Diverse mechanisms for body fluid regulation in teleost fishes

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Short title: Endocrine control of osmoregulation in teleosts

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Abstract

Teleost fishes are the major group of ray-finned fishes and represent more than one-half of the total number of vertebrate species. They have experienced in their evolution an additional third-round whole genome duplication just after the divergence of their lineage, which endowed them with an extra adaptability to invade various aquatic habitats. Thus their physiology is also extremely diverse compared with other vertebrate groups as exemplified by the many patterns of body fluid regulation or osmoregulation. The key osmoregulatory organ for teleosts, whose body fluid composition is similar to mammals, is the gill, where ions are absorbed from or excreted into surrounding waters of various salinities against concentration gradients. It has been shown that the underlying molecular physiology of gill ionocytes responsible for ion regulation is highly variable among species. This variability is also seen in the endocrine control of osmoregulation where some hormones have distinct effects on body fluid regulation in different teleost species. A typical example is atrial natriuretic peptide (ANP); ANP is secreted in response to increased blood volume and acts on various osmoregulatory organs to restore volume in rainbow trout as it does in mammals, but it is secreted in response to increased plasma osmolality, and specifically decreases NaCl, and not water, in the body of eels. The distinct actions of other osmoregulatory hormones such as growth hormone, prolactin, angiotensin II and vasotocin among teleost species are also evident. We hypothesized that such diversity of ionocytes and hormone actions among species stems from their intrinsic differences in body fluid regulation that originated from their native habitats, either fresh water or seawater. In this review, we summarized remarkable differences in body fluid regulation and its endocrine control among teleost species, although the number of species is still limited to substantiate the hypothesis.

Key words: environmental adaptation; aquatic vertebrates; transporters/channels; osmoregulatory hormones
Body fluid regulation, or osmoregulation, continues to be one of the major topics in comparative physiology (138). Ample studies have been performed in tetrapods, particularly in endothermic mammals and birds, as they are constantly exposed to the threat of dehydration due to the high respiratory water loss in the terrestrial environment. However, fishes are more vulnerable to body fluid changes because their extracellular fluids closely contact environmental waters of varying salinities across the thin respiratory epithelia of the gills (35). Thus, the gills are the primary osmoregulatory organ for teleost fishes, where active uptake and extrusion of ions occur against the concentration gradients imposed by environmental fresh water (FW) or seawater (SW) (Fig. 1). In addition to the gills, major osmoregulatory sites are drinking and subsequent absorption of ions and water by the intestine, particularly for acquisition of water in hyperosmotic SW, and the kidney where copious urine was excreted in FW and divalent ions are excreted in SW (Fig. 1). The osmoregulatory mechanisms are maximally flexible in euryhaline or migratory species, which experience drastic salinity changes during their life cycle and must switch ion and water regulation to achieve opposite directions of active transport. Thus the mechanisms of osmoregulation has been the subject of intensive research in euryhaline teleost fishes (34). These studies have revealed that various hormones play pivotal roles in adaptation to fluctuating environmental salinities (106, 158). In these studies, we frequently found great differences in osmoregulatory mechanisms and hormone actions among species. There are two migratory species that have been frequently used in the study of osmoregulation, anadromous salmonids and catadromous eels, and such differences were apparent in body fluid regulation, particularly in terms of contrasting hormone actions between the two species. Several euryhaline species such as tilapia, killifish, flounder, sea bass, medaka, etc., are also used frequently as model species of osmoregulation research, and we often found similar differences in the regulatory mechanisms among these species. Thus, we compared differences in the mechanisms of body fluid regulation within each group of vertebrates and found that the diversity is greater in teleosts compared with the other groups including tetrapods (155).

The causes of such rich diversity in teleosts may be multifold. According to Nelson (116), the total number of teleost species (ca. 27,000) are greater than the sum of all species of tetrapods (mammals, birds, reptiles and amphibians) and the class Teleostei is most successful and diversified group among vertebrates. This is due in part to the extra whole genome duplication that seems to have occurred after teleosts were diverged ca. 250 Mys ago (110). The additional genome duplication event in teleosts was first suggested by the extra Hox cluster genes identified in this group, but its occurrence is
now confirmed by the genome database of several teleost species (76). The redundancy of the genes may have facilitated diversification and speciation of this ray-finned fish group (167).

Based on these accumulating data, we thought it a good time to summarize the diverse mechanisms of body fluid regulation among teleost fishes and highlight such rich diversity in this fish group. We also attempted to identify the underlying evolutionary history of such diversity. We chose two topics to introduce the diversity. One is molecular physiology of gill ionocytes since these cells are the key to the teleost’s ability to adapt to diverse salinity environments, and several different types of ionocytes have been identified and characterized in several species. The other is endocrine control of osmoregulation since hormones play key roles in body fluid regulation but at the same time their roles are so different among teleost species. In the final section of this review, we attempted to identify the cause of such rich diversity in this vertebrate group in relation to their ‘intrinsic’ habitat (FW or SW), which includes various aspects such as ‘in which environment they spawned and spent the early life stages’, ‘in which environment they prefer or need to consume less energy’, and ‘from which environment their group originated during evolution’. Of course, there are basic mechanisms common to all teleost species, but it is evident that the diversity of body fluid regulation is greater than other vertebrate groups and other regulatory systems such as cardiovascular regulation, probably because of their adaptation to various salinity environments. We will attempt to relate such rich diversity of body fluid regulation to the ‘intrinsic’ habitat as mentioned above. We expect that this review will afford new insights into the vertebrate body fluid regulation not only for comparative physiologists but also for general physiologists including those in the medical field.

**Diverse transporter topology of gill ionocytes**

Euryhaline teleosts are able to acclimate to both FW and SW, primarily by means of specialized epithelial cells referred to as “chloride cells” or “mitochondrion-rich cells”, but the term “ionocytes” is now recommended as a specific and functional name for this cell type (107). Recent advances in cellular/molecular physiological approaches have revealed that teleost ionocytes are composed of several subsets with different ion-transport functions (29, 54, 59, 60), and the proliferation, differentiation and function of ionocytes are regulated by several hormones (15, 16, 91, 158). Before functional classification of ionocytes and comparison among three euryhaline species, Mozambique tilapia (*Oreochromis mossambicus*), rainbow trout (*Oncorhynchus mykiss*) and killifish (*Fundulus heteroclitus*), let us refer to the classification of the
cation-chloride cotransporter family, the members of which are the key players in ion
regulation by the branchial ionocytes.

**Cation-chloride cotransporter family** Cation-chloride cotransporters, such as \( \text{Na}^+{-}\text{Cl}^- \)
cotransporter (NCC) and \( \text{Na}^+{-}\text{K}^+{-}\text{2Cl}^- \) cotransporter (NKCC), are key components of
ion-transport functions by ionocytes. However, there seems to be some confusion
among fish researchers about their nomenclature. Thus we first attempt to reconcile the
history of identification of these cotransporters. Since the first suggestion by Renfro
(129) about the presence of electrically neutral cation-chloride cotransporter(s) in the
urinary bladder of winter flounder (*Pseudopleuronectes americanus*) in 1975, various
attempts have been made to identify the genes, which resulted in isolation of a cDNA
encoding NCC from the urinary bladder of this species (43) and of one encoding
NKCC1 from the rectal gland of spiny dogfish (*Squalus acanthias*) (178). The fish
genes were subsequently used to “fish out” mammalian counterpart genes, and currently
the mammalian solute carrier (SLC) 12 family consists of SLC12A1 (NKCC2),
SLC12A2 (NKCC1), SLC12A3 (NCC), SLC12A4-7 (KCCs) and SLC12A8-9 (orphan
transporters) (5). However, little information has yet been available on this family in
fishes despite the pioneering study.

To detect NKCC1 in various teleost species, a mouse monoclonal antibody, named T4,
raised against human colonic NKCC1 (96) has been frequently used. The T4 antibody
resulted in basolateral staining in ionocytes of several euryhaline teleosts acclimated to
both FW and SW (e.g., salmonids and eels; 53, 123, 144), but in contrasting basolateral
localization in SW ionocytes and apical localization in FW ionocytes of tilapia, killifish,
sailfin molly (*Poecilia latipinna*), Indian medaka (*Oryzias dancena*) and European sea
bass (*Dicentrarchus labrax*) (75, 80, 95, 177, 180). The latter results misled fish
researchers to assume the basolateral and apical immunoreactants represents NKCC1
and NKCC2 proteins, respectively (e.g. 55), because the T4 antibody was known to
recognize NKCC2 as well as NKCC1 (96). However, molecular cloning from tilapia
gills revealed that the SW-type cotransporter is NKCC1 as expected, but the FW-type
was NCC, not NKCC2, as confirmed by the use of homologous antibodies to each
protein (56).

In a phylogenetic tree of fish and human cation-chloride cotransporters (Fig. 2), all
examined transporters are resolved into either of two major clades, the NKCC clade and
the NCC clade. The NKCC clade is divided into NKCC1 and NKCC2 clades. The NCC
clade is also divided into two clades, a conventional NCC (including human NCC)
clad and a fish-specific NCC clade. The latter NCC was in the FW-type ionocyte of
tilapia (56). Its ortholog was also identified in a subset of ionocytes (NCC cell) of
zebrafish (Danio rerio) and is now grouped as SLC12A10 (18, 171). The conventional NCC is restricted to renal tissues in mammals and teleosts, but the fish-specific NCC is expressed in extrarenal tissues such as the gills and intestine (23, 77, 160, 173). Therefore, we suggest to use the term “NCC1” and “NCC2” for conventional NCC and fish-specific NCC, respectively, as recently suggested (48). It will be important to analyze functional differences of the two NCCs in different clades. All teleosts thus far examined have a single NCC1 gene, but the number of NCC2 genes varies among species, being 3 in zebrafish, 2 in Nile tilapia (O. niloticus), and 0 in tiger pufferfish (Takifugu rubripes) (Fig. 2). NCC2 is localized at the apical membrane of FW ionocytes in tilapia, zebrafish and killifish, but does not seem to be expressed in the gills of salmonids and eels. It thus seems of interest to examine the cause of diversification of the NCC2 gene among teleost species.

Four types of tilapia ionocytes  

Ion-transport functions of ionocytes can be estimated by the localization patterns of multiple ion transporters within the cells. The description of four types of ionocytes in the yolk-sac membrane of tilapia embryos exemplifies this estimation process (54-56). Simultaneous immunofluorescence staining of five ion-transport proteins, NKCC1, NCC2, Na+/K+-ATPase (NKA), Na+/H+ exchanger 3 (NHE3) and cystic fibrosis transmembrane conductance regulator (CFTR) Cl− channel ascertained the apical or basolateral localization of each protein at the single-cell level, and consequently allowed the classification of all observable ionocytes into one of four types; types I, II, III and IV (Fig. 3A, Table 1).

The type-IV ionocyte is defined by basolateral NKA, basolateral NKCC1a and apical CFTR, a typical pattern of NaCl secretory ionocytes in SW teleosts (35, 100, 102). This cell type was not observed in FW fishes, but rapidly appeared and increased in number following SW transfer, and disappeared following transfer back into FW. The type-III ionocyte possesses basolateral NKA and basolateral NKCC1 like type IV ionocytes, but apical CFTR is replaced by NHE3. Further, the type III ionocyte is rarely observed in SW, but rapidly increased in expression following FW transfer and disappeared following transfer back to SW. This inverse regulation suggests that the two types represent the same cells with different apical transporter configurations (56). The type-III ionocyte may be active in ion absorption or acid/base regulation through apical NHE3 in FW, and differentiate rapidly to the type IV by inserting CFTR apically upon encountering SW (56). The type-II ionocyte possesses basolateral NKA and apical NCC2 and is FW-specific. Thus, it seems that the type II ionocyte is an ion-absorptive cell where the apical NCC2 transports Na+ and Cl− together into the cell from the ion-poor FW environment. The type-I ionocytes have only basolateral NKA and were
constant in number irrespective of environmental salinity. This cell type was first assumed to be an immature ionocyte that will develop into other types (55), but subsequent observation suggests that it is an independent cell type with unknown ion-transport functions (see below) (54, 56).

The localization pattern of ion-transport proteins in branchial ionocytes has been updated by recent studies in adult tilapia. Apical NHE3 was restricted to type III in FW embryos, but it was detectable also in type IV of SW adults (63). Na\(^+\)-HCO\(_3\)^− cotransporter 1 (NBC1) was localized to the basolateral membrane of type II, probably responsible for exit of Na\(^+\) (40). Renal outer medullary K\(^+\) channel (ROMK) was detected at the apical membrane of types I, III and IV (41). As ROMK was upregulated after transfer to high-K\(^+\) FW or SW, these ionocytes seem to play a role in K\(^+\) excretion (F. Furukawa and T. Kaneko, personal communication). It is of interest to determine whether or not type-I ionocytes are exclusively involved in K\(^+\) excretion.

**Comparison with other euryhaline teleosts**

Ionocyte types with distinct morphological features have been identified in various teleost species by transmission or scanning electron microscopy (58, 126). Although most of these studies have not been linked to specific ion-transport proteins, we attempted to compare the ionocyte types between tilapia and other euryhaline species (rainbow trout and killifish). Ionocyte types of stenohaline zebrafish have been extensively reviewed elsewhere (59, 60). Salmonids have been widely used as model species in the study of ion transport physiology. Ionocytes of rainbow trout are classified into two types, PNA\(^+\) and PNA\(^-\), which are distinguished by specific binding of peanut agglutinin (PNA) to the apical membrane (29). Basolateral NKA immunoreactivity was found in both PNA\(^+\) and PNA\(^-\) ionocytes, but apical NHE3 immunoreactivity was restricted to the PNA\(^+\) ionocytes (68). Similar results are observable with triple-color immunofluorescence staining for NKA, NKCC1 and NHE3, in which at least two types with or without apical NHE3 are distinguishable (Fig. 3B, Table 1) (54). The trout NHE3-positive ionocytes showed basolateral NKA and basolateral NKCC1, and were found in both FW- and SW-acclimated fish (54). Therefore, NHE3-positive ionocytes seem to be analogous to tilapia type-III and IV ionocytes, and could be equipped with apical CFTR in SW. However, immunocytochemical detection of CFTR in salmonid ionocytes has not been successful even with homologous antibodies (S. D. McCormick, personal communication). Recent studies have shown that there are FW- and SW-type isoforms of NKA \(\alpha\)-subunit, NKA\(\alpha\)1a and NKA\(\alpha\)1b, respectively, in branchial ionocytes of Atlantic salmon (*Salmo salar*) and tilapia (108, 109, 162). Accordingly, it is expected that the basolateral NKA immunoreactivity in the trout NHE3-positive ionocytes is
largely by NKAα1a in FW and NKAα1b in SW. Recent studies suggested that apical NHEs and Rhesus (Rh) glycoproteins link together for ammonia-dependent Na\(^{+}\) uptake (90, 175, 176). Indeed, a preliminary study showed the colocalization of Rhcg1 and NHE3b at the apical membrane of the trout NHE3b-positive ionocytes using homologous antibodies (J. Hiroi, unpublished data). The basolateral NKCC1 in the NHE3b-positive ionocytes may be involved in NH\(_{4}\)\(^{+}\) transport (176). Colocalization of these ion-transport proteins at the single cell level remains to be determined.

The killifish opercular membrane is a popular model for electrophysiology as the tissue contains a rich and dense population of ionocytes (100, 101). Branchial and opercular ionocytes of killifish become larger in FW than in SW (81, 103), opposite in expression pattern to that seen in other teleosts including tilapia and salmonids (54, 58). Branchial ionocytes of SW-acclimated killifish showed the typical localization pattern for type-IV ionocytes of tilapia, basolateral NKA and NKCC1 and apical CFTR (Fig. 3C, Table 1) (79, 80). In contrast, ionocytes of FW-acclimated killifish lost apical CFTR expression, increased basolateral H\(^{+}\)-ATPase expression, and had distinct apical immunoreactivity with anti-human NKCC1 (Fig. 3C, Table 1) (79, 80, 82). The apical immunoreactivity seems to be NCC2 and thus is comparable to tilapia type-II ionocytes, although H\(^{+}\)-ATPase was not detectable in any ionocytes of tilapia embryos but was found at the apical membrane of a subset of respiratory pavement cells (52). Katoh and Kaneko (79) found that 85% of pre-existing SW-type ionocytes were transformed into FW type and 15% were newly differentiated at 3 days after transfer to FW. These results suggest that basolateral NKCC1 and apical CFTR of SW ionocytes are replaced by basolateral H\(^{+}\)-ATPase and apical NCC2, respectively, after FW transfer. Such a functional plasticity of killfish ionocytes is in marked contrast to tilapia ionocytes, in which type IV cells are transformed into NHE3-positive type III cells after SW to FW transfer but not into NCC2-positive type II cells, and NHE3 and NCC2 are not expressed in the same ionocyte (55, 56). NHE2 and NHE3 were detected in killifish gills by Western blotting, real-time PCR or in situ hybridization (21, 32, 33, 140), but the apical localization of these proteins has not yet been confirmed. Immunohistochemical studies are necessary to determine whether NHE and NCC2 are colocalized on the apical membrane of ionocytes in the killifish gills.

**Diverse hyposmoregulatory actions of hormones**

**Cortisol** It is recognized that cortisol, a glucocorticoid in many vertebrates, acts not only as glucocorticoid but also as mineralocorticoid in teleost fishes. Although 11-deoxycorticosterone (DOC) has recently been identified as a more specific ligand for
mineralocorticoid receptor (MR) (148, 149, 152), cortisol, not DOC, exerts its
mineralocorticoid actions primarily by interacting with glucocorticoid receptors (GRs)
rather than MRs (127, 148). Accumulated evidence indicates that cortisol plays a pivotal
role in SW acclimation as a slow-acting hormone in euryhaline fishes (Fig. 4) (152). In
the gills of salmonids, eels, killifish and tilapia, cortisol promotes the differentiation of
ionocytes into SW-type and elevates the activity and/or transcription of key transporters
in SW-type ionocyte (Type-IV in tilapia) such as NKA, NKCC1, and CFTR, resulting in
increased excretion of excess ions (106, 152). In the silver sea bream (Sparus sarba),
cortisol increases the mRNA level of branchial NKA (26). In the intestine of these
species, cortisol also elevates the NKA activity and aquaporin expression, thereby
increasing water absorption across the epithelia to maintain water balance in a
dehydrating SW environment (24, 168, 169). In the esophagus, cortisol stimulates
epithelial apoptosis for the simple epithelium with high permeability to NaCl for
desalination in the mudskipper (Periophthalmus modestus) (154) and medaka (O.
latipes) (151).

In agreement with these actions to promote SW adaptation, plasma cortisol and GR
transcripts in osmoregulatory organs increase during SW acclimation in euryhaline
fishes (106, 152). Plasma cortisol and branchial GR mRNA levels are elevated in
parallel during parr-smolt transformation, supporting salmonid migration into SW (85,
114, 117). These studies indicate that the hyposmoregulatory action of cortisol seems to
be well-conserved among euryhaline fishes. As will be mentioned below, cortisol
secretion is enhanced by atrial natriuretic peptide (ANP) and angiotensin II (Ang II),
both of which are candidates for fast-acting, SW-adapting hormones (Fig. 4).

Growth hormone (GH)  GH, a member of the same hormone family as prolactin
(PRL), has a role in teleost osmoregulation (Fig. 4), in addition to its role in growth
promotion. Smith (146) was the first to observe that GH treatment increased the
capacity of brown trout (Salmo trutta) to tolerate exposure to SW. It was later
determined that the hormone’s primary effect is to increase the number and size of gill
ionocytes and the transcription of ion transporter genes involved in salt secretion not
only in salmonids but in other euryhaline teleosts (122, 131). The GH effect is at least in
part mediated by the action of circulating/local insulin-like growth factor I (IGF-I) (Fig.
4) (131, 161).

The effect of GH/IGF-I on salinity tolerance and differentiation of
hyposmoregulatory mechanisms is not restricted to salmonids, as this effect has been
found at least in two other euryhaline fishes: tilapia and killifish (131, 153). In addition
to the effect of exogenous hormones treatments, changes in gene expression, secretion,
metabolic clearance rate and receptor level of GH/IGF-I also provide evidence for the
hyposmoregulatory actions of GH in several euryhaline species widely separated in
their evolution (14, 64, 72, 111, 115, 130, 132, 134, 179). However, effects in silver
seabream are not consistent with a SW-acclimating impact (83). Pituitary GH and liver
IGF-I mRNA levels were lower after both hyper- and hyposaline challenges (25).

In stenohaline catfish (*Ictalurus punctatus*), plasma GH levels increase following
exposure to 12 ppt SW (27). In zebrafish, however, an increased mortality after transfer
to 11 ppt salinity in the GH-transgenic fish suggests an impaired salinity acclimation by
GH, although the transgene used was the heterologous GH gene from the silverside
(*Odontesthes argentinensis*) (1). Therefore, we now assume that the physiological
impact of the GH/IGF-I axis is variable and versatile among teleosts. ANP is shown to
stimulate GH secretion from dispersed pituitary cells of eels (37) (Fig. 4).

To date, only a relatively small number of teleosts have been examined in the
osmoregulatory actions of endocrine GH. Furthermore, in contrast to the situation for
PRL, there is little information on the osmoregulatory role(s) of extrapituitary GH (132),
which may act as an autocrine/paracrine factor, either directly or via its local induction
of IGF-I (Fig. 4). In higher vertebrates, GH gene expression occurs in gastrointestinal
tissues and in the integumentary and cardiovascular systems, especially prior to
pituitary gland development and after pituitary senescence and somatopause, where GH
may act locally, rather than as an endocrine hormone, to effect proliferation and
differentiation of cells and tissues (49). GH control of the salinity acclimation process
will be discussed further below.

**The natriuretic peptide (NP) family** The NP family consists of two different groups;
A-type (or atrial), B-type and ventricular NPs (ANP, BNP and VNP) synthesized in the
heart, and four C-type NPs (CNP1–4) that are most abundant in the brain (66, 159).
Comparative genomic analyses revealed that CNP4 is the ancestral molecule and the
cardiac NPs were generated by tandem duplication from CNP3 (65). The diversity of
cardiac NPs differs greatly among teleost species; only eels and salmonids have a
complete set of three cardiac NPs.

Accumulated evidence has shown that the cardiac NPs are important for acclimation
to SW in eels (156). Indeed, transfer of eels from FW to SW resulted in a transient
increase in plasma ANP concentration, although it returned to a FW level in a few hours
(69). The stimulus for ANP secretion is not an increase in blood volume (atrial stretch)
as established in mammals, because blood volume decreases transiently after SW
transfer. It was later shown that an increase in plasma osmolality is a major stimulus for
ANP secretion in eels (70). When injected into the circulation, ANP inhibits drinking
and intestinal NaCl (and water) absorption, where these effects are 2-3 orders more potent than in mammals. ANP has a weak antidiuretic effect in the eel kidney (157) and a strong stimulatory effect on cortisol section from the eel interrenal (Fig. 4) (94). As ANP causes profound diuresis in mammals, the hormone has opposite effects on renal water excretion in eels. The renal and interrenal effects of ANP were found only in SW eels but not in FW eels. ANP stimulates GH secretion from the pituitary cells of Mozambique tilapia (37), further supporting its action on promoting SW acclimation (Fig. 4). In contrast to mammals, ANP increases plasma Ang II concentration in eels (164). All of these data strongly suggest that ANP is an important SW-adapting hormone, which is secreted in response to an increase in environmental salinity and acts on various organs to ameliorate sudden increases in plasma ion concentrations to allow initial acclimation to SW (156).

In contrast to the results obtained in eels, initial studies in rainbow trout demonstrated ANP actions quite similar to those of mammals. For instance, an increase in blood volume caused by injection of isotonic saline was a profound stimulus for ANP secretion (22), and exogenous injections of ANP into the circulation induced potent diuresis and natriuresis (28). We initially thought that the differences in trout are due to the use of heterologous (mammalian) hormone and radioimmunoassay; as the cardiac NPs are similar among different members, it is possible that radioimmunoassay for human ANP might measure trout BNP/VNP and that rat ANP might mimic the effect of trout BNP/VNP. However, eel ANP, BNP and VNP had similar osmoregulatory effects, although the potency and efficacy differ among the three peptides (112). Therefore, we now assume that the effects of ANP are versatile among teleost species. Unfortunately, the stimulus for ANP secretion and the renal effect of ANP have not yet been examined in teleost species other than eels and trout. Other difference between eels and trout is the steroidogenic effect; ANP has direct action on interrenal in SW trout (4) but only permissive action to validate the ACTH effect in eels (170). Another interesting difference between species is that rat ANP stimulates Cl⁻ secretion by the opercular skin of SW killifish (139) but eel ANP failed to increase ²²Na excretion from gills and skin of SW eels (165). Interestingly, the steroidogenic effect was demonstrated in stenohaline FW carp (87), though ANP was effective only in SW-acclimated eels and trout. As ANP decreased NaCl in the body in all animals thus far examined from fishes to mammals, the essential action of ANP is on NaCl extrusion but not water (156, 159).

**The renin-angiotensin system (RAS)** The renin-angiotensin cascade is initiated and regulated principally by the release of renin from the juxtaglomerular cells in the renal afferent arteriole, which is stimulated by hypovolemia and subsequent decreases in
perfusion pressure at the arteriole (88). Therefore, principal action of the active
principle of the system, angiotensin II (Ang II), is to restore blood volume, that is, to
retain both NaCl and water. Ang II stimulates vasopressin and aldosterone secretion,
thereby further contributing to volume retention. These Ang II actions are obviously
opposite to those of ANP (3). As mentioned above, however, ANP increases plasma Ang
II concentration in eels (164).

In teleost fishes, plasma Ang II also increases in response to hypovolemia (88).
Concerning biological actions, Ang II induces drinking in the flounder, eel, and goldfish,
causes glomerular antidiuresis and increases cortisol secretion in eels, trout, toadfish, or
flounder (17, 88, 118, 158). These data suggest that the RAS is important for
acclimation to SW (Fig. 4). In fact, transfer of eels from FW to SW resulted in a small,
transient increase in plasma Ang II concentration in parallel with plasma osmolality
(121). However, similar hypovolemia or hypernatremia given alone induced greater
increases in plasma Ang II concentration in eels (158). Thus, it is possible that exposure
to SW itself may be inhibitory for renin release. Ang II stimulates the secretion of PRL
but not GH in vivo (93) and in vitro (30) in Mozambique tilapia (Fig. 4), which further
favors its role in FW adaptation. Further researches are required in more species to
determine whether the RAS is important for SW or FW acclimation.

The dipsogenic effect of Ang II has been examined extensively in various teleost
species (89), although the effect was generally much weaker than in mammals (155).
These studies show that potency and efficacy of the Ang II effect differ profoundly
among species depending on their ecological and physiological need for drinking. Ang
II induced drinking in euryhaline or migratory species that start drinking when they
encounter the hyperosmotic environment such as trout, eels, medaka, mullet (Mulgi
cephalus) and goby (Glossogobius giuris), but Ang II was ineffective in stenohaline FW
species such as carp (Cyprinus carpio), loach (Cobitis anguillicaudatus), landlocked
char (Salvelinus leucomaenis) and bittering (Rhodeus ocellatus) and in stenohaline SW
species such as horse mackerel (Trachurus japonicus), grass pufferfish (Takifugu
nephobles), rockfish (Sebastes inermis) and file fish (Rudarius ercodes). Stenohaline
FW fishes scarcely drink water but those in SW drink constantly to cope with osmotic
loss of water. It seems that stenohaline marine fishes drink surrounding SW only by a
swallowing reflex and the drinking is not motivated by thirst induced by Ang II.

**Diverse hyperosmoregulatory actions**

**Cortisol** In most euryhaline fishes, cortisol plays important roles through GR not only
in SW adaptation but also in FW adaptation (Fig. 4). In salmonids, eels, killifish and
tilapia, cortisol stimulates differentiation of FW-type ionocytes in the gills and elevates the branchial influx of Na\(^+\) and Cl\(^-\) in FW (106, 152). In the Atlantic salmon, cortisol stimulates the expression of claudin 27a and 30 genes to increase epithelial tightness in FW (163). In the esophagus of mudskipper and medaka in FW, cortisol also induces cell proliferation in the epithelia to stratify the epithelium, resulting in reduced permeability (151, 154). Changes in the levels of plasma cortisol and GR transcripts in osmoregulatory organs during acclimation to ion-poor water further support the importance of the cortisol-GR system in acclimation to hyposmotic environment. In many euryhaline marine teleosts such as mullet (\textit{Mugil cephalus}), sea bass (\textit{Dicentrarchus labrax}), gilthead sea bream (\textit{Sparus aurata}) and starry flounder (\textit{Platichthys stellatus}), similar increases in plasma cortisol and GR transcript levels are reported after transfer to lower salinity environments (98, 106, 151). Similar hyper-osmoregulatory effects have also been shown in stenohaline FW fishes. In zebrafish larvae, cortisol elevated Na\(^+\) uptake and reduced the epithelial water permeability (91, 92). In the gills of goldfish and brown bullhead catfish (\textit{Ictalurus nebulosus}), cortisol increased the surface area of FW-type ionocytes and elevated the branchial influx of Na\(^+\) and Cl\(^-\) (19, 124).

Collectively, the cortisol-GR system appears to have substantial physiological impacts on adaptation to both hypo- and hyperosmotic environments (Fig. 4). However, the relative importance of the system for adaptation to either environment is diverse among species. Particularly, the degree of increase in the plasma cortisol level during acclimation to different salinities varies. Transfer of rainbow trout and eel from FW to SW resulted in a transient increase in plasma cortisol, whereas no significant changes were observed after the reverse transfer (7, 86). Plasma cortisol increased after transfer from SW to FW in Mozambique tilapia, but not as large as that after transfer from FW to SW (71). In the killifish, the increase in plasma cortisol concentration was similar in fish transferred from FW to SW or vice versa (104, 141), but in mudskipper, the increase was higher after SW to FW transfer than after FW to SW transfer (152). We assume that these differences in plasma cortisol responses among species are dependent on the developmental status, habitat preference and/or life history as discussed below. Our study in medaka esophagus showed that low-dose cortisol administration stimulated differentiation to SW-type esophagus, but FW-type esophagus was differentiated at a higher dose. These differences in effective doses of cortisol are comparable to the increased ranges of plasma cortisol in SW and FW medaka after transfer from isotonic one-third SW (151). To generalize the dose-dependent dual action of cortisol for acclimation to different salinities, further analyses in other species are
required. The dual actions of cortisol may be related to the distinct action on multiple
GR isoforms with different sensitivities to cortisol for trans-activation and
trans-repression activities (127, 148).

**Prolactin (PRL)**  PRL is an important regulator with multiple biological functions in
vertebrates (12, 47, 133). In teleosts, PRL has been viewed as important for increasing
ion uptake as well as reducing ion and water permeability of osmoregulatory surfaces in
FW fishes (51, 99, 135). Using hypophysectomized killifish, Grace Pickford was the
first to conclusively demonstrate that PRL has an essential role in ion uptake
mechanisms of teleost fishes in FW (125). Since then, evidence for PRL as an important
FW-adapting hormone in teleost fishes comes from studies on exogenous PRL treatment
and PRL dynamics in a relatively limited number of FW and euryhaline fishes, using
salmonids, goldfish, tilapia, and mudskipper (Fig. 4), where the homologous PRLs and
their receptors were identified and assays for their quantification were developed (131).
Recently, mechanisms for PRL secretion from the pituitary cells in response to
hypotonic stimulus have been intensively studied in Mozambique tilapia, and PRL
action on the development of FW-type ionocytes has been suggested (142). Including
these studies, evidence points to PRL playing a primary role in hyperosmoregulation,
although there is species variability in the actions of PRL as is the case for other aspects
of fish PRL physiology (99). Indeed, the relatively primitive eels, catfish and salmonids
can survive for long periods in FW after hypophysectomy, whereas hypophysectomized
killifish and tilapia, advanced teleosts, appear to be unable to survive in FW (51).
Relative importance of extra-pituitary PRL might be a possibility in the primitive
species, since production of PRL seems to have been centralized into the pituitary
during evolution (131,135). Such high extra-pituitary expression has been reported only
in teleosts (62, 132). Furthermore, the role of different isoforms of extrapituitary PRL
and their receptors may also be associated with the species variation (137). In addition
to the PRL isoforms originated from recent gene duplication event(s) in some particular
lineages (or species), including the third round teleost-specific whole genome
duplication (e.g., 147), the novel extrapituitary-expressed isoforms from the second
round of genome duplication event have been discovered in non-mammalian vertebrates,
but neither their variations among teleost species nor their relations to osmoregulation
or habitat use are clear (57).

In stenohaline SW teleosts such as anglerfish and pipefish, which have aglomerular
kidneys, marine origin and limited capacity to hyperosmoregulate, PRL is thought to
have little stimulatory effect on ion uptake. Such different limitations in ion regulatory
capacity and/or strategies for ion regulation according to species may affect to what
extent PRL is involved in osmoregulation. However, studies using the stenohaline SW teleosts are limited (115) and analyses of their PRL receptors are needed to determine the full scope of osmoregulatory actions of PRL among teleost fishes. And phylogenetic histories and evolutionary pressures must be considered in explaining observed patterns. In order to delineate a clearer picture for the role of extrapituitary PRL, further research on gene expression dynamics after hypophysectomy and the effects of PRL gene removal using gene knockout technologies in fishes are necessary (8, 67).

PRL and GH belong to a super-family of cytokines and produce multiple biological effects on embryogenesis, growth, metamorphosis and reproduction in teleosts as in other vertebrates by regulating cell turnover in the respective target organs (131). Genes encoding GH/PRL and their receptors are actively transcribed and translated at different developmental stages among teleost species. For example, the GH/IGF-I axis is important in the preparatory physiological adaptations that comprise the parr-smolt transformation of anadromous salmonids. This transformation includes a number of changes that are adaptive for growth during migration in SW. Distinction among osmoregulatory actions like the autocrine/paracine actions of IGF-I on gill ionocytes and renal epithelia, and actions on growth promotion is limited (136). There is also an important additive/synergistic interaction with cortisol (44, 97) whose osmoregulatory roles are also diverse as explained in detail above. Some of the interaction of GH and cortisol may be permissive through GH/IGF-I’s capacity to upregulate the number of gill cortisol receptors (143, 145, 161). Cortisol and GH also increased drinking rate, possibly interacting with the RAS in juvenile salmonids (38, 39). Relationships among such (modes of) osmoregulatory actions and other functions of these hormone systems, as well as the complexity of factors involved in regulating their secretion (42, 133), also appear to mirror the variability in their actions. Again, cortisol exerts regulatory actions on secretion of GH and PRL. Therefore, we anticipate that future research on diversity of endocrine control of osmoregulation will be focused on how the hormones interact at different tissues involved in ion and water transport (Fig. 4).

Neurohypophysial hormones Vasopressin/vasotocin (VT) is an essential antidiuretic hormone in all tetrapod species, and its gene mutation in human and rodents results in severe dehydration (6, 9). VT is also an important stimulator of cutaneous water absorption in amphibians as part of the water-balance response in that group (150). It seems therefore that VT may play an important role in adaptation by teleosts to a desiccative SW environment. However, accumulating evidence suggests that the effect of VT on salinity adaptation is species dependent. An initial in situ hybridization study in trout showed that VT gene transcripts in the magnocellular preoptic nucleus were
down-regulated after transfer to hyperosmotic 80% SW and restored after back transfer
to FW (61). In primary culture of gill pavement cells from the sea bass, VT inhibited
NaCl secretion as measured by a decreased short-circuit current (46), although the cells
do not contain ionocytes. Recent data in medaka showed that VT mRNA in the
hypothalamus and VT receptor mRNA in the anterior segment of distal tubules, where
NaCl absorption occurs via NCC1, were down-regulated after hyperosmotic challenges,
and exogenous VT administration increased NCC1 mRNA at the distal tubules (Konno
N and Uchiyama M, personal communication). All of these changes support a role for
VT in FW adaptation (Fig. 4). However, transfer of flounder (Platichthyes flesus) to FW
decreased plasma VT concentration (13), while transfer from FW to SW increased
hypothalamic VT mRNA and plasma VT levels (172), which favors the role of VT in
SW adaptation. VT infusion into the trout in vivo was diuretic as a consequence of
increased blood pressure, but in perfused trout trunk preparation in which pressure to
the glomeruli was maintained constant, VT decreased urine flow rate due to glomerular
intermittency (2). It is not known whether physiological increase of plasma VT level is
antidiuretic in trout as VT is highly potent in vasopressor effect. In the hypothalamus of
gilthead sea bream (S. aurata), VT mRNA levels are up-regulated after transfer from
SW to both lower and higher salinity environment (105). The increase is most likely due
to the increase in plasma cortisol concentration after transfer to either media. Thus VT
responses to environmental osmotic challenges differ among species.

It is interesting to note that VT inhibited drinking, but isotocin (IT) was strongly
dipsogenic in the eel (119). IT has stronger effect on branchial NaCl secretion than does
VT in the sea bass (46), and the hypothalamic IT mRNA levels increased after transfer
to hypersaline media but not to hyposaline media in the sea bass (105). These results
suggest that IT is involved in hyposmoregulation. In zebrafish, however, IT mRNA
expression is stimulated after transfer to deionized water and IT gene knockdown
resulted in impaired differentiation of FW-type ionocytes and expression of related
transporters (20). In mammals, oxytocin, the IT ortholog, has a natriuretic effect but the
effect is much weaker than the antidiuretic effect of vasopressin. However, IT appears to
be a major osmoregulatory hormone secreted from the posterior pituitary in teleost
fishes (158).

What is the cause of such diversity?

In this section, we first itemize the major differences in molecular physiology of gill
ionocytes and hormonal control of osmoregulation among teleost species in order to
provide materials for identifying possible causes of such differences. Although the
number of species examined is still too small to suggest any hypothesis, the regulatory
mechanisms seem to have evolved rather quickly to maintain body fluid homeostasis
when teleosts expanded their habitats to various osmotic environments. It seems that
primitive species such as eels and salmonids tend to be more euryhaline, but the
diversity cannot be explained by the taxonomic position because there are species with
diverse osmotic adaptability among species of an advanced group such as killifish,
tilapia, flounder, and pufferfish. As mentioned above, an additional doubling of the
genes that occurred only in the teleost lineage may be one of the major causes of
diversity, but possible other causes are considered and discussed here.

(1) Ion-secretory ionocytes in SW seem to be only one type, which is equipped with
NKA, NKCC1 and CFTR, in all teleost species. However, it seems likely that the
number of ionocyte types in FW varies among teleost species. For instance,
NCC2-positive ionocyte in FW has been confirmed at the molecular level with
Mozambique tilapia and zebrafish, but the NCC2-positive ionocyte has not been
observed in eels and salmonids (54). It would be interesting to determine whether the
common ancestor of Elopomorpha (including eel), Ostariophysi (including zebrafish),
Protacanthopterygii (including salmonids) and Acanthopterygii (including tilapia) had
the NCC2-positive ionocyte before divergence, or whether the NCC2-positive ionocyte
was independently acquired in Ostariophysi and Acanthopterygii. The more ionocyte
types in FW may be due to the higher variability of FW environment than SW.

(2) The size of gill ionocytes increases greatly after transfer from SW to FW in
killifish (81, 103) but ionocytes enlarge after transfer from FW to SW in other teleosts
including salmonids and tilapia (54, 58). This may be a reflection of the historical SW
origin of this killifish species (F. heteroclitus), which lives along the coast of Atlantic
Ocean (Fig. 5). Since the distal tubules that are responsible for NaCl reabsorption in FW
are poorly developed in the nephron of the killifish, this species is apparently SW origin
and needs to be distinguished in its osmoregulatory scheme from other euryhaline
teleosts of FW origin such as salmonids (31, 74, 80).

(3) ANP is a volume-depleting hormone that decrease both NaCl and water in trout
as it is in mammals, but it more specifically deplete NaCl in the body to promote SW
acclimation in eels. As generally thought, salmonids are FW species and some are
land-locked, staying in the river or the lake throughout their lives (128). By contrast,
eels are basically SW fish. In support is a recent study showing that eels breed in the
ocean and some do not enter the river (FW) and spend their whole life in SW as judged
by the Sr/Ca ratio in the otoliths (166). Sr is incorporated into the otoliths only when
fish are in SW. Therefore, it is possible that ANP is diuretic and natriuretic in trout.
whose kidney has been developed to excrete excess water, while ANP is antidiuretic in
the SW eel kidney to save water (157). The principal function of marine teleost kidney
is to excrete divalent ions at the proximal tubule (10, 78, 174), and some species such as
toadfish and seahorse lost glomeruli (11).

(4) The killifish cannot survive in FW without PRL but the trout and other FW
fishes can live normally without the pituitary. These findings signal the importance of
the SW or FW origins, respectively, of these species. An exception is the eel which can
live in FW after hypophysectomy (51). Thus it is not possible to explain the difference
only by their FW or SW origin, but Japanese eels used in these experiments were
cultured in FW and could be different in their physiology from natural eels. Culture eels
do not undergo silvering even after SW transfer, it will be interesting to examine
whether or not silver eels caught during downstream migration can survive in FW
without pituitary.

(5) VT gene expression is down-regulated after transfer of trout from FW to SW, but
after similar transfer it is up-regulated in the euryhaline flounder that lives in estuaries
(Fig. 5). Furthermore, the gene expression is up-regulated in the sea bream after transfer
to both hypo- and hyper-saline media (105). Although it is yet undetermined whether
VT is important for FW or SW adaptation, it seems that its function may differ
according to species whose osmoregulation fits more to FW or SW.

Based on these observations and findings, we hypothesize that the diverse
osmoregulatory mechanisms among teleost species are basically due to the differences
in their intrinsic habitat, FW or SW (Fig. 5). The word ‘intrinsic’ used here includes
various factors such as the environment where they hatch and spend their early life
stages, where they preferred to stay (like salt preference), where they need to consume
less energy, and where they originate phylogenetically. We compared the oxygen
consumption rate when euryhaline fishes are in FW or SW from previous reports, but
we could not find any clear tendency among species (e.g., killifish, 84; tilapia, 73; sole,
50; stickleback, 45; medaka, 120). However, each fish group may have an environment
of its origin phylogenetically and developmentally in either FW or SW. For instance,
salmonids and medaka spawn in FW and their groups are basically FW fishes, while
eels and killifish spawn in SW and their group of fishes are mostly SW fishes although
they are all migratory or euryhaline species (Fig. 5). Therefore, we must interpret the
data with caution because variety of ionocyte types and hormonal responses often
depend not on their current habitat but on the habitat of origin. We must note here that
the response to a hormone differ among physiological parameters. For instance, the
cardiovascular effect of ANP is consistent in all teleost species thus far examined (vasodilatory and hypotensive) and ANP acts to protect the heart in both eels and trout as in mammals (36). Furthermore, cardiac NPs are important for the normal development of the heart in teleosts, as knockdown their genes resulted in abnormal development of the heart in medaka embryos (113). It seems that basic or essential function of a hormone is conserved throughout vertebrate species, but diversified functions become evident in body fluid regulation after diversification of habitats.

We are aware that more species need to be examined to delineate more definite picture for the diverse osmoregulation among teleosts and to extract the cause of the diversity. Since next generation sequencing technologies have accelerated the availability of genome databases in various teleost species, we can compare the genes between species with different osmoregulatory mechanisms and identify genes responsible for such diversity in the near future. There are close species within the same genus that has distinct ability to acclimate to SW such as medaka (O. marmoratus and O. dancena), killifish (F. heteroelitus and F. lima), and tilapia (O. mossambicus and O. niloticus) (158). By comparing the known osmoregulatory genes and their regulatory elements between the two species will afford new insights into the different adaptability to SW in the two species. Furthermore, the parallel increases in sequencing technology and bioinformatics techniques enhance the sensitivity and reliability of the transcriptomic analyses such as RNA-seq. Application of such techniques to the two akin species after salinity challenges will accelerate the identification of the key and novel genes for the difference in FW or SW adaptation. More data with traditional physiological techniques combined with new technologies will deepen our understanding of the body fluid regulation in teleosts and other vertebrate groups.

Acknowledgements

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Table 1. Classification of ionocyte types of three euryhaline teleost fishes, Mozambique tilapia, rainbow trout and killifish, defined by immunofluorescence patterns for five major ion transport proteins, Na⁺/K⁺-ATPase (NKA), Na⁺/K⁺/2Cl⁻ cotransporter 1 (NKCC1) Na⁺/Cl⁻ cotransporter 2 (NCC2), Na⁺/H⁺ exchanger 3 (NHE3) and cystic fibrosis transmembrane conductance regulator Cl⁻ channel (CFTR).

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Figure Legends

Fig. 1. Osmoregulatory sites and ion/water movement at the sites in teleost fishes in fresh water (FW) or seawater (SW). The size of the arrow shows the amount of ion/water movement. The gills are the major site of active NaCl uptake in FW and of active NaCl excretion in SW. For details, see text.

Fig. 2. A phylogenetic tree of full-length amino acid sequences of fish and human SLC12 cation-chloride cotransporters, constructed by the maximum likelihood method. Numbers at the nodes are bootstrap support values from 100 maximum likelihood replicates using RAxML Blackbox (http://phylobench.vital-it.ch/raxml-bb/). Human KCC1 was used as an outgroup to root the tree. Bar, evolutionary distance of 0.5 amino acid substitutions per site. The GenBank accession numbers are as follows: Mosambique tilapia (NKCC1a, AY513737; NKCC1b, AY513738; NKCC2, AY513739; NCC2, EU518934), European eel (NKCC1a, AJ486858; NKCC1b, AJ486859; NKCC2a, AJ564602; NKCC2b, AJ564603; NCC1, AJ564604; NCC2, AJ564606) and shark (Squalus acanthias NKCC1, U05958; S. acanthias NKCC2, AF521915; Carcharhinus leucas NCC1, BAN42613). Amino acid sequences of lamprey, coelacanth, spotted gar, zebrafish, Nile tilapia, fugu and human were retrieved from the Ensembl website (http://www.ensembl.org).

Fig. 3. Schematic diagrams of ionocyte types in three euryhaline teleost fishes,
Mosambique tilapia (A), rainbow trout (B) and killifish (C). A: Ionocytes of Mozambique tilapia were originally classified into four types (types I-IV) by simultaneous immunofluorescence staining for NKA, NKCC and CFTR, and then the localization patterns of several ion-transport proteins, such as NKCC1a, NCC2, NHE3 and ROMKα were demonstrated in each ionocyte type by developing homologous antibodies. Shown on the right are quintuple-color immunofluorescence staining for NKA (red), NKCC1a (blue), NCC2 (cyan), NHE3 (yellow) and CFTR (green) [Modified from Ref. 56.]. Scale bar, 10 µm. B: Ionocytes of rainbow trout are known to be classified into PNA⁺ and PNA⁻ ionocytes, by examining the specific binding of peanut agglutinin to their apical membrane. Immunolocalization of ECaC, NHE3b, Rhcg 1, NKA and NKCC1 has been examined with the PNA⁺ and PNA⁻ ionocytes. Shown on the right are triple-color immunofluorescence staining for NKA (red), NKCC1 (blue) and NHE3b (green) [Modified from Ref. 54.]. Trout ionocytes are classified into at least two types: cells possessing apical NHE3b, basolateral NKA and...
basolateral NKCC1 (+); cells showing only apical NKA (-). The nuclei were

counterstained with DAPI (gray). Scale bar, 10 µm. C: Ionocytes of killifish possess

apical CFTR and basolateral NKCC1 in seawater, and apical NCC2 and basolateral HA

in freshwater. AC, accessory cell; NKA, Na+/K+-ATPase; NKCC, Na+-K+-2Cl⁻
cotransporter; NCC, Na+-Cl⁻ cotransporter; NHE, Na+/H⁺ exchanger; CFTR, cystic

fibrosis transmembrane conductance regulator Cl⁻ channel; ROMK, renal outer

medullary K⁺ channel; NBC, Na⁺-HCO₃⁻ cotransporter; ECaC, epithelial Ca²⁺ channel;

Rh, Rhesus glycoprotein; HA, H⁺-ATPase. For details, see text.

Fig. 4. Schematic diagram showing interactions of fast- and slow-acting hormones

included in this review for acclimation to fresh water (FW) and seawater (SW)
environments. The hormones are conveniently categorized as FW- or SW-adapting

hormones by different colors, but their actions are too diverse among species to
categorize in a group as detailed in the text. For instance, cortisol act as both FW- and

SW-adapting hormone via glucocorticoid receptors, possibly in a

concentration-dependent manner. Fast-acting hormones often regulate secretion of

slow-acting hormones as shown by arrows, and the interactions within each group of

hormones are also reported. In addition, some slow-acting hormones regulate the gene

expression of fast-acting hormones in mammals, but such evidences are still limited in

fishes. These interactions among the hormones may mirror the variability in their

actions. The diverse actions may also depend on the developmental states, habitat

preference and/or life history of many species. Accumulating evidence indicates

important osmoregulatory roles of isotocin in addition to vasotocin (VT) in teleost. ANP,

atrial natriuretic peptide; GH, growth hormone; IGF-I, insulin-like growth factor I; PRL,

prolactin; RAS, renin-angiotensin system.

Fig. 5. Schematic drawing of ‘intrinsic’ habitat of teleosts that are described in this

review. The relative position of the fish to fresh water (FW), brackish water (BK) or

seawater (SW) indicates the degree of ‘intrinsic’ habitat to FW or SW fish. Zebrafish

and catfish are stenohaline FW species and tiger puffer is a stenohaline SW species, but

others are euryhaline species. Eels and salmonids are migratory species between FW

and SW. Mozambique tilapia, flounder and mudskipper live in BK in the river mouth,

and sea bass and killifish move along the coast and sometimes enter the river. Japanese

medaka is in FW but can survive in the natural salt pan with concentrated SW.


A Mozambique tilapia

Freshwater

Seawater

Fresh & Seawater

Type II

Type III

Type IV

Type I

Modified from Hiroi et al., 2008

B Rainbow trout

Freshwater

Seawater

Freshwater

Seawater

NKA (α1a?)

NKA (α1b?)

NKA (α1b?)

NKA (α1a?)

NHE3 (PNA–)

NHE3 (PNA–)

NHE3 (PNA–)

NHE3 (PNA–)

3x immunostaining

Modified from Hiroi & McCormick 2012

C Killifish

Freshwater

Seawater

NKA

NKA

NKA

NKA

NHE3?

CFTR

CFTR

CFTR

NCC2 (SLC12A10)

NBC1

NKCC1

NKCC1a

ROMKa

NHE3

AC

AC

AC

AC

FW type

(= tilapia type II?)

SW type

(= tilapia type IV)

Modified from Hiroi et al., 2008

Modified from Hiroi & McCormick 2012