrane (Larsen, 1991). While the exchanger mechanism for Cl^- uptake has been postulated to occur in sea lamprey (Fig. 2A), to date no direct characterization of a Cl^-/HCO_3^- exchanger system has been done in the context of the sea lamprey lifecycle.

Another plausible ion uptake mechanism in sea lamprey is the Na^+:Cl^- cotransporter (NCC/*slc12a3*). Ferreira-Martins and coworkers (2016a) have demonstrated that *slc12a3* together with *scnn1* were downregulated in the gill of sea lamprey after transfer from FW to brackish water. Supporting evidence is found in some teleost fishes (Hiroi et al., 2008; Hiroi and McCormick, 2012) although there is still the question of the mechanism driving this symporter (Parks et al., 2008). Further work is necessary to fully establish the cellular location and expression pattern of gill NCC in ion uptake by lamprey because the monoclonal antibody T4 clone used in teleost fishes is not cross-reactive in lamprey (Shaughnessy and McCormick, 2020). Together, these results indicate that more than one uptake mechanism exists for Na^+ as well as Cl^- uptake in lamprey.

Paracellular ion transport and tight junction (TJ) proteins in FW

The TJ complex regulates paracellular solute movement between cells of vertebrate epithelia (Farquhar and Palade, 1963). The TJ complex consists of (i) transmembrane TJ proteins that span through the cell membrane and occlude the paracellular cleft between adjacent cells, and (ii) cytosolic scaffolding proteins that, amongst other things, connect the transmembrane TJ protein assembly to the cellular cytoskeleton (Furuse et al., 1993, 1998; Ikenouchi et al., 2005; Stevenson et al., 1986). Transmembrane TJ proteins of vertebrates include claudin (Cldn) and occludin (Ocln) proteins associated with the bicellular TJ (bTJ, i.e., regions where two epithelial cells are linked) as well as tricellulin (Tric) and several angulin proteins associated with the tricellular TJ (tTJ, i.e., regions of tricellular contact where three epithelial cells are linked) (Günzel and Fromm, 2012). Compared to terrestrial vertebrates, a multiplied array of TJ proteins have been reported in teleost fishes, which is primarily attributed to the expansion of the Cldn family of proteins in teleosts in association with genome and gene duplication events (e.g. Loh et al., 2004; Mohindra et al., 2019; Sun et al., 2015). Teleost fish genomes studied to date encode more than twice the number of Cldns found in other vertebrate groups (i.e., often >50 in each described fish species). The diversity of TJ proteins has been proposed to have contributed to the success of teleost fishes in diverse aquatic settings (Loh et al., 2004), and studies on the molecular physiology of TJ proteins in teleost fish epithelia (or organs that contribute to homeostasis) typically support this idea (for reviews see Chasiotis et al., 2012; Kolosov et al., 2013). In contrast, little is known about the molecular components of the epithelial TJ complex in other fish groups, including Petromyzontiformes. Nevertheless, alterations in the morphology of the lamprey TJ complex are well documented, particularly in association with environmental change such as alterations in water ion content (Bartels and Potter, 1991, 1993). Despite this, the sea lamprey genome has been found to encode only half of all Cldn orthologues of other vertebrate clades (~1/4 of all teleost Cldns) (Mukendi et al., 2016), but it also encodes an additional Ocln paralogue, Ocln-a (Kolosov et al., 2017b). Recent studies have linked organ-specific alterations in the molecular physiology of the sea lamprey TJ complex of both larvae and transformed animals with iono/osmoregulatory strategies that allow lamprey to maintain salt and water balance in water of differing ionic content (Kolosov et al., 2020, 2017b).

In lamprey, gill epithelium TJ heterogeneity and alterations in TJ morphology in association with changes in water ion composition are well established (Bartels and Potter, 1991, 1993; Mallatt et al., 1995; Peek and Youson, 1979a), which supports the idea that significant alterations in the molecular physiology of the lamprey gill TJ complex most likely occur in association with both environmental change and metamorphosis. In terms of the organ-specific presence of TJ proteins in the branchial tissue of lamprey, recent studies using custom antibodies and immunohistochemical observations reported that the gill of FW *P. marinus* larvae express Ocln in the vasculature and mucous cells, while Ocln-a and Tric reside in the epithelial lining of the gill pouch and the gill epithelium (Kolosov et al., 2017b). Transcripts encoding TJ proteins Cldn-3b, -4, -10, -18 and -19 have also been found in larvae gill where *cldn-3b* is the most abundant transcript and *cldn-10* is the least abundant (Kolosov et al., 2020). A transcript encoding Cldn-14 was not detected in branchial tissue of *P. marinus* larvae (Kolosov et al., 2020). In postmetamorphic juveniles of *P. marinus* residing in FW, the expression patterns (most abundant to least abundant) of genes encoding Cldn TJ proteins in the gill remained the same as those of larvae, with *cldn-14* also being absent (Kolosov et al., 2020). When larvae were acclimated to ion-poor water (IPW), which would require the gill epithelium to mitigate passive ion loss, the increased abundance of Tric/*tric* as well as *cldn-3b, -4, -10* and *-19* have been observed in the gill (Kolosov et al., 2020, 2017b). Furthermore, under the same IPW conditions, a reduction in branchial Ocln/*ocln* was reported (Kolosov et al., 2017b). Using a model primary cultured epithelium, it was suggested by pharmacological and loss of function studies that Tric is a barrier-forming TJ protein in the teleost fish gill (Kolosov and Kelly, 2013, 2018). Therefore, data indicate that lamprey larvae likely restrict passive ion loss across the gill epithelium, at least in part,

by employing barrier forming Tric. It also seems likely that increased abundance of select Cldns will play a role in gill epithelium tightening in larvae as this relationship, via loss of function studies, has also been established for select Cldns in the teleost fish gill (see Kolosov et al., 2017a; Kolosov and Kelly, 2017). An interesting additional idea, however, is that an observed reduction in gill Ocln/*ocln* in IPW-exposed larvae gill (see Kolosov et al., 2017b) may represent an attempt to reduce ion loss by restricting permeability of the gill vasculature.

Transcellular ion transport and ion transport proteins in SW

In SW, the gill ionocytes of sea lamprey are salt secretory cells that actively secrete excess monovalent ions such as Na^+ and Cl^- (Fig. 2B). The SW-type ionocyte is characterized by an abundance of mitochondria and the presence of an internal tubular system (Bartels and Potter, 2004; Hiroi and McCormick, 2012) that greatly amplifies the surface of the basolateral membrane. In teleost fishes, the basolateral membrane is rich in NKA and Na^+:K^+:2Cl^- cotransporter (NKCC) (Eriksson et al., 1985; Karnaky et al., 1976; Karnaky, 1980, 1986). The NKA moves Na^+ into the intercellular space which generates a gradient that provides the driving force for Cl^- movement via NKCC into the cell through the basolateral membrane. The accumulation of intracellular Cl^- then facilitates Cl^- exit into a cup-shaped apical crypt by passive diffusion via an apical cystic fibrosis transmembrane conductance regulator (CFTR; Marshall, 2002; Singer et al., 1998; Wilson et al., 2000b) chloride anion channel. Finally, the Cl^- movement is coupled with paracellular Na^+ secretion down its electrochemical gradient into the same apical crypt microenvironment via selectively permeable, shallow TJs that reside between teleost ionocytes and adjacent accessory cells (ACs) (Bartels and Potter, 2004). Because CFTR supports salt secretion from the rectal gland of elasmobranchs (Forrest, 2016) to the nasal glands of sea birds (Hildebrandt, 2001) it has been widely assumed to be conserved in the vertebrate lineage with a function associated with salt secretion. CFTR has recently been identified in the genome of sea lamprey and the Japanese lamprey (*Lampetra japonica*) using an RNA-Seq approach (Ren et al., 2015). However, no clear role in ion secretion in the gill epithelia of lamprey has yet been established and no studies have addressed if CFTR in the sea lamprey is even localized to SW-type ionocyte.

In sea lamprey gill, NKA transcript, protein abundance and activity are high (Reis-Santos et al., 2008). NKA has also been immunolocalized to the tubular system of the SW-type ionocytes (Ferreira-Martins et al., 2016a; Reis-Santos et al., 2008), which suggests a role for NKA in the extracellular accumulation of Na^+ for paracellular secretion though the leaky pathway between SW-type ionocytes (see next section). In addition to NKA, Ferreira-Martins et al. (2016a) observed that *nkcc1* mRNA abundance increases in the gill after transfer of adult sea lamprey from FW to 25 ppt brackish water which suggests that NKCC also plays an important role in Cl^- secretion by the gill epithelia of sea lamprey. In a more recent study, NKCC1 has been fully sequenced and characterized in the context of the sea lamprey life cycle, where it has been shown to be localized to SW-type ionocytes and increase in abundance late in metamorphosis and further increase upon acclimation of postmetamorphic juveniles to SW (Shaughnessy and McCormick, 2020).

Paracellular ion transport and tight junction (TJ) proteins in SW

Juveniles of *P. marinus* acclimated to a hyperosmotic environment (60% SW; 21 ppt) exhibit reduced mRNA abundance of *cldn-3b* and *-10* in the gill (Kolosov et al., 2020). As in larvae, *cldn-3b* is the most abundant *cldn* transcript in the gill of transformed *P. marinus* (Kolosov et al., 2020). Reduced gill *cldn-3a* and *-3c* transcript abundance in association with acclimation to hyperosmotic conditions has been reported in more derived euryhaline teleosts such as the puffer fishes *Tetraodon nigroviridis* and *T. biocellatus*, but curiously *cldn-3b* is not present in the gill of these animals (Bagherie-Lachidan et al., 2008; Duffy et al., 2011). Nevertheless, phylogenetic analysis suggests shared identity between *P. marinus cldn-3b* and mammalian *cldn-3*, the latter of which encodes a barrier forming TJ protein. In addition, through whole-genome and tandem gene duplication events in teleosts, ancestral *cldn-3* and *-4* gave rise to over 17 different *cldns* including several isoforms of *cldn-27, -28, -29* and *-30* specific to the teleost lineage (Loh et al., 2004). A number of these *cldns* have been suggested to play a barrier forming role in the gill epithelium (Kolosov et al., 2013). Therefore, changes in *cldn-3b* would suggest a leakier gill in *P. marinus* acclimated to hyperosmotic conditions. Reduced abundance of a barrier-forming TJ protein would be in line with morphological observations of the gill in SW-acclimated young adults of the anadromous lamprey *Geotria australis*, where shallow TJs, that are not present in FW, link adjacent ionocytes (Bartels and Potter, 1991). Alterations in *cldn-10* in the gill are of special significance. Phylogenetic analysis indicates that the closest ortholog of sea lamprey *cldn-10* in the teleost lineage is *cldn-10a* (Baltzegar et al., 2013; Loh et al., 2004). In teleosts *cldn-10* radiated into five forms, with *cldn-10a* being a pseudogene in more than one species. Interestingly, other Cldn-10 TJ proteins in teleosts, in particular Cldn-10d and -10e, have been reported to be either enriched or to exclusively occur within ionocytes in the gill epithelium (Bui et al., 2010; Bui and Kelly, 2014; Kolosov et al., 2014). In hyperosmotic conditions, teleost Cldn-10 TJ proteins in the gill increase in abundance and all evidence suggests that they play a role in facilitating Na^+ secretion under hyperosmotic conditions (Bui et al., 2010; Bui and Kelly, 2014; Kolosov et al., 2014; Marshall et al., 2018; Tipsmark et al., 2008). In contrast, observations of reduced lamprey gill *cldn-10* abundance in hyperosmotic conditions coupled with increased *cldn-10* abundance in IPW-acclimated larvae gill suggests a barrier-forming role for lamprey cldn-10 and shared ancestry with teleost *cldn-10a* (currently a pseudogene).

Osmoregulatory mechanisms of the kidney

Lamprey kidney morphology

In the lamprey prolarvae, the pronephric kidney in the pericardial region of the body is believed to be the renal excretory organ until the opisthonephric kidney develops around the time of the transition to the burrowing larvae (Youson 1981b). In sea lamprey the pronephric kidney persists in a degenerate form after metamorphosis, lacking tubules (Ellis and Youson, 1989). The paired, elongate strap-like opisthonephric kidneys in lamprey are attached to the roof of the distal half of the coelomic cavity and show life-stage dependent differences. The larval kidney degenerates during metamorphosis and is replaced by the 'adult' or definitive kidney (Ooi and Youson, 1979). The morphology of the adult kidney is highly ordered with similarities to other vertebrates but also some unusual differences. A notable difference is the compound glomerulus, or glomus, that runs the length of each kidney and the lack of typical Bowman's capsules (Youson and McMillan, 1970a). In the case of larvae, the glomus is discontinuous. Other vertebrates have an individual glomerulus and Bowman's capsule to provide filtrate for each nephron. However, in adult lamprey the nephrons that receive the filtrate from the glomus have the capsule subdivided into separate individual urinary spaces (Logan et al., 1980b; Miyoshi et al., 1978). The blood supply to the glomus is from numerous renal arteries via shared short affer-

ent arterioles. Blood leaves the glomus via efferent arterioles to supply the tubules via shared capillaries and sinuses. The peritubular circulation of lamprey is lacking a renal portal system seen in teleost fishes and other vertebrates except for mammals (Logan et al., 1980b; Youson and McMillan, 1971d). The nephron or tubule is composed of five regions: neck, proximal, intermediate, distal and collecting segments (Youson, 1981b). The proximal and distal tubules are also subdivided into convoluted and straight regions. The neck is short and ciliated, which helps draw filtrate into the proximal tubule that is long and distinguished by a well-developed brush border and is followed by a short intermediate segment that unlike in teleosts is not ciliated (Youson and McMillan, 1970b). In the adult kidney, the distal tubule forms a distinctive hairpin loop and is composed of mitochondrion-rich cells having an intracellular tubular system continuous with the basolateral membrane similar to the tubular system of branchial ionocytes in teleost fishes and SW-type in lamprey and have been described as renal 'chloride' cells (Youson and McMillan, 1971b), likely playing a role in urine formation. Distal tubules drain into shared collecting tubules and ducts, that are lined by columnar cells lacking the intracellular tubular system. Finally, the tubular fluid passes to the large archinephric duct (ureter) that runs the length of each kidney along the ventral margin for storage and elimination as urine, since lamprey lack a urinary bladder. The archinephric duct is lined with mucous secretory cells and is contractile to aid in the expulsion of urine (Youson and McMillan, 1971c).

Urine is produced from glomerular filtration at the glomus, which is modified by the reabsorption and/or secretion of water and ions by the different regions of the nephron. The glomus design and blood flow patterns mean that lamprey lack glomerular intermittence or recruitment for regulating glomerular filtration rate (GFR), which also correlates with the absence of a renal portal system (Logan et al., 1980b; Youson and McMillan, 1971d). The portal system otherwise allows peritubular blood flow so tubular secretion/reabsorption can continue when glomerular perfusion is reduced or shutdown and thus the post-glomerular blood flow to the tubules.

TJs play an important role in vertebrate kidney function by contributing to spatial differences in nephron permeability. In this regard, TJ morphology varies regionally along the vertebrate nephron as does the presence and/or abundance of TJ proteins (Günzel and Yu, 2013). Ultrastructural differences in TJ morphology also occur along the nephron of the lamprey and morphological alterations occur in association with metamorphosis (Youson and Ogilvie, 1990). Youson and Ogilvie (1990) describe a general decrease in proximal tubule TJ (zonulae occludentes) strand number and apical-basal depth as sea lampreys progress through metamorphosis, concluding that at stage 7 of metamorphosis, junctions of lamprey proximal tubules may be less tight, which is consistent with the properties of this nephron region in other vertebrates. In contrast, from stage 4 in metamorphosis the distal segment of the nephron has two cell populations (type I and II) that exhibit different junction properties. Type I cells possess fewer TJ strands and a shorter apical-basal depth and do not exhibit alterations during the remaining stages of metamorphosis, whereas type II cells exhibit a modest (but significant) increase in TJ strand number (Youson and Ogilvie, 1990). These changes reflect progressive regional specialization of the nephron in association with metamorphosis. The result of this would be a functional renal unit that is capable of contributing to osmoregulatory homeostasis under more challenging physiological circumstances such as those associated with changes in environmental activity, diet and/or size. Despite the fact that region-specific presence and abundance of TJ proteins in the mammalian nephron is well documented, very little is known about the distribution of TJ proteins along the nephron of fishes. We do know that it occurs (Chasiotis and Kelly, 2008; Kwong et al., 2013) and that salinity-induced alterations in fish nephron morphometrics and nephron region TJ depth match up well with changes in the transcript abundance of select TJ proteins (Duffy et al., 2011). However, most of our current knowledge on the molecular physiology of TJ proteins in fish kidney and how they might play a role in the regulation of salt and water balance is based on studies using the whole organ (Kolosov et al., 2013).

Ion transport and ion transport proteins in FW

In FW adapted lamprey, a large volume of highly dilute urine is formed by the kidneys. The GFR and urinary flow rate (UFR) are significantly higher than in FW teleost fishes (Hickman and Trump, 1969), which was necessitated by the lamprey's higher water permeability of the body surfaces (Rankin, 2002). *In situ* nephron micropuncture studies revealed that approximately 40% of filtered water is reabsorbed and 90% of filtered Na^+, Cl^- and K^+ is reabsorbed primarily along the distal tubule and collecting ducts (Logan et al., 1980a). The abundance of ionocytes in the distal tubule was linked to ion transport and significantly higher NKA expression (activity and α subunit protein) in FW acclimated adults of sea lamprey (Ferreira-Martins et al., 2016a). Ferreira-Martins et al. (2016a) reported that *scnn1* and *ncc* mRNA expression were also higher in FW providing potential mechanisms for Na^+ and Cl^- reabsorption. Shakhmatova (1977) found that furosemide, an NKCC inhibitor, depressed Na^+ reabsorption and NKCC1 mRNA has been detected in the kidney although salinity did not alter expression levels (Ferreira-Martins et al., 2016a). In the mammalian collecting duct, NKCC1 is found in collecting duct intercalated cells and is involved in H^+, NH_4^+, and Na^+ fluxes (Wall and Fischer, 2002). The VHA E subunit mRNA was also detected in kidney but was not responsive to salinity either. In teleost fishes, renal VHA has a role in acid-base regulation and may aid in Na^+ reabsorption through indirect uptake via the ENaC (Perry et al., 2003). The urine that is produced has 10% the osmolality of plasma allowing the maintenance of body fluid osmolality, with the gills compensating for the loss. Physiological data on larvae are lacking.

The kidney of larvae was found to express Ocln, Ocln-a, and Tric (Kolosov et al., 2017b). Indeed, transcript abundance of *ocln* and *tric* was greatest in the kidney of larvae versus all other organs examined (Kolosov et al., 2017b). A higher transcript abundance of *ocln* and *tric* would suggest that these TJ proteins play an important role in larval kidney function which is consistent with an increase in *ocln*/Ocln abundance following acclimation of larvae to IPW (Kolosov et al., 2017b). Tric abundance did not alter following IPW acclimation of larvae (Kolosov et al., 2017b), but it does remain abundant following metamorphosis (Kolosov and Kelly, unpublished observations). In the larval kidney the pattern of *cldn* transcript abundance was found to be similar to the gill (i.e., *cldn-3b* > *-10* > *-19* > *-18* > *-4* > -14) except that *cldn-10* is much more abundant and *cldn-14* is present (Kolosov et al., 2020). In juveniles, *cldn-19* became the second most abundant transcript, but otherwise kidney *cldn* expression followed the same pattern between FW-dwelling larvae and juveniles. Higher relative *cldn-10* abundance in the kidney compared to other tissues suggests that *cldn* may be functionally similar to *cldn-10b* in teleosts, which is typically associated with the kidney (Baltzegar et al., 2013; Kolosov et al., 2013; Loh et al., 2004). In IPW-acclimated larvae, Ocln-a abundance decreased as did transcript abundance of *cldn-4, -14* and *-18* (Kolosov et al., 2020, 2017b), that seems to be different from teleosts (at least those studied) where acclimation to IPW was typically associated with an increase in *cldn* mRNA abundance (Duffy et al., 2011). However, exceptions did occur, such as *T. biocellatus cldn-3b* which decreased in renal tissue following acclimation to IPW. However, this view may be limited by the few *cldns*

studied to date in other species as well as the limited number of species studied. The observations to date suggest that modulation of Ocln as well as Cldn-4, -14 and -18 may be important for the production of dilute urine in FW-dwelling larvae.

Ion transport and ion transport proteins in SW

Because water conservation becomes a major consideration at higher salinities as urine production contributes to water loss, GFR and consequently UFR negatively correlate with environmental salinity (Rankin, 1997). As mentioned earlier glomerular intermittency (recruitment) has little or no importance in lamprey (Brown and Rankin, 1999) and the reduced GFR results from a decrease in individual nephron filtration. However, tubular water reabsorption (90% of filtrate water) accounts for the majority of the significant 94% reduction in UFR in SW lamprey compared to their FW counterparts (Logan et al., 1980c).

A noticeable difference between larval and definitive kidneys, is the greater degree of regionalization found in the latter (Youson and McMillan 1971a, 1971b). In the definitive kidney, part of the straight proximal tubule, the intermediate segment and hairpin loop formed by the straight distal tubule run parallel to collecting tubules and ducts in the ventral zone of the kidney (Youson and McMillan, 1971b). There is also an apparent counter current blood and fluid flow for at least part of their length (Logan et al., 1980b; Youson and McMillan, 1971b). Such an arrangement is reminiscent of the loop of Henle, collecting duct and vasa recta arrangement in the medulla of the kidneys of birds and mammals which are capable of producing concentrated urine (Hentschel and Elger, 1987). In SW-acclimated animals, Youson (1982) observed a dilation of the tubules in this ventral region with an elongation in the intermediate segment and flattening of the tubular epithelial cells to take on a more squamous appearance. The larval kidney lacks this loop and parallel arrangement of tubular elements, which may reflect why it is FW stenohaline (Youson and McMillan, 1971a). The persistence of the loop structure in the definitive kidney of the landlocked lamprey is interpreted as a reflection of the relatively recent FW invasion (Youson, 1981b).

Logan et al. (1980c) demonstrated in the anadromous river lamprey (*L. fluviatilis*) that SW-acclimated adults were capable of producing hyperosmotic urine (389 mOsm urine vs. 309 mOsm plasma). The urine had higher [Cl^-], [Mg^{2+}], [Ca^{2+}] and [SO_4^{2+}], although [Na^+] was lower reflecting a need for electroneutrality. These results were largely confirmed in a follow up study (Rankin, 1997). The role of the kidney in divalent ion secretion is similar to marine teleost fishes; however, the production of a hyperosmotic urine is unique among fishes. *In situ* nephron micropuncture studies revealed a high water reabsorption rate (89%) that was largely accounted for by the distal tubule (Logan et al., 1980c). Using the same technique Rankin (1980) showed that magnesium secretion occurs in the proximal tubule; however, the very low GFR and significant water reabsorption in the distal tube and collecting duct present a technical limitation for fluid collection that has precluded the investigation of the site of urine concentration and the role of the loop (Rankin, 1997). The urine precipitates a chalky material (Youson, 1982) in the archinephric duct fluid of SW-adapted sea lamprey that has tentatively been identified as $CaPO_4$, but the duct itself does not appear to be involved in modifying the ductal fluid (Rankin, 1997).

Relatively little is known about ion transport proteins in the kidney of SW-acclimated lamprey. However, in recent studies in juveniles, Tric abundance was elevated in response to hyperosmotic conditions (Kolosov and Kelly, unpublished observations) but the only kidney *cldn* response to hyperosmotic conditions was a decrease in *cldn-3b* (Kolosov et al., 2020). It is hypothesized that the decreased abundance of Cldn-3b, and increased abundance of Tric, may underlie divalent cation excretion in the kidney of juveniles in SW.

Osmoregulatory mechanisms of the gut

The importance of the intestine to osmoregulation has been clearly established in teleost fishes (Grosell, 2013). Although secondary to active ion uptake by the gill in FW, uptake of ions from dietary sources can play a significant role in ion balance of feeding fishes (Wood and Bucking, 2010). To date, a role of the gut in ion uptake by FW lamprey remains largely unexamined as well as contributions from ingested water during feeding.

A role of the gut in SW osmoregulation of lamprey has received more attention. As in teleost fishes, the drinking rate of lamprey in SW is substantially higher when compared to sea lamprey in FW. Rankin (2002) found that drinking rate increased by 3-fold in adult sea lamprey exposed to 50% SW, which was the highest salinity they could tolerate at that stage. However, drinking rate of recently metamorphosed juveniles increased following exposure to increased salinity; at 10 ppt their drinking rate was 4-fold higher than in FW, and 26-fold higher at 35 ppt SW (Barany et al., 2020).

Several investigators have observed changes in the morphology of the gut during lamprey metamorphosis (see Youson, 1981a for review). Mitochondrion-rich cells with a smooth tubular network appear in the anterior sections of the intestine in mid-metamorphosis and become more abundant in late metamorphic stages (Youson and Horbert, 1982). It is unclear if these changes relate to the osmoregulatory changes that occur during metamorphosis or the anticipated change in diet that comes with a parasitic mode of feeding. To our knowledge, there are no extensive published studies on the impact of salinity itself on gut morphology. In an informal analysis of impacts of SW exposure on intestinal morphology of recently postmetamorphic juveniles, Youson (1981a) found no substantial changes in gross morphology, but did observe changes in the cells of the anterior and posterior intestine, including enlarged and spherical mitochondria, greater vacuolation and a more extensive network of smooth endoplasmic reticulum, all of which are hallmarks of greater ion regulatory activity. Heinig (1993) used transmission and scanning electron microscopy to find morphological changes in absorptive cells from the anterior intestine which included the formation of apical blebs in anadromous sea lamprey exposed to SW. Freeze-fracture observations also revealed a decrease in apical-basal depth, strand number, and alterations in the morphology of cellular junctions in sea lamprey acclimated to full strength SW (Heinig, 1993).

Direct physiological studies on gut osmoregulatory function in newly metamorphosed sea lamprey exposed to SW have recently been conducted by Barany et al. (2020). They demonstrated both ion and water uptake in isolated (ex vivo) sections of the intestine, at similar or slightly higher rates than have been shown for teleost fishes. Also, these ion and water uptake rates are higher in SW-acclimated postmetamorphic juveniles compared to those of adult sea lamprey in 50% SW (Pickering and Morris, 1973). Differences between the two studies are perhaps due to the lower salinity used and the fact that adults have a more limited capacity for osmoregulation in SW. Both Pickering and Morris (1973) and Barany et al. (2020) observed a greater maximum ion and water uptake capacity in the anterior than posterior intestine, suggesting regionalization of ion and water uptake within the gut, similar to observations in many teleost fishes (Grosell, 2013).

An important driver in ion and nutrient coupled transport is the NKA which has a basolateral distribution in columnar enterocytes (Wilson and Castro, 2010). Studies in estuarine upstream migrating sea lamprey showed that NKA activity and protein and mRNA expression levels are at their highest in the anterior section of the intestine compared to middle and posterior regions

(Ferreira-Martins et al., 2016a), which is consistent with the importance of this region in transport in teleost fishes (Grosell, 2013). In sea lamprey capable of hypo-osmoregulation, anterior intestinal NKA activity was stable, but in sea lamprey that were osmocompromised, activity levels decreased. In adult lamprey, after long term acclimation to FW, NKA activity was lowered as well as its protein levels (Ferreira-Martins et al., 2016a), which might be due to the senescence of the gut during sexual maturation. During sea lamprey metamorphosis there are large (20-fold) increases in intestinal NKA activity that correlate with the development of SW tolerance (Barany et al., 2020). Intestinal NKA activity is greater in the anterior relative to posterior intestine, the former also having the greater maximum ion and water uptake capacity. Exposure to SW results in further (though moderate) increases in intestinal NKA activity. Exposure of adult lamprey to 17.5 and 25 ppt brackish water resulted in increases in anterior intestinal NKA mRNA levels (Ferreira-Martins et al., 2016a). Exposure of isolated (*ex vivo*) sections of the anterior intestine to ouabain, a specific inhibitor of NKA, resulted in significant decreases in Na^+, Cl^- and water uptake (Barany et al., 2020; Pickering and Morris, 1973). These findings support the idea that processes of ion and water absorption are coupled in sea lamprey, and that a major driving force for water absorption across the intestinal epithelium is NKA driven ion uptake.

The VHA has an apical localization (VHA B subunit) in intestinal ciliated cells (Wilson and Castro, 2010). The intestinal VHA E subunit (*atp6v1e*) mRNA level was found to be lower than those in gill and kidney and unchanged after transfer to a hyperosmotic environment, suggesting it may have a role in nutrient uptake rather than osmoregulation. As for passive ion transporters in FW, NKCC1 and NCC were found at lower expression levels at the mRNA level compared to gill and unaltered after transfer to a hyperosmotic environment (Ferreira-Martins et al., 2016a).

Changes in intestinal TJ proteins following metamorphosis and acclimation to 60% SW suggests active regulation of intestinal permeability. The intestine of FW larvae expresses *ocln, ocln-a* and *tric*, as well as several *cldns* (Kolosov et al., 2017b, 2020). Larval intestine exhibited an approximate *3b ~ 19 > 18 ~ 4 > 14 > 10 cldn* expression pattern. In response to IPW acclimation, mRNA abundance of *cldn-3b, -4, 10* and *-19* increased, while *cldn-14* decreased. In contrast, intestine of juveniles demonstrated a much higher expression of *cldn-19* compared to other *cldns* and no detectable levels of *cldn-10*, resulting in a *19 > 3b > 18 > 14 > 4* abundance pattern. In response to 60% SW acclimation, transcript abundance of *cldn-3b* and *-14* decreased, while transcript abundance of *cldn-18* increased in the intestine of juveniles (Kolosov et al., 2020).

Increased drinking in SW, active uptake of ions and absorption of water by the intestine, and alteration of permeability by salinity provide evidence this is a conserved strategy among most fishes with an osmoregulatory strategy (Grosell, 2013), with the exception of elasmobranchs that utilize a different strategy. Further parallels can be drawn between the development of salinity tolerance during metamorphosis of anadromous sea lamprey and smolt development of anadromous salmonids (McCormick, 2013). Both appear to involve increased abundance and activity of gill and intestinal NKA (and other hyperosmoregulatory mechanisms) that are likely to be preparatory for SW entry. Such preparatory changes allow for high ion regulatory capacity, allowing rapid movement and high survival during the FW-SW transition.

There are several areas ripe for investigation of intestinal functions in lamprey. As noted above, there is currently no direct evidence for the capacity of the gut to take up ions in FW. The esophagus is important for initial desalination of imbibed SW in teleosts (Grosell, 2013) but little analogous information is available for lamprey. Precipitates in the gut of SW lamprey have been observed (Barany et al., 2020), but their composition and the role of alkalinisation in their formation has not been established. The specific transporters involved in initial apical movement of Na^+ and Cl^- across the gut in SW (and FW) have also not yet been established.

Osmoregulatory mechanisms of the skin

The skin is commonly the largest organ in vertebrates and provides a barrier that regulates passive paracelullar solute movement between the environment and the animal. While recent studies have reported on molecular endpoints that may reflect this barrier role, to date no studies have demonstrated an active role for sea lamprey skin in either ion acquisition or secretion.

The skin of FW larvae expresses Ocln, Ocln-a and Tric, as well as several *cldns* (Kolosov et al., 2020, 2017b). Interestingly, the relative abundance of the TJ makeup in the skin in larvae and juveniles was strikingly similar to that of the gill, highlighting the barrier function of both tissues, where *cldn* abundance followed the *3b > 19 > 18 > 4 > 10* pattern. However, alterations in skin TJ mRNA and protein abundance in animals acclimated to IPW and SW were quite different from the changes observed in the gill. Transcript abundance of *ocln* and *cldn-10*, as well as protein abundance of Tric increased in the skin of IPW-acclimated larvae, while *cldn-3b, -4, -10* and *-19* decreased in abundance in the skin of SW-acclimated juveniles. The observed change in transcript abundance of *ocln* and *cldn-10* and increase in the protein abundance of Tric in the skin suggests that Ocln, Tric and Cldn-10 may play an important role in restricting passive paracellular ion loss through larvae skin.

Rhesus (Rh) glycoproteins play a role in ammonia secretion in fish and recent studies have indicated that they also play a role on ion transport (Wright and Wood, 2009). In sea lamprey, Rh glycoprotein isoforms Rhbg, Rhcg1, and Rhcg2 have been detected in all stages of sea lamprey's lifecycle and differentially expressed in distinct life stages and play a role on ammonia excretion (Blair et al., 2017). Nevertheless, more studies on Rh glycoproteins are needed in sea lamprey to assess their role in ion regulation.

Hormonal control of lamprey osmoregulation

Corticosteroid hormones play a key role in the control of osmoregulatory mechanisms and their development in addition to the control of metabolism and the stress response (Mommsen et al., 1999). Due to the diversity and abundance of teleost species it is no surprise that most studies on the corticosteroid control of osmoregulation in fishes have been performed in this group. In teleosts, cortisol is the main active corticosteroid, known to have both glucocorticoid and mineralocorticoid actions, and to promote SW adaptation (McCormick, 2001). However, lamprey lack the enzyme 11β-hydroxylase which converts 11-deoxycortisol to cortisol, and Close and co-workers (2010) identified 11-deoxycortisol as the main corticosteroid in sea lamprey. Moreover 11-deoxycortisol has a direct action on the upregulation of NKA (Shaughnessy and McCormick, 2020), an enzyme that has been shown to be directly correlated with the development of SW tolerance in sea lamprey (Ferreira-Martins et al., 2016a; Reis-Santos et al., 2008).

Other hormones such as prolactin and growth hormone/insulin-like growth factor I have been described as FW- and SW-adapting hormones in teleosts (Takei and McCormick, 2013). Nevertheless, to date prolactin has not been identified or characterized in lamprey; and, although growth hormone has been fully cloned and molecularly characterized (Kawauchi et al., 2002), it has not been

described in the context of the sea lamprey's life cycle and promotion of SW acclimation. In fast acting hormones, the renin-angiotensin system (RAS) plays an important role in salt and water balance control and produces an antidiuretic effect decreasing urinary flow rate (UFR) in river lamprey as it does in most other vertebrates (Cobb et al., 2010). A decrease in UFR is associated with the production of a low volume of highly concentrated urine as water is reabsorbed by the kidneys to counteract an increase of internal osmotic pressure (Ferreira-Martins and Reis-Santos, 2018 for a more complete review).

Remarks and future directions

A substantial amount of effort has been made to improve our understanding of sea lamprey osmoregulation in the past decade. The availability of sea lamprey and Arctic lamprey annotated genomes has allowed for a significant advancement of the characterization of key ion regulatory mechanisms from a molecular perspective. Nonetheless, the details of the function and cellular localization of most of these mechanisms have yet to be determined.

On the western coast of Europe and eastern coast of North America, anadromous populations of sea lamprey are listed as threatened or endangered mainly due to barriers to their upstream spawning migration and overfishing (Hume et al., 2021). On the other hand, in the Great Lakes in North America, every year millions of dollars are spent to control landlocked populations that threaten recreational and commercial fish stocks (Brant, 2019). Most of the control of these landlocked populations of sea lamprey have been achieved through the use of chemicals and barriers to migration, which have been shown to be enormously effective, but still cannot overcome the challenge of a total eradication of this species. In order to complement these control strategies, new approaches have been proposed such as sex bias and sterilization to more ground-breaking and controversial genetic manipulations (Ferreira-Martins et al., 2021; York et al., 2021). However, we have learned that no natural system is completely closed, and methods used for the control of landlocked populations pose a risk to the conservation efforts being conducted for the anadromous ecotype. In order to minimize these risks, "security triggers" have been suggested to be applied, and osmoregulatory mechanisms such as complete loss of salinity tolerance that would confine genetically modified lamprey to FW have a promising potential in this regard (Ferreira-Martins et al., 2021). If such genetic control options are even to be considered, there is a need to expand our understanding of the basic biology of sea lamprey, including its physiology, to assure a safe and successful management of this species. The origin of sea lamprey in some of the Great Lakes in North America is still a subject of debate. Inter-population of sea lamprey physiological studies, particularly in the field of osmoregulation, could prove a useful tool to assess the evolutionary differences at the functional level thus complementing molecular evolutionary studies. Genetic based studies using two types of neutral genetic markers provided evidence that the sea lamprey is native to Lake Ontario and Lake Champlain (Bryan et al., 2005); however, historical and ecological information questions the native status of lamprey in these two lakes (Eshenroder et al., 2009). In landlocked populations of species that are traditionally anadromous such as salmon and alewife, a decrease in the ion secretory capacity which translates into lower salinity tolerance is observable after hundreds to thousands of years of isolation (Nilsen et al., 2007; Velotta et al., 2014). If the native scenario of sea lamprey to some of the Great Lakes in North America is correct, then similar loss of SW tolerance of these populations would be expected, but to date this remains largely unexamined.

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.

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