8-2015

Water Transport and the Functional Dynamics of Aquaporins in Osmoregulatory Organs of Fishes

Steffen S. Madsen
University of Southern Denmark, steffen@biology.sdu.dk

Morton B. Engelund
University of Southern Denmark

Christopher P. Cutler
Georgia Southern University, ccutler@georgiasouthern.edu

Reference: *Biol. Bull.* **229:** 70–92. (August 2015)

Water Transport and Functional Dynamics of Aquaporins in Osmoregulatory Organs of Fishes

STEFFEN S. MADSEN[1,*], MORTEN B. ENGELUND[1], AND CHRISTOPHER P. CUTLER[2]

[1]Department of Biology, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark; [2]Department of Biology, Georgia Southern University, P.O. Box 8042, Statesboro, Georgia 30460

Abstract. Aquaporins play distinct roles for water transport in fishes as they do in mammals—both at the cellular, organ, and organismal levels. However, with over 32,000 known species of fishes inhabiting almost every aquatic environment, from tidal pools, small mountain streams, to the oceans and extreme salty desert lakes, the challenge to obtain consensus as well as specific knowledge about aquaporin physiology in these vertebrate clades is overwhelming. Because the integumental surfaces of these animals are in intimate contact with the surrounding milieu, passive water loss and uptake represent two of the major osmoregulatory challenges that need compensation. However, neither obligatory nor regulatory water transport nor their mechanisms have been elucidated to the same degree as, for example, ion transport in fishes. Currently fewer than 60 papers address fish aquaporins. Most of these papers identify "what is present" and describe tissue expression patterns in various teleosts. The agnathans, chondrichthyans, and functionality of fish aquaporins generally have received little attention. This review emphasizes the functional physiology of aquaporins in fishes, focusing on transepithelial water transport in osmoregulatory organs in euryhaline species – primarily teleosts, but covering other taxonomic groups as well. Most current knowledge comes from teleosts, and there is a strong need for related information on older fish clades. Our survey aims to stimulate new, original research in this area and to bring together new collaborations across disciplines.

Received 4 February 2015; accepted 30 March 2015.

* To whom correspondence should be addressed. E-mail: steffen@biology.sdu.dk

Abbreviations: CFTR, cystic fibrosis transmembrane conductance regulator; ClC, chloride channel; Glp, aquaglyceroporin; GLUT, glucose transporter; LIS, lateral intercellular space; MC, mucus cell; MRC, mitochondrion-rich cell; NCC, Na^+,Cl^-cotransporter; NHE, Na^+/H^+-exchanger; NKA, Na^+,K^+-ATPase; NKCC, Na-K-Cl-cotransporter; NPA, asparagine-proline-alanine; PC, pavement cell; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase polymerase chain reaction; SGLT, Na^+-glucose cotransporter; TMAO, trimethylamine oxide; WGD, whole genome duplication.

Introduction

Living in a "world of water", fishes are exposed to major osmotic challenges in freshwater and marine environments. In both cases, obligatory water fluxes due to osmotic gradients across respiratory surfaces threaten the stability of the internal milieu and must be compensated for by bulk flow of water in the opposite direction. While the ion-regulatory mechanisms that generate the osmotic driving force for such water flows have been known and investigated in increasing detail for decades, the molecular pathways of compensatory water fluxes are still largely unresolved. Current models suggest that water passes hydrophobic epithelia by transcellular and/or paracellular pathways, the former determined by the serial permeability of apical and basolateral cellular membranes, the latter being defined by the characteristics of intercellular junction complexes. However, the relative significance of these two pathways is still debated—not only in the case of fishes but in vertebrates generally. Transcellular water transport may occur by simple diffusion through lipid bilayers or become markedly improved by the insertion of integral channel proteins (aquaporins) in the plasma membrane. Thus, aquaporins should truly be viewed as the plumbing system of cells (Agre *et al*., 1998).

In Mammalia, 13 aquaporin subfamilies (Aqp0 - Aqp12) are each represented by one gene; several of these have been investigated structurally and functionally in a vast number of publications (>8500, January 2015, Web of Science; Thomson Reuters, Philadelphia, PA)

since their discovery in 1992 by P. Agre and colleagues (Preston *et al*., 1992). A recent extensive phylogenetic analysis of deuterostome genomic data, however, revealed that the vertebrate superfamily contains 17 classes of aquaporins (Aqp0 - Aqp16; Finn *et al*., 2014). The first report on aquaporins in fishes appeared in 2000 (Cutler and Cramb, 2000), but since then surprisingly few papers have addressed aquaporins in fishes and other non-mammalian vertebrates. Tingaud-Sequeira *et al*. (2010) established that zebrafish *Danio rerio* (Hamilton, 1822) and other teleosts retain up to 18 aquaporin genes with homologies to all of the mammalian orthologs except Aqp2, -5, and -6. However, as discussed below, the recently published genomes of the Atlantic salmon *Salmo salar* Linnaeus, 1758 (Davidson *et al*., 2010) and rainbow trout *Oncorhynchus mykiss* (Walbaum 1792) (Berthelot *et al*., 2014) have added even more paralogs to this list. One of the major challenges is to describe and understand the differentiated functionality of such extreme diversity.

Osmoregulation and Water Balance in Euryhaline Fishes

Euryhaline fishes make up less than 10% of all known fish species (McDowall, 1988). They display a number of different lifestyles but share the ability to survive and, indeed, thrive in a broad range of environmental salt concentrations. There are two principal ways of dealing with the osmotic conditions of a given aquatic environment: 1) conforming to the extracellular osmotic concentration or 2) keeping internal homeostasis by regulating the exchange of ions and water with the surrounding environment. Osmoconforming fishes usually use the former strategy at the higher end of the salinity range and switch to some degree of hyperosmoregulation if the salinity decreases to below approximately 300 mOsm/kg (10 parts per thousand). Osmoregulation generally requires a higher energy input than osmoconformation and is dependent on coordinated processes by multiple organs and molecular mechanisms, which together maintain internal osmolality of the organism either above or below that of the surroundings. Among euryhaline fishes, most teleosts and petromyzontid agnathans (lampreys) are regulators, while chondrichthyans and the myxinoid agnathans (hagfishes) are conformers (see "Water Balance in Elasmobranchs" below). In conforming species, fluid loss to the marine environment is minimal, whereas osmoregulating species that maintain an internal homeostasis of around 320–360 mOsm/kg (Evans *et al*., 2005; Whittamore, 2012) experience large osmotic gradients across the integument in both fresh water (FW) and seawater (SW). Thus, relatively large obligatory water fluxes occur in these species primarily across the gill epithelium, which need compensatory water fluxes by either the kidney (in FW) or the intestine (in SW). In the chondrichthyans, an additional extrarenal organ—the rectal gland – participates in salt and, to some degree, water exchange (Evans and Claiborne, 2009).

Teleost Fishes

The gills

The most studied osmoregulatory organ in fishes is the gill, which plays important roles in ion regulation in both FW and SW. In FW, excess water accumulates across the large surface area of the gill and must be excreted as hypotonic urine by the kidney; in SW, osmotic loss of water across the gill is compensated for by oral ingestion and intestinal absorption of (salt)water and net secretion of surplus salts in the gills. The gill epithelium is largely composed of pavement cells (PC), mucus cells (MC), and various types of ion-transporting, mitochondrion-rich cells (MRC), the function of which depends on the osmotic environment. In SW, there is consensus that MRCs maintain transcellular secretion of Cl^- and establish the conditions for paracellular electrochemical diffusion of Na^+. The molecular mechanisms are well described in several species and include basolateral Na^+,K^+-ATPase and Na^+,K^+,$2Cl^-$-cotransporter (NKCC1), apical cystic fibrosis transmembrane conductance regulator (CFTR), and cation leaky tight junctions between neighboring cells, which are structurally and functionally defined by various claudin isoforms. In FW, current knowledge suggests that many different strategies and molecular mechanisms are used to solve compensatory active ion uptake (Hwang *et al*., 2011; Hiroi and McCormick, 2012). Basolaterally, Na^+,K^+-ATPase drives Na^+ into the extracellular fluid and K^+ into the intracellular fluid. The activity of the pump and the subsequent efflux of K^+ together create intracellular electronegativity. Apically, Na^+ enters *via* a Na^+,Cl^--cotransporter (NCC) or Na^+/H^+-exchanger (NHE), aided by the activity of a V-type H^+-ATPase. Apical chloride uptake is mediated by a NCC and/or Cl^-/HCO_3^--exchanger, whereas the basolateral exit pathway has not been fully resolved. In spotted green puffer fish *Tetraodon nigroviridis* (Marion de Procé, 1882), it is mediated by a chloride channel (CIC-3) (Tang *et al*., 2010). Due to the large surface area and "osmo-respiratory compromise" (Nilsson, 1986; Gonzales and McDonald, 1992), the gills are the main determinant of obligatory fluxes of water entering or leaving the fish in FW and SW, respectively. Ideally, water permeability should be kept to a minimum and, therefore, it would not be expected that aquaporins are involved in creating transcellular pathways for water movement here. Aquaporins have been localized in the gill epithelium, but their role has been associated mostly with cellular volume regulation and CO_2 elimination.

The intestine

As early as 1930, it was shown that SW-acclimated fishes drink considerable amounts of SW to compensate for osmotic water primarily across the gill (Smith *et al*., 1930). It was speculated that this water was absorbed by the intestine in combination with salts and the salts were then excreted through other pathways. We now know that when SW enters the gastrointestinal tract of marine fish, the fluid is initially desalinated by removal of salts in the esophagus (Parmelee and Renfro, 1983; Grosell, 2006, 2011). Some efflux of water may also occur here. Past the stomach, the bulk absorption of fluid in the intestine is believed to be driven by active uptake of salts into the lateral-intercellular space (Diamond and Bossert, 1967), but the molecular mechanism is debated, and is discussed below (see "The Dogma of Transepithelial Water Transport"). Fluid absorption takes place through/between the principal enterocytes of the intestinal epithelium along the entire length of the intestine, including its pyloric appendices (in salmonids). Several ion transport proteins aid in establishing the driving osmotic gradient. Briefly, the basolateral Na^+,K^+-ATPase sets up the Na^+-gradient needed for apical absorption of this and other ions through cotransport mechanisms such as NCC, NKCC2, and possibly NHE2/3. Chloride is absorbed by the Cl^-/HCO_3^- exchanger SLC26a6, NCC, and NKCC2. The basolateral exit of Cl^- is provided by anion channels. Fluid and ion absorption are tightly linked to acid/base balance *via* CO_2, which is metabolized to HCO_3^- by cytosolic carbonic anhydrase. HCO_3^- exchanged to the lumen of the intestine precipitates with Ca^{2+} from the imbibed SW, thus lowering luminal osmolality and further aiding transepithelial fluid absorption (see Grosell, 2006, 2011; Sundell and Sundh, 2012). During the smoltification period in salmonids, the capacity of the intestine to absorb saline fluid steadily increases until the peak of this transition (Nielsen *et al*., 1999; Sundell *et al*., 2003). Transepithelial resistance (TER) of the intestine increases during the same period, most likely reflecting a remodeling of the tight junctions. This action is believed to facilitate water transport across the epithelium by alleviating the build-up of high osmotic pressure inside the lateral intercellular space (Sundell and Sundh, 2012). The intestine of fish is, therefore, essential in maintaining water homeostasis in SW, whereas the kidney is of limited importance in this environment.

The kidney

The mesonephric kidney of teleosts essentially evolved as a water-secreting organ with relatively big glomeruli. It lacks discrete cortical and medullary zonation and the loop of Henle of the metanephric kidney of birds and mammals, which allow these animals to produce hypertonic urine (Hickman and Trump, 1969). A high glomerular filtration rate (GFR) in FW fishes ensures that excess water gained by diffusion is removed from the bloodstream. The filtrate passes through the proximal tubule, where reabsorption of salts and water originally was believed to occur (Hickman and Trump, 1969). However, net secretion of both ions and water is also observed in this segment of both FW- and SW-acclimated fish, and is linked to the active secretion of $MgSO_4$ (Cliff and Beyenbach, 1992). In the distal tubules of FW fish, important salts including NaCl secreted in the proximal tubules, are eventually reabsorbed. This action prevents excessive loss of precious salt. Finally, in collecting tubules and ducts and in the urinary bladder, NaCl is further reabsorbed, leaving the unwanted divalent ions in the urine (Beyenbach, 2004). The apical absorptive NKCC type-2 cotransporter plays an important role in this process (Cutler and Cramb, 2008; Katoh *et al*., 2008; Kato *et al*., 2011). For the distal segments to act as diluting segments in FW fishes, they also need to be relatively water-impermeable, which would allow for the excretion of strongly hypotonic urine. The unavoidable salt loss associated with urination in FW fishes is rectified by the active uptake of ions across the gills.

In SW, kidney function is refined to excrete Mg^{2+} and SO_4^{2-} in a much reduced urine volume (Beyenbach, 2004), while the gill and intestine maintain monovalent ion excretion and fluid uptake, respectively, as described above. This specialization has led to an evolutionary secondary loss of glomeruli in a few stenohaline marine fish species, as filtration of the blood is no longer necessary and comes at too high a price in the form of fluid loss. In both glomerular and aglomerular marine teleosts, Mg^{2+}, SO_4^{2-}, and NaCl are secreted directly into the proximal tubules with water following passively (Schmidt-Nielsen and Renfro, 1975; Cliff and Beyenbach, 1992; Beyenbach, 2004), as explained above. A generalized model of water and salt transport in the teleost kidney is summarized in Figure 1.

The GFR is highly variable in relation to salinity, with SW-acclimated fish generally decreasing GFR to approximately 10% of the FW rate (Brown *et al*., 1978; Beyenbach, 2004). In addition to adjustment of single-nephron GFR, glomerular intermittency is widespread in fishes both in FW and in SW. In FW, approximately 20% of the nephrons were non-filtering in an *in situ* perfused kidney of a FW-acclimated rainbow trout; this fraction increased to 95% in kidneys from SW-acclimated trout (Brown *et al*., 1978; Amer and Brown, 1995). Thus, GFR is highly adjustable in teleosts, in contrast to mammals, where GFR is relatively constant, and an important component of fluid homeostasis by the kidney is expected to be maintained at the level of glomerular filtration.

The Dogma of Transepithelial Water Transport

Water may traverse a permeable epithelium whenever an osmotic gradient is established at a micro- or macro-scale.

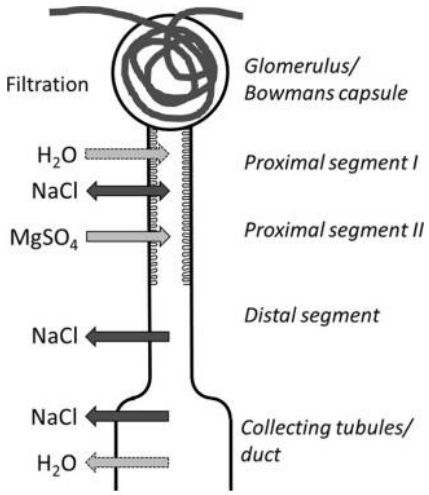

Figure 1. Generalized model summarizing water and salt transport processes in FW and SW teleost nephron. Dotted arrows indicate passive transport of water; solid arrows indicate active transport of ions. Distal segments exist only in stenohaline and diadromous FW species.

Even though water transport overall may appear as isosmotic on a macroscopic scale, local osmotic pressure differences are necessary to drive unidirectional movement. This is called solute-linked water transport. Thus, the issues related to transepithelial water transport are 1) how is the osmotic gradient established and maintained? and 2) how is water permeability established? Based on the current view of water absorption in the intestine of SW-acclimated fish, water may move in transcellular fashion through aquaporins positioned in apical and basolateral membranes and cross the lipid bilayer membranes directly and/or through the paracellular pores between epithelial cells (Murakami *et al*., 2006; Fischbarg, 2010). The diffusion of water across lipid bilayers is now recognized as a less important pathway than the other options (see Paula *et al*., 1996). Paracellular water flow is discussed below, but the relative contribution of paracellular versus transcellular flow is generally not understood, primarily due to the technical difficulty of measuring each component.

Solute-Linked Water Transport

There is general consensus that asymmetrical pumping of Na^+ and K^+ and Cl^- is a major factor in creating osmotic gradients between intra- and extracellular compartments, and also between mucosal and serosal compartments on a macroscopic scale; however, the molecular pathway for water movement is still a topic of debate. The current theory was first proposed by Curran and Solomon (1957), based on investigations of the rat ileum. A fine-tuned model emerged ten years later, when Diamond and Bossert presented "The standing gradient osmotic flow hypothesis" (Diamond and Bossert, 1967). The basic principle of this model is that transport of ions across the apical and lateral membranes, into the lateral intercellular space (LIS) beneath the tight junction, generates a hyperosmotic solution that moves in a serosal direction due to the constant efflux of intracellular water into the LIS (see Fig. 2). The theory was widely accepted but a major problem was to explain how water crossed both apical and lateral plasma membranes. This difficulty was resolved with the identification of aquaporins. However, the tight junction at the apical boundary of the LIS to the lumen is also of great importance for overall absorption of water by the tissue, and the ability of this tight junction to discriminate between water and solutes may greatly affect transepithelial water transport, as suggested by Larsen *et al*. (2007). Secondary active transport of water also has been suggested as a part of solute-linked water transport. This theory is based on experimental findings that certain ion- and metabolite channels such as the mammalian Na^+-glucose cotransporter (SGLT1) and glucose transporter (GLUT) increase water permeability when expressed in *Xenopus* oocytes (Loike *et al*., 1996; Loo *et al*., 1996; Meinild *et al*., 1998). The calculations made by the authors showed that approximately 260 water molecules are transported with each molecule of sugar. These transporters could thus account for a significant amount of water being transported in association with nutrient uptake. However, this theory was later challenged by Duquette *et al*. (2001), who showed that water transport induced by SGLT1 is primarily due to its creation of a local intracellular hyperosmotic environment that drives water through both endogenous and SGLT1-induced pathways. Thus, irrespective of the pathway of water transport, it can still be concluded that significant water transport (absorption and secretion) ultimately requires transport of ions.

The Paracellular Pathway

Two models have been proposed for paracellular water transport, the "osmo-sensor model" of Hill and Shachar-Hill (2006), and "electro-osmosis," by Fischbarg (2010). The "osmo-sensor model" incorporates aquaporins as sensors of osmotic concentrations, in which tetrameric organization of the aquaporins changes with osmolality and initiates signals to the tight junctions, mediating paracellular fluid transport. This action, the authors propose, occurs through a junctional fluid transport system more permeable to water than to ions. The system sets up hydrostatic pressure by micro peristaltic movements of the tight junction, thereby shuttling water across the epithelium (Hill and Shachar-Hill, 2006; Murakami *et al*., 2006).

The "electro-osmosis model" has been tested mainly in corneal endothelia and is not readily applicable to other osmoregulatory tissues. However, the concept is interesting; it involves communication with, and modification of, the tight junctions in such a way that these are lined by negative

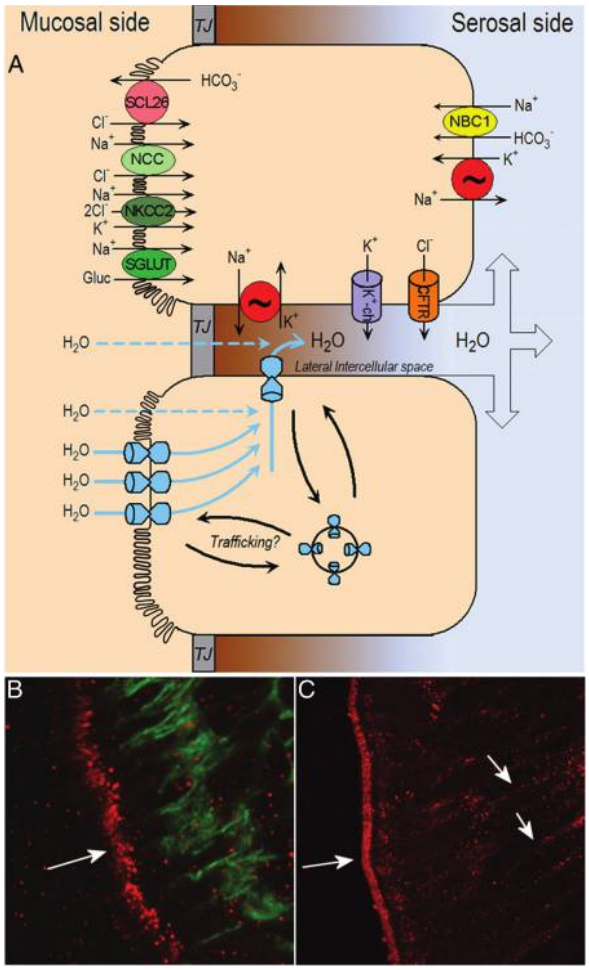

Figure 2. (A) Tentative model of solute-linked water transport mechanisms across the teleost intestinal epithelium (based on Diamond and Bossert's standing gradient hypothesis; see also Grosell 2011 for more detail). Transport of solute (primarily NaCl) is driven by ATP-consuming basolateral Na^+, K^+-ATPase activity and leads to build-up of high osmotic pressure in the lateral intercellular space (LIS), as shown by the increased color intensity. Water is drawn along the osmotic gradient from cell to LIS and hydraulically moves toward the serosal side of the epithelium. The pathway of water movement is not finally resolved, but may involve (1) apical-basolateral passage through aquaporins and/or solute-cotransporters such as the sodium-glucose transporter (SGLUT1) or the Na^+, K^+, $2Cl^-$ cotransporter; (2) movement across the tight junction (TJ) and/or (3) passage through the lipid bilayer of enterocytes. There is ample evidence for (1), some evidence for (2), and very little evidence for (3) as the major pathway. Aquaporins may be recycled to/from the cell membranes but this action remains to be shown. Hourglass symbols represent aquaporins of various classes that may be present both apically and basolaterally facing the lateral intercellular space (*e.g.,* Aqp1a and Aqp8ab). (B) and (C) show confocal images of intestinal sections from SW trout labeled as Aqp1a (red in (B)), as Aqp8ab (red in (C)), and Na^+,K^+-ATPase alpha 5 (green in (B)) antibodies. Apical (Aqp1a and Aqp8ab) and basolateral (Aqp8ab) staining indicate localization of the two aquaporins (arrows).

Abbreviations: NBC1, sodium-bicarbonate cotransporter; SLC26, chloride-bicarbonate exchanger; NCC, sodium-chloride cotransporter; NKCC2, sodium-potassium-2 chloride cotransporter type-2 (absorptive); SGLUT, sodium-glucose transporter type 1; CFTR, cystic fibrosis transmembrane conductance chloride channel; K^+-ch, potassium channel; ~, electrogenic Na^+,K^+-ATPase.

charges and a very small potential across the endothelium facilitates movement of water and ions through paracellular pores (Fischbarg, 2010). The ions moving along with water through the tight junction are recycled back to their starting point and can accompany further water transport. Recent research in tight junction proteins has focused on the charged residues in the extracellular loops of these proteins, which define charge selectivity of the paracellular pore, and it may be relevant to couple research in this field with the electro-osmosis hypothesis of water transport. Recently, the mammalian tight junction protein claudin-2 was found to increase water permeability of a polarized cell monolayer (Rosenthal *et al*., 2010). Further studies have shown that paracellular water absorption in the mouse proximal tubules may account for up to 25% of the total absorbed fluid, supposedly mediated by claudin-2 (Schnermann *et al*., 2013).

Only a few attempts have been made to characterize the transepithelial route of water absorption in fish osmoregulatory organs. Wood and Grosell (2012) concluded that the pathway was mainly transcellular in killifish intestine (*Fundulus heteroclitus*; Linnaeus, 1766), which is in strong contrast to the situation in the mouse jejunum, which Rehman *et al*. (2003) estimated to be approximately 80% paracellular.

Discovery and Structure of Aquaporins and the Basis for Transcellular Water Flow

With the discovery of aquaporins in 1992, the question of how water moved across single hydrophobic cell membranes seemed answered (Preston *et al*., 1992). However, the debate as to how water traverses epithelia is ongoing (Fischbarg, 2010; Wood and Grosell, 2012). Aquaporins were first discovered from red blood cell membranes during an attempt to characterize a polypeptide from the antigens of the Rhesus blood group (Borgnia *et al*., 1999). After cDNA of CHIP28 (a channel-like integral protein of 28 kDa) was isolated and compared with other known sequences, the protein product was tested to determine if it generated water permeability in *Xenopus* oocytes, which have very low native water permeability (Agre and Kozono, 2003). In *Xenopus* oocytes, CHIP28, now known as AQP1, induced high water permeability, confirming that the isolated protein was a water channel (Preston *et al*., 1992). However, to eliminate the possibility that injected cRNA of AQP1 affected endogenous proteins within the oocyte, which then increased water permeability, it was necessary to express AQP1 in reconstituted liposomes. Data confirmed the initial hypothesis and AQP1 was concluded to be responsible for a major part of the water permeability in red blood cells (Zeidel *et al*., 1992). It was resolved that AQP1 facilitated water transport along the osmotic gradient and that the protein had no effect on ion conductance across the

oocyte membrane, thereby showing that only water (H_2O and D_2O, Mamonov *et al*., 2007)—and not even protons (H_3O^+)—were able to permeate the channel. Aquaporin 1 exists in the membrane as a tetrameric assembly (Jung *et al*., 1994). Each protein has six alpha helices spanning the plasma membrane and is flipped to create a central pore with two asparagine-proline-alanine (NPA) motifs. These motifs, in addition to a cysteine (C189) and an aromatic arginine residue (R195), are highly conserved across most families of aquaporins and create the two restriction sites of the pore that allow water to pass. When water or other molecules enter the channel, they are restricted in size at the narrowest point of the channel, which is only 2.8 Ångström (Å) wide and is lined by two residues, a histidine (H180) and an arginine (R195), which bear positive charges and thus repulse protons. At the same location, the conserved cysteine is present; this is responsible for the ability of Hg^{2+} to block the water permeability of AQP1. Moving onwards through the channel, which is 20 Å long, the water molecule gets reoriented by the presence of the two asparagines present in the NPA motifs. The water molecule is believed to form partial hydrogen bonds with these two residues, undergoing a dipole reorientation that would not be possible for other molecules (Agre and Kozono, 2003). More recent studies have confirmed the overall structure of aquaporins throughout most phyla, but have also found that in some organisms the conserved residues have been lost, with possible new or altered functions arising for these proteins (Ishibashi *et al*., 2011).

As more aquaporins were discovered, additional permeabilities were also assigned and aquaporins were divided into different subfamilies based on their sequence homology and solute preferences (King *et al*., 2004; Xu *et al*., 2010). In humans, AQP0, -1, -2, -4, and -5 are termed "true aquaporins" and are (mostly) only permeable to water. AQP3, -7, -9, and -10 are "aquaglyceroporins," or Glps, with additional permeability to glycerol and urea. The remaining aquaporins, AQP6, -8, -11, and -12, are termed "unorthodox aquaporins" due to their different characteristics such as NH_3 permeability for AQP8 (Saparov *et al*., 2007), ability to transport anions (AQP6) (Yasui *et al*., 1999), or intracellular locations and modified NPA motifs (AQP11 and -12) (Itoh *et al*., 2005; Gorelick *et al*., 2006). Aquaporins are expressed in a wide range of tissues in mammals and, where subcellular localization data exist, multiple aquaporins in one tissue are often spatially distributed between different organelles and cell membranes (Nielsen *et al*., 2002; King *et al*., 2004). Aquaporins have been investigated in a wide variety of fish species, from the strictly marine osmoconforming hagfish to the highly euryhaline Mozambique tilapia *Oreochromis mossambicus* (Peters, 1852). In the following sections, the focus turns to the role of aquaporins in euryhaline fishes, and includes examples from stenohaline fishes.

Diversity of Aquaporins and Genomic Duplication Events in Fishes

The vertebrate genome is believed to have duplicated two times near the origin of the group and a third time in fish (Brunet *et al*., 2006). In addition, a fourth round of whole genome duplication (WGD) has probably occurred in the ancestor to the salmonids (Davidson *et al*., 2010), which theoretically would supply these species with four times as many (aquaporin) genes as humans. In practice however, WGDs are often followed by loss of some or all of the duplicated genes, or even chromosomes, in a process referred to as diploidization (see Wolfe, 2001). Shortly after a WGD, the duplicated genes might also attain new functions through neo- and subfunctionalization (Semon and Wolfe, 2007). Subfunctionalization occurs if the duplicated paralogs divide the functions of the ancestral gene between them, whereas neofunctionalization occurs if any of the duplicated paralogs attains new functions in comparison to the ancestral gene.Until recently, the number of aquaporin genes in the teleost fish species with mapped genomes counted between 13–18 paralogs; the stenohaline zebrafish had 18 paralogs and was thus the vertebrate with the highest numbers of identified aquaporins at that time (Tingaud-Sequeira *et al*., 2010). However, with the recent sequencing of the salmonid genomes (Atlantic salmon: Davidson *et al*., 2010; rainbow trout: Berthelot *et al*., 2014), these species have taken the lead in the number of aquaporin paralogs due to the more recent WGD. Indeed, a recently published analysis of deuterostome aquaporins suggests that the Atlantic salmon may express more aquaporin paralogs than any other animal analyzed (Finn *et al*., 2014). The Atlantic salmon genome comprises an impressive 42 paralogs distributed in 12 aquaporin classes, and only a tiny fraction of these classes have been studied. The diversity, localization, and – whenever possible – functional significance of aquaporins in the primary osmoregulatory organs of euryhaline teleost species and more ancestral fish classes are reviewed below.

The Outer Barrier

Branchial and dermal aquaporins

Even though the gill and skin do not share much structural similarity, they both constitute a direct interface between the interior and exterior environments. This means that they are asymmetrically exposed to physiological saline on the inside and either FW or SW on the outside. In addition to specialized functions such as gas transport, mucus secretion, and ion transport, these epithelia share the challenge of regulatory cell volume adjustments to a higher degree than probably any other cell type within the fish.

Aquaporins could therefore be expected to be asymmetrically expressed in apical and basolateral membrane domains to allow for volume-regulatory fluxes of water.

The first report of aquaporins in fish came from Cutler and Cramb (2000), who cloned an *aqp3b* paralog from the gill of European eel *Anguilla anguilla* Linnaeus, 1758. They characterized the effect of salinity on *aqp3b* mRNA in the gill and found that the level decreased significantly when both indigenous "yellow eels" and migratory "silver eels" were acclimated to SW. Since Aqp3b is an aquaglyceroporin, the authors suggested that the protein played a role in nitrogen metabolism in the gill by acting as a channel for NH_3 or urea. An accompanying report from the same laboratory showed that the Aqp3b protein colocalized with Na^+,K^+-ATPase in the basolateral membranes of chloride cells and occupied a subapical area in these cells (Lignot *et al*., 2002). They also noted that the observed decrease in Aqp3b might be due to decreased expression in cells in the serosal part of the filament epithelium as well as in cells of the branchial arch. Since then, the effect of salinity on gill *aqp3* expression has been investigated in other species and most reports have supported the findings of Cutler and Cramb, *i.e., aqp3* expression is down-regulated in the gill of SW-acclimated European seabass (*Dicentrarchus labrax,* Linnaeus, 1758; Giffard-Mena *et al*., 2007, 2008), silver seabream (*Sparus sarba* Forsskål, 1775; Deane and Woo, 2006), Mozambique tilapia (Watanabe *et al*., 2005), Japanese eel (*Anguilla japonica*, Temminck & Schlegel, 1846; Tse *et al*., 2006), Atlantic salmon (Tipsmark *et al*., 2010b), Japanese medaka (*Oryzias latipes*, Temminck and Schlegel, 1846; Madsen *et al*., 2014), marine medaka (*Oryzias dancena*, Hamilton, 1882; Kim *et al*., 2014), and killifish (Jung *et al*., 2012). The studies in tilapia, Japanese eel, killifish, and rainbow wrasse (*Coris julis*, Linnaeus, 1758; Brunelli *et al*., 2010) also found Aqp3 in the basolateral membranes of chloride cells, supporting the initial findings made by Lignot *et al*. (2002). Remarkably, in killifish the transcript was down-regulated in the SW gill as a whole but the protein level was unchanged. Jung *et al*. (2012) found that the amount of Aqp3 in pillar cells of the lamellae decreased at the same time that Aqp3 in MRCs increased in SW, suggesting that Aqp3 may play a role in SW-osmoregulation in this species. Preliminary evidence from Atlantic salmon gills also shows colocalization of Aqp3 and Na^+,K^+-ATPase in chloride cells (M.B. Engelund, unpubl. data). A recent report on sockeye salmon (*O. nerka*, Walbaum, 1792) showed up-regulation of *aqp3* in SW-acclimated parr, while it was down-regulated in smolts (Choi *et al*., 2013). However, the subtype of this paralog is unknown, though most similar to Aqp3a. Western blots in this study with one FW- and one SW-acclimated parr and smolt also indicated that total Aqp3 protein changed in the same direction as mRNA for both parr and smolts. As with the gill, mRNA of *aqp3* is also expressed in the skin and fin of marine medaka (Kim *et al*., 2014; Madsen *et al*., 2014) and is down-regulated in SW in both tissues.

Functional characterization of the eel Aqp3 has shown that the protein can transport water and glycerol, and that the transport rate is pH-dependent, with pH 6.5 significantly decreasing transport rate. In addition, eel Aqp3 is inhibited by mercury, copper, zinc, and nickel (MacIver *et al*., 2009). The general finding of Aqp3 protein in the basolateral membrane of chloride cells suggests that this aquaporin is participating in cell volume regulation and may take part in nitrogen excretion as well. Urea permeability has been shown for the zebrafish Aqp3a paralog (Tingaud-Sequeira *et al*., 2010) and the European eel Aqp3b paralog (MacIver *et al*., 2009), whereas evidence of NH_3 permeability has not been published. In Japanese eel, a urea transporter has been cloned that is up-regulated in SW (Mistry *et al*., 2001). A plausible hypothesis is that in Japanese eel, Aqp3 transports urea in FW while a specific urea transporter takes over in SW, but this theory needs additional investigation. Ammonia exposure (470–1700 μmol l^{-1} total ammonia nitrogen) for 22 d was recently shown to down-regulate expression of *aqp3a1* and urea transporter mRNA in Atlantic salmon, while prolonged exposure for 105 d increased the expression of *aqp3a1* compared to controls (Kolarevic *et al*., 2012). These findings suggest that the Aqp3a protein may play a role in nitrogen metabolism in Atlantic salmon, but further studies, especially those investigating ammonia permeability of fish Aqp3, are warranted. Hirata *et al.* (2003) found that acid exposure increased branchial Aqp3 expression in chloride cells of the Osorezan dace *Tribolodon hakonensis* Günther, 1877. They suggested that this action might supply water for the hydration of CO_2 as acid excretion was increased.

Paralogs of *aqp1* have been found in the branchial epithelium of several teleost species but were reported to be absent in others (catfish *Heteropneustes fossilis* Block, 1794; Chaube *et al*., 2011). In black porgy (*Acanthopagrus schlegelii* Bleeker, 1854; An *et al*., 2008), river pufferfish (*Takifugu obscurus* Abe, 1949; Jeong *et al*., 2014), and marine medaka (Kim *et al*., 2014), gill *aqp1* expression is higher in FW than in SW. In sea bass and climbing perch (*Anabas testudineus* Bloch, 1792), an *aqp1a* paralog was present in the gills but mRNA expression did not change with salinity (Giffard-Mena *et al*., 2007; Ip *et al*., 2013). In SW-acclimated gilthead seabream (*Sparus aurata* Linnaeus, 1758), *aqp1aa* was also detected and the Aqp1a protein was found on the surface of the gill lamellae (Cerdà and Finn, 2010), whereas Aqp1 in rainbow wrasse (in SW) was found in chloride cells, colocalizing basolaterally with Na^+,K^+-ATPase (Brunelli *et al*., 2010). The latter study used rat antibodies to detect both Aqp1 and Aqp3 in rainbow wrasse. In zebrafish larvae, Aqp1a1 was localized in the basolateral membrane of ionocytes of the yolk sac skin, where they were assigned a role in transcellular water transport (Kwong et al., 2013).

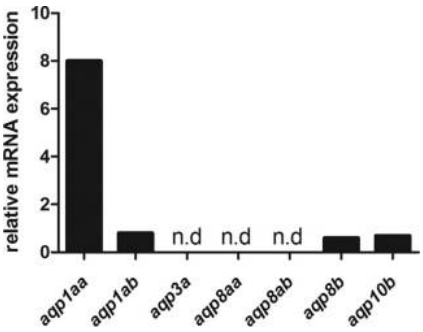

Figure 3. Aquaporin mRNA expression as determined by quantitative PCR and normalized to elongation factor 1a in mixed blood cells from rainbow trout. N=2, n.d., not detected. Data show mean values. (M. B. Engelund, unpubl. data).

In Atlantic salmon, two paralogs (*aqp1aa* and *aqp1ab*) are present in the gill (Tipsmark *et al*., 2010b). *aqp1aa* mRNA is down-regulated upon SW acclimation while *aqp1ab* mRNA is up-regulated. The cellular localization of these aquaporins is unknown. The apical lamellar localization of Aqp1a in seabream is surprising since significant water permeability of these cells might lead to dehydration in a marine environment. However, a recent study of zebrafish suggests that branchial Aqp1a may play an alternative and fundamental role in gas transport, as significant CO_2 (and NH_3) permeability was found for this protein (Chen *et al*., 2010). An interesting note is that *aqp1aa* expression decreased in the gill of climbing perch following exposure to 100 mmol l^{-1} NH_4Cl for 6 d (Ip *et al*., 2013). The authors suggested that decreased expression of *aqp1aa* is a defense mechanism to avoid excessive loading of ammonia, which is supposedly transported through the Aqp1aa protein. They further proposed that up-regulation of *aqp1aa* when fish are exposed to terrestrial conditions satisfies the need to excrete additional ammonia across the gill surface. However, it has yet to be determined if these changes are carried on to the protein level, and if these fish species accept the risk of increased water permeability in favor of increased permeability to CO_2 and/or NH_3. Other studies have also highlighted the role of Rhesus proteins in gas transfer across the gill of fish (Wright and Wood, 2009), and calculations have shown that in human red blood cells these proteins together with AQP1 may account for all CO_2 transport (Chen *et al*., 2010). A major bias regarding *aqp1a* in highly vascularized tissues such as the gill's is the expression of this protein in endothelia of the microvasculature (Rehn *et al*., 2011), as well as red blood cells (Fig. 3). This bias is hard to avoid and, therefore, whole-tissue mRNA data should be interpreted alongside localization data.

Several other aquaporins have been detected in the gill of fishes but their participation in osmoregulation has yet to be confirmed. For example, *aqp10b*, *-11a*, *-8ab,* and *-12* were all detected in the gill of two medaka species (*Oryzias dancena*: Kim *et al*., 2014; *O. latipes*: Madsen *et al*., 2014), and *aqp10b* and *aqp11a* were found at slightly lower levels in SW- than in FW-acclimated fish. AQP10-like paralogs have also been found in Japanese eel (Kim *et al*., 2010) and gilthead seabream (Santos *et al*., 2004) but were not investigated for salinity-dependent effects. In European eel and Atlantic salmon, *aqp10b* was reported absent and it seems that the role of this aquaglyceroporin in osmoregulation in the gill is minimal. In the stenohaline zebrafish, one *aqp10a* paralog was found as well as additional paralogs of aquaporins and aquaglyceroporins, but neither protein expression nor localization has yet been investigated (Tingaud-Sequeira *et al*., 2010).

In summary, several aquaporin paralogs have been detected in the gill. Some, Aqp3, especially, have been localized in specific cell types—consistently in the basolateral membrane—and show a consistent response to salinity, as described above. Gill and skin aquaporins are believed to be more important in cell volume regulatory responses than in transepithelial water exchange.

Internal Exchange Surfaces

Intestinal aquaporins

The saltwater drinking response and water transport across the fish intestine have been studied for decades, and the discovery of aquaporins has naturally stimulated investigation of the molecular mechanism in a range of euryhaline species. Several studies have identified aquaporins that are expressed in one or more segments of the intestine and, not surprisingly, the expression pattern often varies according to intestinal segment. A few studies have localized the expression to cell type and subcellular domain. By far, most studies have found aquaporin expression in the apical brush border and only two studies have given evidence for a basolateral expression, which is needed to create a functional transcellular pathway for water absorption.

The first studies were reported in eels by Lignot *et al.* (2002) and Aoki *et al.* (2003), who found that SW acclimation stimulated intestinal aquaporin mRNA and protein expression. In Japanese eel, there was an up-regulation of *aqp1aa* upon SW transfer, which was confirmed in other species in at least one intestinal segment (European eel: Martinez *et al*., 2005b; sea bass: Giffard-Mena *et al*., 2007, 2012; gilthead seabream: Raldua *et al*., 2008; Atlantic salmon: Tipsmark *et al*., 2010b). In climbing perch, there was a tendency toward an increased level of *aqp1aa* after 6 d, although this finding is non-significant (Ip *et al*., 2013). The Aqp1aa protein was localized in the apical brush-border membrane in the intestinal epithelium in several SW-acclimated fish species, suggesting an entry pathway for transcellular water transport (Aoki *et al*., 2003; Martinez *et al*., 2005b; Raldua *et al*., 2008; Madsen *et al*., 2011). In zebrafish, the permeability characteristics of Aqp1aa have

been investigated; the pore only allows for transport of water and gases (Chen *et al*., 2010; Tingaud-Sequeira *et al*., 2010). Thus, Aqp1aa might certainly take part in exchange of water but also of CO_2, which is transferred to the gut lumen.

In the study of gilthead seabream, Aqp1aa was expressed in both apical and lateral membrane domains, indicating that this protein may create a functional transcellular pathway for water exchange in this species (Raldua *et al*., 2008). The tandem duplicated paralog *aqp1ab* was also found in intestinal tissues and showed similar regulation to *aqp1aa*, although the effect of salinity was less pronounced. The transcript of *aqp1ab* was more abundant in the rectum of SW-acclimated fish, whereas the other intestinal segments had equal levels in SW and FW (Raldua *et al*., 2008). In Atlantic salmon, *aqp1ab* was at higher levels in the middle intestine of SW-acclimated fish compared to FW fish (Tipsmark *et al*., 2010b). The subcellular localization of Aqp1ab was similar to that of Aqp1aa, *i.e.,* it was abundant in the brush border membrane of enterocytes in both gilthead seabream and Atlantic salmon (Raldua *et al*., 2008; Madsen *et al*., 2011). Interestingly, in the posterior intestine of Atlantic salmon, the Aqp1ab protein was located in a subapical domain, possibly reflecting a dynamic pool, which can be inserted into the plasma membrane when needed. This is in line with the recent data presented for Aqp1ab in kidney tubules, as explained below.

As an exception to the rule, *aqp1a* was down-regulated following FW-SW transfer in black porgy (An *et al*., 2008) and in Japanese medaka (Madsen *et al*., 2014). Madsen *et al*. (2014) verified that a decrease also occurred at the protein level, and showed that Aqp1a was localized in the brush border membrane of FW-acclimated fish. They suggested that the onset of drinking was accompanied by species-specific responses in the intestinal water uptake mechanism. By comparison, Kim *et al*. (2014) found that *aqp1a* was unaffected by salinity in marine medaka.

The studies by Lignot *et al*. (2002), which initiated investigation of aquaporins in fish intestines, focused on localization and expression of aquaporin 3 in the European eel. They found no effect of salinity on Aqp3b expression; Aqp3b was localized in macrophage-like cells deep within the intestinal epithelium and in goblet cells of the eel rectum, suggesting that this aquaglyceroporin was not a primary water channel in fluid absorption in the eel (Cutler and Cramb, 2002). Instead, it was thought to play a role in mucus production (Lignot *et al*., 2002). A recent study found up-regulation of *aqp3b* mRNA in the rectum of Japanese eel in SW (Kim *et al*., 2010). However, the cellular localization was not reported and its role is unclear. More recent immunohistochemical studies found Aqp3b in the esophagus of the European eel with most staining in goblet cells, and an additional signal in apical and basal parts of the epithelial cells (Cutler *et al*., 2007). In the esophagus of Atlantic salmon, *aqp3a* is also expressed (Tipsmark *et al*., 2010b), but its response to salinity in this tissue has yet to be determined. *aqp3a* did not change upon SW transfer in middle intestine of Atlantic salmon, whereas in Mozambique tilapia and sea bass, *aqp3a* expression, as determined by RT-PCR or Northern blot, was higher in a SW sample than in the FW sample (Watanabe *et al*., 2005; Giffard-Mena *et al*., 2007). Finally, in sockeye salmon, *aqp3(a)* mRNA was also elevated in the intestine following transfer to SW (Choi *et al*., 2013), suggesting that either mucus production is elevated in SW or Aqp3a participates in transcellular transport of water or small organic molecules.

Aquaporin 7 (Aqp7) is found apically in the mammalian gastrointestinal tract, where it is assigned a role in transcellular water transport (Laforenza *et al*., 2005; Tritto *et al*., 2007). It is expressed in the intestine of zebrafish and is permeable to water, glycerol, and urea (Tingaud-Sequeira *et al*., 2010). Recently, Aquaporin 7 was also reported in the Japanese medaka (Madsen *et al*., 2014), but it does not consistently respond to salinity and its cellular localization is so far unknown.

Another aquaglyceroporin found in the intestine of fishes is Aqp10a. This paralog was first reported in zebrafish, where it is expressed at relatively high levels throughout the intestine (Tingaud-Sequeira *et al*., 2010). It was later analyzed in the intestine of Japanese and marine medaka (Kim *et al*., 2014; Madsen *et al*., 2014). In both studies, the mRNA level was higher in FW- than in SW-acclimated fish, which was verified at the protein level in the Japanese medaka by both Western analysis and immunofluorescence microscopy. Intense labeling of the brush border was found in FW medaka; labeling was much less in SW specimens and was mainly located in intracellular pools.

The related paralog *aqp10b* is also present in zebrafish intestine (Tingaud-Sequeira *et al*., 2010), and in European silver eels *aqp10b* was elevated in SW compared to FW, but only in the middle intestine (Martinez *et al*., 2005b). A similar result was reported for *aqp10b* in Atlantic salmon (Tipsmark *et al*., 2010b) and for the posterior intestinal segment in Japanese eels (Kim *et al*., 2010). The cellular localization of *aqp10b* has been studied directly only in seabream, where *in situ* hybridization detected this paralog in the muscle layers and *lamina propria* (Santos *et al*., 2004). In contrast, expression was higher in mucosal scrapings than in the residual layer from the intestine of Atlantic salmon, indicating that this paralog might be expressed in the enterocytes (Tipsmark *et al*., 2010b), where it may participate in transcellular water transport.

The unorthodox aquaporin 8 (termed "ammoniaporin" in Finn *et al*., 2014) has been investigated in the intestine of the stenohaline zebrafish and the Japanese eel, European eel, sockeye salmon, Atlantic salmon, and in two medaka species. In eel and salmon, *aqp8* increases in response to SW transfer (Cutler *et al*., 2009; Kim *et al*., 2010; Tipsmark

```
asAqp8b   GCTVIINILAGGDVSGTCLNPARALGPAIVANYWTYHWVYWVGPITGGLVAAALVRLLLGDRKTRILMK  69
ssAqp8    GCTVIINILAGGDVSGTCLNPARALGPAIVANYWTYHWVYWVGPITGGLVAAALVRLLLGDRKTRILMK  69
asAqp8ab  GCTVIINILAGGDVSGTCLNPARALGPAVMANYWTYHWVYWVGPIAGGLLAAALVRLVLGDRKTEIIMK  69
          ****************************::***************:***:*******.******.*:**
```

Figure 4. Alignment of C-terminal amino acid residues from Atlantic salmon aquaporin Aqp8ab and Aqp8b to sockeye salmon aquaporin 8 (SS8). (*), (:), and (.) show identical, conserved, and semi-conserved residues, respectively. Accession numbers for the paralogs are Aqp8ab: KC626879, Aqp8b: KC626880 (Engelund *et al.*, 2013), and SS8: JX183098.1 (Choi *et al*., 2013).

et al., 2010b; Choi *et al*., 2013). In the study by Choi *et al*. (2013), the increase was only observed in parr, whereas a decrease was seen in smolts, suggesting that the developmental stage is important for the response to SW. The published sequence of the sockeye salmon *aqp8* paralog is most similar to the Atlantic salmon *aqp8b* (Fig. 4) rather than the *aqp8ab* paralog, which increases at both mRNA and protein levels in response to SW (Tipsmark *et al*., 2010b; Engelund *et al*., 2013). The presence of multiple *aqp8* paralogs in the intestine of fishes complicates comparison of studies across species. The multiplicity of paralogs is most likely due to both a WGD event, which gave rise to *aqp8b*, as well as a local tandem genomic duplication that gave rise to *aqp8aa* and *aqp8ab*. This evolutionary mechanism was recently revealed by Cerdà and Finn (2010), who analyzed chromosomal synteny across a wide range of fish species. In the pyloric caeca and middle intestine of Atlantic salmon, protein expression of Aqp8ab, but not Aqp8b, was elevated following 7 d in SW (Engelund *et al*., 2013). Aqp8ab and Aqp8b are both localized at the apical membrane of the intestinal epithelium, but while Aqp8ab also extends along the lateral membrane, the third paralog, Aqp8aa, is restricted to a subapical area (Madsen *et al*., 2011; Engelund *et al*., 2013). In addition, there is Aqp8b immunoreactivity in a subset of goblet cells in the middle intestine. These results suggest that Aqp8ab is the main Aqp8 paralog participating in increased water absorption across the intestine of the SW-acclimated salmon, while Aqp8b may play a supporting role and Aqp8aa may be concerned with maintaining fluid homeostasis in intracellular compartments. Electron microscopy from the closely related rainbow trout has confirmed the presence of Aqp8ab and Aqp8b at the brush border membrane of enterocytes. Besides transporting water, these paralogs were also found to be permeable to urea and glycerol, a quality that may extend their function to aid uptake of small organic molecules from processed food (Engelund *et al*., 2013). Aqp8 phylogeny has reached its maximal complexity in Atlantic salmon with the recent identification of eight paralogs (Aqp8aa1, -aa2, -ab1, -ab2, -bb1, -bb2, -ba1, and -ba2; Finn *et al*., 2014).

In the recent investigations of Japanese and marine medaka (Kim *et al*., 2014; Madsen *et al*., 2014), *aqp8ab* was also found in the intestine. In Japanese medaka, *aqp8ab* is the only *aqp8* paralog found in the genome. In both species, the *aqp8ab* FW level was higher than the SW level, as confirmed at the protein level in Japanese medaka. Immunofluorescence microscopy localized this paralog to the brush border in the Japanese medaka, most intensely in FW, and moving to a subapical position in SW.

Paralogs of aquaporin 11 and 12 also have been reported in the intestine of zebrafish and medaka species (Tingaud-Sequeira *et al*., 2010; Kim *et al*., 2014; Madsen *et al*., 2014). The expression of *aqp12* is higher in SW than in FW marine medaka, but the role of these two "super-aquaporins" is speculative since they induce very little water permeability when expressed in *Xenopus* oocytes (Tingaud-Sequeira *et al*., 2010).

Renal aquaporins

In contrast to mammals, few studies have examined aquaporin expression in fish kidneys and their response to external salinity, and even fewer reports have tried to localize the expression to specific tubular segments. Most papers report aquaporins present in "a subset of tubules" or in "tubules of unknown origin." Considering the fundamental change in water handling by this organ in FW and SW, it should be expected that aquaporin expression is highly dynamic at the tubular cell membranes. In contrast to the studies in the gill, the first aquaporin reported in fish kidney was an AQP10-like paralog in gilthead seabream, expressed in renal tubules of "undefined origin" (Santos *et al*., 2004). These tubules are likely proximal, as gilthead seabream is a marine fish, with tolerance to low-salinity, brackish water (Mancera *et al*., 2002). This report later found support in European eel, where *aqp10b* was expressed in kidneys of indigenous yellow eels and migratory silver eels. The level decreased significantly when yellow (but not silver) eels were transferred to SW (Martinez *et al*., 2005a). In contrast, *aqp10b* increased in response to SW transfer in Atlantic salmon (Tipsmark *et al*., 2010b), though a later study using both parr and smolt stages of this species could not reproduce this effect (Engelund and Madsen, 2015). Instead, they found a general decrease in *aqp10b* in both SW- and sham-transferred fish, suggesting a stress-related effect rather than an effect of salinity *per se*. This effect was not carried on to the protein level, which remained unchanged. However, a significant increase in *aqp10b* from parr to the smolt stage was observed, with a tendency of the protein levels to be higher in smolts, although not to a statistically significant difference. Engelund and Madsen (2015) were among the

first researchers to carry out systematic mapping of aquaporin proteins in tubular segments. Aqp10b is mainly located in the apical cytosolic space of proximal tubule epithelial cells extending from the apical end of the nucleus to the apical membrane. It is also present at lateral locations extending to the basal side of the cells. This localization suggests an important role in transcellular water transport acting as both entry and exit pathway. Another putative role of Aqp10b is to mediate transport of small organic solutes such as glycerol and urea. This theory has been confirmed for eel and zebrafish Aqp10b, as well as the eel Aqp10b paralog (MacIver *et al*., 2009; Tingaud-Sequeira *et al*., 2010). Finally, because secretion of Mg^{2+} is believed to occur through generation and exocytosis of cytoplasmic vesicles (Renfro, 1999), it is possible that intracellular Aqp10b is incorporated into these vesicles, where it mediates water transport.

Aqp3 paralogs have also been investigated in the kidney of a few fishes (Cutler *et al*., 2007; Engelund and Madsen, 2011). The *aqp3b* level decreased in whole kidney homogenates from European eel when transferred from FW to SW (Martinez *et al*., 2005a), whereas total kidney *aqp3a* increased in Atlantic salmon (Tipsmark *et al*., 2010b; Engelund and Madsen, 2015), sea bass (Giffard-Mena *et al*., 2007), and Mozambique tilapia (Watanabe *et al*., 2005) upon acclimation to SW. In European eel, Aqp3b was located apically in "undefined" tubule cells in both FW and SW silver eels, and in Atlantic salmon Aqp3a was found apically in proximal tubule cells with occasional labeling extending laterally toward the basal membrane (Engelund and Madsen, 2015). Surprisingly, the increase in *aqp3a*, which was very consistent in response to SW transfer and independent of life stage, was not translated to the protein level in the plasma membrane. Thus, the role of Aqp3a in Atlantic salmon kidney may be secondary to transcellular water transport. As for Aqp10b, the role of Aqp3a may be to transport organic solutes or maintain fluid balance in intracellular compartments.

Both *aqp1aa* and *aqp1ab* are present in the kidney of Atlantic salmon and European eel (Martinez *et al*., 2005a; Tipsmark *et al*., 2010b). In eel, the transcript level of both paralogs decreased following SW transfer, whereas *aqp1aa* increased and *aqp1ab* decreased in Atlantic salmon. In sea bass and marine medaka, *aqp1a* increased in response to SW transfer (Giffard-Mena *et al*., 2007, 2012; Kim *et al*., 2014), and in climbing perch a transient increase in *aqp1aa* occurred after 24 hours in SW (Ip *et al*., 2013). An *et al*. (2008) found increased levels of *aqp1aa* in the kidney of black porgy when moved from FW to 10 ppt SW, whereas transfer to full strength SW decreased the transcript level compared to FW. In the kidney of the Japanese eel, *aqp1a* was not detected (Aoki *et al*., 2003), and in silver seabream salinity had no effect on *aqp1a* expression (Deane *et al*., 2011), which adds extra complexity to the functional role of these paralogs. The Aqp1a protein was located in the apical membrane of "undefined" tubule cells in European (silver) eels (Martinez *et al*., 2005a) and gilthead seabream (Cerdà and Finn, 2010). In rainbow trout and Atlantic salmon, Aqp1aa occupied apical and basolateral positions in the cells of the proximal tubules (Engelund and Madsen, 2011, 2015), whereas the related Aqp1ab paralog was intracellular in proximal tubules of Atlantic salmon and, in rainbow trout, only occasionally found in distal tubule cells. It seems, therefore, that salmonid Aqp1aa most likely participates in transcellular water transport in proximal tubules, whereas Aqp1ab either is concerned with water homeostasis of intracellular vesicles (Anderson and Loewen, 1975), or it represents a cytosolic pool, which may be trafficked to/from the plasma membrane on a rapid time scale. This specific paralog plays a vital role in oocyte hydration in marine teleosts, where phosphorylation status influences its distribution between cytosolic and membrane domains (Fabra *et al*., 2005; Cerdà *et al*., 2013).

In addition to tubular cells and vascular tissue, the fish kidney also contains hematopoietic tissue, which may express aquaporins at various stages of development. This finding may interfere with conclusions based on whole kidney analyses. In addition, cells destined to become erythrocytes will most likely express Aqp1aa (Fig. 3), which may explain some of the variation seen in mRNA data across different fish species. Immunohistochemical staining for Aqp1a in European eel kidney and Aqp1aa and -1ab in Atlantic salmon also show staining of some cells in the hematopoietic tissue that may represent developing or mature erythrocytes expressing these proteins.

Two paralogs of aquaporin 8 (*aqp8aa* and *aqp8ab*) are expressed in the stenohaline zebrafish kidney, but their localization has not been analyzed. In Japanese medaka, only one aquaporin 8 paralog, *aqp8ab*, is present in the genome and it is expressed in the kidney; in response to salinity change, it remains unchanged in the Japanese medaka and decreases slightly in the marine medaka (Kim *et al*., 2014; Madsen *et al*., 2014). In rainbow trout and Atlantic salmon, Aqp8b was recently shown to be abundant in the basolateral membrane of proximal tubule cells (Engelund and Madsen, 2011, 2015). The permeability characteristics of this paralog were also recently investigated; it was shown that besides transporting urea, as some mammalian AQP8s do, Aqp8b can transport significant amounts of glycerol (Engelund *et al*., 2013). The *aqp8b* transcript was elevated in FW smolts compared to parr, but no clear effects were seen in response to SW transfer. This finding suggests that, although Aqp8b might be important in preparation of the kidney to produce iso-osmotic urine, it is not regulated directly in response to salinity. All in all, the detailed study by Engelund and Madsen (2015) found that transcript levels

of the investigated paralogs varied in response to development and salinity, but protein levels remained stable, suggesting that tubular water transport either is not regulated or paracellular pathways play a significant role. In medaka, *aqp11a* and *aqp12* were both expressed in the kidney, as in most other organs examined, and showed no response to salinity (Kim *et al*., 2014; Madsen *et al*., 2014). In zebrafish, *aqp11b* was not detected in kidney RNA whereas *aqp12* was expressed at a high level (Tingaud-Sequeira *et al*., 2010).

Aquaporin Responses to Salinity

Table 1 summarizes all available studies that have probed for salinity-dependent regulation of aquaporins in fish osmoregulatory tissues. In zebrafish, more paralogs have been identified in osmoregulatory and non-osmoregulatory organs than those described in Table 1 (see Tingaud-Sequeira *et al*., 2010 for a complete survey). The table shows the present status of subcellular localization of the investigated aquaporins. It is apparent that most paralogs display tissue-dependent subcellular location. For instance, Aqp3b is expressed in the basolateral membrane of chloride cells, but it is located apically in the kidney tubule cells of European eel; Aqp8b is localized in the apical membrane of enterocytes but is basolateral in proximal tubule cells of Atlantic salmon. For the few paralogs in which multiple species have been investigated, there is consensus about subcellular localization in specific tissues; *e.g.,* Aqp3 is basolateral in chloride cells in the gills of most species investigated while also being expressed at the lamellae in some species. There are also overall trends of expression of aquaporins within different tissues. Most paralogs investigated are up-regulated, at least at mRNA level, in the intestine of SW-acclimated fishes, whereas the consensus is less clear in the kidney and gill, where some are up-regulated and some are down-regulated in response to SW transfer. It is obvious from current research that there is a high degree of redundancy of aquaporin expression in osmoregulatory organs. Several of these are regulated in response to osmotic challenges, suggesting that they play important roles in transcellular water transport across the transporting epithelium. However, it is still controversial as to which degree other routes of water transport play a role, *e.g.,* diffusional paracellular transport directly through cell-cell junctions (Hill and Shachar-Hill, 2006; Murakami *et al*., 2006; Fishbarg, 2010) and secondary active water transport through cotransporters for ions and metabolites (see Zeuthen, 2002).

Aquaporins in Other Fish Taxonomic Groups

There is a limited amount of information about aquaporins in other non-teleost, older taxonomic groups of fish species. What little information there is largely comes from genomic or gene expression studies. Genomic information is now available for the actinopterygian holostean spotted gar, which has 13 paralogs (copies of Aqp0, -1, -3, -4, -8, -9, -12, -14, and -15, with two copies each of Aqp 10 and 11; Finn *et al*., 2014). Similar to the gar, Sarcopterygii Actinistia coelacanths have a single copy of the aquaporins found in gar, but also a copy of Aqp7 and three duplicated copies of Aqp2. Indeed, these three copies of Aqp2 are thought to be basal forms of the triad of Aqps (Aqp2, -5, and -6) otherwise found in land animals (Dipnoi, lungfish also have single copies of Aqp2; Finn *et al*., 2014). The presence of Aqp2 in renal tubules was one of the key developments that allowed amphibious animals to conserve water and, hence, move away from inhabiting aquatic environments.

Water Balance in Elasmobranchs

One of the interesting things about marine sharks is that, unlike teleost fish, they have body fluids whose osmotic concentration is similar to that of SW, with a tendency toward hyperosmoregulation (body fluids 1018-1118 mOsm kg^{-1}, seawater 930-1050 mOsm kg^{-1}; Karnaky, 1998; Evans *et al*., 2004, 2005; Marshall and Grosell, 2005; Evans and Claiborne, 2009). However, there are major deviations in body fluid ionic and other solute concentrations compared to the external medium. This is because urea (Anderson *et al.,* 2005) and trimethylamine oxide (TMAO; Karnaky, 1998) are used as osmolytes; hence, the concentration of most major ions is below what is found in SW. Because of the near iso-osmotic body fluids, it might be hard to see a role for aquaporin water channels in shark tissues, but, as in other animals, there is still the need for cell volume regulation and the production of fluids in various parts of the body where aquaporins could be utilized. Additionally, the fact that marine shark body fluids have a slightly higher osmolarity than SW has a major impact on ion and water homeostatic and fluid volume regulatory mechanisms. Essentially, there is continuous endosmosis and, due to lower levels of body fluid NaCl, a net influx of sodium ions across the gills. The inflow of water allows for the production of urine without any additional need to acquire water for that purpose. In fact, sharks produce urine that is significantly more dilute than their body fluids (800 mOsm kg^{-1} in spiny dogfish *Squalus acanthias* Linnaeus, 1758; Evans *et al*., 2004) with 60%–85% of the glomerular filtrate being reabsorbed (so that net tubular water absorption must occur), although some fluid associated with Mg^{2+} and SO_4^{2-} ion secretions may also occur in proximal tubule segments (Marshall and Grosell, 2005). Consequently, some of the body fluid dilution caused by water ingress across the gills is counteracted by urine production. Urine production is also increased in response to increases in body fluid volume (Anderson *et al*., 2007). The renal tubule of sharks is more complex than the mammalian or teleost counterparts, with an additional intermediate loop/segment compared to these

Table 1

Summary of all available studies that have probed for salinity-dependent regulation of aquaporins in teleost fish osmoregulatory tissues.

	Gill			Intestine			Kidney			
Aquaporin	Expression	Cell type	Subcell	Expression	Cell type	Subcell	Expression	Cell type	Subcell	Reference
1aa										
Atlantic salmon	m+	NI	NI	m+, p	ENT	A	m+, p	PT, H	A, C, BL	18, 22, 27
Black porgy	m-	NI	NI	m-	NI	NI	m-	NI	NI	11
Climbing perch	m	NI	NI	m+	NI	NI	m	NI	NI	26
European eel	NI	NI	NI	m+, p	ENT	A	m-, p	RT, E	A	5, 6
Gilthead seabream	p	LA	A	m+, p	ENT	A, L	p	RT	A	14, 16
Japanese eel	n.d.	n.d.	n.d.	m+, p	ENT	A	n.d.	NI	NI	3
Japanese medaka	m	NI	NI	m-, p-	ENT	A(FW), SA (SW)	m	NI	NI	30
Marine medaka	m-	NI	NI	m	NI	NI	m+	NI	NI	29
Rainbow trout	NI	NI	NI	m+, p	ENT	A	m, p	PT, H	A,C, BL	20, 22, 31
Rainbow wrasse	p	LA, CC	C, BL (CC's)	NI	NI	NI	NI	NI	NI	12
River pufferfish	m-	NI	NI	m+	NI	NI	m+	NI	NI	28
Sea bass	m	LA	A,C	m+, p	ENT, SM	A	m+	NI	NI	10, 21
Silver seabream	m-	NI	NI	m	NI	NI	m	NI	NI	19
1ab										
Atlantic salmon	m +	NI	NI	m+, p	ENT	A +SA	m-, p	PT, H	SA	18, 22, 27
European eel	NI	NI	NI	NI	NI	NI	m-	NI	NI	5, 6
Gilthead seabream	NI	NI	NI	m-, p	ENT	A	NI	NI	NI	14
Rainbow trout	NI	NI	NI	m+, p	ENT	A + SA	m, p	PT, H	SA	20, 22, 31
3a										
Atlantic killifish	m-, p-	CC	BL	NI	NI	NI	NI	NI	NI	23
Atlantic salmon	m-, p	CC	BL	m	NI	NI	m+, p	PT, H	A, C, L	18, 27, 31
Japanese medaka	m-	NI	NI	m	NI	NI	m	NI	NI	30
Marine medaka	m-	NI	NI	m	NI	NI	m	NI	NI	29
Mozambique tilapia	m+, p	CC	BL	m+	NI	NI	m+	NI	NI	7
Rainbow wrasse	p	CC, AC	BL	NI	NI	NI	NI	NI	NI	12
River pufferfish	m+	NI	NI	m+	NI	NI	m-	NI	NI	28
Sea bass	m-	NI	NI	m	NI	NI	m+	NI	NI	10, 13
Silver seabream	m-	NI	NI	NI	NI	NI	m	NI	NI	8
Sockeye salmon	m+	NI	NI	m+	NI	NI	m	NI	NI	24
3b										
European eel	m-	LA, CC	BL	m, p	MLC, GC	C	m, p	RT	A	1, 2, 9
Japanese eel	m	NI	NI	m+	NI	NI	n.d.	NI	NI	17
7										
Japanese medaka	m	NI	NI	m	NI	NI	m	NI	NI	30
8aa										
Atlantic salmon	n.d.	NI	NI	m	ENT	SA	m	NI	NI	25
European eel	NI	NI	NI	m+	NI	NI	NI	NI	NI	15
Japanese eel	n.d.	NI	NI	m+	NI	NI	n.d.	NI	NI	17
8ab										
Atlantic salmon	n.d.	NI	NI	m+, p+	ENT	A, L	n.d.	NI	NI	18, 22, 25
Japanese medaka	m	NI	NI	m-, p	ENT	A(FW), SA (SW)	m	NI	NI	30
Marine medaka	m	NI	NI	m-	NI	NI	m-	NI	NI	29
Rainbow trout	n.d.	NI	NI	m+, p	ENT	A, L	n.d.	NI	NI	25, 31
8b										
Atlantic salmon	m	NI	NI	m, p	ENT, GC	A, C	m, p	PT	BL	25, 27
Rainbow trout	NI	NI	NI	m, p	ENT, GC	A, C	m, p	PT	BL	20, 25
Sockeye salmon	n.d.	NI	NI	m+	NI	NI	m	NI	NI	24
10a										
Japanese medaka	m+	NI	NI	m-, p-	ENT	A	m	NI	NI	30
10b										
Atlantic salmon	n.d.	NI	NI	m+	NI	NI	m, p	PT	A, C, L	18, 27
European eel	n.d.	NI	NI	m+	NI	NI	m-	NI	NI	5, 6
Gilthead seabream	m	CC	ISH	m	LP, M	ISH	m	RT	ISH	4
Japanese eel	m	NI	NI	m+	NI	NI	n.d.	NI	NI	17

(Continued)

Table 1 (*Continued*)

	Gill			Intestine			Kidney			
Aquaporin	Expression	Cell type	Subcell	Expression	Cell type	Subcell	Expression	Cell type	Subcell	Reference
Japanese medaka	m	NI	NI	m	NI	NI	m	NI	NI	30
Marine medaka	m-	NI	NI	m-	NI	NI	m	NI	NI	29
11a										
Japanese medaka	m	NI	NI	m	NI	NI	m	NI	NI	30
Marine medaka	m-	NI	NI	m+	NI	NI	m	NI	NI	29
12										
Marine medaka	m	NI	NI	m+	NI	NI	m	NI	NI	29

Abbreviations: SC, subcellular localization; m (+/-), mRNA detected and up- (+) or down- (-) regulated in response to SW; p, protein by immunohistochemistry or western blotting; NI, not investigated; n.d., not detected; PT, proximal tubules; RT, unspecified renal tubules; H, hematopoietic tissue; E, endothelium; ENT, enterocytes; SM, submucosa; GC, goblet cells; MLC, macrophage-like cells; M, muscle; LP, *Lamina propria;* LA, lamellae; CC, chloride cells; AC, accessory cells; A, apical membrane; SA, subapical domain; C, cytoplasmic; L, lateral membrane; BL, basolateral membrane; ISH, paralog mRNA located by *in situ* hybridization. References in the last column cover information gathered for some or all of the tissues. 1, Cutler and Cramb (2002); 2, Lignot *et al.* (2002); 3, Aoki *et al.* (2003); 4, Santos *et al.* (2004); 5: Martinez *et al.* (2005a); 6, Martinez *et al.* (2005b); 7, Watanabe *et al.* (2005); 8, Deane and Woo (2006); 9, Cutler *et al.* (2007); 10, Giffard-Mena *et al.* (2007); 11, An *et al.* (2008); 12, Brunelli *et al.* (2010); 13, Giffard-Mena *et al.* (2008); 14, Raldua *et al.* (2008); 15, Cutler *et al.* (2009); 16, Cerda and Finn (2010); 17, Kim *et al.* (2010); 18, Tipsmark *et al.* (2010b); 19, Deane *et al.* (2011); 20, Engelund and Madsen (2011); 21, Giffard-Mena *et al*. (2011); 22, Madsen *et al.* (2011); 23, Jung *et al.* (2012); 24, Choi *et al.* (2013); 25, Engelund *et al.* (2013); 26, Ip *et al.* (2013); 27, Engelund and Madsen (2015); 28, Jeong *et al.* (2014); 29, Kim *et al.* (2014); 30, Madsen *et al.* (2014); 31, Engelund and Madsen (unpubl. data).

other species, but even including FW elasmobranchs (Lacy and Reale, 1995). There are also parts of the kidney where renal tubules are densely packed together (lateral bundle zone) and other regions where renal tubules are within the space of blood sinuses (sinus zone; Lacy and Reale, 1995). Friedman and Herbert (1990) have suggested that the marine shark intermediate IV bundle zone segment is a "diluting segment" with low water permeability but high NaCl re-absorption *via* a NKCC2 cotransporter protein in the apical membrane of renal tubule cells. They also suggested that the shark intermediate VI sinus zone segment has high water permeability, which would lead to water reabsorption and elevation of the concentration of urea, with subsequent urea reabsorption in more distal renal tubule segments.

In addition to the normal renal route for eradicating water from the body, sharks also have a rectal gland, which is attached to the posterior end of the gastrointestinal tract. The rectal gland produces fluid that is iso-osmotic to body fluids, but in which the osmolytes are almost all NaCl (around 524 mmol l^{-1}; Evans and Claiborne, 2009) and little urea (14.5 mmol l^{-1}; Karnaky, 1998) or TMAO. Solomon *et al*. (1984) demonstrated that the rectal gland is stimulated principally by an expansion of body fluid volume rather than by elevated plasma NaCl levels, and that the principal role of the rectal gland presumably is the eradication of excess fluid (*i.e.,* water) rather than directly excess Na^+ and Cl^- ions. Of course, as the movement (excretion) of water is passive, it necessitates that solutes must be actively transported to allow the movement of water to follow by osmosis. The mechanism used for water transport is unclear; measurements of purified rectal gland membranes show low water permeability (Zeidel *et al*., 2005), suggesting an absence of aquaporin water channels in this organ.

The last route that may have an impact on elasmobranch water balance involves drinking or swallowing of SW during eating. Little has been done to investigate the effect of these actions on water balance, but evidence exists that shows that elasmobranchs do drink when needed and probably imbibe SW when eating (Hazon *et al*., 2003; Hammerschlag, 2006). This action would bring water and salts into the gastrointestinal tract where both may be absorbed (Anderson *et al*., 2007). Assuming that absorption occurs, the additional extracellular fluid volume would probably be eradicated mainly by elevated rectal gland secretions in marine elasmobranchs and/or *via* greater urine production in elasmobranchs inhabiting FW (where urea loss through higher urine production is a lesser issue).

Aquaporins in Elasmobranchs

Chondrichthyians are one of the few taxonomic groups of animals for which there is still a paucity of genomic information. The closest genomes to those of elasmobranch sharks are those of a member of the chondrichthyian Holocephali, the chimaera elephant shark, or ghost shark (*Callorhinchus milii* Bory de Saint-Vincent, 1823). There is also genomic information on aquaporins from the elasmobranch rajiform little skate (*Leucoraja erinacea* Mitchill 1825) (Finn *et al*., 2014). Both species have partial or complete copies of Aqp0, -1, -4, -9, -10, -12, -14, and -15 and two copies of Aqp3. The little skate genome also has a copy of Aqp11. Compared to other fish, the elasmobranchs' aquaporin complement does not include Aqps7 and -8; both

aquaporins are found in coelacanths and teleosts, although only Aqp8 is present in lampreys or older invertebrates (Finn *et al*., 2014). As a consequence of the limited genomic information available on chondrichthyian species, very few studies of the role of aquaporins in water balance have been made.

Branchial aquaporins

In the gill of sharks, there are two types of mitochondrion-rich cells (MRCs) that are thought to be engaged in ion transport (Evans *et al*., 2004, 2005; Evans and Claiborne, 2009). The Na^+,K^+-ATPase-rich cells that express the sodium-hydrogen exchanger (NHE3) are also thought to be involved in acid extrusion across the gills, associated with acid-base regulation. The second category of MRCs, expressing V-type H^+-ATPase and a pendrin-like chloride bicarbonate exchanger, are thought to be involved in base extrusion (Evans *et al*., 2004, 2005; Evans and Claiborne, 2009). More recently, in the spiny dogfish Aqp4 was colocalized with Na^+,K^+-ATPase and V-type H^+-ATPase to cells within the branchial epithelium (Cutler *et al*., 2012a,b). This showed that almost all Aqp4 immunoreactivity was located in either gill Na^+,K^+-ATPase and V-type H^+-ATPase MRCs, with Aqp4 expression present in all MRCs. The V-type H^+-ATPase-rich MRCs were principally, but not exclusively, located in the primary gill epithelium, whereas the Na^+,K^+-ATPase-rich MRCs were principally, but not exclusively, located in the secondary gill lamellae (Cutler *et al*., 2012b). Using species- and paralog-specific polyclonal antibodies, spiny dogfish gill cells were shown to exhibit Aqp3 and Aqp15 (formerly Aqp1e/Aqp1e2 in sharks; Cutler *et al*., 2005; Cutler, 2006a, 2007a; or drAqp1/5, then Aqp1b in the zebrafish, Tingaud-Sequeira *et al*., 2010; Zapater *et al*., 2011) immunoreactivity (using species-specific and paralog-specific polyclonal antibodies; C.P. Cutler, unpubl. data). Although the distribution of each of these antibodies differs from those of Aqp4, without colocalization studies any overlap with the cellular location of Aqp4 expression is unknown but may be related to any fluid transport that MRCs engage in. Perhaps Aqp3 and Aqp15 may be involved in cell volume regulation in these cells. *aqp0*, *-1*, and *-9* all show RT-PCR amplification in gill tissue samples (Fig. 5), but the mRNA abundance of those genes is hard to determine using that technique. Antibody studies suggest that branchial Aqp1 may be partly located in red blood cells within the tissue (C.P. Cutler, unpubl. data).

Renal aquaporins

In shark kidney, Aqp4 is expressed in a select range of renal tubule segments (Cutler *et al.*, 2012b), including those segments located in both the sinus and bundle zones. The exact segmental identity of Aqp4 immunoreactivity was not determined. However, the fact that the intermediate VI segment cells in the sinus zone are thought to have highly water-permeable membranes suggests that the localization of at least one Aqp4-expressing segment might be intermediate VI within the sinus zone, and that other connected renal tubule bundle zone segments (*e.g.,* intermediate V or IV) might, therefore, also express Aqp4. Colocalization experiments using species- and paralog-specific polyclonal antibodies have shown that Aqp4 has overlapping expression with Aqp3 and Aqp15 in renal tubules, with Aqp3 noted mainly in sinus zone segments and Aqp15 in both sinus and bundle zone segments (C.P. Cutler, unpubl. data). The expression of both Aqp3 and Aqp15 is less extensive (less renal tubule staining within a cross-section) than that of Aqp4 staining. *aqp0*, *-1*, and *-9* cDNAs all amplify from renal tissue samples using RT-PCR (Fig. 5), but the absolute abundance of mRNAs for these genes is currently unknown.

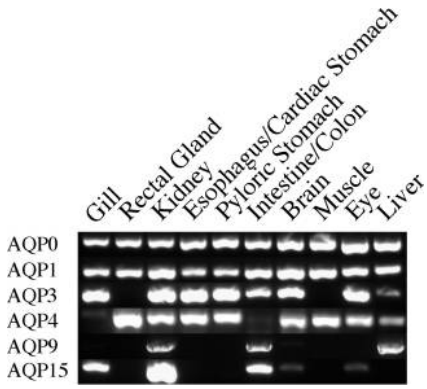

Figure 5. Reverse transcriptase polymerase chain reaction (RT-PCR) samples run on an agarose electrophoresis gel. PCR was performed with specific primers for each aquaporin using cDNAs generated from various spiny dogfish (*Squalus acanthias*) tissue sample total RNAs. These amounts of the same cDNAs were identical to what was used in Cutler *et al*. (2012a). Images were generated using a Syngene GBox gel documentation system (Syngene, Frederick, MD).

Rectal gland aquaporins

Although Zeidel *et al*. (2005) suggested that the rectal gland expressed no aquaporin genes, it was a surprise when significant amounts of *aqp* mRNAs were identified in tissue samples (Cutler *et al*., 2012a). Aqp4 was found in all rectal gland tubules/cells, with either more staining in the apical pole of the cell or the basal membrane region, depending on which Aqp4 antibody was used for immunohistochemistry (Cutler *et al*., 2012b). In most species, Aqp4 is basolaterally located in epithelial cells (*e.g.,* see Kortenoeven and Fenton, 2014), which is more consistent with the results for the dogfish Aqp4/2 antibody (Cutler *et al*., 2012b). However, as most of the Aqp4 staining with either antibody is cytoplasmic, that finding is not totally inconsistent with the Zeidel *et al*. (2005) study. One possibility is that Aqp4 is regulated *via* protein trafficking into the membrane (in a similar

fashion to mammalian renal AQP2; Kortenoeven and Fenton, 2014), in response to rectal gland hormonal stimulation by C-type natriuretic peptide (CNP) or vasoactive intestinal peptide (VIP), or other secretagogs (Marshall and Grosell, 2005). To date, attempts to stimulate the gland and investigate changes in Aqp4 localization have been equivocal (C.P. Cutler, unpubl. data). In addition to a significant amount of *aqp4* mRNA in the rectal gland, there are even greater amounts of *aqp1* mRNA, while antibody studies show only low levels of Aqp1 protein abundance in a selection of secretory tubules. RT-PCR studies also show amplification of *aqp0* and very low levels of *aqp3* (Fig. 5). The significance of this finding is unknown, but no protein staining is seen with the dogfish Aqp3-specific antibody that gives strong signals in renal tubule cells (see above; C.P. Cutler, unpubl. data).

Gastrointestinal tract aquaporins

The gastrointestinal tract of sharks such as the spiny dogfish is significantly different morphologically from those of teleost fish or, indeed, mammals (see Gilbert, 1973 for a more complete anatomical description). The esophageal luminal surface is covered in white cartilaginous, cone-shaped papillae structures. This morphology transitions sharply into a smooth, light brown, folded epithelium of the cardiac/fundic stomach about halfway along the length of the esophagus, toward the pyloric stomach. The intestine itself is valvular in nature, with multiple internal folds or flaps that probably allow this organ to be compartmentalized. Distally there is a short straight rectum/colon segment.

The most significant aquaporin localization within the gastrointestinal tract is that of Aqp4 within the cardiac/fundic stomach luminal surface epithelium. It has apparent secretory tubule structures whose cells stain strongly for Aqp4 (Cutler *et al*., 2012b). There is also some lower-level sporadic Aqp4 staining in cells deeper within the tissue. Further immunohistochemical studies show that Aqp4 is also expressed strongly in unidentified swirls of cells within the wall of the intestine (Fig. 6a); these may represent some kind of unusual muscle morphology, but their identity is unknown. In the rectum/colon, there is also low-level expression of Aqp4 protein in the rectal/colon luminal epithelium in comparison to control sections (Fig. 6 b, c, d). Furthermore, there is intense Aqp1 staining in a layer of cells just underneath the rectum/colon luminal epithelial surface layer (not shown). RT-PCR studies further show *aqp0*, *-1*, and *-3* cDNA amplification from a combined sample from the esophagus and cardiac/fundic stomach. They also show *aqp0*, *-1, -3,* and *-4* in the pyloric stomach and *aqp0, -1, -3, -9*, and *-15* amplification in a combined intestine/rectum/colon sample (Fig. 5). The significance of these *aqp* mRNAs in these gastrointestinal tract segments is unknown.

Aquaporins in Agnathans

The two main taxonomic groups of agnathan or cyclostome fish are Hyperoartia, lampreys and Hyperotreti, and hagfish (for a review of agnathan ion and water homeostasis see Karnaky, 1998; Evans and Claiborne, 2009). Lampreys are anadromous euryhaline osmoregulators and include some landlocked FW populations. Hagfish, in comparison, are stenohaline marine species that are more osmoconformers as their body fluids are isotonic, but with some divergence of ion concentrations from those found in SW. These profound differences in life style and ion and water homeostatic strategies are probably reflected by the fact that the hagfish and lamprey taxonomic groups have been separate for at least 500 million years. One of the more interesting morphological differences between lampreys and hagfish is that lampreys have eyes and hagfish do not.

A recent study of aquaporin homologs in lampreys, by Finn *et al*. (2014), showed that these animals possess a gene that has some exons with homology to *aqp0* and some that have higher homology to *aqp1*. Earlier invertebrate species do not possess either gene, and thus these two *aqp* genes, which are derived from *aqp4*, likely originated in an agnathan ancestor. This fact is of interest because lamprey species represent the most ancient extant ancestral chordates with eyes, and AQP0 is also known as MIP (major intrinsic protein (of the lens of the eye)). It may be that the development of eyes was facilitated by the generation of *aqp0* (Finn *et al*., 2014). The other aquaporins in the complement present in lamprey genomes include two copies of *aqp3* and *-10*, and single copies of *aqp4, -8, -12*, and *-14*. Nothing further is known about the role of these genes in lamprey water balance and osmoregulation.

In hagfish, while there is no genomic information available for the Hyperotreti, two aquaporin genes have been isolated and studied. An Aqp4 homolog was initially isolated from the Atlantic hagfish (*Myxine glutinosa*, Linnaeus, 1758; Cutler, 2006a, 2007b), but was subsequently isolated from the Pacific hagfish (*Eptatretus stoutii* Lockington, 1878); this gene was studied much more extensively (Nishimoto *et al*., 2007). Pacific hagfish *aqp4* shows only mRNA and protein expression within the gill, where it is located in the basolateral membranes of pavement cells. Given that hagfish are iso-osmotic to the environment, the usual roles that aquaporins play in higher chordate species are mostly moot. However, for aquaporins to have survived from invertebrates to higher vertebrates during evolution, they must have a function. In hagfish gill pavement cells, it was suggested that Aqp4 plays a role in the hydration requirements needed for mucus secretion by these cells. Another possible role for Aqp4 was to be in cell-cell contact or adhesion (Nishimoto *et al*., 2007). Indeed, Aqp4 in other species is involved in cell-cell adhesion processes (Hiroaki *et al*., 2006). A role for Aqp4 in mucus secretion was

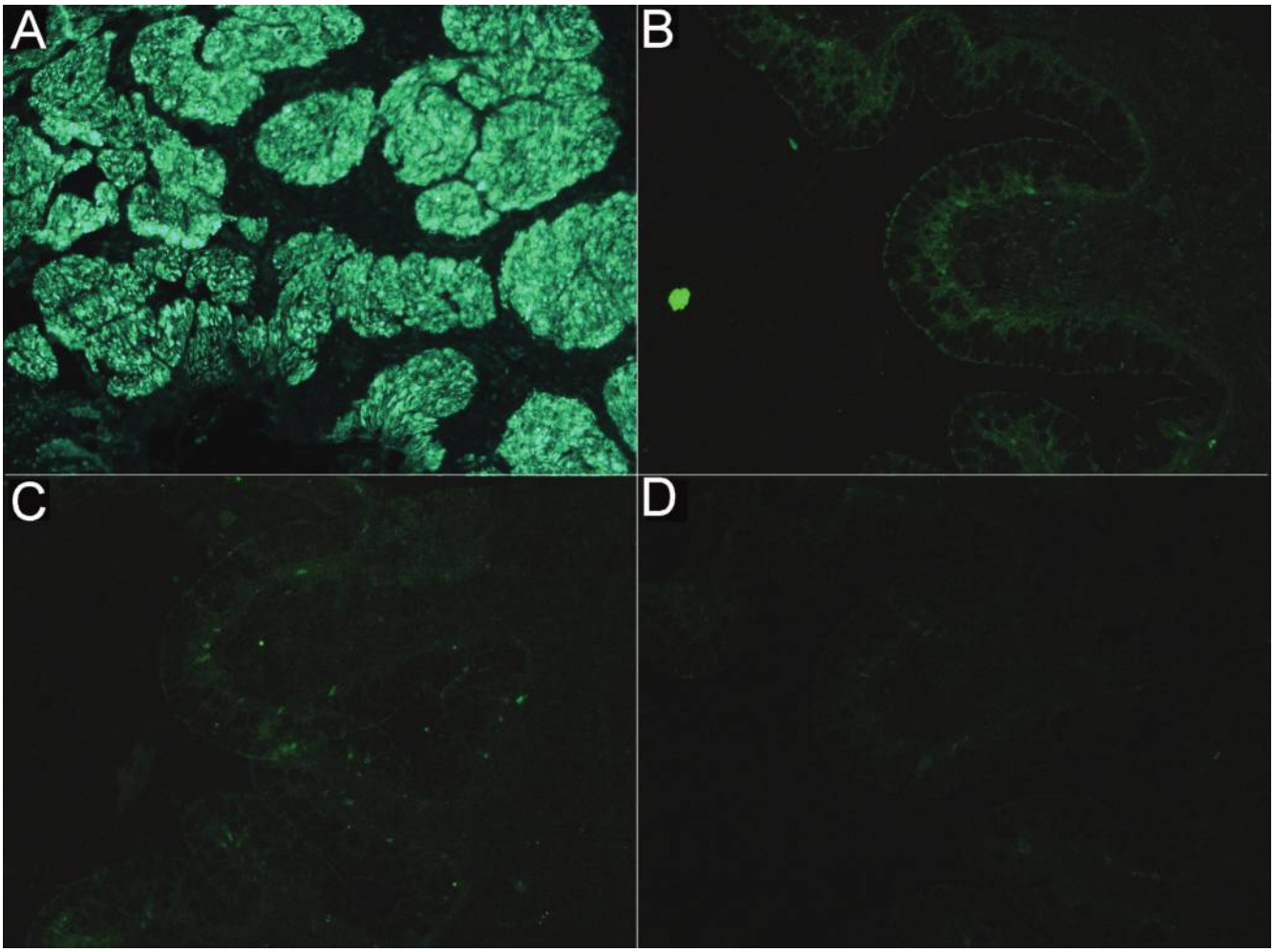

Figure 6. Immmunohistochemical localization of spiny dogfish (*Squalus acanthias*) AQP4/1 antibody (see Cutler *et al*., 2012b) in the side wall of the spiral valve intestine (A) and in the colon/rectum (B-D). All images are at 100x magnification using a Zeiss Axiovert 200 microscope (Carl Zeiss, Göttingen, Germany). The strong staining in A was not verified with subsequent control sections. The apparent AQP4 staining appears to be located in swirls of muscle-like clusters of cells. (B) Weak staining of the AQP4/1 antibody in the surface epithelial cells of the colon/rectum. Staining appears in the apical membrane and in the basal pole of these cells. This staining was substantially reduced when the antibody was pre-blocked/negated with 50 μg/ml peptide antigen (C). Non-specific staining is indicated in a similar section incubated with a similar dilution (1 in 100) of pre-immune serum instead of the AQP4/1 antibody (D). The images in (B), (C), and (D) were taken with the same camera exposure conditions and show the same part of the colon/rectum in different sections. All sections used a highly cross-absorbed, anti-rabbit Alexa 488-labeled secondary antibody and were otherwise prepared, as in Cutler *et al*., 2012b.

corroborated by work on the other known hagfish Aqp. This Aqp is an aquaglyceroporin (Glp), and it was originally isolated from the Atlantic hagfish. It was described as an ortholog of Aqp9 based on a marginally higher level of amino acid homology to human AQP9 compared to its homology to other human AQP aquaglyceroporins (AQP3, -7, -10; Cutler, 2006b). More recently, another aquaglyceroporin homolog was identified in the Pacific hagfish. Due to more extensive phylogenetic analysis, it was described as an Aqp3 homolog (Herr *et al*., 2014). However, as the amino acid homology of these two hagfish sequences is 93.3%, these aquaglyceroporins most likely represent copies of the same gene in the two hagfish species (for comparison, human AQP3 and -9 amino acid homology is only 46.3%, and that is within the same species). As noted above, there are two copies of Aqp3 in the agnathan lamprey genome (sea lamprey *Petromyzon marinus* Linnaeus, 1758; Finn *et al*., 2014) but the amino acid homology of these sequences is only 34.2%. Exhaustive phylogenetic analysis of probably all currently known animal Aqp sequences concluded that the hagfish Glp sequence identity could not be determined; hagfish Glp associated with the new branch

of Aqp13 Glps, but this was thought to be due to long-branch attraction as a result of the low homology of hagfish Glp to Glps in other species (Finn *et al*., 2014). Pacific hagfish Glp is expressed in the slime gland and is speculated to be involved in the rupture of mucin vesicles in this tissue (Herr *et al*., 2014). The same study (in contrast to the work of Nishimoto *et al*., 2007) curiously also showed that hagfish Aqp4 is expressed in the slime gland and thus, Aqp4 might also be involved in the rupture of mucin vesicles in this organ (Herr *et al*., 2014). Identification of other Aqps in hagfish probably awaits a hagfish genomic or transcriptomic sequencing effort.

Transepithelial Water Transport in Fish Osmoregulatory Organs: Future Perspectives

It is apparent that there is no consensus regarding the molecular pathway that water travels across epithelia. One route or mechanism may be preferred in one tissue whereas another route is taken in other tissues, but it seems more likely that that both transcellular and paracellular routes may act in concert. Water permeability (osmotic as well as diffusional) of eel gills was highest in FW and lowest in SW (Motais *et al.,* 1969; Isaia, 1984). Several aquaporins have been expressed in this tissue (Table 1) and are associated with chloride cells in many fish species. Redistribution of chloride cells in this tissue occurs upon SW acclimation (Evans *et al*., 2005), which may greatly affect aquaporin abundance. The elevated expression of, for example, Aqp3 in FW gill chloride cells, lends support to the hypothesis that aquaporins contribute to the increased water permeability of gills of FW fishes, and links nicely back to the early conclusion of Motais *et al*. (1969), that "The surprisingly low diffusional and osmotic permeabilities of the gill epithelium in sea-water fish may be possibly related to the absence of water-filled pores." However, the primary role of aquaporins in the gill of fishes may likely be cell volume regulation and/or gas transport. Basolateral Aqp3, in particular, in chloride cells of several euryhaline species may regulate cell volume of these highly active specialized cells, whereas the lamellar location of Aqp1 in sea bass and gilthead seabream indicates a role for this aquaporin in gas transport (Table 1). Water permeability of the gill is also under the influence of calcium (Ogasawara and Hirano, 1984), which indicates that the tight junctions are modulated in response to water hardness and salinity. Gills of rainbow trout have higher transepithelial resistance in FW than in SW (Wood and Part, 1997). This change most likely reflects an alteration of the tight junction complex between chloride cells and accessory cells (see Chasiotis *et al.*, 2012) and may also affect conditions for paracellular water transport, even though there is no straightforward relationship between ionic tightness and water permeability of any epithelium. Some tight junction proteins, such as mammalian claudin-2, create water-permeable pores (Rosenthal *et al*., 2010), but, to date, there is no information on similar properties of any fish tight junction.

Intestinal water absorption rates and transepithelial resistance increase when salmon are acclimated to SW, as explained earlier (Sundell *et al*., 2003; Sundell and Sundh, 2012). In a recent review, J. M. Whittamore discussed the relevance of the different molecular pathways of water absorption for the marine fish intestine and suggested that both transcellular and paracellular pathways may be important (Whittamore, 2012). The components needed for the solute-coupled water transport to function in the fish intestine are present considering the available data on aquaporins and ion transporters in the intestine of marine fish (Cerdà and Finn, 2010; Grosell, 2011). Only the undisputable localization of aquaporins in the lateral membrane is lacking, since the data so far only have shown localization by immunofluorescence of seabream Aqp1aa and Atlantic salmon Aq8ab in the lateral membrane of enterocytes (Raldua *et al*., 2008; Madsen *et al*., 2011). Localization by immunoelectron microscopy with multiple antibodies per aquaporin would rigorously confirm the presence of these aquaporins and establish a basis for their important role in transepithelial water absorption. The molecular evidence indirectly supports a transcellular route of water absorption in most fish species, as many aquaporins are up-regulated in response to SW transfer in the intestine (Table 1). However, in eel intestine, analysis of purified brush border membrane vesicles showed very low water permeability of the apical membrane, and water transport also was insensitive to Hg. This finding indicates that water transport is not mediated by aquaporins but occurs through diffusion of the lipid bilayer of the vesicles (Alves *et al*., 1999). A more recent mammalian study found a wide range of water permeabilities when analyzing brush border membrane vesicles from the rat whole small intestine. The authors urge caution when extending the findings of the vesicles to the native tissue (Tritto *et al*., 2007). There also may be differences in lipid composition and, thus, water permeability between fish and mammalian enterocytes, and indeed between individual intestinal segments in these species. This theory complicates further comparisons of intestinal water permeability across vertebrate classes.

The paracellular route also may be important in the absorption of water across the intestinal epithelium. The precipitation of $CaCO_3$ along the intestinal tract of marine teleost may enhance both transcellular and paracellular water transport by lowering the osmotic concentration of the luminal fluid. A recent study found up-regulation of Atlantic salmon claudin-15 and -25b mRNA upon SW acclimation, which supported a modification of the paracellular barrier in SW-acclimated fish intestine (Tipsmark *et al*., 2010a). If these claudins modulate the tight junction, allowing more water than solutes to flow, and if the hyperosmotic

condition of the LIS fluid is maintained, a significant amount of water transport may occur through this pathway. In contrast, a recent study on killifish intestine probed for changes in transcellular and paracellular water permeability in response to feeding and various inhibitors of trans- and paracellular transport. The authors concluded that water moved by a transcellular pathway, whereas the radio-labeled PEG oligomers that were used moved strictly through the paracellular pore (Wood and Grosell, 2012).

In the kidney of fishes, not much is known about which molecular mechanism is responsible for the water secretion seen in the proximal tubules of SW-acclimated fish, in particular. In the mammalian nephron, transcellular absorption of fluid by aquaporins occurs due to their distribution along the nephron, and results using AQP knockout mice have shown a decreased ability of the animals to concentrate urine (Nielsen *et al*., 2002). However, recent studies have shown water permeability of claudin-2 and indicated that the paracellular pathway may add an important and hitherto overlooked contribution to fluid reabsorption in the proximal tubules of mammalian kidneys (Rosenthal *et al*., 2010; Schnermann *et al*., 2013).

Since the proximal tubules of fish nephrons are involved in both reabsorption and secretion of ions, the net fluid transport, which is secretion in most cases (Cliff and Beyenbach, 1992), may be the result of large amounts of water passing through the epithelium in both directions. Ample evidence shows that apical aquaporins (Table 1) and few examples of basolateral aquaporins (Engelund and Madsen, 2015) supply the epithelium with a transcellular route for water transport in this particular example.

Aside from the recent study by Engelund and Madsen (2015), there is a general lack of knowledge about distribution of aquaporins and their contribution to urine formation in the fish kidney. A starting point would be to firmly determine, by high-resolution microscopy, the subcellular localization of aquaporins already found in fish kidneys. Investigation of aquaporins and fluid handling in aglomerular marine fish species may be an important avenue to shed light on the importance of aquaporins in renal function. Finally, more studies of aquaporins in the kidneys of agnathans and elasmobranchs would allow comparative hypotheses to be tested regarding the role and evolution of aquaporins in fish kidneys. The role of aquaporins in elasmobranch branchial MRCs and in rectal gland tubule cells also needs further investigation. Unlike in the mammalian kidney, claudins in the kidney of fish and their modulation in response to salinity have been sparsely investigated (*e.g.,* Duffy *et al*., 2011). To resolve the issues of transepithelial water transport, we encourage integrated research into both transcellular and paracellular mechanisms and how these mechanisms respond to environmental challenges.

Acknowledgments

SSM received support from the Danish Council for Independent Research/Natural Sciences (09-070689). MBE is supported by the Danish Council for Independent Research/Medical Sciences (4004-00619). CPC received support from the National Science Foundation (NSF IOS 0844818, 1041885, 1241312) and four summer research fellowships from the Mount Desert Island Biological Laboratory (MDIBL).

Literature Cited

Agre, P., and D. Kozono. 2003. Aquaporin water channels: molecular mechanisms for human diseases. *FEBS Lett.* **555:** 72–78.

Agre, P., M. Bonhivers, and M. J. Borgnia. 1998. The aquaporins, blueprints for cellular plumbing systems. *J. Biol. Chem.* **273:** 14659-14662.

Alves, P., G. Soveral, R. I. Macey, and T. F. Moura. 1999. Kinetics of water transport in eel intestinal vesicles. *J. Membr. Biol.* **171:** 177–182.

Amer, S., and J. A. Brown. 1995. Glomerular actions of arginine vasotocin in the *in situ* perfused trout kidney. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **269:** R775–R780.

An, K. W., N. N. Kim, and C. Y. Choi. 2008. Cloning and expression of aquaporin 1 and arginine vasotocin receptor mRNA from the black porgy, *Acanthopagrus schlegeli:* effect of freshwater acclimation. *Fish Physiol. Biochem.* **34:** 185–194.

Anderson, B. G., and R. D. Loewen. 1975. Renal morphology of freshwater trout. *Am. J. Anat.* **143:** 93–114.

Anderson, W. G., J. P. Good, R. D. Pillans, N. Hazon, and C. E. Franklin. 2005. Hepatic urea biosynthesis in the euryhaline elasmobranch *Carcharhinus leucas. J. Exp. Zool.* **303:** 917–921.

Anderson, W. G., J. R. Taylor, J. P. Good, N. Hazon, and M. Grosell. 2007. Body fluid volume regulation in elasmobranch fish. *Comp. Biochem. Physiol.* **148:** 3–13.

Aoki, M., T. Kaneko, F. Katoh, S. Hasegawa, N. Tsutsui, and K. Aida. 2003. 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.

Berthelot, C., F. Brunet, D. Chalopin, A. Juanchich, M. Bernard, B. Noel, P. Bento, C. Da Silva, K. Labadie, A. Alberti *et al.* 2014. The rainbow trout genome provides novel insights into evolution after whole-genome duplication in vertebrates, *Nat. Commun.* **5:** 3657. doi: 10.1038/ncomms4657.

Beyenbach, K. W. 2004. Kidneys sans glomeruli. *Am. J. Physiol. Renal Physiol.* **286:** F811–F827.

Borgnia, M., S. Nielsen, A. Engel, and P. Agre. 1999. Cellular and molecular biology of the aquaporin water channels. *Annu. Rev. Biochem.* **68:** 425–58.

Brown, J. A., B. A. Jackson, J. A. Oliver, and I. W. Henderson. 1978. Single nephron filtration rates (SNGFR) in the trout, *Salmo gairdneri.* Validation of the use of ferrocyanide and the effects on environmental salinity. *Pflug. Arch.* **377:** 101–108.

Brunelli, E., A. Mauceri, F. Salvatore, A. Giannetto, M. Maisano, and S. Tripepi. 2010. Localization of aquaporin 1 and 3 in the gills of the rainbow wrasse *Coris julis. Acta Histochem.* **112:** 251–258.

Brunet, F. G., H. R. Crollius, M. Paris, J. M. Aury, P. Gibert, O. Jaillon, V. Laudet, and M. Robinson-Rechavi. 2006. Gene loss and evolutionary rates following whole-genome duplication in teleost fishes. *Mol. Biol. Evol.* **23:** 1808–1816.

Cerdà, J., and R. N. Finn. 2010. Piscine aquaporins: an overview of recent advances. *J. Exp. Zool.* **313A:** 623–650.

Cerdà, J., C. Zapater, F. Chauvigné, and R. N. Finn. 2013. Water homeostasis in the fish oocyte: new insights into the role and molecular regulation of a teleost-specific aquaporin. *Fish Physiol. Biochem.* **39:** 19–27.

Chasiotis, H., D. Kolosov, P. Bui, and S. P. Kelly. 2012. Tight junctions, tight junction proteins and paracellular permeability across the gill epithelium of fishes: a review. *Respir. Physiol. Neurobiol.* **184:** 269–281.

Chaube, R., F. Chauvigné, A. Tingaud-Sequeira, K. P. Joy, A. Acharjee, V. Singh, and J. Cerdà. 2011. Molecular and functional characterization of catfish (*Heteropneustes fossilis*) aquaporin-1b: changes in expression during ovarian development and hormone-induced follicular maturation. *Gen. Comp. Endocrinol.* **170:** 162–171.

Chen, L. M., J. Zhao, R. Musa-Aziz, M. F. Pelletier, I. A. Drummond, and W. F. Boron. 2010. Cloning and characterization of a zebrafish homologue of human AQP1: a bifunctional water and gas channel. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **299:** R1163–R1174.

Choi, Y. J., H. S. Shin, N. N. Kim, S. H. Cho, Y. Yamamoto, H. Ueda, J. Lee, and C. Y. Choi. 2013. Expression of aquaporin-3 and -8 mRNAs in the parr and smolt stages of sockeye salmon, *Oncorhynchus nerka:* effects of cortisol treatment and seawater acclimation. *Comp. Biochem. Physiol.* **165A:** 228–236.

Cliff, W. H., and K. W. Beyenbach. 1992. Secretory renal proximal tubules in seawater- and freshwater-adapted killifish. *Am. J. Physiol. Renal Physiol.* **262:** F108–F116.

Curran, P. F., and A. K. Solomon. 1957. Ion and water fluxes in the ileum of rats. *J. Gen. Physiol.* **41:** 143–168.

Cutler, C. P. 2006a. Cloning of aquaporin 1e gene homologues in the dogfish shark (*Squalus acanthias*) and hagfish (*Myxine glutinosa*). *Bull. Mt. Desert Isl. Biol. Lab.* **45:** 40–41.

Cutler, C. P. 2006b. Cloning of an aquaporin 9 gene orthologue from the hagfish (*Myxine glutinosa*). *Bull. Mt. Desert Isl. Biol. Lab.* **45:** 42–43.

Cutler, C. P. 2007a. Cloning and identification of four aquaporin genes in the dogfish shark (*Squalus acanthias*). *Bull. Mt. Desert Isl. Biol. Lab.* **46:** 19–20.

Cutler, C. P. 2007b. Nucleotide and derived-amino acid sequence of a putative Atlantic hagfish (*Myxine glutinosa*) aquaporin 4 cDNA. *Bull. Mt. Desert Isl. Biol. Lab.* **46:** 21–22.

Cutler, C. P., and G. Cramb. 2000. Water transport and aquaporin expression in fish. Pp. 431–441 in *Molecular Biology and Physiology of Water and Solute Transport,* S. Hohmann, and S. Nielsen, eds. Kluwer Academic Press, London.

Cutler, C. P., and G. Cramb. 2002. Branchial expression of an aquaporin 3 (AQP-3) homologue is downregulated in the European eel *Anguilla anguilla* following seawater acclimation. *J. Exp. Biol.* **205:** 2643–2651.

Cutler, C. P., and G. Cramb. 2008. Differential expression of absorptive cation-chloride cotransporters in the intestinal and renal tissues of the European eel (*Anguilla anguilla*). *Comp. Biochem. Physiol.* **149:** 63–73.

Cutler, C. P., L. Meischke, and G. Cramb. 2005. Evolutionary and comparative analysis of aquaporin water channel genes in fish. *Bull. Mt. Desert Isl. Biol. Lab.* **44:** 55.

Cutler, C. P., A. S. Martinez, and G. Cramb. 2007. The role of aquaporin 3 in teleost fish. *Comp. Biochem. Physiol.* **148A:** 82–91.

Cutler, C. P., C. Philips, N. Hazon, and G. Cramb. 2009. Aquaporin 8 (AQP8) intestinal mRNA expression increases in response to salinity acclimation in yellow and silver European eels (*Anguilla anguilla*). *Comp. Biochem. Physiol.* **153A:** S78.

Cutler, C. P., B. MacIver, G. Cramb, and M. L. Zeidel. 2012a. Aquaporin 4 is a ubiquitously expressed isoform in the dogfish (*Squalus acanthias*) shark. *Front. Physiol.* **2:** 107.

Cutler, C. P., S. Harmon, J. Walsh, and K. Burch. 2012b. Characterization of aquaporin 4 protein expression and localization in tissues of the dogfish (*Squalus acanthias*). *Front. Physiol.* **3:** 21.

Davidson, W. S., B. F. Koop, S. J. M. Jones, P. Iturra, R. Vidal, A. Maass, I. Jonassen, S. Lien, and S. W. Omholt. 2010. Sequencing the genome of the Atlantic salmon (*Salmo salar*). *Genome Biol.* **11:** 403. doi:10.1186/gb-2010-11-9-403.

Deane, E. E., and N. Y. Woo. 2006. Tissue distribution, effects of salinity acclimation, and ontogeny of aquaporin 3 in the marine teleost, silver sea bream (*Sparus sarba*). *Mar. Biotechnol. (NY)* **8:** 663-671.

Deane, E. E., J. C. Luk, and N. Y. Woo. 2011. Aquaporin 1a expression in gill, intestine, and kidney of the euryhaline silver sea bream. *Front. Physiol.* **2:** 39. doi: 10.3389/fphys.2011.00039.

Diamond, J. M., and W. H. Bossert. 1967. Standing-gradient osmotic flow: a mechanism for coupling of water and solute transport in epithelia. *J. Gen. Physiol.* **50:** 2061–2083.

Duffy, N. M., P. Bui, M. Bagherie-Lachidan, and S. P. Kelly. 2011. Epithelial remodeling and claudin mRNA abundance in the gill and kidney of puffer fish (*Tetraodon biocellatus*) acclimated to altered environmental ion levels. *J. Comp. Physiol.* **181B:** 219–238.

Duquette, P. P., P. Bissonnette, and J. Y. Lapointe. 2001. Local osmotic gradients drive the water flux associated with Na^+/glucose cotransport. *Proc. Natl. Acad. Sci. USA* **98:** 3796–3801.

Engelund, M. B., and S. S. Madsen. 2011. The role of aquaporins in the kidney of euryhaline teleosts. *Front. Physiol.* **2:** 51. doi: 10.3389/fphys.2011.00051.

Engelund, M. B., and S. S. Madsen. 2015. Tubular localization and expressional dynamics of aquaporins in the kidney of seawater-challenged Atlantic salmon. *J. Comp. Physiol.* **185:** 207–223.

Engelund, M. B., F. Chauvigné, B. M. Christensen, R. N. Finn, J. Cerdà, and S. S. Madsen. 2013. Differential expression and novel permeability properties of three aquaporin 8 paralogs from seawater-challenged Atlantic salmon smolts. *J. Exp. Biol.* **216:** 3873–3885.

Evans, D. H., and J. B. Claiborne. 2009. Osmotic and ionic regulation in fishes. Pp. 295-366 in *Osmotic and Ionic Regulation: Cells and Animals,* D. H. Evans, ed. CRC Press, Boca Raton, FL.

Evans, D. H., P. M. Piermarini, and K. P. Choe. 2004. Homeostasis: osmoregulation, pH regulation and nitrogen excretion. Pp. 247–268 in *Biology of Sharks and their Relatives,* J. C. Carrier, J. A. Musick, and M. R. Heithaus, eds. CRC Press, Boca Raton, FL.

Evans, D. H., P. M. Piermarini, and K. P. Choe. 2005. The multifunctional fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. *Physiol. Rev.* **85:** 97–177.

Fabra, M., D. Raldua, D. M. Power, P. M. T. Deen, and J. Cerdà. 2005. Marine fish egg hydration is aquaporin-mediated. *Science* **307:** 545.

Finn, R. N., F. Chauvigné, J. B. Hlidberg, C. P. Cutler, and J. Cerdà. 2014. The lineage-specific evolution of aquaporin gene clusters facilitated tetrapod terrestrial adaptation. *PLoS One* **9(11):e**113686. doi:10.1371/journal.pone.0113686.

Fischbarg, J. 2010. Fluid transport across leaky epithelia: central role of the tight junction and supporting role of aquaporins. *Physiol. Rev.* **90:** 1271–1290.

Friedman, P. A., and S. C. Hebert. 1990. Diluting segment in kidney of dogfish shark. I. Localization and characterization of chloride absorption. *Am. J. Physiol.* **258:** R398–R408.

Giffard-Mena, I., V. Boulo, F. Aujoulat, H. Fowden, R. Castille, G. Charmantier, and G. Cramb. 2007. Aquaporin molecular characterization in the sea-bass (*Dicentrarchus labrax*): the effect of salinity on AQP1 and AQP3 expression. *Comp. Biochem. Physiol.* **148A:** 430–444.

Giffard-Mena, I., C. Lorin-Nebel, G. Charmantier, R. Castille, and V. Boulo. 2008. Adaptation of the sea-bass (*Dicentrarchus labrax*) to

fresh water: role of aquaporins and Na^+/K^+-ATPases. *Comp. Biochem. Physiol.* **150A:** 332–338.

Giffard-Mena, I., V. Boulo, C. Abed, G. Cramb, and G. Charmantier. 2012. Expression and localization of aquaporin 1a in the sea-bass (*Dicentrarchus labrax*) during ontogeny. *Front. Physiol.* **2:** 34. doi: 10.3389/fphys.2011.00034.

Gilbert, S. G. 1973. *Pictorial Anatomy of the Dogfish.* University of Washington Press, Seattle, WA.

Gonzales, R. J., and D. G. McDonald. 1992. The relationship between oxygen consumption and ion loss in a freshwater fish. *J. Exp. Biol.* **163:** 317–332.

Gorelick, D. A., J. Praetorius, T. Tsunenari, S. Nielsen, and P. Agre. 2006. Aquaporin-11: a channel protein lacking apparent transport function expressed in brain. *BMC Biochem.* **7:** 14. doi: 10.1186/1471-2091-7-14.

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

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

Hammerschlag, N. 2006. Osmoregulation in elasmobranchs: a review for fish biologists, behaviourists and ecologists. *Mar. Freshw. Behav. Physiol.* **39:** 209–228.

Hazon, N., A. Wells, R. D. Pillans, J. D. Good, W. G. Anderson, and C. E. Franklin. 2003. Urea based osmoregulation and endocrine control in elasmobranch fish with special reference to euryhalinity. *Comp. Biochem. Physiol.* **136:** 685–700.

Herr, J. E., A. M. Clifford, G. G. Goss, and D. S. Fudge. 2014. Defensive slime formation in Pacific hagfish requires Ca^{2+}- and aquaporin-mediated swelling of released mucin vesicles. *J. Exp. Biol.* **217:** 2288–2296.

Hickman, C. P., and B. F. Trump. 1969. The kidney. Pp. 91-239 in *Fish Physiology,* Vol. 1, W.S. Hoar and D. J. Randall, eds. Academic Press, New York.

Hill, A. E., and B. Shachar-Hill. 2006. A new approach to epithelial isotonic fluid transport: an osmosensor feedback model. *J. Membr. Biol.* **210:** 77–90.

Hirata, T., T. Kaneko, T. Ono, T. Nakazato, N. Furukawa, S. Hasegawa, S. Wakabayashi, M. Shigekawa, M.-H. Chang, M. F. Romero, and S. Hirose. 2003. Mechanism of acid adaptation of a fish living in a pH 3.5 lake. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **284:** R1199–R1212.

Hiroaki, Y., K. Tani, A. Kamegawa, N. Gyobu, K. Hishikawa, H. Suzuki, T. Walz, S. Sasaki, K. Mitsuoka, K. Kimura, A. Mizoguchi, and Y. Fujiyoshi. 2006. Implications of the aquaporin-4 structure on array formation and cell adhesion. *J. Mol. Biol.* **355:** 628–639.

Hiroi, J., and S. D. McCormick. 2012. New insights into gill ionocyte and ion transporter function in euryhaline and diadromous fish. *Respir. Physiol. Neurobiol.* **184:** 257–268.

Hwang, P. P., T. H. Lee, and L. Y. Lin. 2011. Ion regulation in fish gills: recent progress in the cellular and molecular mechanisms. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **301:** R28–R47.

Ip, Y. K., M. M. L. Soh, X. L. Chen, J. L. Y. Ong, Y. R. Chng, B. Ching, W. P. Wong, S. H. Lam, and S. F. Chew. 2013. Molecular characterization of branchial aquaporin 1aa and effects of seawater acclimation, emersion or ammonia exposure on its mRNA expression in the gills, gut, kidney and skin of the freshwater climbing perch, *Anabas testudineus. PLoS One* **8:** e61163. doi: 10.1371/journal-.pone.0061163.

Isaia, J. 1984. Water and nonelectrolyte permeation. Pp. 1-38 in *Fish Physiology,* Vol. 10*, Gills. Part B. Ion and Water Transfer,* W.S. Hoar and D. J. Randall, eds. Academic press, New York.

Ishibashi, K., S. Kondo, S. Hara, and Y. Morishita. 2011. The evolutionary aspects of aquaporin family. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **300:** R566–R576.

Itoh, T., T. Rai, M. Kuwahara, S. B. Ko, S. Uchida, S. Sasaki, and K. Ishibashi. 2005. Identification of a novel aquaporin, AQP12, expressed in pancreatic acinar cells. *Biochem. Biophys. Res. Commun.* **330:** 832–838.

Jeong, S.-Y., J.-H. Kim, W.-O. Lee, H.-U. Dahms, and K.-N. Han. 2014. Salinity changes in the anadromous river pufferfish, *Takifugu obscurus,* mediate gene regulation. *Fish Physiol. Biochem.* **40:** 205–219.

Jung, J. S., G. M. Preston, B. L. Smith, W. B. Guggino, and P. Agre. 1994. Molecular structure of the water channel through aquaporin CHIP: the hourglass model. *J. Biol. Chem.* **269:** 14648–14654.

Jung, D., J. D. Sato, J. R. Shaw, and B. A. Stanton. 2012. Expression of aquaporin 3 in gills of the Atlantic killifish (*Fundulus heteroclitus*): effects of seawater acclimation. *Comp. Biochem. Physiol.* **161A:** 320–326.

Karnaky, K. J., Jr. 1998. Osmotic and ionic regulation. Pp. 157-176 in *The Physiology of Fishes,* 2nd ed., D. H. Evans, ed. CRC Press, Boca Raton, FL.

Kato, A., T. Muro, Y. Kimura, S. Li, Z. Islam, M. Ogoshi, H. Doi, and S. Hirose. 2011. Differential expression of Na^+-Cl^- cotransporter and Na^+-K^+-Cl^- cotransporter 2 in the distal nephrons of euryhaline and seawater pufferfishes. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **300:** R284–R297.

Katoh, F., R. R. Cozzi, W. S. Marshall, and G. G. Goss. 2008. Distinct $Na^+/K^+/2Cl^-$ cotransporter localization in kidneys and gills of two euryhaline species, rainbow trout and killifish. *Cell Tissue Res.* **334:** 265–281.

Kim, Y. K., S. Watanabe, T. Kaneko, M. D. Huh, and S. I. Park. 2010. Expression of aquaporins 3, 8 and 10 in the intestines of freshwater- and seawater-acclimated Japanese eels *Anguilla japonica. Fish. Sci.* **76:** 695–702.

Kim, Y. K., S. Y. Lee, B. S. Kim, D. S. Kim, and Y. K. N. Kim. 2014. Isolation and mRNA expression analysis of aquaporin isoforms in marine medaka *Oryzias dancena,* a euryhaline teleost. *Comp. Biochem. Physiol.* **171:** 1–8.

King, L. S., D. Kozono, and P. Agre. 2004. From structure to disease: the evolving tale of aquaporin biology. *Nat. Rev. Mol. Cell Biol.* **5:** 687–698.

Kolarevic, J., H. Takle, O. Felip, E. Ytteborg, R. Selset, C. M. Good, G. Baeverfjord, T. Asgard, and B. F. Terjesen. 2012. Molecular and physiological responses to long-term sublethal ammonia exposure in Atlantic salmon (*Salmo salar*). *Aquat. Toxicol.* **124-125:** 48–57.

Kortenoeven, M. L. A., and R. A. Fenton. 2014. Renal aquaporins and water balance disorders. *Biochim. Biophys. Acta* **1840:** 1533–1549.

Kwong, R. W. M., Y. Kumai, and S. Perry. 2013. The role of aquaporin and tight junction proteins in the regulation of water movement in larval zebrafish (*Danio rerio*). *PLoS One* **8:** e70764. doi: 10.1371/journal.pone.0070764.

Lacy, E. R., and E. Reale. 1995. Functional morphology of the elasmobranch nephron and retention of urea. Pp. 107-146 in *Fish Physiology,* Vol. 14*, Cellular and Molecular Approaches to Fish Ionic Regulation,* C. Wood and T. Shuttleworth, eds. Academic Press, San Diego, CA.

Laforenza, U., G. Gastaldi, M. Grazioli, E. Cova, S. Tritto, A. Faelli, G. Calamita, and U. Ventura. 2005. Expression and immunolocalization of aquaporin-7 in rat gastrointestinal tract. *Biol. Cell* **97:** 605–613.

Larsen, E. H., N. Mobjerg, and R. Nielsen. 2007. Application of the Na^+ recirculation theory to ion coupled water transport in low- and high-resistance osmoregulatory epithelia. *Comp. Biochem. Physiol.* **148A:** 101–116.

Lignot, J. H., C. P. Cutler, N. Hazon, and G. Cramb. 2002. Immunolocalisation of aquaporin 3 in the gill and the gastrointestinal tract of the European eel *Anguilla anguilla* (L.). *J. Exp. Biol.* **20:** 2653–2663.

Loike, J. D., S. Hickman, K. Kuang, M. Xu, L. Cau, J. C. Vera, S. C. Silverstein, and J. Fishbarg. 1996. Sodium-glucose cotransporters display sodium- and phlorizin-dependent water permeability. *Am. J. Physiol. Cell Physiol.* **271:** C1774–C1779.

Loo, D. D., T. Zeuthen, G. Chandy, and E. M. Wright. 1996. Cotransport of water by the Na^+/glucose cotransporter. *Proc. Natl. Acad. Sci. USA* **9:** 13367–13370.

MacIver, B., C. P. Cutler, J. Yin, M. G. Hill, M. L. Zeidel, and W. G. Hill. 2009. Expression and functional characterization of four aquaporin water channels from the European eel (*Anguilla anguilla*). *J. Exp. Biol.* **212:** 2856–2863.

Madsen, S. S., J. H. Olesen, K. Bedal, M. B. Engelund, Y. M. Velasco-Santamaria, and C. K. Tipsmark. 2011. Functional characterization of water transport and cellular localization of three aquaporin paralogs in the salmonid intestine. *Front. Physiol.* **2:** 56. doi: 10.3389/fphys.2011.00056.

Madsen, S. S., J. Bujak, and C. K. Tipsmark. 2014. Aquaporin expression in the Japanese medaka (*Oryzias latipes*) in freshwater and seawater: challenging the paradigm of intestinal water transport? *J. Exp. Biol.* **217:** 3108–3121.

Mamonov, A. B., R. D. Coalson, M. L. Zeidel, and J. C. Mathai. 2007. Water and deuterium oxide permeability through aquaporin 1: MD predictions and experimental verification. *J. Gen. Physiol.* **130:** 111–116.

Mancera, J. M., R. L. Carrion, and M. D. M. del Rio. 2002. Osmoregulatory action of PRL, GH, and cortisol in the gilthead seabream (*Sparus aurata* L.). *Gen. Comp. Endocrinol.* **129:** 95–103.

Marshall, W. S., and M. Grosell. 2005. Ion transport, osmoregulation and acid-base balance. Pp. 177-230 in *The Physiology of Fishes,* 3rd ed., D. H. Evans and J. B. Claiborne, eds. CRC Press, Boca Raton, FL.

Martinez, A. S., C. P. Cutler, G. D. Wilson, C. Phillips, N. Hazon, and G. Cramb. 2005a. Cloning and expression of three aquaporin homologues from the European eel (*Anguilla anguilla*): effects of seawater acclimation and cortisol treatment on renal expression. *Biol. Cell* **97:** 615–627.

Martinez, A. S., C. P. Cutler, G. D. Wilson, C. Phillips, N. Hazon, and G. Cramb. 2005b. Regulation of expression of two aquaporin homologs in the intestine of the European eel: effects of seawater acclimation and cortisol treatment. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **288:** R1733–R1743.

McDowall, R. M. 1988. *Diadromy in Fishes: Migrations Between Freshwater and Marine Environments.* Timber Press, Portland, OR.

Meinild, A., D. A. Klaerke, D. D. Loo, E. M. Wright, and T. Zeuthen. 1998. The human Na^+-glucose cotransporter is a molecular water pump. *J. Physiol.* **508:** 15–21.

Mistry, A. C., S. Honda, T. Hirata, A. Kato, and S. Hirose. 2001. Eel urea transporter is localized to chloride cells and is salinity dependent. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **281:** R1594–R1604.

Motais, R., J. Isaia, J. C. Rankin, and J. Maetz. 1969. Adaptive changes of the water permeability of the teleostean gill epithelium in relation to external salinity. *J. Exp. Biol.* **51:** 529–546.

Murakami, M., K. Murdiastuti, K. Hosoi, and A. E. Hill. 2006. AQP and the control of fluid transport in a salivary gland. *J. Membr. Biol.* **210:** 91–103.

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

Nielsen, S., J. Frøkiaer, D. Marples, T. H. Kwon, P. Agre, and M. A. Knepper. 2002. Aquaporins in the kidney: from molecules to medicine. *Physiol. Rev.* **82:** 205–244.

Nilsson, S. 1986. Control of gill blood flow. Pp. 87-101 in *Fish Physiology: Recent Advances,* S. Nilsson and S. Holmgren, eds. Croom Helm, London.

Nishimoto, G., G. Sasaki, E. Yaoita, M. Nameta, H. Li, K. Furuse, H. Fujinaka, Y. Yoshida, A. Mitsudome, and T. Yamamoto. 2007. Molecular characterization of water-selective AQP (EbAQP4) in hagfish: insights in ancestral origin of AQP4. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **292:** R644–R651.

Ogasawara, T., and T. Hirano. 1984. Changes in osmotic water permeability of the eel gills during seawater and freshwater adaptation. *J. Comp. Physiol.* **154:** 3–11.

Parmelee, J. T., and J. L. Renfro. 1983. Esophageal desalination of seawater in flounder: role of active sodium transport. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **245:** R888–R893.

Paula, S., A. G. Volkov, A. N. Van Hoek, T. H. Haines, and D. W. Deamer. 1996. Permeation of protons, potassium ions, and small polar molecules through phospholipid bilayers as a function of membrane thickness. *Biophys. J.* **70:** 339–348.

Preston, G. M., T. P. Carroll, W. B. Guggino, and P. Agre. 1992. Appearance of water channels in *Xenopus* oocytes expressing red cell CHIP28 protein. *Science* **256:** 385–387.

Raldua, D., D. Otero, M. Fabra, and J. Cerdà. 2008. Differential localization and regulation of two aquaporin-1 homologs in the intestinal epithelia of the marine teleost *Sparus aurata. Am. J. Physiol. Regul. Integr. Comp. Physiol.* **294:** R993–R1003.

Rehman, K. U., M. A. Wingertzahn, S. Teichberg, R. G. Harper, and R. A. Wapnir. 2003. Gum arabic (GA) modifies paracellular water and electrolyte transport in the small intestine. *Dig. Dis. Sci.* **48:** 755–760.

Rehn, K., K. S. Wong, D. Balciunas, and S. Sumanas. 2011. Zebrafish enhancer trap line recapitulates embryonic *aquaporin 1a* expression pattern in vascular endothelial cells. *Int. J. Dev. Biol.* **55:** 613–618.

Renfro, J. L. 1999. Recent developments in teleost renal transport. *J. Exp. Zool.* **283:** 653–661.

Rosenthal, R., S. Milatz, S. M. Krug, B. Oelrich, J. D. Schulzke, S. Amasheh, D. Gunzel, and M. Fromm. 2010. Claudin-2, a component of the tight junction, forms a paracellular water channel. *J. Cell Sci.* **123:** 1913–1921.

Santos, C. R., M. D. Estevao, J. Fuentes, J. C. Cardoso, M. Fabra, A. L. Passos, F. J. Detmers, P. M. Deen, J. Cerdà, and D. M. Power. 2004. Isolation of a novel aquaglyceroporin from a marine teleost (*Sparus auratus*): function and tissue distribution. *J. Exp. Biol.* **207:** 1217–1227.

Saparov, S. M., K. Liu, P. Agre, and P. Pohl. 2007. Fast and selective ammonia transport by aquaporin-8. *J. Biol. Chem.* **282:** 5296–5301.

Schmidt-Nielsen, B., and J. L. Renfro. 1975. Kidney function of the American eel, *Anguilla rostrata. Am. J. Physiol.* **228:** 420–431.

Schnermann, J., Y. Huang, and D. Mizel. 2013. Fluid reabsorption in proximal convoluted tubules of mice with gene deletions of claudin-2 and/or aquaporin1. *Am. J. Physiol. Renal. Physiol.* **305:** F1352–F1364.

Semon, M., and K. H. Wolfe. 2007. Consequences of genome duplication. *Curr. Opin. Genet. Dev.* **17:** 505-512.

Smith, H. W., N. Farinacci, and A. Breitweiser. 1930. The absorption and excretion of water and salts by marine teleosts. *Am. J. Physiol.* **93:** 480–505.

Solomon, R., M. Taylor, J. S. Stoff, P. Silva, and F. H. Epstein. 1984. *In vivo* effect of volume expansion on rectal gland function. I. Humoral factors. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **246:** R63–R66.

Sundell, K., F. Jutfelt, T. Agustsson, R. E. Olsen, E. Sandblom, T. Hansen, and B. T. Björnsson. 2003. Intestinal transport mechanisms and plasma cortisol levels during normal and out-of-season parr-smolt transformation of Atlantic salmon, *Salmo salar. Aquaculture* **222:** 265–285.

Sundell, K. S., and H. Sundh. 2012. Intestinal fluid absorption in

anadromous salmonids: importance of tight junctions and aquaporins. *Front. Physiol.* **3:** 388. doi: 10.3389/fphys.2012.00388.

Tang, C. H., L. Y. Hwang, and T. H. Lee. 2010. Chloride channel ClC-3 in gills of the euryhaline teleost, *Tetraodon nigroviridis:* expression, localization and the possible role of chloride absorption. *J. Exp. Biol.* **213:** 683–693.

Tingaud-Sequeira, A., M. Calusinska, R. N. Finn, F. Chauvigné, J. Lozano, and J. Cerdà. 2010. The zebrafish genome encodes the largest vertebrate repertoire of functional aquaporins with dual paralogy and substrate specificities similar to mammals. *BMC Evol. Biol.* **10:** 38. doi:10.1186/1471-2148-10-38.

Tipsmark, C. K., K. J. Sorensen, K. Hulgard, and S. S. Madsen. 2010a. Claudin-15 and -25b expression in the intestinal tract of Atlantic salmon in response to seawater acclimation, smoltification and hormone treatment. *Comp. Biochem. Physiol.* **155A:** 361-370.

Tipsmark, C. K., K. J. Sorensen, and S. S. Madsen. 2010b. Aquaporin expression dynamics in osmoregulatory tissues of Atlantic salmon during smoltification and seawater acclimation. *J. Exp. Biol.* **213:** 368–379.

Tritto, S., G. Gastaldi, S. Zelenin, M. Grazioli, M. N. Orsenigo, U. Ventura, U. Laforenza, and M. Zelenina. 2007. Osmotic water permeability of rat intestinal brush border membrane vesicles: involvement of aquaporin-7 and aquaporin-8 and effect of metal ions. *Biochem. Cell Biol.* **85:** 675–684.

Tse, W. K., D. W. Au, and C. K. Wong. 2006. Characterization of ion channel and transporter mRNA expressions in isolated gill chloride and pavement cells of seawater acclimating eels. *Biochem. Biophys. Res. Commun.* **346:** 1181–1190.

Watanabe, S., T. Kaneko, and K. Aida. 2005. Aquaporin-3 expressed in the basolateral membrane of gill chloride cells in Mozambique tilapia *Oreochromis mossambicus* adapted to fresh water and seawater. *J. Exp. Biol.* **208:** 2673–2682.

Whittamore, J. M. 2012. Osmoregulation and epithelial water transport: lessons from the intestine of marine teleost fish. *J. Comp. Physiol.* **182B:** 1–39.

Wolfe, K. H. 2001. Yesterday's polyploids and the mystery of diploidization. *Nat. Rev. Genet.* **2:** 333–341.

Wood, C. M., and M. Grosell. 2012. Independence of net water flux from paracellular permeability in the intestine of *Fundulus heteroclitus,* a euryhaline teleost. *J. Exp. Biol.* **215:** 508–517.

Wood, C. M., and P. Part. 1997. Cultured branchial epithelia from freshwater fish gills. *J. Exp. Biol.* **200:** 1047–1059.

Wright, P. A., and C. M. Wood. 2009. A new paradigm for ammonia excretion in aquatic animals: role of Rhesus (Rh) glycoproteins. *J. Exp. Biol.* **212:** 2303–2312.

Xu, G. Y., F. Wang, X. Jiang, and J. Tao. 2010. Aquaporin 1, a potential therapeutic target for migraine with aura. *Mol. Pain* **6:** 68. doi: 10.1186/1744-8069-6-68.

Yasui, M., A. Hazama, T. H. Kwon, S. Nielsen, W. B. Guggino, and P. Agre. 1999. Rapid gating and anion permeability of an intracellular aquaporin. *Nature* **402:** 184–187.

Zapater, C., J. Chauvigné, B. Norberg, R. N. Finn, and J. Cerdà. 2011. Dual neofunctionalization of a rapidly evolving aquaporin-1 paralog resulted in constrained and relaxed traits controlling channel function during meiosis resumption in teleosts. *Mol. Biol. Evol.* **28:** 3151–3169.

Zeidel, M. L., S. V. Ambudkar, B. L. Smith, and P. Agre. 1992. Reconstitution of functional water channels in liposomes containing purified red cell CHIP28 protein. *Biochemistry* **31:** 7436–7440.

Zeidel, J. D., J. C. Mathai, J. D. Campbell, W. G. Ruiz, G. L. Adopaca, J. Riordan, and M. L. Zeidel. 2005**.** Selective permeability barrier to urea in rectal gland. *Am. J. Physiol. Renal Physiol.* **289:** 83–89.

Zeuthen, T. 2002. General models for water transport across leaky epithelia. *Int. Rev. Cytol.* **215:** 285–317.