
EXTREME ENVIRONMENTS: HYPERSALINE, ALKALINE, AND ION-POOR WATERS

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The physiological mechanisms required for fish to live in freshwater and seawater are well described for some species and are the same mechanisms exploited in amphihaline species that migrate between freshwater and seawater. Many fish not only tolerate, but can acclimate and adapt to conditions outside conventional freshwater and seawater conditions, specifically salinities greater than seawater, alkaline waters (up to pH 10), and ion-poor waters. These environments exist around the globe and in many instances can support recreational and commercial fisheries. This chapter will describe the chemical characteristics of these water types, the physiological challenges associated with living in these extreme

environments, and the physiological solutions that permit fish not only to survive, but in some cases to thrive, in hypersaline, alkaline, and ion-poor waters.

1. INTRODUCTION

A great deal is known about the physiological mechanisms that permit fish to live in freshwater (FW) and seawater (SW; typically 33–35 ppt), and in fish that migrate between, which is the focus of this volume. However, fish can also acclimate or have adapted to environments that lie outside the range of salinities considered “normal” for either FW or SW, specifically salinities greater than those of SW (referred to as hypersaline waters from this point forward), alkaline waters, and low ionic content dilute FW (referred to as ion-poor waters from this point forward). These water types exist throughout the world, supporting a large biomass and biodiversity of fish, and in some cases recreational and even commercial fisheries. Much less is known about the mechanisms that permit fish to acclimate to or adapt and live in these waters and the focus of this chapter will be to review what is known.

Hypersaline waters are commonly found in inland saline lakes, coastal lagoons, embayments, inverted or closed estuaries, and tidal flats and pools. The main physiological challenge for fish in these environments is to minimize ion gain from and water loss to the hypersaline water, which is not different from that for SW fish; however, hypersaline water creates greater osmotic and ionic gradients (potentially leading to faster and greater exchanges), as well as an altered ionic composition relative to SW and a variable nature of both the abiotic and biotic environment.

In general, hypersaline waters are associated with low fish biodiversity and productivity and tend to have dynamic populations. Yet, some of these systems have relatively stable fish populations. Many saline lakes are also alkaline, with water pH reaching values as high as 9–10. This imposes an additional physiological challenge to these fish, primarily related to nitrogenous waste excretion. But again, certain fish species can inhabit and, in some cases, thrive in extreme environments up to pH 10. Finally, ion-poor waters create ionoregulatory challenges for fish owing to low ion availability for uptake and the potential for increased diffusional ion loss. Remarkably, this physiological challenge has been overcome by many fish in the ion-poor waters of the Amazon, which is characterized by its tremendous fish biodiversity.

This chapter will describe the environments and the fish fauna that survive and thrive in hypersaline, alkaline, and ion-poor waters. Furthermore, it will describe the physiological challenges associated with living in these environments as well as the physiological solutions and limitations that ultimately dictate species abundance in these systems.

2. HYPERSALINE WATERS

Hypersaline waters are commonly found in saline lakes, coastal lagoons, embayments, inverted and closed estuaries, and tidal flats and pools. Saline lakes by definition have an ionic content above 3 ppt and in some cases exceed 400 ppt (Hammer, 1986). Saline lakes are found on every continent and the total volume has been estimated at 104,000 km³, which is just less than that of the world's freshwaters, illustrating that they are much more widespread than commonly appreciated (Hammer, 1986). Some of these saline lakes are very large, such as the Caspian and Aral Seas and Lake Balkhash in Central Asia, but, numerically, most are much smaller and in many cases ephemeral. Saline lakes are generally shallow. Only about 25% of saline lakes exceed 10 m in depth and only 25% of saline lakes have a surface area greater than 200 km² (Hammer, 1986).

The accumulation of salts defines these systems and thus specific climatic and geographic characteristics are required for their existence. In particular, saline lakes are generally, but not exclusively, found in drainage basins with no outflow (endorheic basins) where evaporation matches or exceeds precipitation and incoming salts accumulate. This tends to occur in relatively arid, semi-arid, and subhumid regions. While most of these lakes lie below 1500 m, some even below sea level, there are also saline lakes that occur at elevations of 3500–5000 m on the Tibetan plateau and South American Altiplano (Hammer, 1986).

Although there are areas of the oceans (tropical seas and the Mediterranean Sea) and open coastal zones (e.g. Great Barrier Reef and Australian Bight) that are experiencing increases in salinity of 0.4–1.5 ppt (Curry et al., 2003; Andutta et al., 2011), these increases are relatively small in magnitude and likely to have relatively minor physiological consequence for fish. However, when exchange with open waters is limited, such as in coastal lagoons or lakes, embayments, and estuaries (Bayly, 1972; Potter et al., 2010), water may become substantially more hypersaline (40–164 ppt) as evaporative water losses exceed FW inputs from surface or groundwater flow or precipitation. (An estuary is defined by Potter et al., 2010, as

“a partially enclosed coastal body of water which is either permanently or periodically open to the sea and within which there is a measurable variation of salinity due to the mixture of seawater with freshwater derived from land drainage”.) These conditions tend to be met in arid to semi-arid regions and may only occur seasonally; however, an extreme example of this is the salt evaporation ponds of San Francisco Bay which range in salinity from SW to over 300 ppt, with ponds of different salinities sustaining different communities.

In the case of estuaries, hypersalinization can result during natural and anthropogenic, seasonal or perennial droughts when FW input is insignificant, evaporation high, and exchange with the sea limited. Inversion of the salinity gradient (inverted estuary) where salinity is greatest at the head of the estuary can occur, as well as complete closure of the estuary from sandbar formation across the mouth of the estuary (Potter et al., 2010). Seasonally closed estuaries include the Wellstead and Beaufort estuaries in Australia, and the Sine-Saloum estuary in West Africa is an example of an open inverted estuary adversely affected by decades of drought. Some well-studied hypersaline coastal lagoons include the Coorong Lagoon (Australia), Lago de Araruama (Brazil), Sivash Sea (Ukraine), Laguna Ojo de Liebre (Mexico), and Laguna Madre (US–Mexico), as well as large embayments such as Shark Bay and Spencer Gulf in western and southern Australia, respectively (Javor, 1989).

2.1. Environmental Characteristics of Hypersaline Waters

The ionic composition of saline lakes is largely determined by that of the incoming water (influent water and rainfall), dissolution of materials from the rocks, soils, and sediments of the drainage and lake basin, and the differential precipitation or solution of salts as the lake water becomes more concentrated or dilute through subsequent water loss (usually by evaporation) or addition. In general, the ionic composition of saline lakes varies dramatically globally, but tends to be consistent regionally. For a detailed description of the ionic composition of the main saline lakes of the world, see Hammer (1986). Of 167 lakes where total salinity was reported, 84 had salinities greater than that of SW (33 ppt), indicating that hypersaline environments are relatively common among saline lakes. In SW, the dominant ions are Na^+ (followed by $\text{Mg}^{2+} > \text{Ca}^{2+}, \text{K}^+$) and Cl^- (followed by $\text{SO}_4^{2-} > \text{HCO}_3^- \text{CO}_3$) (Table 9.1). Of the 154 lakes summarized, the dominant cation is Na^+ (136 lakes), with Mg^{2+} the second most prominent cation and varying levels of Ca^{2+} and K^+ . The dominant anion is Cl^- (80 lakes); however, there is a large number that are $\text{HCO}_3^- \text{CO}_3$

Table 9.1

Physicochemical water parameters of select saline and alkaline lakes, and ion-poor waters.

Water body	pH	Alkalinity (titratable mM)	Salinity (ppt)	Na ⁺ (mM)	Mg ²⁺ (mM)	Ca ²⁺ (mM)	Cl ⁻ (mM)	SO ₄ ²⁻ (mM)	HCO ₃ ⁻ (mM)	References
Seawater	8.1	2.4	35	469	52.8	10.3	546	28	1.8	Department of Energy (1994)
Saline/alkaline lakes										
Lake Magadi (Kenya); Fish Spring Lagoon	9.9	290	21.3	356	0.04	0.65	112	0.8	67	Wood et al. (2012) ; Jones et al. (1977)
Lake Qinghai (China)	9.4	30	9	200	18.7 36	0.3 0.23	173			Wang et al. (2003) Wood et al. (2007)
Lake Van (Turkey)	9.8	151	22.7	337.9	4.4	0.1	160.6	24.3		Danulat (1995) ; Oguz (personal communication) (Yuzuncu Yil University)
Pyramid Lake (USA)	9.4	23	4.4	58	7.3	0.2	60	1.7		Wright et al. (1993) ; Iwama et al. (1997)
Walker Lake (USA)	9.5	33	12	287.1	8.6	0.2	121.3	45.8	26.2	Bigelow et al. (2010)
Salton Sea (USA)	8.2	2	44	538	58	24	486	109	4	Holdren and Montano (2002)
Aral Sea (Uzbekistan) 1952	8		10	95.3	21.4	11.5	97.3	32.4	2.5	Zavialov et al. (2009)
South Aral Sea Western basin (Uzbekistan) 2007	8.1		127	1364.5	324.0	19.0	1400.7	360.0	10.4	Gertman and Zavialov (2011)
Ion-poor waters										
Rio Negro (Brazil)				16.5		5.3	47.9			Furch (1984)
World rivers				270		370	220			Wetzel (1983)

dominated (31 lakes), both of which have varying amounts of SO_4^{2-} (Table 9.1). There are many other minor ions not mentioned here. The implications of these different ionic compositions relative to that of SW are not well studied in fish.

The ionic composition of saline lakes can vary spatially and temporally, the magnitude of which is system dependent. The former is largely a result of the location and magnitude of the incoming FW source that may result in a horizontal salinity gradient and in some cases may act as a salinity refuge for fish. Temporal variation is dependent upon the relative magnitudes of lake water dilution, predominantly through water addition from rain or snow melt, and lake water concentration, largely due to evaporative water loss or extensive ice formation, both of which exclude salts and reduce the water volume (Hammer, 1986).

The climate of saline lakes is mostly influenced by temperature, insolation, evaporation, precipitation, humidity, and winds of the region, and further influenced by altitude, among other factors. Given that most saline lakes tend to be shallow and in relatively windy areas, water temperatures tend to parallel air temperatures (Hammer, 1986). Depending upon the season, there may be thermal stratification, the degree of which generally increases with lake depth (Hammer, 1986). However, there is a tendency for turnover and destratification, at least seasonally, which may dramatically alter the water chemistry, resulting in either hypoxia or anoxia and elevated sulfides, in some cases leading to large-scale mortality of fish that live in these systems (see Section 2.5).

In the case of hypersaline SW bodies, the dominant ions are Na^+ and Cl^- , as is the case in SW. With increasing salinity due to evaporation the proportions of the major ions remain relatively constant, with the exception of Ca^{2+} , HCO_3^- , and SO_4^{2-} , which precipitate out as CaCO_3 (at a salinity of 70 ppt or with 50% evaporation) and as CaSO_4 (at salinities above 90 ppt) (Fernandez et al., 1982; Marion et al., 2009). In hypersaline coastal waters, pelagic primary production (phytoplankton) tends to be low and, instead, is dependent on benthic and microphytobenthic production (e.g. seagrass, Laguna Madre; or algal mats, Hamelin Pool stromatolites). Changes in salinity are common with seasonal differences in rainfall, river flow, and evaporation rates, as well as storms that can inundate coastal areas.

2.2. Fishes that Inhabit Hypersaline Waters

Saline lakes are often characterized by a high density of bacteria, algae, and invertebrates, but fish are not as common. In general, the fish fauna in saline lakes is limited by the fact that many lakes are ephemeral and/or

occupy endorheic drainage basins. Thus, there are no direct water links to other basins for fish migrations. Mechanisms of fish dispersal are much more limited relative to invertebrates and consequently fish fauna in many saline lakes reflect human introductions to establish new or to enhance existing fisheries. Adequate records of introduction are often lacking. Thus, it is often unknown whether the native populations in permanent lakes are natural or stocked (Bayly, 1972; Hammer, 1986).

While there is a general trend for decreasing species numbers with increasing salinity (Hammer, 1986), it is generally thought that a given saline lake can support more fish species than it does. Species diversity is limited by dispersal opportunities. Saline lakes with a salinity of 3–5 ppt tend to have the most fish species, generally around 10, regardless of the lake's geographic location. Lakes with salinities greater than 20 ppt may have a single species or no species at all. Hammer (1986) provides detailed descriptions of documented fish species in many of the saline lakes, including introduced species. At extreme hypersalinities (80–120 ppt) generally only cichlid (tilapias), mugilid, and antherinid species are successful.

As described above, the ionic composition of saline lakes varies dramatically. Based upon species composition and biomass of fish living in saline lakes, whether intentionally introduced or not, the greatest fish successes appear to be in waters where Na^+ and Cl^- dominate relative to SO_4^{2-} -rich or alkaline waters (Hammer, 1986). Regardless, several species have adapted to the latter waters (see Section 3.2).

In coastal hypersaline waters, euryhaline species are typically better represented than SW or FW species (Bayly, 1972; Potter et al., 1990; Zampatti et al., 2010). However, these waters can act as spawning or nursery grounds for SW and FW species. The antherinids are particularly well adapted to these conditions (*Allanetta mugiloides*, *Atherinosoma microstoma*, *A. wallacei*, and *A. elongata*) (Potter et al., 1986; Wedderburn et al., 2008), as well as the Mugilidae (*Mugil cephalus*). Potter and Hyndes (1999) propose that closure of estuaries has led to the selection of SW species that are capable of completing their life cycles within the estuary. Their review on ichthyofaunas in permanently open, intermittently open, and seasonally closed estuaries of south-western Australia shows that estuarine-spawning species are generally more prevalent in the latter two (>95% vs. <34% total fish numbers in permanently open estuaries). Atherinids and gobies, with their short life cycles, contribute significantly to these numbers. In contrast, in the permanently saline Lago de Araruama (Brazil; 46–56 ppt), only an artisanal mullet fishery exists (Kjerfve et al., 1996).

2.3. Physiological Challenges of Hypersaline Waters

2.3.1. ION AND WATER BALANCE RELATIVE TO SEAWATER

The fundamental physiological mechanisms associated with living in hypersaline waters are those used by SW fishes, but at higher salinities these mechanisms, which are detailed in Chapter 1 of this volume (Edwards and Marshall, 2013), are upregulated and modified as described below.

In SW, teleost fishes regulate osmotic pressure of internal body fluids below that of the surrounding medium. Therefore, water is lost across the large, permeable surface area of the gill epithelium. To compensate for water loss, teleosts drink SW. However, to create an osmotic gradient favorable for water absorption across the gut, they must first take up most of the Na^+ and Cl^- in the imbibed water. Secretion of HCO_3^- into the gut lumen raises the pH of the gut fluid and precipitates most Ca^{2+} and some Mg^{2+} (Wilson et al., 1996, 2002), which prevents absorption of these divalent salts as well as significantly reducing the osmotic pressure of the gut fluid, facilitating water absorption. It is estimated that over 90% of Na^+ , Cl^- , and K^+ of imbibed water in the gut is transferred into the blood, which then drives water uptake (Hickman, 1968; Shehadeh and Gordon, 1969; Kirschner, 1997). Much of the Na^+ and Cl^- may enter the blood passively in the esophagus owing to a favorable ionic gradient, but some, if not most, must be actively transported across the intestinal epithelium, a process driven by basolateral Na^+/K^+ -ATPase (NKA) and facilitated by apical $\text{Na}^+/\text{K}^+/2\text{Cl}^-$ (NKCC) and Na^+/Cl^- (NCC) cotransporters (Grosell, 2010). In addition to the Na^+ and Cl^- taken up across the gut, fish in SW experience large concentration gradients which drive diffusive uptake of ions across the gill epithelium. It seems likely that Cl^- enters across the gills, but because the transepithelial potential (TEP) across the gills is slightly greater than the equilibrium potential for Na^+ (around +25 mV, plasma relative to SW), gill Na^+ entry may be minor (Evans, 1993; Marshall, 2002).

In Gulf toadfish (*Opsanus beta*), intestinal Na^+ concentrations in hypersaline-acclimated fish (50 and 70 ppt) are lower than in SW-control fish while Cl^- levels are higher (McDonald and Grosell, 2006). Luminal Mg^{2+} , the dominant cation, was significantly elevated, although only at 70 ppt. All salts entering the body via the gut and gill must be excreted to prevent overall body salt accumulation. The small amounts of divalent salts that enter (primarily Mg^{2+} and SO_4^{2-}) are excreted in small volumes of isosmotic urine (Hickman, 1968; Genz et al., 2011), while monovalent salts, primarily Na^+ and Cl^- , are excreted across the gill (see review by Evans et al., 2005; Edwards and Marshall, 2013, Chapter 1, this volume). In brief, ionocytes in the branchial epithelium and opercular epithelium utilize NKA

and NKCC on the basolateral membrane to excrete Cl^- through apical chloride channels, which are homologous to the human cystic fibrosis transmembrane conductance regulator (CFTR) (Singer et al., 1998; Hiroi et al., 2005, 2008). Excretion of Cl^- generates the positive TEP that drives Na^+ out through leaky paracellular channels.

In hypersaline water, both water and salt balance using the above mechanisms become more challenging. At 70 ppt, for instance, the osmotic gradient across the gills more than doubles. If the rate of water loss also doubles then fish must double their drinking rate to keep pace. Furthermore, to absorb the ingested water fish must absorb more Na^+ and Cl^- per unit volume of water imbibed. So a doubling of the drinking rate at 70 ppt could quadruple salt absorbed across the gut. Furthermore, at 105 ppt ($3 \times \text{SW}$) if drinking rate triples then salt absorption across the gut must increase nine-fold. All this additional Na^+ and Cl^- taken in must be excreted against a much greater concentration gradient. In addition, elevated salinities require higher rates of HCO_3^- secretion in the gut to precipitate the additional Ca^{2+} ; the renal excretion of extra divalent salts, Mg^{2+} in particular, is another challenge. To date, for fish the consequences of hypersaline waters with altered ion composition relative to SW have been poorly studied.

2.4. The Physiology of Hypersaline-Tolerant Fishes

2.4.1. EARLY LIFE STAGES

Early life stages in fish are often very sensitive to environmental challenges, both natural and anthropogenic (Alderdice, 1988); hypersaline waters are no exception. While some information on the mechanisms and thresholds of hypersaline tolerance in juvenile and adult fish exists, little is known for developing fish. Sensitivity to high salinity arises from the complex and dynamic processes that occur during development, which if disrupted lead to deformity or mortality.

In general, increased salinity tolerance starts between 45 and 70 days posthatch in a number of tilapia species. In fact, 39 days posthatch is the minimum recommended age to begin successful SW acclimation after hatching in FW (Watanabe et al., 1997). Similarly, in the mangrove red snapper (*Lutjanus argentimaculatus*), salinity tolerance is also lowest during the first 7 days following fertilization, and then salinity progressively increases over the next several weeks (Estudillo et al., 2000). Salinity exposure (brackish waters rather than full-strength SW or higher salinities) during spawning and hatching can increase salinity tolerance of larval fish (Watanabe et al., 1997). In the mudskipper (*Boleophthalmus pectinirostris*), prelarval development can occur in 10–40 ppt, but the optimal salinity is

25 ppt, and early juveniles prefer a lower salinity in behavioral choice experiments (Chen et al., 2008).

Understanding the physiological limitations of fishes in the Salton Sea, which has a current salinity of 47 ppt, has generated considerable attention because of a recreational fishery as well as general scientific interest (Hurlbert et al., 2007). Most fish in the Salton Sea are of SW origin and have been transplanted into that system. The upper lethal salinity tolerance for embryos and larvae of two such species, bairdiella (*Bairdiella icistia*) and sargo (*Anisotremus davidsoni*), is 40 ppt (Brocksen and Cole, 1972; Lasker et al., 1972; May, 1975), and large free-breeding populations of these species existed in the Salton Sea until a decade ago when salinity was 43 ppt. The most dominant fish in the Salton Sea is the “California” Mozambique tilapia (*Oreochromis mossambicus* × *O. urolepis hornorum*) hybrid. As adults, they have an acute salinity tolerance in excess of 95 ppt (Sardella et al., 2004a); however, salinities well below this have a large effect on larval growth and development. In F₂ larval tilapia spawned from Salton Sea, a more or less dose-dependent effect of salinity on growth exists, where 9 weeks postrearing at 35–55 ppt halved body mass compared with rearing at 5 ppt (Sardella et al., 2007). The mechanism for this stunting of growth is unknown.

Desert pupfish (*Cyprinodon macularius*) are extremely saline tolerant and embryos can be transferred from FW to salinities of 35, 45, 55, 70, and 85 ppt within just 4 h postfertilization. However, developmental rate, eye development, and pigmentation were most rapid in FW and progressively decreased with salinity, and no hatch occurred at 70 or 85 ppt (Kinne and Kinne, 1962). Clearly, high salinities can be tolerated, but not without a pronounced effect on development.

Newly hatched larval California killifish (*Fundulus parvipinnis*) tolerate salinities up to 70 ppt, depending on the egg incubation salinity (Rao, 1975), while adults tolerate salinities up to 128 ppt (Feldmeth and Waggoner, 1972). The killifish *Austrofundulus limnaeus*, which inhabits ephemeral lakes in Venezuela that probably become hypersaline, have diapausing embryos that are able to defend internal osmolality up to a salinity of 50 ppt, primarily by reducing ion and water permeability. The water permeability of *A. limnaeus* embryos is 1000 times less than that of zebrafish embryos (Machado and Podrabsky, 2007). However, other developmental stages of *A. limnaeus* are fairly sensitive to elevated salinity, indicating that if the water salinity has not been reduced at hatch, these fish are likely to die.

2.4.2. JUVENILE AND ADULT FISH

When saline-tolerant fishes are incrementally exposed to hypersaline waters (e.g. a 5 ppt increase every 5–7 days), ionoregulatory ability is better compared with an abrupt transfer to a high salinity (Nordlie, 1985; Hotos

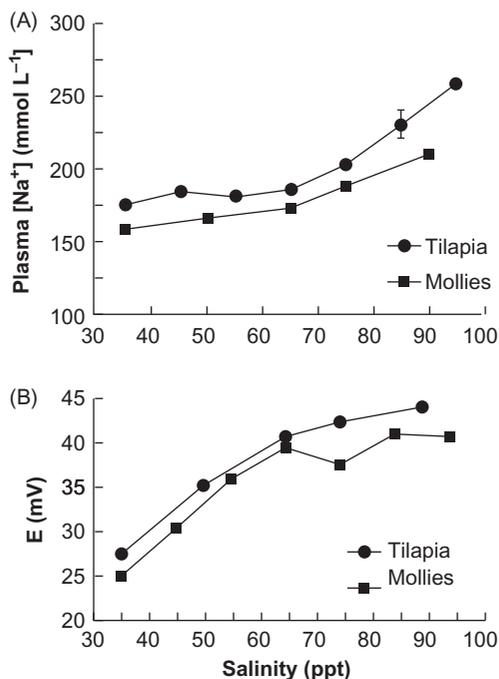


Fig. 9.1. Effect of salinity on (A) plasma Na^+ concentration and (B) equilibrium potential (E) for Na^+ across the branchial epithelium for “California” Mozambique tilapia hybrids (data from Sardella et al., 2004b) and sailfin mollies (data from Gonzalez et al., 2005a). Values for E were calculated from plasma Na^+ and water Na^+ concentrations from individual fish. Values are means \pm SE. Error bars that are not visible are contained within the symbols.

and Vlahos, 1998; Sardella et al., 2004a, b; Gonzalez et al., 2005a). Gradual salinity transfer up to about 70–75 ppt has either no effect or only a modest effect on plasma osmolality or salt concentrations; however, above 75 ppt, values increase considerably (Figs. 9.1 and 9.2) (Valentine and Miller, 1969; Lotan, 1971; Griffith, 1974; Nordlie, 1985; Nordlie and Walsh, 1989; Nordlie et al., 1992; Jordan et al., 1993; Sardella et al., 2004b; Gonzalez et al., 2005a). Muscle water content, when measured as an indicator of overall water balance status (Sardella et al., 2004a, b; Gonzalez et al., 2005a), decreases only slightly (5%) or not at all, even at 85–95 ppt, indicating that despite the elevations in plasma ion concentrations hypersaline-tolerant fishes avoid problematic internal fluid shifts. One study found elevated levels of the osmolyte glycine in muscle tissue and *myo*-inositol in brain tissue of Mozambique tilapia at 70 ppt (Fiess et al., 2007), which is an area clearly worthy of further investigation given the possibility of a physiological mechanism to maintain the water volume of these tissues.

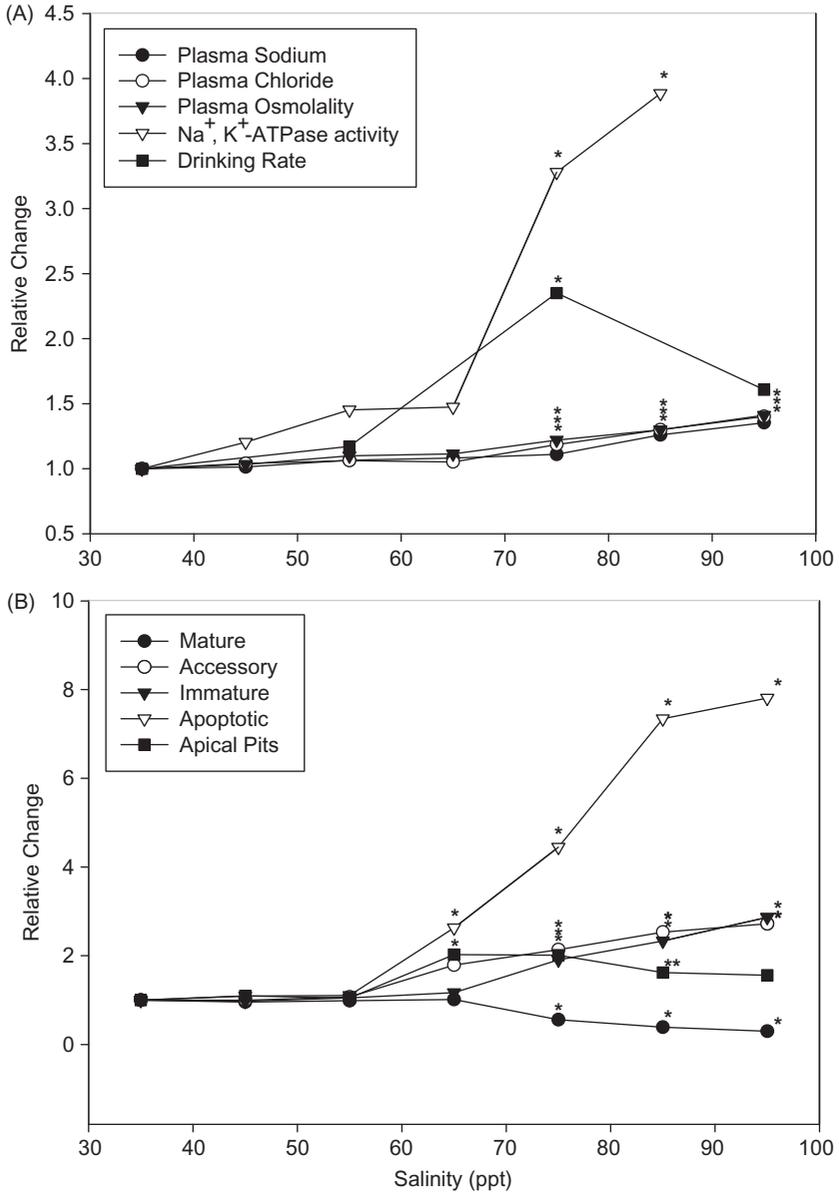


Fig. 9.2. Effect of salinity on relative changes (standardized to 35 ppt values) in (A) plasma [Na⁺], [Cl⁻], osmolality, gill Na⁺/K⁺-ATPase activity and drinking rate, and (B) gill ionoregulatory cell developmental stages (mature, immature, and apoptotic) and characteristics (apical pits, accessory cells) in “California” Mozambique tilapia hybrids. Reproduced from [Sardella et al. \(2007\)](#) with permission from Taylor and Francis.

Hypersaline exposure is initially associated with water loss. Thus, an important adaptation of hypersaline fishes is a reduction in branchial water permeability. This can generally be inferred from drinking rate, which is a proxy for the rate of water loss. Drinking rate increases with salinity, but not in proportion to the osmotic gradient to which the fish are exposed (Fig. 9.2) (Skadhauge and Lotan, 1974; Sardella et al., 2004b; Gonzalez et al., 2005a; Genz et al., 2008). For example, sailfin mollies (*Poecilia latipinna*) exposed to 60 ppt, elevated drinking rate by only 35%, despite a doubling of the osmotic gradient across the gills (Gonzalez et al., 2005a). The lower than expected increase in drinking rate at 60 ppt indicates that water permeability has probably been reduced. However, the 35% increase in drinking rate at 60 ppt, where salt concentrations are 70% higher than SW, may double salt entry into the gut. Water uptake from the gut is accomplished by an elevation in gut NKA activity (Sardella et al., 2004b; Gonzalez et al., 2005a) which drives NaCl uptake into the blood along with osmotically obliged water. Furthermore, there is an increased secretion of HCO_3^- into the gut, resulting in Ca^{2+} and Mg^{2+} precipitation, reducing osmolality, and further enhancing water uptake from the gut (Genz et al., 2008).

The plasma ion load incurred by drinking hypersaline water must be excreted across the branchial epithelium. An increase in ionocyte density and/or size (Kültz and Onken, 1993; Kültz et al., 1995; Uchida et al., 2000; Sardella et al., 2004b; Ouattara et al., 2009), although not always (Kültz et al., 1992; Wilson et al., 2007), is likely to facilitate this, and there appears to be a greater turnover of ionocytes as indicated by a progressive increase in apoptotic cells with salinity, in conjunction with an increase in immature and decrease in mature ionocytes with salinity (Fig. 9.2). Branchial NKA can increase two- to five-fold with a doubling of salinity (Fig. 9.2) (Karnaky et al., 1976; Kültz et al., 1995; Uchida et al., 2000; Sardella et al., 2004b; Gonzalez et al., 2005a; Fiess et al., 2007). In addition, branchial expression of NKCC and CFTR can increase with hypersaline exposure (Wilson et al., 2007; Ouattara et al., 2009). Tine et al. (2010), studying tilapia (*Sarotherodon melanotheron*) in the Sine-Soloum inverted estuary, observed a higher branchial heat shock protein 70 (hsp70) messenger RNA (mRNA) and NKA (ATP1A1) expression in hypersaline environments.

Increasing ionocyte density and size, as well as elevating gill NKA activity, are necessary not only to excrete more salt in a hypersaline environment, but to do so against an elevated gradient. This is evident from the calculated equilibrium potential (E) for Na^+ across the gills, as illustrated for sailfin mollies and “California” Mozambique tilapia hybrids (Fig. 9.1). Using plasma and water Na^+ concentration data in the Nernst equation, E rises markedly between 35 and 70 ppt because external Na^+ levels rise while internal Na^+ concentrations do not. At salinities ≥ 70 ppt, E

plateaus (particularly in tilapia) owing to the rising internal Na^+ concentrations. It appears that a larger TEP cannot be generated at salinities above 70 ppt and plasma levels rise until E falls below the TEP and Na^+ excretion resumes. This observation may explain the “biphasic” response of plasma ion concentrations to increasing salinities that has been observed (Sardella et al., 2004a). *In vitro* measurements across opercular epithelia of *Oreochromis mossambicus* showed that TEP rose linearly in fish acclimated from 35 to 60 ppt (Kültz and Onken, 1993).

Little is known about renal function in the most hypersaline-tolerant species. A few studies have examined renal function of Gulf toadfish (McDonald and Grosell, 2006; Genz et al., 2008, 2011), discovering that urine production predictably decreases and divalent cation concentrations increase. Similar observations were made in European flounder (*Platichthys flesus*) (R. W. Wilson, personal communication). However, neither species is very salt tolerant and the toadfish possesses atypical glomerular nephrons. It would be interesting to examine kidney function in salt-tolerant species such as the cichlids and cyprinodonts.

2.4.3. THE ENERGETIC COSTS OF IONO-OSMOREGULATION

An obvious metabolic implication of life in different salinities has to do with the cost of iono-osmoregulation, which has been surprisingly difficult to determine. As discussed above, the regulation of ionoregulatory and osmoregulatory status at a given salinity is dependent upon a host of energy-consuming transporters at the gills, gut, and kidney, depending upon the salinity inhabited. In a comprehensive and elegant analysis, Kirschner (1993, 1995) determined the theoretical thermodynamic cost of ionoregulation in the FW fish gill to be about 1.6% of resting metabolic rate, similar to that calculated by Eddy (1982). In an isolated perfused FW gill preparation, Morgan and Iwama (1999) determined that the metabolic cost of gill NaCl uptake was surprisingly close to these theoretical values (1.8% of estimated resting metabolic rate). Estimates for the metabolic cost of ionoregulation across the SW gill were slightly higher (5.7%) (Kirschner, 1993), but in general, these estimates reveal fairly low values.

In contrast, many studies have used oxygen consumption rates ($\dot{M}\text{O}_2$) in fish transferred to different salinities to calculate the whole animal cost of iono-osmoregulation. The assumption of these studies is that $\dot{M}\text{O}_2$ will be lowest in an isosmotic environment (where the cost of maintaining osmoregulatory status would be minimal), and an elevation in $\dot{M}\text{O}_2$ at salinities above or below isosmotic is indicative of additional costs associated with iono-osmoregulation. However, iono-osmoregulation costs estimated in this manner range from 20 to 68% of resting metabolic rate, depending upon species, life history stage, acclimation duration, and

experimental design, and therefore must be interpreted with caution (see [Boeuf and Payan, 2001](#); [Soengas et al., 2007](#), for reviews). Some studies have even found costs estimated in this manner close to zero ([Morgan and Iwama, 1991](#); [Pérez-Robles et al., 2012](#)).

It is not unreasonable to expect that resting $\dot{M}O_2$ would increase as salinity is elevated above SW values to deal with the metabolic costs associated with increased drinking and ion exchanges, to maintain water and ion balance as described above. [Gonzalez and McDonald \(1992\)](#) have hypothesized that maintaining osmotic balance places limitations on metabolic scope (difference between maximal and resting metabolic rate), which will thus affect activity levels. However, hypersaline fishes commonly have a metabolic rate up to 40% lower than resting values in SW (see [Sardella and Brauner, 2007a](#), for a review). For example, resting metabolic rate of the euryhaline milkfish (*Chanos chanos*) ([Swanson, 1998](#)) was reduced by 25% after acclimation from SW to 55 ppt. Swimming activity was also reduced, an observation consistent with the hypothesis of [Gonzalez and McDonald \(1992\)](#) that minimization of osmoregulatory disturbance takes priority over activity. In the “California” Mozambique tilapia hybrid, 2 week acclimation to salinities from 60 to 95 ppt reduced resting metabolic rate by about 40%, which was associated with an increase in plasma osmolality and gill NKA activity. A reduction in brain NKA and liver total ATPases suggests tissue-specific metabolic suppression during hypersaline exposure ([Sardella et al., 2004a](#); [Sardella and Brauner, 2008](#)). Thus, there appears to be at least some control over metabolic rate, at least during initial exposure to elevated salinity, which may be beneficial during acclimation to these high salinities ([Sardella and Brauner, 2008](#)). However, this possibility needs further investigation.

2.4.4. THE OSMORESPIRATORY COMPROMISE IN HYPERSALINE WATERS

The fish gill is a multipurpose organ. It is responsible for gas exchange, acid–base balance, and nitrogenous waste excretion, as well as ionoregulation, as described above. Therefore, there is a tradeoff among gill functions, which in relation to ionoregulation and gas exchange has been referred to as the osmorepiratory compromise ([Randall et al., 1972](#); [Nilsson, 1986](#)). Conditions that are beneficial to gas exchange, such as a large gill surface area, thin diffusion distance, and high water and blood flows, are the very characteristics that are detrimental to maintaining ion and water balance and must be actively counteracted ([Sardella and Brauner, 2007a](#)). Conversely, alterations in gill morphology to enhance ion excretion, such as increased ionocyte density and increased gill diffusion distance, may directly impair oxygen uptake. Life in hypersaline waters may have implications for the osmorepiratory compromise through

salinity effects on metabolic rate (and thus the cost of iono-osmoregulation and metabolic suppression; see above) and gill morphology, and through seasonally variable water temperature, which directly affects metabolic rate and oxygen solubility of the water, all of which will briefly be discussed here.

Most shallow saline lakes and hypersaline coastal water bodies are greatly influenced by atmospheric temperatures. Furthermore, they are often located in semi-arid or arid locations where air temperatures, and thus water temperatures, are high. An increase in temperature of 10°C generally doubles or triples a fish's metabolic rate ($Q_{10} = 2-3$). Both temperature and salinity compound matters by decreasing the oxygen solubility of water and hence oxygen availability. So, for a given oxygen extraction from water, ventilation volume must be greater in warmer, hypersaline water, which then has implications for osmoregulation, but few studies have investigated this interaction. The "California" Mozambique tilapia hybrid acclimated to 35 ppt and 25°C and transferred directly to 43, 51, and 60 ppt for 24 h was able to maintain plasma osmolality constant over this salinity range. However, when conducted at 35°C, which presumably elevated metabolic rate and reduced oxygen solubility, plasma osmolality progressively increased with salinity, reaching values at 60 ppt that were 50% higher than those at 35 ppt (Sardella and Brauner, 2008). Altered membrane fluidity and permeability could easily contribute to these clear and large effects of elevated temperature on osmoregulation in hypersaline waters; however, this remains to be specifically investigated.

2.5. Life on the Edge: Fish Population Dynamics in Hypersaline Environments

Although hypersaline waters exist on every continent and comprise a relatively large volume even in comparison with the Earth's total FW volume, only some of these sustain fish populations. The following are some specific examples of systems that currently sustain, or have historically sustained, fish populations in hypersaline waters.

2.5.1. SALTON SEA

In North America, there are many saline lakes, many of which are located in the western USA in the rain shadows of the Great Basin, Mojave, Sonoran, or Chihuahuan deserts. One system that has been quite extensively studied in terms of its fish population dynamics over the past century is that of the Salton Sea, which is the largest lake in California (980 km²) and resides below sea level in the Imperial Valley. Its most recent formation (it has historically existed and dried up repeatedly) occurred in 1905 when the

Colorado River accidentally breached a diversion structure, filling the Salton Sea basin over the following 16 months before the river was diverted back to its regular channel. Starting with Colorado River water, the Salton Sea rapidly increased salinity to SW values over a relatively short 15-year period owing to the combination of dissolution of salts from the sea floor, a high evaporative water loss associated with this arid region, and inflow of mildly saline (2–4 ppt) agricultural waters (Hurlbert et al., 2007). Currently, the salinity is 47 ppt and is increasing at 0.3 ppt annually. Coupled with dramatic changes in oxygen, temperature, and hydrogen sulfide (Watts et al., 2001), the Salton Sea presents very challenging conditions for fish inhabitants.

Fish such as the common carp (*Cyprinus carpio*), striped mullet (*Mugil cephalus*), humpback sucker (*Xyrauchen texanus*), and rainbow trout (*Oncorhynchus mykiss*) entered the Salton Sea with the Colorado River water. However, with salinity rapidly increasing, most of these species perished and fish biomass was greatly reduced (Fig. 9.3). The California Department of Fish and Game introduced as many as 35 species of SW fish from 1929 to 1956 (Whitney, 1967). The most successful stocking effort occurred in 1950–1951 when fish introduced from the Gulf of California successfully established relatively large populations of bairdiella, sargo, and orange mouth corvine (*Cynoscion xanthulus*). These species formed the basis of a successful recreational fishery beginning in the 1960s (Hurlbert et al., 2007). Some time in the 1960s it is thought that two species of tilapia entered the Salton Sea and by the early 1980s, one of these, the “California” Mozambique tilapia hybrid, became the most abundant fish species.

Pronounced fish mortality events have occurred in the Salton Sea throughout the past century owing to its dynamic environment. Those from the late 1980s and onwards (Hurlbert et al., 2007) are well documented and show a large reduction in fish biomass followed by a recovery in the mid-1990s. In the early 2000s a series of mortalities, referred to as the “millennium crash”, resulted in the majority of fish perishing. Some recovery of tilapia has occurred, but other species, if present, are caught only in very low numbers. The basis for these high mortalities is not well understood, but they probably stem from a combination of mixing events and associated anoxia and high sulfide levels which tend to occur in the summer (Hurlbert et al., 2007), and winter kills of tilapia which are probably a result of increasing salinity and low temperature (Sardella and Brauner, 2007b). With the latter, the ability of tilapia to regulate plasma ion levels at 15°C or lower is greatly affected through the direct effect of temperature on gill NKA, as described above (Sardella et al., 2004b).

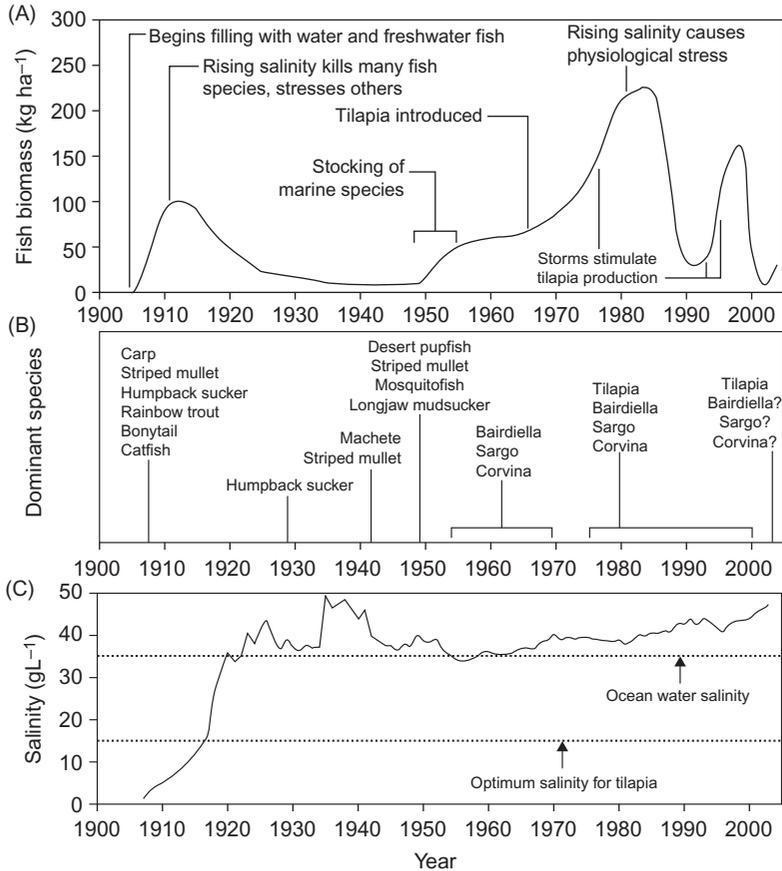


Fig. 9.3. Estimated changes in fish biomass, dominant fish species, and salinity of the Salton Sea in the twentieth century. Reproduced from [Hurlbert et al. \(2007\)](#) with permission from Taylor and Francis.

2.5.2. ARAL SEA

The Aral Sea in central Asia (Kazakhstan, Uzbekistan), once the fourth largest lake in the world (surface area of 66,100 km² and volume of 1064 km³) with a salinity of 8–10 ppt, is endorheic and has been isolated since the last ice age (10,000 years ago). Although characterized by low biodiversity and productivity it supported 20 endemic fish species, 12 of commercial importance including the Fringebarbel sturgeon (*Acipenser nudiventris*) and Aral barbell (*Barbus brachycephalus brachycephalus*) ([Aladin and Potts, 1992](#)). Since 1927, a total of 21 species have been

introduced either intentionally to improve fisheries or accidentally. However, of these only the Baltic herring (*Clupea harengus membras*), silverside (*Atherina boyeri caspia*), bubyr (*Pomatoschistus caucasicus*), monkey goby (*Neogobius fluviatilis*), and round goby (*Neogobius melanostomus*) established populations. Commercial fisheries were also developed for the introduced grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*), black carp (*Mylopharyngodon piceus*) and, later, the European flounder (Aladin and Potts, 1992). Despite these introductions, overall productivity did not increase and native fauna were negatively impacted (Aladin et al., 2004).

The Aral Sea is most noted as an anthropogenic, ecological disaster (Mickle, 2007). In this arid region, the Amudarya (North) and Syrdarya (South) Rivers that drain into the Aral Sea were diverted for large-scale, Soviet-era (1960s), irrigation projects, which ultimately reduced the Aral Sea to less than 10% of its original volume (Mickle, 2007). Desiccation increased salinity and dramatically changed the ichthyofauna, causing extinction of species of FW origin by the 1970s (12–14 ppt) and the majority of brackish water species by the 1980s (22–24 ppt), resulting in the collapse of once important fisheries (Aladin and Potts, 1992). In 1989, falling water levels resulted in a division of what is now the North and South Aral Seas. At this time seven fish species were present [including native species: Ukrainian stickleback (*Pungitius platygaster*) and introduced species: Baltic herring, European flounder, silverside (*Atherina mochon*), monkey goby, and round goby] in both seas, when they were the same salinity (28–30 ppt). The North Aral Sea still receives water from the Amudarya River and has been in positive water balance, and construction of a dam has stabilized water levels, resulting in a decrease in salinity to 11 ppt. The European flounder fishery has been revived and FW species have recolonized the Amudarya River. In contrast, the South Aral Sea continues to receive little water from the Syrdarya River or overflow from the North Aral Sea, and continues its rapid decline with reported salinities of 82–150 ppt due to a strong negative water balance (evaporative loss exceeds input) (Aladin et al., 2004). The dropping water level divided the South Aral Sea into the Western and Eastern basins, with the latter completely drying up in 2009 (Table 9.1). No surviving species were reported by Aladin et al. (2004) when salinities had exceeded 70 ppt and no efforts are being made to save the South Sea. Instead, the dry lakebed is being explored for oil and gas.

2.5.3. SHARK BAY

Shark Bay in Western Australia is situated in the transition zone between temperate and tropical systems, which in combination with extensive seagrass beds and different salinity zones (ranging from 35 to over 65 ppt)

with steep inner gulf salinity gradients, has resulted in an area with a rich biodiversity. Protective islands at the edge of the bay limit water mixing with the open sea and the development of the Fauré Sill 42,000 years ago across the south-eastern portion of Shark Bay has limited tidal mixing. The presence of persistent winds preventing stratification, high evaporative water loss, and an arid climate with little runoff from land have led to hypersalinization of Hamelin Pool and L'Haridon Bight at the bayhead. The hypersaline environment of the Hamelin Pool is famous as the home to stromatolites formed by mats of cyanobacteria.

In contrast to areas in adjacent seagrass beds and sandflats in the Eastern Gulf of Shark Bay, where 58 fish species have been identified (Black et al., 1990), the fish fauna of the hypersaline Hamelin Pool is limited to six species (Lenanton, 1977). Species included in Hamelin Pool are from the Mugilidae (flathead mullet, *Mugil cephalus*) and Antherinidae (few-ray hardyhead, *Craterocephalus pauciradiatus*), which are notably present in other hypersaline environments, as well as Theraponidae (yellowtail trumpeter, *Amphitherapon caudavittatus*), Sparidae (yellowfin seabream, *Acanthopagrus latus*) and Sillaginidae (yellowfin whiting, *Silago schomburgkii*, and golden-lined whiting, *S. analis*). The presence of some of these species in Hamelin Pool appears to be opportunistic as they are more abundant in other lower salinity regions of Shark Bay. However, the presence of both juvenile and adult stages of the few-ray hardyhead and yellowtail trumpeters indicates that these species may complete their life cycle within the pool (Lenanton, 1977). Indeed, the few-ray hardyhead is found in some of the most hypersaline environments in Australia (Potter et al., 1986). Also, in the bay as a whole, a total of 16 species has been recorded at salinities greater than 40 ppt (Bayly, 1972).

2.5.4. HYPERSALINE LAGOONS

The Laguna Madre, Texas, is an interesting example of a highly productive seagrass-based hypersaline lagoon that has been modified by human intervention over the past 70 years (Tunnell and Judd, 2002). Historically, the lagoon experienced cycles of boom-and-bust fisheries that were tied to flushing of the system by wet hurricanes, followed by a few years of high productivity as salinity increased, only to decrease again once the lagoon became extremely hypersaline (80–110 ppt). However, in the 1940s, the US Gulf Intracoastal Waterway (GIWW) was constructed, improving water circulation in the lagoon and eliminating the periods of extremely high salinity but also the posthurricane boom years (Tunnell and Judd, 2002). Subsequently, salinity has rarely exceeded 70 ppt and while stable fish populations exist, fish biodiversity is lower than in nearby non-hypersaline areas such as Corpus Christi Bay (Tunnell and Judd, 2002). The Sciaenidae

(drums) were the most diverse family in Laguna Madre, with 10–13 species present. In the lagoon, 15 species have been recorded at salinities greater than 60 ppt, and an additional 10 are known to be able to tolerate even higher salinities (Bayly, 1972). However, at higher salinities few species dominate and only larger individual fish are found, indicating a lack of recruitment (Tunnell and Judd, 2002).

Another hypersaline lagoon, the Sivash Sea, which borders the Sea of Azov (Ukraine) and is the largest lagoon system in Europe (2500 km²), was once a large hypersaline ecosystem (Siokhin et al., 2000). However, the Eastern and Central Sivash lagoons have been experiencing desalinization from agricultural (rice paddy) runoff and fish ponds, reducing salinity to one-tenth of its original level. The Eastern lagoon salinity ranges from 18 to 20 ppt and is now considered brackish. The decrease in salinity has improved fish productivity (glossa flounder, *Platichthys flesus luscus*, and the introduced grey mullet, *Mugil soiyu*), but suitable fish habitat has been lost owing to the spread of reed beds. The Western Sivash avoided desalinization but was maintained as an industrial reservoir for chemical extraction and has been subjected to industrial pollution (Siokhin et al., 2000). Estimates of ichthyofauna in the Sivash range from 30 to 45 species, with the three-spined stickleback (*Gasterosteus aculeatus*), the European anchovy (*Engraulis encrasicolus*), European flounder, grass goby (*Zostericola ophiocephalus*), and flathead mullet all reported to penetrate into historically hypersaline waters of the Sivash (Hedgpeth, 1959, cited in Bayly, 1972).

2.5.5. INVERTED ESTUARIES

The Sine Saloum estuary in Senegal has been an inverted estuary since the 1970s, when a persistent sub-Saharan drought eliminated the inflowing river and resulted in hypersaline conditions in the upper estuary where salinity is generally greater than 60 ppt, reaching values as high as 130 ppt. The influence of salinity on the life history traits of the hypersaline tolerant black-chinned tilapia (*S. melanotheron*), bongo shad (*Ethmalosa fimbriata*), and several mugilid species has been studied along this inverted salinity gradient. Panfili et al. (2004a) compared chinned tilapia populations in the Saloum with the nearby Gambia estuary, which has a normal head to mouth salinity gradient (0–38 ppt). They found a reduction in growth rate as well as size and age at maturity with increasing salinity in the Saloum, and an increase in fecundity during the wet season when salinity was reduced in the Saloum estuary. The bongo shad is a widely distributed brackish water species that supports important small-scale inland fisheries along the west coast of Africa. It tolerates a wide range of salinities from the FWs of coastal rivers to the hypersaline waters (90 ppt) of the Saloum and Casamance estuaries in Senegal. A comparison between populations from

the Saloum (<60 ppt) and Gambia estuaries indicated higher fecundity and egg size in the former, although growth rate and size at maturity were inversely related to salinity, as in chinned tilapia (Panfili et al., 2004b). Among the six mugilid species found in the Saloum, young-of-the-year exhibited the lowest abundance in the uppermost region of the estuary, although recruitments were observed at salinities as high as 78 ppt (Trape et al., 2009). Salinity had no effect on size at maturity, unlike for chinned tilapia and bongo shad, perhaps indicating an early acquisition of osmoregulatory capacity in Mugilidae, which would be an area worthy of further study.

3. ALKALINE LAKES

Saline lakes tend to be alkaline to some degree, mostly with a pH ranging from 7.5 to 10 (in some cases up to 11) and titratable alkalinity ranging from 2 to 400 (Table 9.1). Of the 108 saline lakes where pH was reported by Hammer (1986), 45 had a pH greater than 9 and 13 had a pH exceeding 10. Perhaps the most studied alkaline lake with endemic fish populations at the extreme of alkalinity is Lake Magadi (Kenya). However, there are examples of other highly alkaline lakes (pH 9.4–10) supporting endemic fish populations, including the other East African rift lakes Natron and Manyara (Tanzania) (Talling and Talling, 1965), as well as Lake Van (Turkey) (Danulat, 1995), Lake Qinghai (China) (Wood et al., 2007), and Pyramid and Walker Lakes (USA) (Galat et al., 1981, Beutel et al., 2001).

3.1. Environmental Characteristics of Alkaline Lakes

Lake Magadi and the other saline–alkaline lakes of the east African rift valley not only have high pH (9.5–10), but also have a Na-HCO₃⁻+CO₃⁻ dominated chemistry (Na⁺ 356 mM, Cl⁻ 112 mM) that results in high alkalinity (>300 mM titratable alkalinity) (e.g. Wood et al., 2012) (Table 9.1). Wilson et al. (2004) have also shown that the physicochemical conditions within the different lagoons of Lake Magadi that support fish populations can vary widely (pH 9.13–10.05; titratable alkalinity 184–1625 mM; Na⁺ 183–978 mM; Cl⁻ 46–693 mM; osmolality 278–1465 mOsm). Talling and Talling (1965) categorize these as Class III saline lakes (conductivity 6000–160,000 μS cm⁻¹; alkalinity >60 mM), which include the more saline lakes such as Lakes Magadi, Natron, Nakuru, Elmenteita, and Manyara. Lake Van has a similar chemistry (22 ppt, pH 9.8, Na⁺ 337 mM, Cl⁻ 154 mM) (Table 9.1) (Danulat, 1995) to the rift lakes although less extreme alkalinity (150 mM titratable alkalinity), while Lake

Qinghai (9 ppt, Na^+ , 200 mM, Cl^- 173 mM) (Wood et al., 2007) and Pyramid Lake (4.4 ppt, pH 9.4, Na^+ 58 mM, Cl^- 60 mM) (Galat et al., 1981) are Na^+/Cl^- dominant. All these lakes are also low in the divalent cation Ca^{2+} (<1 mM), although in Lakes Qinghai and Pyramid Mg^{2+} concentrations are high (18–36 mM and 7 mM, respectively). In addition, in Lake Magadi, over 90% of the surface is covered by a thick layer of trona, the floating precipitate of lake water consisting mainly of NaHCO_3 and Na_2CO_3 , restricting open water to lagoons fed by volcanic saline hot springs along the lake edge. The lagoon waters can reach temperatures of 42°C and abundant cyanobacteria populations result in diurnal fluctuations of dissolved oxygen from 16 to 450 mmHg, during night and day, respectively (Narahara et al., 1996).

3.2. Fish Species that Reside in Alkaline Lakes

Alkaline lakes generally have low species diversity and occasionally only a single species is found. In the most studied example, Lake Magadi, only the cichlid *Alcolapia grahami* (formerly *Oreochromis alcalicus grahami* and *Tilapia grahami*) exists, although different populations of *A. grahami* are found within the isolated lagoons of Lake Magadi and Little Magadi (Wilson et al., 2004). *Alcolapia grahami* has also been transplanted and established in Lakes Nakuru (Vareschi, 1979) and Elmenteita (Owino et al., 2001). In neighboring Lake Natron, which was once (10,000–12,000 years ago) part of the larger paleolake Orolongo encompassing Lake Magadi (and Little Magadi), the closely related cichlids *Alcolapia alcalicus*, *A. latilabris*, and *A. ndalalami* are endemic (Seegers et al., 1999). Lake Manyara, 80 km further south, has an endemic population of cichlid, *Oreochromis amphimelas* (Beadle, 1962). For this species, Nagl et al. (2000) concluded on the basis of phylogenetic analyses that adaptations to saline–alkaline conditions evolved separately from the *Alcolapia* species of Lake Natron and Magadi.

The high-altitude saline–alkaline Lakes Van and Qinghai support endemic cyprinid species, the pearl mullet (*Chalcalburnus tarichi*) (Danulat and Kempe, 1992) and the scaleless carp (*Gymnocypris przewalskii*) (Wang et al., 2003), respectively. Both of these species are unique to their respective lakes and are stream spawners, migrating into FW rivers for breeding and returning to the lake for feeding and development. Food fisheries have existed for both species although both are threatened by population declines due to overfishing.

Pyramid and Walker Lakes are the remnant water bodies of the once larger paleolake Lahontan. They support more diversified ichthyofauna than the previously described lakes. Ten fish species have been reported and

the four most abundant are the tui chub (*Gila bicolor*), Tahoe sucker (*Catostomus tahoensis*), Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*), and cui-tui (*Chasmistes cujus*), which are endemic to these lakes (Galat et al., 1981). Notably, Lahontan cutthroat trout supports an important recreational fishery; they grow to trophy size by feeding on tui chub. Endemic lake breeding populations have been eliminated by damming of the spawning rivers of these obligate stream spawners and the lakes are stocked from hatcheries (Galat et al., 1981; Beutel et al., 2001). Lahontan cutthroat trout has also been successfully introduced into other alkaline lakes outside its native range in the USA (e.g. Omak and Grimes Lakes) (Galat et al., 1985).

3.3. Physiological Challenges of Alkaline Lakes

3.3.1. ION AND WATER BALANCE

The Magadi tilapia *A. grahami* in many respects shows similarities to a SW teleost, given that its environment is hyperosmotic (580 mOsm) to blood. Although *A. grahami* is ureogenic, urea has only a minor role as an osmolyte (2–8% of internal osmolality) (Wood et al., 1994). *Alcolapia grahami* drinks the saline–alkaline water at a high rate ($8 \mu\text{l g}^{-1} \text{h}^{-1}$) even when exposed to dilute lake water (Wood et al., 2002a; Bergman et al., 2003). Of the intestinal water absorption, 70% is coupled with Na^+ and HCO_3^- uptake (consistent with the high imbibed Na^+ and HCO_3^-) and 30% is coupled with Na^+ and Cl^- , the latter of which is typical of SW fishes (Bergman et al., 2003) as described above. The Na^+ , HCO_3^- , and Cl^- load (Bergman et al., 2003) is then excreted across the gills (Eddy et al., 1981). At the cellular level the gills of *A. grahami* possess SW-type ionocytes with neighboring accessory cells and leaky tight junctions (Laurent et al., 1995; Wood et al., 2002a). Wood et al. (2012) proposed that the electrogenic component of TEP in *A. grahami* is due to active HCO_3^- excretion and they found that the gills have an unusually low HCO_3^- permeability. A hypothetical model proposed by Laurent et al. (1995) is very similar to the classic chloride cell model proposed by Silva et al. (1977), in which electrogenic, secondarily active, HCO_3^- excretion is accomplished transcellularly in the gills, resulting in passive Na^+ efflux through the paracellular “shunt” pathway. The essential components in this excretory pathway (NKA, NKCC, and CFTR) have been demonstrated using immunohistochemistry (Fig. 9.4), indicating that they possess an apical NCC that may facilitate Cl^- uptake, similarly to FW-adapted Mozambique tilapia (*Oreochromis mossambicus*) (Hiroi et al., 2008).

One unique characteristic of *A. grahami* is that it has an exceptionally high blood pH (8.6–9.0) and intracellular pH (7.6) (Wood et al., 2002a), and

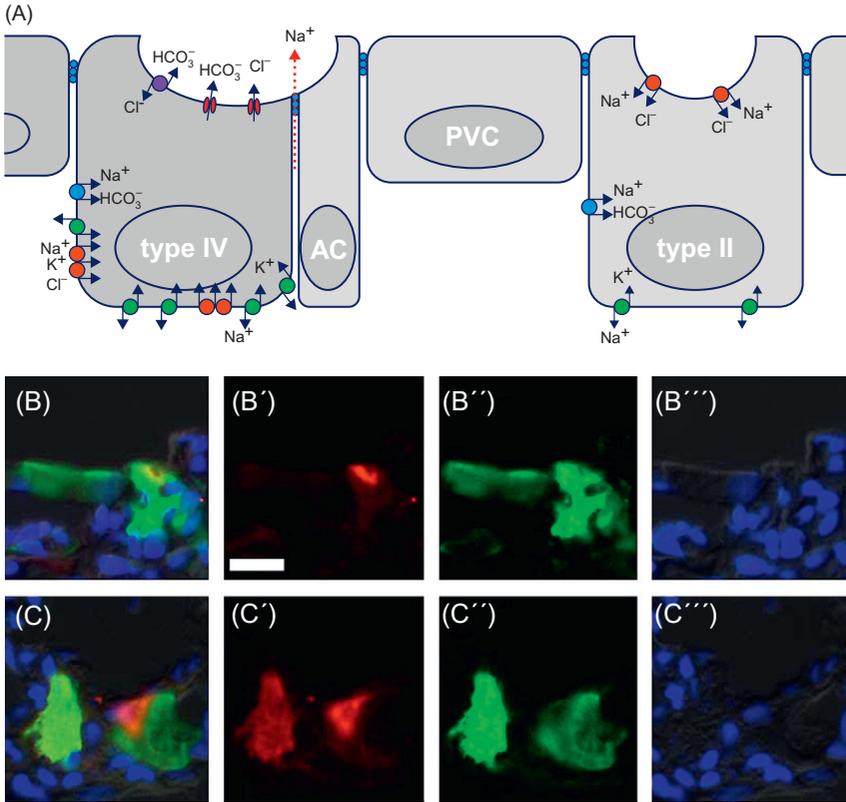


Fig. 9.4. Model of gill ionoregulatory cells in *Magadi tilapia* (*Alcolapia grahami*) (modified from Laurent et al., 1995). A seawater-like ionocyte (type IV*) with apical cystic fibrosis transmembrane regulator (CFTR) anion channel and basolateral Na^+/K^+ -ATPase (NKA) and $\text{Na}^+/\text{K}^+/2\text{Cl}^-$ cotransporter (NKCC) is identified, but rather than functioning in NaCl secretion as in marine teleosts, it facilitates NaHCO_3 secretion with the CFTR operating as an HCO_3^- channel. A freshwater ionocyte (type II*) with apical Na^+/Cl^- cotransporter (NCC) and basolateral NKA uses the inward Na^+ gradient to drive Cl^- uptake. An apical $\text{Cl}^-/\text{HCO}_3^-$ exchanger and a basolateral $\text{Na}^+/\text{HCO}_3^-$ cotransporter (NBC) are also proposed for the type IV cell but their presence has not yet been confirmed. Immunofluorescent co-localization of (B, B', C, C') NKA (green) with either (B, B') CFTR (red) or (C, C') NKCC/NCC (red) in the gill cryosections of *A. grahami*. Sections were counterstained (B, B'', C, C'') with DAPI (blue) to label nuclei and differential interference contrast images also overlain for orientation. PVC pavement cell, AC accessory cell. Scale bar: 10 μm . From J. M. Wilson, P. Laurent, C. M. Wood, C. Chevalier, H. L. Bergman, A. Bianchini, J. N. Maina, O. E. Johannsson, L. F. Bianchini, G. D. Kavembe, M. B. Papah and R. O. Ojoo (unpublished data). *Ionocyte subtype categorized by Hiroi et al. (2008) for *Oreochromis mossambicus*.

it has been estimated that up to 50% of this species' high metabolic rate ($34.5 \mu\text{mol O}_2 \text{ kg}^{-1} \text{ h}^{-1}$) is associated with acid–base regulation (Wood et al., 2002b), a topic clearly worthy of further investigation in this and other species that reside in highly alkaline lakes.

In contrast to *A. grahami*, species from Pyramid Lake, Lake Van, and Lake Qinghai (Lahontan cutthroat trout, tui chub, pearl mullet, and scaleless carp) are far less tolerant to either salinity or alkalinity. Taylor (1972) reported 16 ppt (concentrated by evaporating lake water) to be lethal to Lahontan cutthroat trout and concluded that this species lives close to its current limit in Walker Lake (Beutel et al., 2001). Wilkie et al. (1993) found that exposure to pH 10 was unsustainable and would lead to ionoregulatory failure and ammonia intoxication. In general, cyprinids are not strong osmoregulators. In Lake Van (22 ppt), the pearl mullet maintains very high plasma osmolality (472 mOsm) that is only 15% lower than that of lake water (551 mOsm) (Danulat, 1995). Plasma Na^+ levels (184 mM) are in the range of SW fishes' and given that Cl^- is lower (116 mM), the strong ion difference must be made up by HCO_3^- . Unexpectedly, gill NKA-immunoreactive cells decreased in distribution and size following acclimation to lake water (A. R. Oğuz, Yüzüncü Yıl University, Van, Turkey). In the scaleless carp, plasma ion levels increase towards lake levels following transfer from river water (plasma Na^+ levels were 188 and 222 mM, and Cl^- levels were 130 and 208 mM in river and lake water, respectively), and gill and kidney NKA activity decreases in lake-water acclimated fish, which is associated with up to a 40% reduction in resting metabolic rate (Wang et al., 2003; Wood et al., 2007).

3.3.2. NITROGENOUS WASTE EXCRETION

Teleosts are almost exclusively ammoniotelic, excreting the majority of their nitrogenous wastes as ammonia using an outwardly directed NH_3 partial pressure (PNH_3) gradient that can be enhanced through gill boundary layer acid trapping of NH_3 to NH_4^+ (Wright and Wood, 2009). At the molecular level, ammonia flux is facilitated by Rh glycoprotein ammonia transporters and acidification by either a proton pump or sodium proton exchanger (Wright and Wood, 2009). Urea makes up the remaining smaller portion of nitrogen flux and is generated by arginolysis and/or uricolysis rather than the ornithine urea cycle (OUC), which operates only in early life history stages and in a few adult fish (Wilkie and Wood, 1996). Ammoniotely is usually ideal in an aquatic environment because it is energetically less expensive than conversion to a less toxic molecule such as urea (OUC requires 5 ATP per urea molecule) and may diffuse directly into the water. The high pH of alkaline lakes, however, creates challenges for ammonia excretion by diffusion. The dissociation constant (pK_a) of NH_4^+ is 9.25 and 9.35 in FW and SW, respectively (25°C) (Khoo et al., 1977), so an increase in water pH

from 7 to 10 will increase the percentage of total ammonia that exists as NH_3 from <1 to 85%, greatly impairing NH_3 diffusion across the gills. Furthermore, in highly buffered lake water, boundary layer acidification becomes ineffective, preventing acid trapping of ammonia.

Fish from Pyramid Lake, Lake Van, and Lake Qinghai all show consistent adaptations in nitrogenous waste handling (Wilkie et al., 1993; McGeer et al., 1994; Wang et al., 2003; Danulat, 1995). Positive PNH_3 gradients are accomplished through higher plasma ammonia and/or pH levels (Wilkie et al., 1993; Danulat, 1995; Wood et al., 2007), ammonia excretion rates are reduced, and the relative contribution of urea (through uricolysis and/or arginolysis) to total nitrogen excretion is elevated (up to 35%). In Lake Van, which is markedly more saline (22 ppt), NH_4^+ diffusion may be of importance, although the molecular mechanisms of ammonia excretion in pearl mullet have yet to be studied.

In contrast to the other fishes described above that have adapted to alkaline lake conditions by modifying existing mechanisms of nitrogen excretion, the Magadi tilapia (*Alcolapia grahami*) is remarkable. It is the only teleost that is 100% ureotelic, and it uses the OUC (Randall et al., 1989) to produce urea at very high rates ($7771 \pm 849 \mu\text{mol urea kg}^{-1} \text{h}^{-1}$) (Wood et al., 1989). The OUC enzymes function not only in the liver (Randall et al., 1989), but also in the white muscle (Lindley et al., 1999). The Magadi tilapia, which feeds almost continuously on nitrogen-rich cyanobacteria, has a very high metabolic rate ($\dot{M}\text{O}_2 \sim 34.5 \mu\text{mol g}^{-1} \text{h}^{-1}$ at 36–42.5°C) (Narahara et al., 1996), which contrasts with the hypometabolic responses of the Lahontan trout and scaleless carp under alkaline conditions (Wilkie et al., 1993; Wood et al., 2007). Associated with this high metabolic rate, *A. grahami* has a very high affinity blood oxygen (Narahara et al., 1996) and a large gill diffusing capacity (Maina et al., 1996), as well as a capacity for facultative air-breathing via a physostomous air bladder (Narahara et al., 1996). *Alcolapia alcalicus* from the neighboring Lake Natron has also been shown to be ureotelic and to possess the complete OUC (Wilson et al., 2004). However, it remains to be determined whether the more distantly related *Oreochromis amphimelas*, which adapted independently in Lake Manyara, has also evolved ureotelically to survive under alkaline conditions.

4. ION-POOR WATERS

4.1. Environmental Characteristics of Ion-Poor Waters

Ion-poor waters are found on virtually every continent. A few examples are waters of the Atlantic Coastal Plain of North America, much of the

Canadian Shield, peat bogs of northern Europe and much of Scandinavia, some rivers of Queensland, Australia, and the Rio Negro, a major tributary of the Amazon. They occur typically wherever soils are largely silicate sands, which bind minerals loosely. These soils have long been stripped and the waters draining this region are extremely ion poor, with Na^+ , Cl^- , and Ca^{2+} concentrations well below global averages for rivers (Table 9.1). It is not unusual for ion-poor waters to contain large quantities of dissolved organic acids from partially decayed plant matter (Leenheer, 1980; Ertel et al., 1986; Walker and Henderson, 1996), and since the water has a very low buffer capacity the result is very low pH levels as well. Adaptations to ion-poor, acidic waters have been reviewed recently in this series (Gonzalez et al., 2005b) and therefore the discussion will focus on mechanisms of acclimation to ion-poor waters with some comparisons to species native to ion-poor environments.

4.2. Physiological Challenges in Ion-Poor Waters

4.2.1. ION AND WATER BALANCE RELATIVE TO FRESHWATER

In FW, fish regulate plasma Na^+ and Cl^- concentrations at levels well above those of the surrounding water. Consequently, they experience diffusive salt loss across the branchial epithelium, largely thought to be through paracellular tight junctions, although recent evidence indicates that transcellular pathways may also be involved (Wood et al., 2009; Iftikar et al., 2010; Matey et al., 2011). A number of studies that correlate the magnitude of ion efflux with water Ca^{2+} concentration indicate that permeability of gill epithelia is governed, at least in part, by the binding of Ca^{2+} to tight junction proteins (Hunn, 1985; Freda and McDonald, 1988; Gonzalez and Dunson, 1989) and thus, the lower the water Ca^{2+} the greater the gill permeability. To maintain internal Na^+ and Cl^- levels, both ions are actively taken up from the water across the gills. It is generally believed that Cl^- is taken up in exchange for HCO_3^- , but the mechanisms involved in Na^+ uptake are less clear. There is evidence for the presence of a range of transport systems on the apical membrane of the gills, including Na^+/H^+ exchangers, H^+ -ATPase/ Na^+ channel arrangements, and even Na^+/Cl^- cotransport (see Hwang, 2009, for review), and it is likely that there is no one mechanism common to all fish.

Ion-poor waters pose a range of challenges for ion regulation in teleost fishes. First, low ion concentrations may generate a “substrate limitation” for active Na^+ and Cl^- uptake. Both Na^+ and Cl^- transport, regardless of the mechanism involved, exhibit Michaelis–Menten-type saturation kinetics, and thus uptake tends to be low in ion-poor waters simply owing to the

scarcity of these ions in the bulk medium (Potts, 1994). Potentially more important, however, is that low Ca^{2+} levels increase branchial ion permeability, presumably by removing Ca^{2+} from tight junctions, and stimulate diffusive loss of Na^+ and Cl^- (Hunn, 1985; Freda and McDonald, 1988; Gonzalez and Dunson, 1989). Together, the limitation of uptake and stimulation of efflux results in a net loss of Na^+ and Cl^- and if losses are too great (30–50%) then serious, potentially fatal, internal ionic and osmotic disturbances result (Milligan and Wood, 1982).

4.2.2. ACCLIMATION TO ION-POOR WATERS

For many FW fishes, migration or transfer into ion-poor water disrupts the ability to regulate internal salt levels and they cannot recover (McDonald and Rogano, 1986). However, some species can re-establish ion balance in ion-poor water through upregulation of ion uptake, a reduction in diffusive ion loss, or some combination. Upregulation of ion transport is typically associated with proliferation of ionocytes in the branchial epithelium (Bindon et al., 1994; Greco et al., 1995, 1996; Perry, 1998). FW ionocytes in general are much larger than surrounding pavement cells and are primarily found on the gill filaments and in intralamellar spaces, comprising less than 10% of the total gill surface area. As ionocytes proliferate during exposure to ion-poor water, they appear on the lamellar epithelium and can comprise up to 30% of the total gill surface area (Perry, 1998). There is also some indication of changes occurring in the transporters themselves. For instance, studies of Na^+ transport in zebrafish (*Danio rerio*) during acclimation to ion-poor water suggest that there is a switch from an Na^+/H^+ exchanger in ion-rich water to an H^+ -ATPase/ Na^+ -channel arrangement in dilute water (Boisen et al., 2003; Yan et al., 2007). At the same time, fish may reduce branchial permeability and thus diffusive ion loss (McDonald, 1983; Audet and Wood, 1988; Gonzalez and Dunson, 1987; 1989). The mechanism for reduction in branchial permeability is not known but may involve adjustments in cell volume and/or tight junctions, the latter of which may involve hormones such as prolactin or cortisol. Given enough time, the degree to which efflux is reduced and influx is increased may allow fish to migrate into ion-poor waters; however, this is likely to be species specific and has only been examined in a very limited number of species.

4.2.3. ACCLIMATION VERSUS ADAPTATION

Given the challenges to osmoregulation posed by ion-poor waters it is not surprising that most naturally occurring ion-poor environments are characterized by low piscine diversity. For example, along the Atlantic Coastal Plain of North America relatively few species inhabit these waters, with the exception of a few families such as the Centrarchidae, Ictaluridae,

and Esocidae (Hastings, 1979; Graham and Hastings, 1984). Notably missing are cyprinids, the most species-rich family of fish in North America. In stark contrast to this general pattern is the rich and varied diversity of the Rio Negro. It is estimated that over 1000 species of fish from over 40 different families inhabit the Rio Negro, including a lungfish (*Lepidosiren*), two Osteoglossiformes (*Arapaima gigas* and *Osteoglossum bicirrhosum*), several dozen gymnotids, and many cichlids. The order Characiformes is particularly diverse, represented by 12 families, including the Characidae with almost 800 species (Val and Almeida-Val, 1995).

Species inhabiting ion-poor environments tend to possess specializations for ion regulation that sometimes differ from the acclimatory adjustments described above. The few measurements of plasma ion levels in these species reveal levels typical of FW fishes (Wood et al., 1998, 2002c; Brauner et al., 2004; Gonzalez et al., 2010), indicating that they do not reduce levels to mitigate the physiological challenge to ionoregulation in such a dilute environment. Gonzalez et al. (2002) described two basic patterns of ion regulation in species from the Rio Negro. One group displays high-capacity, high-affinity transport mechanisms that maintain high rates of uptake even in very dilute media. Despite these high rates of transport there is no proliferation of ionocytes on the lamellae as seen in non-native fish. It is not clear what specific transporters are involved in ion uptake in these species, but they possess some of the highest affinities reported and are able to function unhindered at pH 3.0–3.25 (Gonzalez and Preest, 1999; Gonzalez and Wilson, 2001; Preest et al., 2005). The second group has low-capacity, low-affinity transporters that produce low rates of transport in ion-poor water. These fish rely on equally low rates of diffusive loss to maintain ion balance. In both groups, regardless of their rate of uptake, diffusive ion loss is very resilient to ion-poor (or low pH) water. The waters of the Rio Negro have incredibly low Ca^{2+} concentrations ($1\text{--}10\ \mu\text{mol L}^{-1}$), yet this does not seem to interfere with their ability to limit efflux (Gonzalez and Preest, 1999; Preest et al., 2005). Furthermore, there are some indications that dissolved organic carbon material in the waters of the aptly named Rio Negro can substitute for Ca^{2+} at the gills of fish in ion-poor waters, reducing passive ion efflux (Gonzalez et al., 2002; Wood et al., 2003). Such independence from water Ca^{2+} concentration has not been observed in non-native species. For further exploration of this topic see the review by Gonzalez et al. (2005b).

4.2.4. THE OSMORESPIRATORY COMPROMISE IN ION-POOR WATERS

As described above, changes in gill morphology associated with exposure to a changing environment may have implications for gas exchange through

the osmorepiratory compromise. As described above, exposure to ion-poor water results in proliferation of ionocytes to enhance ion uptake; however, this is associated with a thickening of the blood–water diffusion distance of the lamellae in rainbow trout. Within 4 weeks of exposure to soft water, the blood–water diffusion distance was shown to double as a result of ionocyte proliferation (Greco et al., 1996), which negatively affected both oxygen uptake and carbon dioxide excretion. During exposure to progressive hypoxia, fish exhibiting lamellar thickening exhibited lower arterial PO_2 values than control fish exposed to the same water PO_2 (Perry, 1998). To compensate, ventilation rate was greatly elevated in rainbow trout exhibiting lamellar thickening (Greco et al., 1995). In rainbow trout acclimated to soft water, maximal swimming performance was reduced by 14% relative to control fish (Dussault et al., 2008), indicating that the impairment of gas exchange associated with lamellar thickening comes at a cost to whole-animal performance. Clearly, more research is required to understand the nature of the tradeoff between gas exchange and ionoregulation during acclimation to ion-poor waters. In fish that have adapted to ion-poor waters, the response may be quite different. In the only study where this has been investigated, exposure to hypoxia in the Amazonian oscar (*Astronotus ocellatus*) endemic to ion-poor waters was associated with a reduction in branchial ion efflux and water permeability, indicating a rapid and dramatic overall reduction in gill permeability. This was associated with changes in gill ultrastructure that were proposed to result in rapid closure of transcellular channels, thus reducing permeability, without compromising gas exchange capacity and mitigating the impact of the osmorepiratory compromise (Wood et al., 2009). Whether this is a common trait in fish native to ion-poor waters remains to be investigated, but may represent a more general adaptation among fishes to these conditions.

5. CONCLUSIONS AND PERSPECTIVES

Aquatic environments that deviate from “typical” FW or SW values are abundant throughout the world and in many cases are inhabited by fish populations that support recreational and even commercial fisheries. Fish have acclimated or adapted to the physiological challenges associated with these environments; however, we are only just starting to understand the physiological mechanisms involved. In some cases, the mechanisms represent fine-tuning or upregulation of processes that are well described in FW and SW fishes; however, in other cases more unique solutions have been discovered. A great deal remains to be learned, and the following are some areas raised in this chapter that are timely for further investigation.

In hypersaline lakes, upregulation of the mechanisms that are well described in SW fishes are important; however, little is known about the renal function and the role of the kidney in the most hypersaline-tolerant species. Furthermore, many species of hypersaline-tolerant fish exhibit a reduction in whole-animal $\dot{M}O_2$ with an increase in salinity. Is this associated with metabolic suppression (as some data imply) and, if so, how is this controlled? The ionic composition of hypersaline lakes often varies dramatically from SW ratios and little is known about the physiological implications of this. As discussed above, fish have more successfully inhabited lakes where Na^+ and Cl^- dominate over SO_4^{2-} - or HCO_3^- -rich waters. Is there a physiological basis for this and, if so, what are the mechanisms that allow those fish to live in SO_4^{2-} - or HCO_3^- -rich waters?

In alkaline lakes, water pH limits ammonia excretion and in the few species investigated, ammonia excretion is dependent upon an elevation in PNH_3 gradients through higher plasma ammonia and/or pH levels, and an elevation in the relative contribution of urea to total nitrogen excretion, in some cases up to 100%. How widespread is the elevation in blood pH in fishes from alkaline lakes? How widespread is ureotely among fishes that have independently adapted to alkaline lakes? In some fishes, NH_4^+ diffusion may be of importance to nitrogenous waste excretion: what are the mechanisms involved? In one species, *A. graham* (Wood et al., 2002b), it has been proposed that 50% of resting metabolic rate is associated with acid–base regulation. What is the cost of acid–base regulation in fishes in general, and is it higher in fishes adapted to alkaline lakes?

Ion-poor waters in some cases contain among the highest FW biodiversity in the world (such as in the Amazon). However, very little is known about how these fish deal with limited ion availability and the potential for diffusive ion efflux due to low environmental Ca^{2+} . In fishes known to possess high ion uptake capacity and affinity, what transporters are involved and how do they function at low pH values of 3–3.25? In other fishes that exhibit low efflux, what is the basis for low branchial permeability and how can this be accomplished in the face of low environmental Ca^{2+} ? How can dissolved organic carbon substitute for Ca^{2+} at the gills to reduce efflux? In the Amazonian Oscar, which is adapted to ion-poor waters, branchial ion and water permeability can be rapidly reduced during exposure to hypoxia to minimize ionoregulatory disturbances without impairing gas exchange, apparently eliminating the osmorepiratory compromise. Is this a general characteristic of fishes adapted to ion-poor waters?

Clearly, there is a great deal to learn about how fish acclimate and adapt to hypersaline, alkaline, and ion-poor waters. Elucidation of novel physiological mechanisms that may be involved, and the degree to which

described mechanisms can be modified, will give insight into the physiological control of euryhalinity and further our understanding of how fish have been able to inhabit almost every aquatic environment on the planet, and in some cases even those that have been recently drastically altered.

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