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REVIEW

Physiological mechanisms used by fish to cope with salinity stress

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ABSTRACT

Salinity represents a critical environmental factor for all aquatic organisms, including fishes. Environments of stable salinity are inhabited by stenohaline fishes having narrow salinity tolerance ranges. Environments of variable salinity are inhabited by euryhaline fishes having wide salinity tolerance ranges. Euryhaline fishes harbor mechanisms that control dynamic changes in osmoregulatory strategy from active salt absorption to salt secretion and from water excretion to water retention. These mechanisms of dynamic control of osmoregulatory strategy include the ability to perceive changes in environmental salinity that perturb body water and salt homeostasis (osmosensing), signaling networks that encode information about the direction and magnitude of salinity change, and epithelial transport and permeability effectors. These mechanisms of euryhalinity likely arose by mosaic evolution involving ancestral and derived protein functions. Most proteins necessary for euryhalinity are also critical for other biological functions and are preserved even in stenohaline fish. Only a few proteins have evolved functions specific to euryhaline fish and they may vary in different fish taxa because of multiple independent phylogenetic origins of euryhalinity in fish. Moreover, proteins involved in combinatorial osmosensing are likely interchangeable. Most euryhaline fishes have an upper salinity tolerance limit of approximately 2× seawater (60 g kg^{-1}). However, some species tolerate up to 130 g kg^{-1} salinity and they may be able to do so by switching their adaptive strategy when the salinity exceeds 60 g kg^{-1}. The superior salinity stress tolerance of euryhaline fishes represents an evolutionary advantage favoring their expansion and adaptive radiation in a climate of rapidly changing and pulsatory fluctuating salinity. Because such a climate scenario has been predicted, it is intriguing to mechanistically understand euryhalinity and how this complex physiological phenotype evolves under high selection pressure.

KEY WORDS: Osmoregulation, Stress tolerance, Evolution, Phenotypic plasticity

Introduction

Salinity is an inherent physicochemical property of water, representing a measure of its content of dissolved (ionized) salt. By influencing thermodynamic properties of water (e.g. density, heat capacity, solvent capacity for solids and gases, vapor pressure), salinity contributes greatly to defining habitat characteristics for fishes and other aquatic organisms. In addition, biochemical processes inside and outside cells are greatly influenced by salinity. The ionic strength of almost all environmental waters results virtually exclusively from dissolved inorganic ions (table salt – NaCl – in most cases) and is, therefore, commonly expressed as salinity. In contrast, the solute content of aqueous fluids inside organisms is often expressed as osmolality. Osmolality is a measure of all dissolved ions (not just inorganic salts), including organic compounds such as sugars and amino acids that are common in biological fluids. Thus, while salinity and osmolality are virtually identical for the great majority of aquatic habitats, salinity accounts for only a fraction of the overall osmolality of biological fluids.

Habitat salinity represents a major abiotic factor that governs the activity and distribution of fishes and other aquatic animals. A change in the saltiness of habitat water causes salinity stress because, if not compensated for, it interferes with physiological homeostasis and routine biological processes. Most fishes are adapted to tolerate some degree of salinity stress (small for stenohaline and large for euryhaline species). The vast majority of species are restricted to habitats with relatively stable salinity, defined according to the Venice salinity system as either marine at 30–40 parts per thousand salinity (ppt) or freshwater at <0.5 ppt (IAL and IUBS, 1958). According to the most recent Thermodynamic Equation Of Seawater (TEOS-10) convention, salinity is expressed as the mass fraction of salt in water with g kg^{-1} as the unit (IOC et al., 2010). Therefore, salinity values are expressed as g kg^{-1}, which is virtually interchangeable with ppt, in what follows.

As a result of climate change, habitat degradation and anthropogenic activities, the severity and frequency of salinity stress are increasing in many parts of the world and may eventually exceed the coping ability of an unknown number of species. Anthropogenic climate change has already greatly accelerated rises in sea level, a trend that is anticipated to continue. The global sea level rise is predicted to be between 40 cm and 1.2 m by the year 2100 and up to 3 m by the year 2300 (Horton et al., 2014). Rising ocean levels are largely a result of melting polar ice caps and are associated with a mean decrease of ocean salinity (van Wijk and Rintoul, 2014). In contrast to decreasing salinity of the pelagic ocean, rising sea level leads to increased salinization of coastal areas due to flooding and seawater invasion into freshwater aquifers. In addition to such gradual climate-induced salinity changes, severe and acute salinity stress results from extreme pulsatory climate events (tsunamis, hurricanes, etc.), which are predicted to increase in frequency and severity (Nielsen et al., 2012). Such events can cause large and sudden increases or decreases in habitat salinity, e.g. during flooding associated with tsunamis or rainstorms in intertidal, coastal or desert habitats (Illangasekare et al., 2006; Drake et al., 2013; Duggan et al., 2014). Climate change-induced floods have already caused salinity stress resulting in significant mortality in coral reefs and other coastal marine ecosystems (Huang et al., 2014). In addition, drought-induced salinity stress has been shown to cause significant changes in species composition of desert lake and stream habitats (Wedderburn et al., 2014). During droughts, heat-induced evaporation concentrates all solutes (e.g. salts) that are dissolved in the universal solvent water. Therefore, thermal and salinity stress often co-occur during climate-induced droughts, especially in aqueous habitats such as desert lakes that contain large amounts of dissolved inorganic ions. This brief review will summarize what we

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List of abbreviations

CFTR	cystic fibrosis transmembrane conductance regulator
FAK	focal adhesion kinase
MAPK	mitogen-activated protein kinase
MLCK	myosin light chain kinase
NKCC	$Na^+/K^+/2Cl^-$ co-transporter
OSTF1	osmotic stress transcription factor 1
PLC	phospholipase C
ppt	parts per thousand

know about the physiological mechanisms that fish have at their disposal to cope with salinity stress in their habitat.

Maintenance of osmotic homeostasis in fishes

Marine hagfish and elasmobranchs represent a small minority of fishes that are osmoconformers. The concentration of NaCl in body fluids of hagfish is approximately equal to that of seawater (Evans and Claiborne, 2009). However, in elasmobranchs it is less than half that of seawater and the osmotic gap is filled by active accumulation of compatible organic osmolytes (Yancey et al., 1982). Elasmobranchs maintain the difference in NaCl content (relative to seawater) by active NaCl secretion via the rectal gland. In contrast to these primitive fishes, most (>25,000 extant species) fishes are teleosts that osmoregulate. Teleost fishes maintain the osmolality of their extracellular body fluids relatively constant at approximately 300 mosmol kg^{-1} (which is isosomotic to 9 g kg^{-1} salinity), independent of environmental salinity. To achieve osmotic constancy of the internal milieu, teleost species inhabiting freshwater environments have to counter passive loss of salt by active absorption and passive gain of water by excretion of dilute urine. Marine teleosts do the opposite: they actively secrete salt and retain water to maintain osmotic homeostasis. The many physiological mechanisms involved in these processes of steady-state osmoregulation are strikingly different in freshwater and marine teleosts (Table 1). They have been reviewed extensively elsewhere (e.g. Karnaky, 1986; Jürss, 1987; Perry, 1997; Evans and Claiborne, 2009). Many elaborate functional and structural changes take place in gill, kidney and intestine (see Table 1) when euryhaline teleosts switch from plasma hyper-osmoregulation (environmental salinity <9 g kg^{-1}) to plasma hypo-osmoregulation (environmental salinity >9 g kg^{-1}), which illustrates the critical influence of environmental salinity on fish physiology. In this paper, I will review: (1) mechanisms that enable euryhaline teleosts to alter their adaptive strategy between plasma hyper- and hypo-osmoregulation and (2) mechanisms that enable them to cope with large salinity changes that do not reverse osmotic and ionic gradients.

Evolution of high salinity tolerance in euryhaline fishes

Before summarizing the current knowledge on how euryhaline fishes alter their adaptive strategy from plasma hyper- to hypo-osmoregulation, it is critical to consider the evolutionary origin of high salinity tolerance in teleost fishes. If euryhalinity represents a monophyletic trait of all teleosts then we would expect the physiological mechanisms of switching between osmoregulatory strategies to be highly conserved. Alternatively, if euryhalinity has evolved multiple times independently in different lineages of teleosts then those mechanisms would be expected to be

Table 1. Some phenotypic differences that are important for altered osmoregulatory function in teleost gill, kidney and intestine

	<9 g kg^{-1} salinity	>9 g kg^{-1} salinity
Habitat	Freshwater, low brackish	Marine, high brackish, hyperhaline
Adaptive strategy	Plasma hyper-osmoregulation	Plasma hypo-osmoregulation
Salt diffusion	Passive salt loss	Passive salt gain
Water movement	Passive hydration	Passive dehydration
Gill phenotype		
Ionocyte number	Lower	Higher
Ionocyte size	Smaller	Larger
Ionocyte mitochondria	Less abundant	More abundant
Ionocyte apical membrane	Exposed, microvilli	Covered, apical crypts
Ionocyte basolateral membrane	Fewer tubular infoldings	Extensive tubular infoldings
Ionocyte complexes	Uncommon	Common
Accessory cells	Uncommon	Common
NKCC activity	Lower	Higher
CFTR activity	Lower	Higher
V-type H^+-ATPase activity	Higher	Lower
Blood vessels and interstitium	More constricted	More dilated
Epithelial tight junctions	Tighter	Leakier
NaCl permeability	Low	High
Water permeability	Higher	Lower
Active NaCl transport	Absorption	Secretion
Kidney phenotype		
Urine volume	Large	Small
Urine osmolality	Very low	Isosomotic
Distal tubule water reabsorption	Very low	High
Distal tubule Na^+ reabsorption	High	Very low
Functional glomeruli	Many	Few
Renal port system	Less developed	Well developed
Divalent ion excretion	Low (depends on water hardness)	High
GI tract phenotype		
NaCl-coupled water absorption	Absent	High
Drinking rate	Very low (only during feeding)	High
Divalent ion transport	Absorption (depending on water hardness)	Excretion
Water permeability	Low	High

Note that all of these (and many other) phenotypes change when euryhaline fish transverse the isosmolality threshold of 9 g kg^{-1}.
CFTR, cystic fibrosis transmembrane conductance regulator; GI, gastrointestinal; NKCC, $Na^+/K^+/2Cl^-$ co-transporter.

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more diverse. The latter scenario is overwhelmingly supported by the pertinent literature. The physiological phenotype (trait) of euryhalinity is distributed in a mosaic pattern across different orders of fish (Nelson, 2006; Schultz and McCormick, 2013). This pattern could be a consequence of either selective loss or convergent evolution in multiple teleost orders, depending on whether euryhalinity represents an ancestral or derived condition. According to Nelson's phylogeny (Nelson, 2006), the 15 most primitive orders of fish consist almost entirely of strictly marine species. A notable exception are lampreys, which have successfully conquered freshwater habitats. The next 20 moderately advanced (from a phylogenetic perspective) orders are composed mostly of freshwater species (with the exception of Albuliformes, Anguilliformes, Saccopharyngiformes and Clupeiformes). The remaining 27 orders are again mostly composed of marine species (with the exception of Percopsiformes, Atheriniformes, Cyprinodontiformes, Synbranchiformes and Ceratodontiformes). This pattern supports a scenario according to which the earliest fishes evolved in a marine environment, invaded freshwater habitats before the origin of ray-finned fishes (actinopterygii), and reinvaded marine environments during a second wave of evolutionary expansion after bony fishes (teleosts) had already appeared. The fossil record of fishes seems inconclusive with regard to a marine or freshwater origin of the last common ancestor of all fishes (Halstead, 1985; Evans and Claiborne, 2009). However, it has been argued that the internal fluid osmolality (9 g kg^{-1}) of the teleost/tetrapod clade of fishes, which is much lower than that of a marine environment, represents evidence for an origin of this clade in a freshwater or brackish (mesohaline) habitat (Evans and Claiborne, 2009). This notion is supported by trait reconstructions from extant and fossil taxa (Vega and Wiens, 2012). Regardless of their habitat of origin, most fish orders include euryhaline species (Nelson, 2006; Schultz and McCormick, 2013). Moreover, the mosaic-like pattern of euryhalinity at the taxonomic level of orders is also apparent at lower taxonomic levels. For instance, the order Gasterosteiformes and even a single family (Gasterosteidae) within that order contain stenohaline marine, stenohaline freshwater and euryhaline species (Fig. 1). Such an extreme mosaic pattern of euryhalinity even at lower taxonomic levels may be a reflection of a modular mix of ancestral and derived characters giving rise to the physiological phenotype of euryhalinity by a process termed mosaic evolution (Gould, 1977).

Euryhaline fishes have radiated in two principal environmental contexts. First, coastal environments such as estuaries and intertidal zones subject to large and frequent salinity fluctuations harbor many euryhaline fish species (Marshall, 2013). Second, euryhaline fishes are common in arid zones containing desert lakes and creeks (Brauner et al., 2012). The evolution of euryhaline fishes in coastal and arid-zone environments that are characterized by variable salinity was presumably favored by the competitive advantage that euryhalinity provided for occupying new and unique ecological niches. Such a strategy increases fitness of euryhaline species and their competitiveness for resources relative to stenohaline species. A significant benefit (selective driving force) of the evolution of euryhalinity in coastal/intertidal zones is access to one of the most energy-rich ecosystems on the planet. Although characterized by severely fluctuating salinity, estuaries and marshes are extremely productive habitats in which costs associated with osmoregulatory adaptation are apparently well offset by the advantages of access to energy (food) resources. Euryhaline species are often migratory; in fact, migration may have evolved as a behavioral avoidance mechanism for escaping salinity stress. Physiological coping mechanisms may then have co-opted avoidance behavior into exploratory behavior, giving rise to diadromous species (those that migrate between freshwater and marine habitats). Pleiotropic advantages of diadromy (e.g. protection of susceptible juvenile stages from biotic or abiotic environmental stress in more sheltered habitats) may have favored evolutionary fixation of diadromy. Diadromous fishes include anadromous species that spawn in freshwater environments (e.g. *Salmo salar*, *G. aculeatus*, *Acipenser medirostris*, *Petromyzon marinus*) and catadromous species that spawn in marine environments (e.g. *Anguilla rostrata*, *Anguilla*

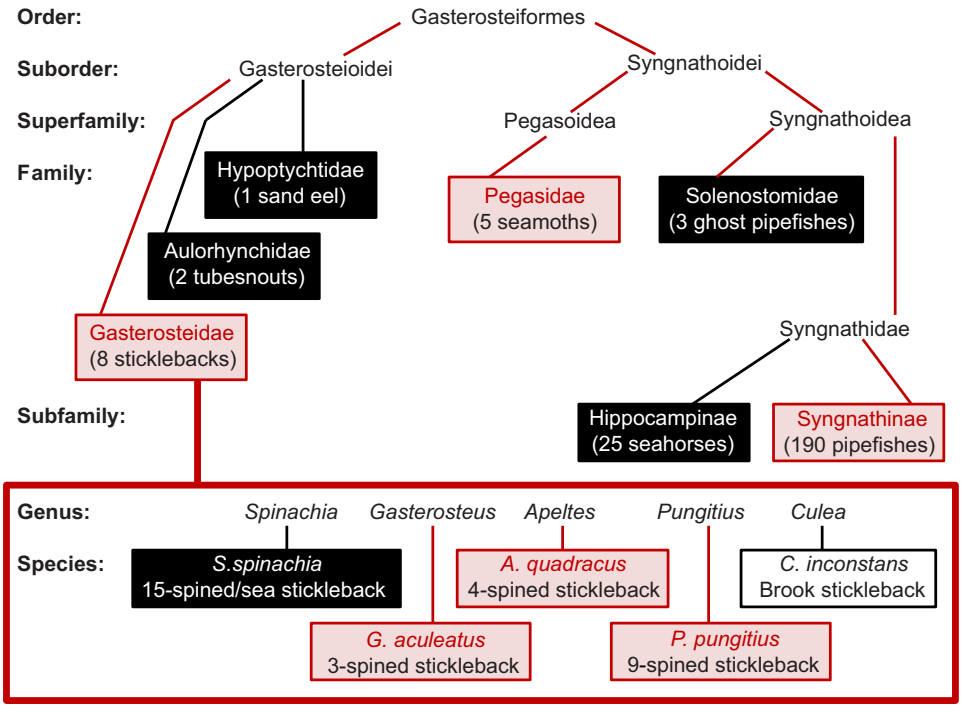

Fig. 1. Salinity tolerance of Gasterosteiformes at different taxonomic levels. Box colors indicate whether the corresponding taxon is composed of stenohaline marine (black), stenohaline freshwater (white) or euryhaline (red) species.

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japonica). The presence of diadromy in diverse phylogenetically primitive (e.g. *P. marinus*, *A. medirostris*) as well as advanced (e.g. *S. salar*, *G. aculeatus*) fishes illustrates that it represents an evolutionarily ancient and convergent strategy that is closely associated with the physiological phenotype of euryhalinity. Diadromy represents an interesting life history strategy that is often associated with remarkably large salinity tolerance differences of juvenile and adult developmental stages. Whether such life history stage-specific differences in salinity tolerance represent a prerequisite (intermediate) for the evolution of euryhalinity or a derived secondary trait is not clear. In any case, diadromy (in particular anadromy) facilitates formation of new ecotypes that may evolve into distinct species when the corresponding populations are genetically isolated for sufficiently long periods. Examples for such ecotypes include landlocked forms of three-spined sticklebacks (*Gasterosteus aculeatus*) and rainbow trout (the land-locked form of steelhead trout, *Oncorhynchus mykiss*) that reside in freshwater throughout their entire life cycle (Schluter and Conte, 2009; Pearse et al., 2014).

Mechanisms that enable teleosts to switch between plasma hyper- and hypo-osmoregulation

In strictly marine or strictly freshwater teleosts that inhabit environments of stable salinity, steady-state osmoregulatory mechanisms are sufficient to maintain physiological homeostasis. However, teleosts that inhabit environments of fluctuating salinity need to have the physiological capacity for adjusting their osmoregulatory strategy to match the variable salinity of the external milieu. Not much is known about such mechanisms or whether and how much they differ in multiple independently evolved clades of euryhaline fishes. Two basic scenarios for salinity stress can be envisaged. First, a reversal of the osmotic gradient between plasma/extracellular body fluids and the environment within a moderate salinity range (e.g. freshwater–seawater) will require a change in osmoregulatory strategy. The problem in this case is: how do euryhaline fish switch between salt secretion and salt absorption and manage to accomplish the many mandatory physiological and structural alterations of osmoregulatory tissues (see examples provided in Table 1)? Second, salinity stress may not require a reversal of the direction and mechanisms of active ion transport but, instead, greatly increase the osmotic gradient. For instance, movements of fish between brackish and hyperhaline water (e.g. in the Salton Sea watershed, California, or Saloum estuary, Senegal) or exposure to rapid salinization resulting from intense evaporation in tide pools or desert ponds can greatly increase the osmotic gradient without reversing it (e.g. from 35 to 60 g kg^{-1} or from 15 to 50 g kg^{-1}, etc.). The main challenge in this case is that active transepithelial ion transport and water retention demands are increased greatly, which comes at the price of disproportionately large energetic costs, as reflected, for instance, in Na^+/K^+-ATPase activity (Karnaky et al., 1976; Kültz et al., 1992; Laverty and Skadhauge, 2012). Increased water retention is achieved by increasing drinking rates, intestinal reabsorption of water via solute-linked transport, and decreased osmotic permeability of gill epithelium (Laverty and Skadhauge, 2012).

How do euryhaline fish accomplish the multitude of qualitative and quantitative physiological changes necessary for coping with salinity stress? Fishes have the ability to sense the osmolality of their environment and to transduce the sensory stimulus to signaling pathways that trigger the many specific changes that are necessary for adjusting osmoregulatory strategy and/or intensity (Kültz, 2011). The mechanism of such osmosensing is poorly understood and likely based on a combinatorial interaction of multiple molecular sensors (Kültz, 2013). Molecular osmosensors include transmembrane proteins such as ion channels, the calcium-sensing receptor, phospholipase A2 and cytokine receptors, proteins that are directly regulated by intracellular calcium and other inorganic cations, and cytoskeletal proteins. In addition, osmosensing is informed by direct osmotic and ionic effects on DNA and protein stability (Kültz, 2012). Each of the molecules involved in osmosensing may be non-specific by themselves with regard to the stimulus by which they are modulated. However, the specific combination and degree of modulation of multiple osmosensors results in triggering the appropriate effector mechanisms (see Table 1) to the extent needed. Osmoregulatory hormones/cytokines and their receptors integrate salinity stress responses at the whole-organism level (Foskett et al., 1983). Recent work has revealed signal transducers that are activated by molecular osmosensors in ionocytes and transport epithelia of euryhaline teleosts. Not surprisingly, post-translational modifications such as phosphorylation represent a prominent mechanism for osmosensory signal transduction. For instance, phospholipase C (PLC) and mitogen-activated protein kinase (MAPK) signaling pathways are involved in osmosensing in tilapia (Loretz et al., 2004). MAPK pathways also play a role in osmosensing in killifish and turbot (Kültz and Avila, 2001; Marshall et al., 2005). Reversible protein phosphorylation provides a link between cytoskeletal strain and osmosensory signal transduction during salinity stress. This link is exemplified by myosin light chain kinase (MLCK), which phosphorylates a tight junction protein, causes F-actin distribution, and is required for hyperosmotic activation of Na^+/Cl^-/taurine co-transporters in primary cultures of Japanese eel gill cells (Chow et al., 2009). Moreover, focal adhesion kinase (FAK) is dephosphorylated in response to hypo-osmotic stress in killifish gill and opercular epithelia (Marshall et al., 2005). FAK dephosphorylation has been shown to regulate the activity of $Na^+/K^+/2Cl^-$ co-transporter (NKCC) and cystic fibrosis transmembrane conductance regulator (CTFR) transport proteins during salinity stress (Marshall et al., 2008, 2009). Another signaling protein participating in osmosensing in a variety of euryhaline fishes is osmotic stress transcription factor 1 (OSTF1), which may have a role in governing changes in expression of ion transporters and channels (Fiol and Kültz, 2005; Fiol et al., 2006; Choi and An, 2008; Tse et al., 2008; Breves et al., 2010; McGuire et al., 2010). Other elements of salinity stress signaling have been identified but their discussion exceeds the scope of this brief review.

Prominent effector proteins regulated by salinity stress signaling networks include NKCC, CFTR, several plasma membrane ATPases, and other transporters. The regulation of these proteins in response to salinity change is reflected at the levels of expression (abundance), compartmentalization and activity (Hiroi and McCormick, 2012). In addition, euryhaline fish achieve a switch from plasma hyper- to hypo-osmoregulation by increasing cell proliferation and turnover and via extensive epithelial remodeling of gills (Conte and Lin, 1967; Laurent and Dunel, 1980; Chretien and Pisam, 1986). The high degree of complexity and the multitude of interacting physiological mechanisms that confer protection of euryhaline fish during salinity stress call for systems-level approaches to study them. Such approaches bear promise for deciphering how the information about external salinity set points is communicated from osmosensors via signal transducers to regulate appropriate effector mechanisms. Systems-level approaches address the genome-to-phenome continuum integratively by attempting to measure and correlate responses of

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transcriptomes, proteomes and metabolomes in cells and tissues with responses at higher-order physiological, morphological and behavioral phenotypes in a given genomic background. In particular, proteomics approaches are primed to reveal functional insight into the mechanistic basis of complex salinity stress responses. The proteome represents the direct functional link at which genomic and environmental input are integrated to give rise to organismal form and function (Fig. 2). Proteomics approaches combined with gene ontology, biochemical pathway and (last but not least) scholarly literature analyses have the power to reveal the mechanisms and underlying regulatory networks that euryhaline fishes utilize for coping with salinity stress. For instance, such approaches have revealed a major role of the *myo*-inositol biosynthesis pathway for tilapia salinity stress responses (Gardell et al., 2013; Kültz et al., 2013; Sacchi et al., 2013). This pathway is critical for maintaining cellular inorganic ion homeostasis during acute salinity stress, when plasma osmolality levels can rise as much as 100 mosmol kg^{-1} above normal (Gardell et al., 2013). The metabolite *myo*-inositol produced by this pathway fills this 'osmotic gap' and its concentration is proportional to environmental salinity (Gardell et al., 2013). Even when euryhaline fish are fully acclimatized to seawater, plasma osmolality is significantly elevated compared with when they are acclimatized to freshwater (Seale et al., 2003). Such plasma osmolality allostasis is supported by elevated concentrations of *myo*-inositol and higher activities of the enzymes involved in its synthesis. We have recently shown that the enzymes involved in *myo*-inositol biosynthesis are regulated at multiple levels (Fig. 2). In addition to large increases in the expression of *myo*-inositol phosphate synthase and inositol monophosphatase at the levels of mRNA and protein, their activity is directly regulated by inorganic ion concentration and pH (Villarreal and Kültz, 2014). This mode of regulation provides a very direct, rapidly responsive and highly efficient feedback loop (Fig. 3). The *myo*-inositol feedback loop depends on elevated intracellular inorganic ion concentration and pH, which are direct consequences of increased plasma osmolality (Kültz, 2012). This example of direct ionic effects on compatible osmolyte synthesizing enzymes illustrates that not all aspects of the salinity stress response network in euryhaline fish depend on complex cascades of information transfer. However, clearly, the subsequent transcriptional and translational induction of the *myo*-inositol biosynthesis enzymes involve additional signal transducers. The need to increase the abundance of these enzymes may be explained by a 'wear and tear' hypothesis as follows: as direct ionic activation of these enzymes greatly increases their catalytic efficiency and enzymatic activity, they may accumulate damage more rapidly during repeated cycles of structural changes associated with catalysis; this would then accelerate their degradation and turnover and explain the need for increased rates of *de novo* synthesis. This hypothesis may also be applicable to and is testable for other salinity-regulated effector proteins.

Osmoregulatory strategies at extreme salinities

In addition to the qualitative change in osmoregulatory strategy that takes place when the osmotic gradient between plasma and the environment reverses, there is also evidence for qualitative changes at extremely high and extremely low (ion-poor water) salinities. At very high salinities (>60 g kg^{-1}), the correlation between increased salinity and increased branchial NaCl permeability reverses (Kültz and Onken, 1993). This is also the case for the leakiness of tight junctions, which promote paracellular Na^+ extrusion across gill epithelium (Karnaky et al., 1977; Degnan and Zadunaisky, 1980). The decreased osmotic permeability of gill and opercular epithelia in fish exposed to strongly hyperhaline environments represents a qualitative shift in the adaptive strategy relative to the increase in osmotic permeability that occurs when euryhaline fish are acclimated from freshwater to regular seawater (35 g kg^{-1}). In euryhaline tilapia, the salinity threshold at which such a shift in osmoregulatory strategy occurs is approximately 2× seawater (60 g kg^{-1}) (Kültz and Onken, 1993). This change in osmoregulatory strategy indicates that during extremely hyperhaline conditions, water retention gains priority over paracellular secretion of Na^+, which relies on leaky tight junctions. Therefore, one might predict that in extremely hyperhaline environments the mechanism of Na^+ secretion is altered (e.g. from para- to trans-cellular routes) to accommodate the decreased osmotic permeability (junctional leakiness) of branchial epithelia. Although I am not aware of any

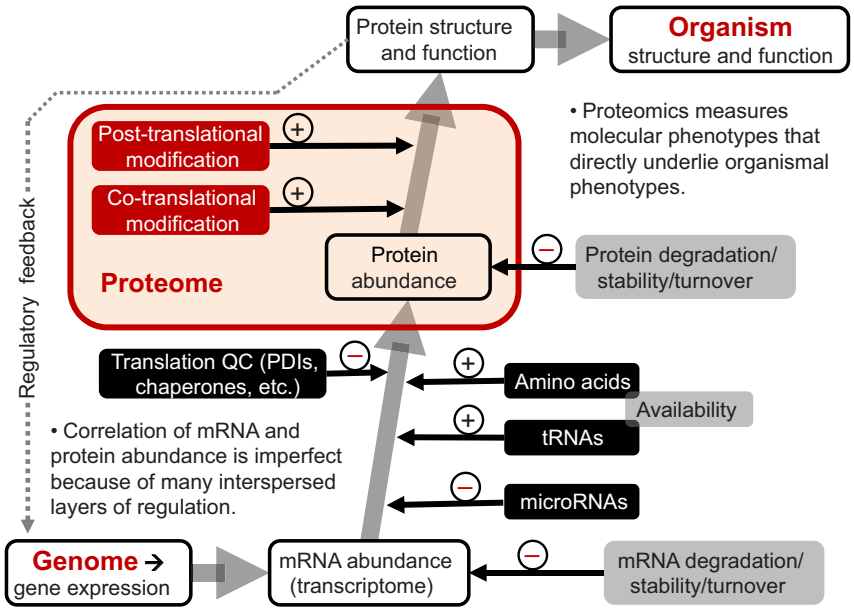

Fig. 2. Central position of the proteome within the genome to phenome continuum. Environmental signals (not shown) are integrated during each step along the genome to phenome continuum and phenotypic outcomes represent the result of complex genotype×environment interactions (QC, quality control; PDI, protein disulfide isomerases).

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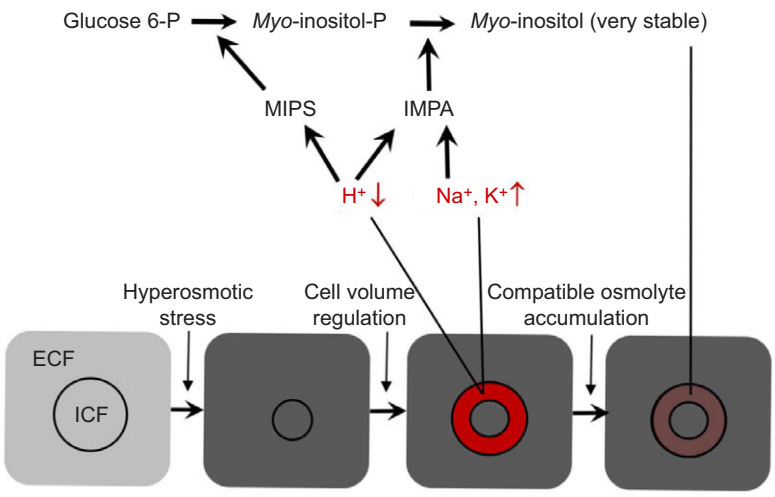

Fig. 3. Direct ionic regulation of the *myo*-inositol biosynthesis (MIB) pathway in euryhaline Mozambique tilapia (*Oreochromis mossambicus*). Upon transfer of fish from freshwater to seawater plasma osmolality increases and causes hyperosmotic stress, which leads to cellular dehydration and shrinkage (hypertonicity). Cell shrinkage is compensated by regulatory volume increase, a process that increases $[Na^+]_i$ and $[K^+]_i$ and decreases $[H^+]_i$ (increases pH). Cells do not tolerate the change in the concentration of these cations (marked in red) for long and, thus, excess ionorganic ions are replaced by *myo*-inositol, which is an organic osmolyte that is compatible with cell function. Elevated $[Na^+]_i$, $[K^+]_i$ and pH_i directly stimulate the enzymatic activity of myo-inositol phosphate synthase (MIPS) and inositol monophosphatase (IMPA), which constitute the MIB pathway. The degree of MIB pathway activation directly depends on $[Na^+]_i$, $[K^+]_i$ and pH_i in this simple feedback loop. ECF, extracellular fluid; ICF, intracellular fluid.

direct evidence for this conjecture it is indirectly supported by osmotic permeability and water balance studies on teleosts acclimated to hyperhalinity (Motais et al., 1966, 1969; Gonzalez et al., 2005; Laverty and Skadhauge, 2012). Interestingly, many species of euryhaline fish have upper salinity tolerance thresholds of about 2× seawater (Schultz and McCormick, 2013), which suggests that only the most euryhaline species that tolerate salinities well above 2× seawater have evolved the capacity for qualitatively changing their osmoregulatory strategy when encountering extremely hyperhaline conditions. The highest upper salinity tolerance limits of euryhaline fishes have been recorded at 114 g kg^{-1} for *Fundulus heteroclitus* (Griffith, 1974), 120 g kg^{-1} for *Oreochromis mossambicus* (Stickney, 1986), 130 g kg^{-1} for *Sarotherodon melanotheron* (Panfili et al., 2004; Ouattara et al., 2009) and 110 g kg^{-1} for *Craterocephalus eyresii* (Glover and Sim, 1978). Additional salinity tolerance ranges for euryhaline fishes are provided in two excellent recent reviews (Brauner et al., 2012; Schultz and McCormick, 2013). The apparent existence of a finite upper salinity tolerance at approximately 120 g kg^{-1} suggests that an insurmountable physiological barrier prevents fish from conquering habitats of higher salinity, e.g. the Great Salt Lake or the Dead Sea. The mechanistic basis for this apparent salinity tolerance barrier is not known and represents an intriguing subject for further scientific inquiry.

A shift in osmoregulatory strategy is apparent not only at extremely high but also at extremely low salinity. At extremely low salinity (deionized water), the size, number and mitochondrial content of ionocytes as well as Na^+/K^+-ATPase activity increase, which is opposite to the general relationship between these parameters and environmental salinity (see Table 1). These adjustments observed in fish exposed to deionized water presumably reflect the steepness of the ionic gradients and the increased energetic demand for active transepithelial NaCl absorption (Lee et al., 1996; Sakuragui et al., 2003, 2007). Both prolactin and cortisol production are greatly stimulated in fish exposed to deionized water and the particular concentrations and ratio of these osmoregulatory hormones may provide a sensory clue for alteration of branchial epithelial ultrastructure and function in ion-poor environments (Parwez et al., 1994). The changes in fish osmoregulation that occur both at extremely low (deionized water) and strongly hyperhaline (>2× seawater) salinities suggest that unique mechanisms are involved in conferring tolerance to extreme salinities. Although such mechanisms may have evolved independently in different lineages of very euryhaline fishes, a common feature seems to be divergence from 'typical' salinity-dependent gill phenotypes (Table 1).

Conclusions and future perspective

Euryhalinity and environmental stress tolerance are physiological traits that enable fish to complete their life cycle in variable habitats of fluctuating salinity. Stenohaline fish, by contrast, inhabit osmotically stable environments (the oceans or freshwater lakes and streams). Although a narrow physiological salinity tolerance range is well documented only for a limited number of these stenohaline species (e.g. 0–15 g kg^{-1} for zebrafish and common carp), lack of evolutionary selection pressure in stable environments antagonizes retention of the physiological capacity for euryhalinity. Many proteins are involved in salinity stress tolerance and they compete for 'real estate' in the crowded cell interior and for energy resources supporting their synthesis and stabilization. Thus, significant costs are associated with being highly stress-tolerant and these costs may decrease competitiveness of euryhaline species in osmotically stable environments. If not needed for other functions then proteins promoting euryhalinity will be selected against in stable, non-challenging environments. From the mosaic evolution history of euryhalinity in fishes it appears that euryhalinity can be acquired relatively easily. Most proteins required for this physiological capacity appear 'ready' in stenohaline species and a few changes in a few proteins may be all that is needed to confer high salinity tolerance. Even so, rates of natural protein evolution seem incompatible with the extent and pace of salinity changes predicted for some parts of the world over the next centuries. Furthermore, selective sweeps favoring euryhaline over stenohaline species may become more prominent as extreme, pulsatory climate events that are associated with acutely severe salinity stress increase in frequency and intensity (see Introduction). During rapid climate fluctuations, future biodiversity may depend greatly on the radiation of species with broad environmental stress tolerance (Jaume, 2008; Zaksek et al., 2009). Consequently, euryhaline fishes can be expected to gain a competitive advantage over their stenohaline relatives in the foreseeable future. Such a trend calls for better understanding of the biochemical and physiological mechanisms that enable teleosts to cope with large salinity fluctuations and extreme salinities.

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