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# Physiological responses of euryhaline marine fish to naturally-occurring hypersalinity

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ABSTRACT

Hypersaline habitats are generally defined as those with salinities in excess of 40 ppt. Well-known hypersaline regions (e.g. salt and soda lakes) have a well-earned reputation for being among the most inhospitable habitats in the world, and fish endemic to these areas have been the subject of much research related to extremophile physiology. Yet, marine coastal hypersalinity is both a common occurrence and a growing consideration in many marine coastal ecosystems, in part owing to human influence (e.g. evaporation, river diversion, desalination effluent). Importantly, any increase in salinity will elevate the osmoregulatory challenges experienced by a fish, which must be overcome by increasing the capacity to imbibe and absorb water and excrete ions. While great attention has been given to dynamic osmoregulatory processes with respect to freshwater to seawater transitions, and to the extreme hypersalinity tolerance that is associated with the adoption of an osmo-conforming strategy, relatively little focus has been placed on the physiological implications of moderate hypersalinity exposures (e.g. $\leq$ 60 ppt). Importantly, these exposures often represent the threshold of osmoregulatory performance owing to energetic constraints on ion excretion and efficiency limitations on water absorption. This review will explore the current state of knowledge with respect to hypersalinity exposure in euryhaline fishes, while placing a particular focus on the physiological constraints, plasticity and downstream implications of long-term exposure to moderate hypersalinity.

## 1. Introduction

As osmoregulating organisms, fish are constantly faced with the challenge of maintaining internal ion concentrations and osmolality against adverse external gradients. In hyperosmotic environments, which are generally those habitats with a salinity above 9–10 ppt, fish are required to drink to offset diffusive water loss, after which they eliminate excess ions via the gills and, to some extent, the renal system (see reviews Edwards and Marshall, 2012; Marshall and Grosell, 2006). The opposite is true in hypo-osmotic environments whereby fish must actively absorb ions from the environment across their gills while offsetting osmotic water gain through the copious production of dilute urine (Edwards and Marshall, 2012; Marshall and Grosell, 2006). The ability of fish species to tolerate various osmotic habitats is loosely categorized as stenohaline, amphihaline or euryhaline. There is considerable flexibility with how these terms are used in the literature – particularly with respect to euryhaline and amphihaline – but for the purposes of this review stenohaline refer to those species that can survive over only a narrow range of salinities, whether freshwater or seawater, while amphihaline species can survive in both freshwater and seawater habitats. Euryhaline species are more variable wherein they can survive across a wide variety of salinities, much like amphihaline species. However, most euryhaline species are adapted either to a brackish/freshwater phenotype with upper salinity thresholds below full-strength seawater, or a brackish/seawater phenotype that in some cases can extend beyond normal seawater. Such habitats are typically referred to as hypersaline environments, and often require special physiological adjustments to offset the more extreme osmotic gradients (Brauner et al., 2012; Gonzalez, 2012).

For many, the term hypersaline will conjure images of salt and soda lakes that are among the most inhospitable aquatic environments in the world. Many of these systems are devoid of fish life, but work on the species that can survive (e.g. tilapia species *Alcolapia graham* or *Oreochromis mossambicus*) serve as preeminent examples of extremophile osmoregulatory physiology in fishes. But the water chemistry characteristics of saline lakes are strongly impacted by local geology, and thus many have unique chemical characteristics beyond high salinity alone. For example, the hypersaline Lake Magadi is also an alkaline

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environment that poses unique challenges to ammonia transport that would not occur in high sodium chloride environments (Randall et al., 1989; Wood et al., 1994; Wood et al., 1989). Furthermore, many extremophile fish species show an ability to qualitatively change their osmotic strategy to osmo-conformers, whereby the plasma and tissue osmotic pressure begins to rise as salinity exceeds 60 ppt (Brauner et al., 2012; Gonzalez, 2012; Kültz, 2015), owing in part to the accumulation of organic osmolytes like *myo*-inositol (Fiess et al., 2007; Gardell et al., 2013). Interest in these extremophile fishes has led to an abundance of physiological research, including previous reviews (e.g. Brauner et al., 2012; Gonzalez, 2012; Kültz, 2015), related to the adaptations that allow for extreme hypersalinity tolerance. However, the interest in extremophile species has overlooked the fact that hypersalinity can be a common stressor in coastal marine habitats, and moreover the conditions that create coastal hypersalinity (e.g. high temperature and low river input) are being exacerbated by climate change and human activities. As such, it seems prudent to fully consider the effects of hypersalinity in the context of the broader euryhaline fish community that most often inhabits coastal regions.

On this background, this review will seek to explore the physiological challenges associated with hypersalinity as an environmental stressor to fishes common to coastal marine habitats, while striving to highlight unanswered questions and areas for future research. I will first outline the occurrences of marine hypersaline habitats, after which I will discuss the physiological limitations imposed by hypersalinity through the lens of osmoregulatory physiology and phenotypic plasticity. Finally, I will discuss the potential downstream consequences of hypersalinity exposure, particularly chronic exposure, while also considering the likely co-occurrence of multiple environmental stressors.

## 2. Global occurrence of marine hypersaline habitats

Hypersalinity in the marine environment is generally constrained to coastal estuaries and lagoons. The definition of an estuary has changed somewhat over time, but today is considered any partially enclosed coastal body of water with riverine input, and which has permanent or periodic exchange with the ocean. In contrast, coastal lagoons have no freshwater input and are separated from the ocean by a barrier (e.g. barrier island), but nonetheless maintain connection to the ocean. Importantly, both of these systems are highly productive ecosystems that are habitat or nursery grounds for many economically important fisheries species. A recent meta-analysis used the scientific literature to identify over 100 lagoons, estuaries and coastal embayments (i.e. estuaries without river input) with documented periods of hypersalinity, which was defined as $\geq$40 ppt (Tweedley et al., 2019). These areas were found on all continents except Antarctica, with the greatest concentration in North America – particularly in the Gulf of Mexico and Gulf of California regions. Overall, the majority of hypersaline systems are associated with shallow lagoons ($\leq$4 m maximum depth) in arid regions of the world, where the lack of ocean circulation and limited riverine input combine with high evaporation rates. These systems account for the majority of hypersaline areas in North America as well as those associated with the Mediterranean, Arabian Gulf and Red Sea (Tweedley et al., 2019). However, hypersalinity can also be found in estuaries and coastal embayments, which make up the bulk of hypersaline areas in Australia and Africa. Of the systems documented by Tweedley et al. (2019), 42 % had maximum salinities below 60 ppt with an additional 22 % having maximum salinities of 60-79 ppt. The remaining third of systems had maximum salinities that reached or exceeded 3$\times$ that of normal seawater, with a documented high of 350 ppt.

## 3. Current models of marine osmoregulation in Teleosts

As mentioned previously, marine teleosts are osmoregulators that maintain a stable composition of their internal body fluid in the face of osmotic water loss to the hyperosmotic environment in which they live. To do this, marine fish have evolved the capacity to drink and absorb seawater, after which they excrete the absorbed sodium and chloride back into the environment via the gills, resulting in net water uptake (Edwards and Marshall, 2012; Marshall, 2012; Marshall and Grosell, 2006). Overall, the entire osmoregulatory process involves the integrated action of three organ systems – the gills, gastrointestinal tract, and renal system – and the mechanisms by which these organs function has largely been cemented in the scientific literature (see reviews Grosell, 2010; Larsen et al., 2014). Here, I will briefly summarize the current state of knowledge regarding the mechanisms of osmoregulation for marine fish in a typical marine saline environment.

### 3.1. The gastrointestinal tract

The first step of gastrointestinal seawater absorption is esophageal desalination, which is the process by which the osmotic pressure of the imbibed seawater is reduced to match that of the plasma (Fig. 1). The best estimates suggest that as much as 50 % of the total sodium chloride load in a given volume of seawater is removed as it moves through the esophagus (Hirano and Mayer-Gostan, 1976; Parmelee and Renfro, 1983), and because the esophagus has very low water permeability the osmotic pressure of the imbibed fluid is greatly reduced. Despite the importance of esophageal desalination, our understanding of the process is based on only a few species, including winter flounder (*Pseudopleuronectes americanus*)(Parmelee and Renfro, 1983), toadfish (*Opsanus beta*)(Esbaugh and Grosell, 2014), and two eel species (*Anguila Anguilla* and *Anguilla japonica*)(Hirano and Mayer-Gostan, 1976; Kirsch and Meister, 1982; Nagashima and Ando, 1993). The available evidence suggests that the process contains both active and passive components. Studies on unidirectional sodium flux have demonstrated sensitivity to ouabain and EIPA, implicating the sodium potassium ATPase (NKA) and sodium proton exchangers (NHE), respectively (Esbaugh and Grosell, 2014; Nagashima and Ando, 1993; Parmelee and Renfro, 1983). Relative transcript abundance data from toadfish show that NHE2 is much more abundant in the esophagus than NHE3 (Esbaugh and Grosell, 2014). Sodium flux was also insensitive to both furosemide and thiazide,

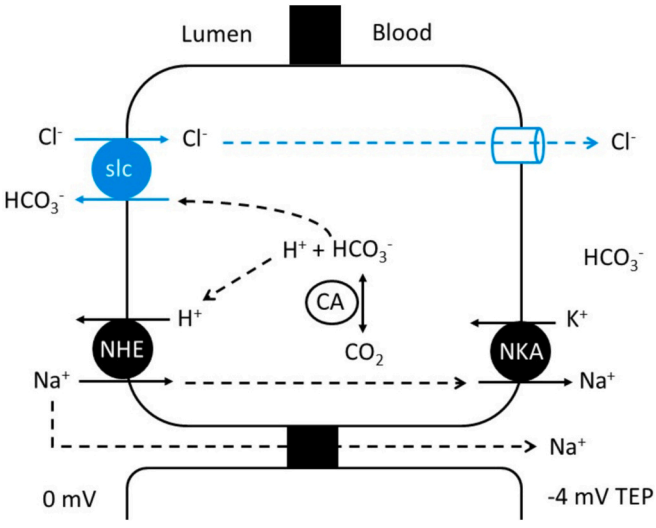


**Fig. 1.** Illustration of the cellular models of esophageal desalination in marine fishes. The pathway in blue denotes transcellular chloride transport, which is mediated by the passive uptake of chloride through an apical anion exchanger (slc) and a basolateral chloride channel. The pathway in black denotes transcellular sodium uptake through an apical sodium proton exchanger (NHE) and basolateral sodium potassium ATPase (NKA). A significant amount of sodium also enters the plasma via paracellular pathways owing to a high concentration gradient and favorable electrical gradient. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

which ruled out any involvement of sodium chloride co-transport despite the fact that chloride-free saline inhibited sodium flux to a magnitude consistent with ouabain treatment (Esbaugh and Grosell, 2014; Parmelee and Renfro, 1983).

Despite the above stated evidence for the active transport of sodium, the short circuit ($I_{sc}$) current of toadfish esophagus was near zero under symmetrical voltage clamp conditions, demonstrating limited capacity for the tissue to maintain active net ion uptake without the benefit of passive electrochemical gradients (Esbaugh and Grosell, 2014). Furthermore, asymmetrical current-clamp preparations revealed significantly reduced epithelial conductance – indicative of reduced paracellular transport – when tested in a chloride-free medium. Similar studies also showed that the esophagus exhibits a serosal side (i.e. plasma side) negative transepithelial potential (TEP) of approximately 5 mV, which indicates marginally greater absorption of chloride than sodium (Esbaugh and Grosell, 2014; Parmelee and Renfro, 1983). Little has been done to explore chloride absorption – beyond the experiments that ruled out sodium chloride co-transport – but gene expression profiles suggest that the esophagus contains the electroneutral slc4a2 anion exchanger along with very low expression of the electrogenic slc26a6 (Esbaugh and Grosell, 2014). Overall, these data suggest a model whereby chloride transport occurs through a transcellular pathway that involves electroneutral apical transport and electrogenic basolateral transport (i.e. a chloride channel). This model would result in a negative blood side TEP that facilitates paracellular sodium uptake, which would work in parallel with transcellular sodium uptake via apical NHE2 and basolateral NKA. The best estimates suggest that paracellular sodium transport accounts for between 40 and 70 % of total transport, if not higher (Esbaugh and Grosell, 2014; Parmelee and Renfro, 1983).

After leaving the esophagus the imbibed fluid has an osmolality close to that of the plasma and travels through the stomach and into the intestine. The stomach plays little to no role in ion and water transport, but the intestine is the primary site of water uptake and has been the subject of intense study (Grosell, 2006, 2011)(Fig. 2). The basis of intestinal water absorption relies on active sodium and chloride uptake, but unlike the esophagus, the intestine is water permeable. Therefore, as ions are absorbed the intestinal fluid will become slightly hypo-osmotic to the plasma, which drives water absorption. There are three main pathways by which the osmotic pressure of the intestinal fluid is lowered: 1) electroneutral sodium chloride co-transport, 2) electrogenic anion exchange-driven bicarbonate secretion, and 3) calcium carbonate precipitation. The combination of these three pathways allows the marine fish intestine to absorb up to 85 % of the imbibed fluid volume (e.g. Genz et al., 2008; Wilson et al., 1996), with the remaining volume excreted through the rectum. Electroneutral sodium chloride co-transport is relatively straightforward and involves either a sodium chloride transporter (NCC) or sodium potassium chloride co-transporter (NKCC2) in the apical membrane coupled to basolateral NKA and a basolateral chloride channel. The pairing of sodium and chloride transport is necessary because the driving force for chloride uptake is greatly reduced in the intestine owing to the lower chloride concentration in the intestinal fluid and the negative inside cellular potential. The evidence for this pathway comes from observations that seawater acclimated fishes often have higher NKA activity and NKCC transcript levels than freshwater acclimated individuals of the same species (e.g. Esbaugh and Cutler, 2016; Seidelin et al., 2000), as well as through Ussing chamber studies that have demonstrated bumetanide-sensitive absorptive $I_{sc}$ values in the intestine of marine fishes (Esbaugh and Cutler, 2016; Martin and Esbaugh, 2021; Tresguerres et al., 2010).

The second pathway for intestinal ion transport is anion exchange-driven bicarbonate secretion, which has been intensively studied and links chloride uptake to bicarbonate secretion through the electrogenic anion exchanger slc26a6 (Grosell, 2011; Grosell et al., 2009b; Grosell et al., 2005; Wilson et al., 2002). This exchanger transports one chloride and three bicarbonate ions per cycle, taking advantage of the intracellular negative potential generated by NKA to allow apical chloride

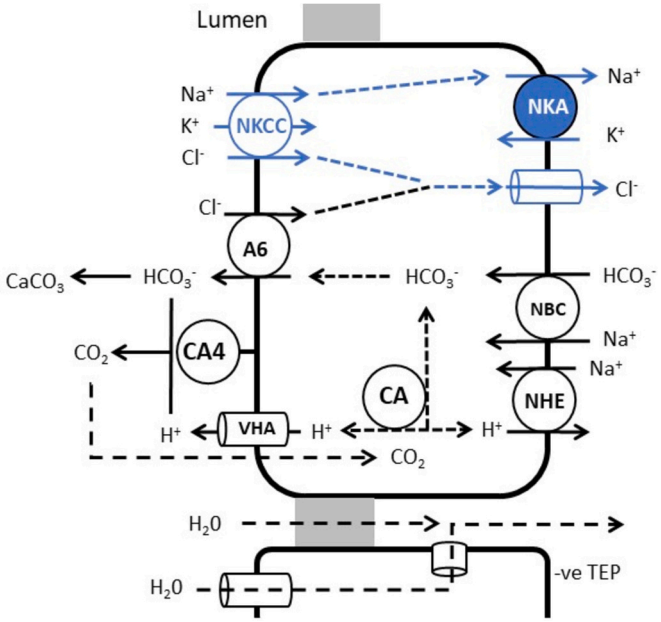


**Fig. 2.** Illustration of the intestinal epithelia ion transport pathways that drive water absorption in marine fishes. The first pathway (blue) represents the sodium chloride co-transport pathway mediated by NKCC2, NCC (not shown) and NKA. The second pathway (black) denotes the bicarbonate-dependent pathway that exchanges plasma or intracellularly produced bicarbonate for luminal chloride via apical slc26a6 (A6), while simultaneously promoting calcium carbonate precipitation to reduce luminal osmotic pressure and increase water absorption. See Section 3a for full details. Abbreviations: NKCC, sodium potassium two chloride co-transporter 2; NKA, sodium potassium ATPase; NBC, sodium bicarbonate co-transporter; NHE, sodium proton exchanger; CA, cytoplasmic carbonic anhydrase; CA4, membrane-bound carbonic anhydrase 4. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

absorption (Grosell and Genz, 2006). The chloride ions subsequently move across the basolateral membrane using the mechanisms described above. The intracellular bicarbonate can be formed from endogenous $CO_2$ production (Dixon and Loretz, 1986; Grosell, 2006; Wilson and Grosell, 2003), whereby $CO_2$ is hydrated by cytoplasmic carbonic anhydrase (CA) and the resulting proton is removed from the cell by basolateral NHE (Grosell and Genz, 2006). The intestinal epithelium also takes up bicarbonate from the plasma via sodium bicarbonate co-transporters (NBC)(Kurita et al., 2008) with the rate of uptake proportional to the plasma bicarbonate concentration (Taylor et al., 2010). To use the gulf toadfish as an example, a typical plasma bicarbonate concentration of 3.5 mM (Esbaugh et al., 2012) would result in a total bicarbonate secretion rate of approximately 5.8 μmol $cm^{-2}$ $h^{-1}$ with approximately 60 % being the product of CA-mediated endogenous $CO_2$ hydration (Taylor et al., 2010). Bicarbonate secretion can also be modulated on the apical epithelium through the combined actions of V-type $H^+$ ATPase (VHA) and membrane-associated CAIV (Grosell et al., 2009a; Guffey et al., 2011; Sattin et al., 2010). In this case, protons are shuttled into the lumen by VHA, which combines with luminal bicarbonate to form $CO_2$ – a process catalyzed by CAIV – which then diffuses back into the intestinal epithelium. This process effectively allows bicarbonate to be recycled and elevate chloride uptake.

A final osmoregulatory process that occurs in the intestine is the formation of calcium carbonate precipitates (see review Grosell and Oehlert, 2023). The fluid of the intestinal lumen has an alkaline pH and contains 10–115 mM bicarbonate (Grosell et al., 2001; Wilson et al., 2002), which when combined with calcium from imbibed seawater will promote the formation of calcium carbonate precipitates. While it might be assumed that this is a passive process that is simply a by-product of

intestinal anion exchange, there is evidence that fish actively contribute to carbonate formation by means beyond bicarbonate secretion. Using a proteomics approach, Schauer et al. (2016) demonstrated the presence of an organic matrix associated with carbonates that was enriched relative to the surrounding fluid, and subsequently demonstrated that capacity of this matrix to regulate the rate of carbonate formation, as well as the characteristics of crystal formation (Schauer and Grosell, 2017). This has since been extended to additional species (Schauer et al., 2018). The functional significance of carbonate formation lies in the effect of precipitation on fluid osmotic pressure. Precipitation effectively removes calcium and carbonate ions from the luminal fluid, which has been shown to reduce fluid osmotic pressure by up to 70 mOsm (Wilson et al., 2002) and thus drive water absorption in the absence of ion uptake by the animal. Interestingly, some estimates have suggested that fish carbonate production can represent up to 15 % of the total carbonate production in the ocean, and this production has also been shown to be significant in the context of global carbon cycling (Folkerts et al., 2024; Oehlert et al., 2024; Wilson et al., 2009). In short, sinking fish carbonates transport surface inorganic carbon to the deep ocean where it resolubilizes and contributes to the titratable alkalinity reservoir. Trapped organic dietary carbon associated with the carbonates – which accounts for up to 40 % of the total carbon – is also transported to depth and becomes bioavailable upon solubilization, which contributes to the ocean biological pump (Oehlert et al., 2024).

While the processes summarized above can apply throughout the intestine, it is important to note that there may be significant heterogeneity in the absorptive capacities of different intestinal segments and thus their contribution to ion and water absorption. As will be discussed in Section 5, this is particularly important in the context of hypersalinity acclimation and physiological plasticity. From the species that have been tested, it seems clear that the anterior intestine is the most active with respect to ion absorption, with estimates suggesting that sodium and chloride uptake rates may exceed those of the posterior intestine by over 10× (Grosell, 2006). Furthermore, both NBC and slc26a6 gene expression is highest in the anterior intestine and declines toward the distal regions (e.g. Grosell et al., 2009b; Taylor et al., 2010), and NKA activity is also generally higher in the anterior than posterior intestine (e.g. Esbaugh and Cutler, 2016; Guffey et al., 2011). Conversely, there is little difference in VHA between intestinal segments (Esbaugh and Cutler, 2016; Guffey et al., 2011) while CA activity and expression shows varying patterns depending on species and isoform (Grosell et al., 2007; Sattin et al., 2010). Yet, despite the evidence that the anterior intestine is generally more absorptive, isolated tissue preparations using similar conditions generally show comparable absorptive capacities between anterior and posterior regions, both with respect to bicarbonate secretion rates (Grosell, 2006) and absorptive $I_{sc}$ (Esbaugh and Cutler, 2016; Martin and Esbaugh, 2021). The reason for this is not immediately clear, but likely resides in the fact that the luminal fluid of the anterior and posterior intestines is quite different, with the posterior having lower sodium chloride concentrations and much higher bicarbonate, and as such the difference in transporter abundance may be tailored to luminal fluid conditions the tissue is likely to experience (Grosell, 2006).

### 3.2. The gills and operculum

Marine fish drinking rates in normal strength seawater typically range from 2 to 3 ml $kg^{-1}$ $h^{-1}$. This equates to a combined sodium chloride absorption rate of approximately 1.6–2.4 μmol $kg^{-1}$ $h^{-1}$, all of which must be excreted into the environment along with any additional diffusive ion gain to maintain net salt and water balance. Further, ion excretion must be performed against a strong inward diffusive gradient as the concentration of sodium and chloride are approximately 3× higher in normal strength seawater than the plasma of marine teleosts. How fish accomplish this feat has been the continued subject of research for over 90 years (Smith, 1930), and centers around a population of "chloride cell" ionocytes found on the gill and opercular epithelium

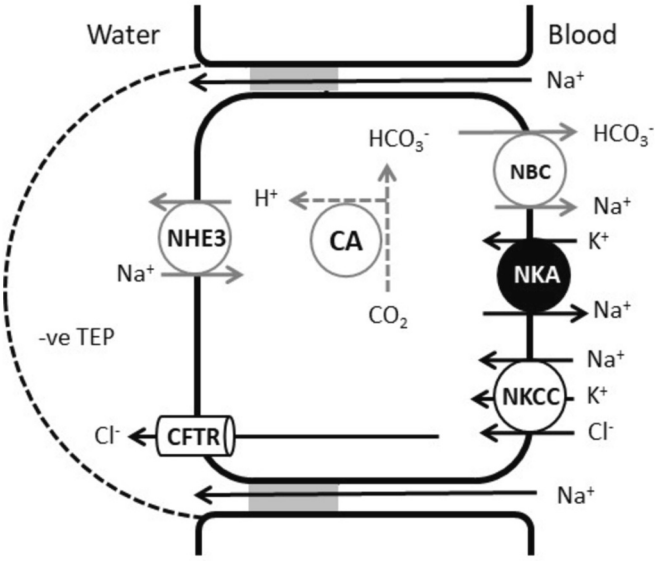


**Fig. 3.** Illustration denoting the role of branchial ionocytes in salt (black) and acid (gray) excretion in marine fishes. Transcellular chloride excretion is mediated by NKA, NKCC1 and CFTR, which generates a negative outside potential (TEP) associated with the ionocyte apical crypt, as shown by the dotted semicircle. This TEP powers paracellular sodium excretion. Acid excretion occurs via apical sodium proton exchange (NHE3). The excreted proton is produced endogenously by cytoplasmic carbonic anhydrase (CA) with the bicarbonate re-entering the plasma via sodium bicarbonate co-transport (NBC). For further details see Section 3b. Abbreviations: NKCC, sodium potassium two chloride co-transporter 1; NKA, sodium potassium ATPase; CFTR, cystic fibrosis transmembrane conductance regulator channel.

(Fig. 3) – or skin of larval fish. In marine teleosts, these cells are characterized by an extensive basolateral tubular network, a high mitochondrial density, and an apical crypt that defines the cell to water interface. The basic mechanism is well established (see reviews Evans et al., 2005; Marshall, 2002; Marshall, 2012; Marshall and Grosell, 2006) and starts with basolateral NKA activity that is responsible for maintaining a negative intracellular potential and low intracellular sodium concentration – often cited as approximately 15 mM (e.g. Marshall, 2012). The low sodium concentration allows basolateral electroneutral NKCC1 to move chloride into the cell in relatively high concentrations, which combines with the negative intracellular potential to power chloride excretion through the apical CFTR chloride channel. Importantly, the excretion of chloride ions creates an outside negative potential relative to the plasma (i.e. transepithelial potential; TEP), which drives sodium excretion through paracellular pathways. Measurements of the trans-body potential as a proxy for gill TEP have almost universally reinforced this model as the trans-body potential exceeds the reversal potential of sodium ($E_{Na}$), although some notable exceptions including Gulf toadfish and seahorse (*Hippocampus erectus*) exist (reviewed by Marshall, 2012).

While the underlying basis of this model has been understood for nearly half a century, there continue to be new advances; particularly with respect to the functional characteristics of paracellular transport. The paracellular pathway of marine ionocytes consists of intercellular cation-specific tight junctions, and characteristics of cation-specificity appear to be related to the relative expression of claudin (cldn) tight junction proteins. Specifically, cldn-10 isoforms are highly expressed in marine fish gills, and exhibit isoform-specific expression and distribution patterns as a consequence of hyperosmotic salinity transfer (Bui and Kelly, 2014; Kolosov et al., 2013; Marshall et al., 2018; Tipsmark et al., 2008). The particular characteristics and composition of cldn-10 isoforms have been suggested to confer increased paracellular conductance of sodium and potassium as compared to other monovalent cations

(Marshall et al., 2018).

### 3.3. The renal system

Seawater fish generally have very low glomerular filtration rates, and in fact several marine species have lost the glomerulus completely (see review Beyenbach, 2004). This is largely due to the fact that fish kidneys lack a loop of Henle and cannot produce hyperosmotic urine, meaning that renal ion excretion will also result in water loss. However, the renal system still plays an important role in ion balance in seawater fishes by regulating plasma magnesium and sulfate, neither of which can be effectively excreted by the gills. Magnesium and sulfate are generally impermeable across the intestinal epithelium (Grosell, 2011); however, both gain entry in marine fishes via passive diffusive uptake. Urine flow rates in marine fishes tend to be very low with published values ranging from 30 to 440 μl $kg^{-1}$ $h^{-1}$, and the urine composition tends to be highest in magnesium (80–152 mM), sulfate (42–116 mM) and chloride (74–132 mM) (Beyenbach, 2004). It is also worthwhile to note that the urine is the major site of calcium efflux in marine fishes, accounting for approximately two thirds of total excretion (4.2 vs. 6.2 μmol $kg^{-1}$ $h^{-1}$ in *Gadus morhea*)(Bjornsson and Nilsson, 1985). But the overall calcium efflux rates are very low relative to magnesium and sulfate.

The mechanisms by which magnesium and sulfate are eliminated via the urine remain unresolved, but current evidence suggests that it primarily takes place through secretion by the proximal tubule (Beyenbach, 2004; Kato et al., 2022). Obviously, secretion is a requirement for any aglomerular species. The models of sulfate secretion have implicated a pair of anion exchange transporters, slc26a6 and slc26a1. While anion exchangers are commonly considered chloride bicarbonate exchangers in the context of fish ion transport, slc26a6 and slc26a1 have both been shown to mediate sulfate transport, with the former performing chloride sulfate exchange and the latter showing bicarbonate sulfate exchange. Work from pufferfish and Japanese eel has demonstrated slc26a6 is present in the apical membrane of the proximal tubule, and also increases in expression following transfer from freshwater to seawater salinities (Kato et al., 2009; Watanabe and Takei, 2011). In contrast, the slc26a1 transporter localizes to the basolateral membrane (Nakada et al., 2005; Watanabe and Takei, 2011). This pairing leads to a model whereby sulfate enters the proximal tubule cells through basolateral slc26a1 in exchange for intracellular bicarbonate – presumably formed by CA – and is subsequently transported into the lumen in exchange for chloride. This would require sulfate transport to be paired with tubular chloride secretion, which is evident from the high urine chloride concentrations.

Much less is known about magnesium secretion; however, it does appear to occur via apical exocytosis as the proximal tubules of seawater fish have been shown to have magnesium rich vesicles (see reviews Kato et al., 2022; Takvam et al., 2021). Immunohistochemistry has also localized slc41a1 – a sodium magnesium exchanger in mammals – to these vesicles (Islam et al., 2013; Kodzhahinchev et al., 2017), although it is not immediately clear how the transport stoichiometry of this protein could be leveraged to concentration magnesium within the vesicles. Furthermore, expression of the Cnnm family of purported magnesium transport proteins has also been demonstrated in the basolateral membranes of proximal tubules of pufferfishes, with the expression of Cnnm3 being up-regulated during seawater transfer (Islam et al., 2014). While the exact transport characteristics remain unknown, this represents an intriguing potential pathway for future study.

## 4. Physiological limitations of hypersalinity

When a fish is exposed to hypersaline waters, generally defined as ≥40 ppt (Anonymous, 1958), the osmotic gradient between their body fluid and the environment is increased and thus all of the processes described in Section 3 become more difficult. At the gill this is most easily demonstrated through the calculation of transepithelial sodium reversal potential ($E_{Na}$)(Fig. 4). Using the red drum as an example, normal seawater would require an $E_{Na}$ of +24 mV (blood side positive), while a hypersaline 40 ppt would require +32 mV. While species-specific, the upper salinity tolerance of seawater euryhaline fishes is generally considered to be around 50–60 ppt (Gonzalez, 2012; Schultz and McCormick, 2012), which would equate to an $E_{Na}$ of +38 to +43 mV, respectively. This would be lower in stenohaline seawater fishes and freshwater dominant euryhaline fishes. Actual determinations of TEP have been made in many species capable of surviving in hypersaline conditions (see Section 5), but it is challenging to directly attribute TEP limitations to setting tolerance thresholds in sensitive species because of uncertainty around intracellular ion concentrations. Here, I will instead attempt to derive energetic limitations on the basis of the energy available from ATP hydrolysis, using data from red drum as the basis for the calculations.

Hydrolysis of ATP releases approximately 63 kJ/mol of energy, which powers NKA and generates the driving force for NKCC-mediated transport of chloride into the branchial ionocyte. Importantly, the energy barrier on NKA function is defined by the intra- and extracellular concentrations of sodium, as well as the efficiency of energy utilization by NKA. In short, the energy from ATP will define the lower limit for intracellular sodium, assuming NKA activity itself is not limiting (see Section 5). The lower limit for intracellular sodium can then be used to predict the highest possible intracellular chloride concentration on the basis of NKCC1 stoichiometry; assuming a typical potassium intra- to extracellular ratio of 30:1 (Esbaugh and Cutler, 2016; Watson et al., 2014), which can also be used to estimate intracellular voltage (-87 mV). It is then possible to compare the maximum outward $E_{Cl}$ to the required TEP to overcome $E_{Na}$, described in Fig. 5 as the theoretical excess potential. What is immediately clear from this exercise is that there is plenty of excess energy from ATP hydrolysis for sodium chloride excretion in normal strength seawater. This will have implications later when discussing branchial plasticity (Section 5). It is also apparent that the upper salinity limit based on ATP constraints would reach as high as 61 ppt assuming 85 % energy conversion efficiency of NKA, which admittedly may be unrealistically high. At salinities above this point a fish would no longer be able to regulate plasma ion concentrations effectively. Coincidently, the estimates here match well with observed

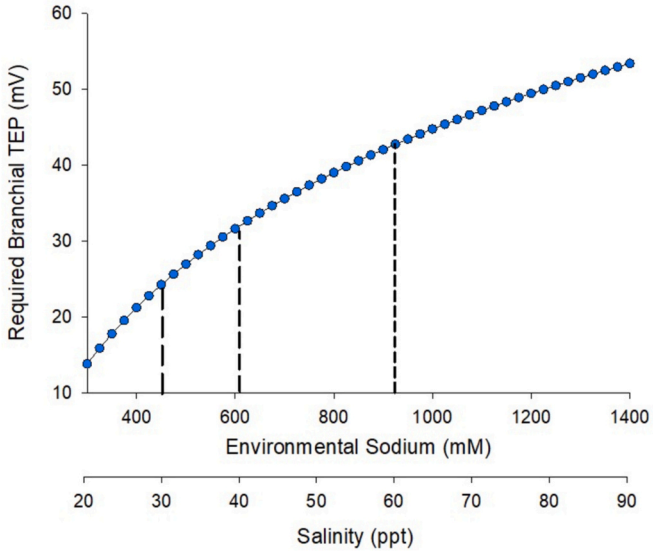


**Fig. 4.** An analysis of the branchial TEP (blood side positive) required to maintain sodium excretion across the gills at different salinities and sodium concentrations, assuming a constant 175 mM plasma sodium concentration. Note that plasma values were based on work from red drum, *Sciaenops ocellatus* (Esbaugh and Cutler, 2016). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

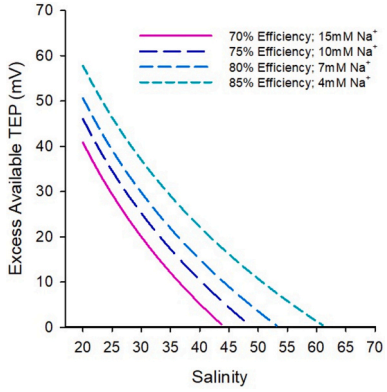


**Fig. 5.** An analysis of the energetic constraints on branchial ion excretion at elevated salinities. The data represent the theoretical excess transepithelial voltage (TEP) that can be generated from the hydrolysis of ATP. The excess available TEP is determined as the difference between the TEP required to maintain sodium excretion across salinities, and the maximum TEP possible given the energy available from ATP hydrolysis. Estimates are made on the basis that the energy from ATP and the efficiency with which NKA can use that energy will dictate the lowest possible intracellular sodium concentration, which subsequently affects NKCC1 function and chloride efflux through CFTR (see Section 4 for complete description). The different lines represent various levels of ATPase efficiency (i.e. ability to capture the energy from ATP hydrolysis) and the subsequent effect on intracellular sodium concentrations.

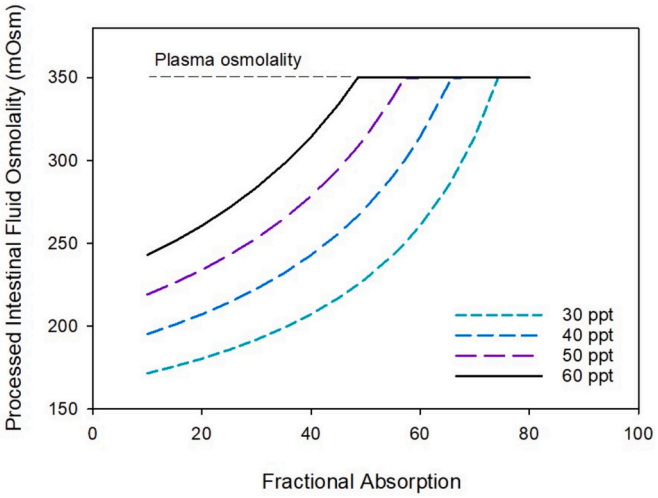


**Fig. 6.** An analysis of the impact of imbibed magnesium and sulfate and fractional absorption efficiency on intestinal fluid osmolality. As the salinity of imbibed water increases (denoted by the different lines) the maximum intestinal fluid osmolality is reached at lower fractional absorption values. This demonstrates that the efficiency of intestinal fluid absorption is constrained by impermeable magnesium and sulfate at higher salinities. Note that intestinal fluid osmolality cannot exceed that of the plasma.

salinity tolerances in seawater euryhaline teleosts following direct transfer methods (Schultz and McCormick, 2012). In fact, even in those fish that can tolerate salinities above 60 ppt, exposure is almost always accompanied by a significant rise in plasma osmolality (Brauner et al., 2012; Genz et al., 2011; Gonzalez, 2012; Nordlie, 1985; Sardella et al., 2007).

Another potential physiological limitation of hypersalinity occurs in the intestine. As mentioned in Section 3b, the fractional absorption of intestinal water absorption in marine fishes is variable, but has been shown to be as high as 85 % (Genz et al., 2008). Importantly, there are chemical constituents of seawater that will limit fractional absorption – specifically magnesium and sulfate – because they are functionally impermeable in the intestine. Thus, they remain in the lumen and as their concentration rises the ability to develop an absorptive osmotic water gradient will decline. This is demonstrated by observations that intestinal magnesium and sulfate becomes more concentrated as fluid is sampled from more distal regions of the intestinal tract. In fact, fluid sampled from the posterior intestine and rectum can contain a combined concentration of over 300 mM (300 mOsm equivalent) (McDonald and Grosell, 2006). Furthermore, an elegant series of experiments using "gut-sac" preparations from gulf toadfish demonstrated that a mucosal saline high in magnesium sulfate will exhibit less water transport to the serosal compartment than a sodium chloride saline of the same osmotic pressure (Genz et al., 2011). This means that fractional absorption efficiency has diminishing returns as the imbibed seawater increases in salinity. For example, Fig. 6 provides estimates of rectal fluid osmolality as a consequence of ingested seawater salinity and fractional absorption efficiency, assuming a consistent contribution of bicarbonate, chloride and calcium of 100 mM (McDonald and Grosell, 2006). While the merits of the latter assumption are debatable, this exercise is intended only to demonstrate the limitations of intestinal water absorption as a consequence of imbibed magnesium and sulfate. For example, in normal seawater (30 ppt), the fractional absorption efficiency would peak at about 75 %, which coincides nicely with literature observations (e.g. Genz et al., 2008; Hickman, 1968). However, as the salinity of imbibed seawater increases to 60 ppt, the maximum fractional absorption would drop to below 50 %, and this is likely an underestimate because higher salinity values also coincide with increased rectal chloride and bicarbonate concentrations (McDonald and Grosell, 2006). Put simply, higher salinities require fish to drink seawater at increasing rates both because of the greater osmotic gradient driving diffusive water loss across the gills, and because intestinal water absorption itself becomes less efficient due to impermeable magnesium and sulfate.

## 5. Organismal responses and phenotypic plasticity

In many ways, the responses of euryhaline marine fish exposed to hypersalinity can be predicted from the responses exhibited by euryhaline fishes that transition from hypo-osmotic to hyperosmotic environments (see review Marshall, 2012). The first response is to increase drinking rate, which has been routinely demonstrated to accompany hypersalinity exposure (Genz et al., 2008; Gonzalez et al., 2005; Sardella et al., 2004). Yet, the drinking response is often lower than what would be expected by the observed change in osmotic gradient. For example, gulf toadfish exhibit an increased drinking rate of approximately 50–60 % when exposed to 50 ppt (Genz et al., 2008), and sailfin mollies only elevated drinking by 35 % when exposed to 60 ppt (Gonzalez et al., 2005). This discrepancy may indicate a significant reduction in branchial water permeability coincident with hypersaline acclimation (Brauner et al., 2012; Gonzalez, 2012); however, the mechanisms by which this would be achieved remain unclear. The branchial epithelium of marine fish has paracellular tight junctions that limit paracellular water movement, and the expression of aquaporin proteins is low in seawater fish gills with no change in expression as a result of hypersalinity exposure (Deane and Woo, 2006; Lema et al., 2018). Moreover, it seems counterintuitive that marine fish would not always limit branchial water permeability to the largest extent possible, as it would serve to reduce the costs of osmoregulation. It is possible that the inferred reduction in branchial permeability is instead the product of reduced functional gill surface area. Euryhaline fish exposed to higher salinities have shown this response (Blair et al., 2017; Blair et al., 2016; Delompré et al., 2019), and at least one species (black-chinned tilapia; *Sarotherodon melanotheron*) exposed to true hypersaline conditions shows a similar response as compared to seawater (Ouattara et al., 2024). Importantly, any reduction in functional surface area would reduce oxygen uptake and carbon dioxide excretion, as described by the osmorespiratory compromise (see reviews Randall et al., 1972; Wood and Eom, 2021). As such, understanding the hypersalinity associated

decline in branchial water permeability is a clear area for future research as it has implications for how hypersalinity will interact with other environmental stressors common to the coastal environment (e.g. hypoxia and warming) (see Section 7).

The gastrointestinal tract also exhibits substantial plasticity when transitioning from normal seawater to hypersaline conditions, and again this appears to mirror the physiological changes exhibited by euryhaline fishes that transition from freshwater to seawater. The list of observed changes includes elevated NKA, VHA (Guffey et al., 2011) and cytoplasmic CA activity (Sattin et al., 2010), as well as elevated NKCC2 (Martin and Esbaugh, 2021) and NBC transporter expression (Taylor et al., 2010); however, all of these responses differ by species and intestinal segment. Nonetheless, when the data are examined holistically, patterns begin to emerge. The first notable observation is that despite transcriptional and protein level responses, the ability of isolated tissue to elevate bicarbonate secretion following acclimation is inconsistent. For example, toadfish exhibit elevated CA activity in all portions of the intestine, increased VHA in the posterior intestine, and elevated NBC transcript abundance in the mid-intestine, yet pH-stat experiments yielded no effect on base section as well as a significant increase in base secretion (Guffey et al., 2011). Experiments on *Fundulus heteroclitus* yielded no significant effects of 70 ppt acclimation on base secretion from the anterior intestine (Genz and Grosell, 2011). In contrast, elevated NKA activity is a relatively consistent response following hypersaline acclimation, and in at least one species (red drum) this was paired with elevated NKCC2 expression that resulted in a dramatic increase in the absorptive short circuit current in the posterior intestine (Martin and Esbaugh, 2021). This also agrees with rectal collection sac studies from toadfish that demonstrated that while rectal base excretion did not increase between 35 and 50 ppt acclimations, there was a significant increase in sodium and chloride ion absorption (Genz et al., 2008). A third generalizable observation is that the posterior intestine appears to exhibit greater plasticity than the anterior intestine (e.g. Cao et al., 2022; Guffey et al., 2011; Laverty and Skadhauge, 2012; Martin and Esbaugh, 2021). This is surprising as the anterior intestine is thought to be responsible for a much greater amount of ion and water absorption (Grosell, 2006), and because the anterior intestine generally exhibits significant plasticity when fishes transition from freshwater to seawater (Cutler and Cramb, 2008; Esbaugh and Cutler, 2016; Grosell et al., 2007; Martinez et al., 2005). It seems likely that the significant plasticity that occurs in the posterior intestine is intended to increase the absorptive capacity along the entire length of the GI tract. Finally, it is also noteworthy that the plasticity exhibited by the intestine following hypersalinity acclimation does not appear to extend to the esophagus (Esbaugh and Grosell, 2014), likely owing to the large contribution of passive transport processes in this tissue.

There has been substantial work documenting branchial plasticity following exposure to hypersaline habitats, particularly in relation to extremophile species, such as the Mozambique and California-hybrid tilapia (Kültz et al., 1995; Kültz et al., 2013; Kültz and Onken, 1993; Sardella et al., 2007; Sardella et al., 2004; Uchida et al., 2000). In general terms, hypersalinity acclimation coincides with an increase in NKA, NKCC and CFTR abundance (e.g. Gonzalez et al., 2005; Karnaky Jr et al., 1976; Martin and Esbaugh, 2021; Wilson et al., 2007), as well as a suite of mitochondrial proteins (Kültz et al., 2013) with the likely intent of increasing the branchial TEP and thus enhancing sodium and chloride excretion. Furthermore, recent work in killifish has demonstrated that hypersalinity acclimation results in an increased prevalence of a punctate CFTR distribution, which is indicative of additional accessory cell attachment points to the ionocyte apical crypt (i.e. increase paracellular junctions) (Cozzi et al., 2015). Opercular membranes have also been shown to increase in ionocyte density with exposure to hypersaline conditions (Kültz and Onken, 1993), although this observation did not extend to the gills where mature ionocyte density was unaffected by hypersaline acclimation (Sardella et al., 2004). Despite all of these observed responses, it is interesting to note that when tested, isolated opercular membranes often do not show significant improvements in their ability to generate a driving force for salt excretion. For example, the opercular membrane of Mozambique tilapia acclimated to a range of salinities showed no difference in short circuit current between 35 and 60 ppt, and in fact showed a significant decrease in current when normalized to ionocyte density (Kültz and Onken, 1993). Similarly, open circuit measurements of TEP from killifish opercular membranes showed no difference between animals acclimated to normal seawater and 2× seawater salinity (Cozzi et al., 2015). In both of these cases, the leak conductance – indicative of the paracellular shunt pathway – was also significantly reduced in hypersalinity (Cozzi et al., 2015; Kültz and Onken, 1993). It is important to note that the electrical measurements of whole epithelia may not reflect the exact properties associated with an ionocyte complex. However, if the above results are taken at face value, it appears that the observed changes in ionocyte protein abundance may simply be an indication of osmotic stress as opposed to a physiological adaptation that promotes survivability. In support of this notion, analysis of the gill proteome in Mozambique tilapia following hypersalinity exposure (70 and 90 ppt) specifically highlighted up-regulation of NRDG1, which is a multifunctional stress response protein (Kültz et al., 2013).

## 6. Metabolic costs

The metabolic cost of osmoregulation has been a topic of extensive research and discussion in the literature (see review Ern et al., 2014). Despite this effort, the available estimates show substantial variability ranging from ≤5 to ≥30 % of standard metabolic rate (SMR), with SMR denoting the cumulative cost of an animal's vital functions excluding activity, digestion and reproduction. Much of this variability likely stems from the different technical approaches that include energetic modeling, isolated tissue measurements, and whole-body measurements. The latter is without doubt the most commonly applied approach, and assumes that the cost of osmoregulation can be calculated as the differences in SMR of fish acclimated to isosmotic conditions relative to the treatment salinity. Unfortunately, this approach has been shown to be suspect with the isosmotic salinities often not showing the lowest SMR values (e.g. Ern and Esbaugh, 2018; Ern et al., 2014). The modeling approach has been applied sparingly (Kirschner, 1993; Little et al., 2023), but is grounded in well-established mechanistic principles and thus appears to provide reasonable estimates. These methods use chloride uptake rates in the gastrointestinal tract and subsequent branchial excretion requirements to estimate ATP utilization, and provide estimates of 7.1 to 7.5 % for three different seawater acclimated species. Interesting, the available data from isolated tissue preparations of gills, esophagus and intestines seems to provide comparable values for seawater fishes (Little et al., 2023; Morgan and Iwama, 1999). Nonetheless, it is important to note that these remain imperfect estimates as neither incorporates renal magnesium and sulfate excretion, and assumptions around the active and passive contributions to esophageal desalination can be debated.

The more relevant question for this review is how hypersalinity exposure will impact the metabolism of fishes. From the perspective of osmoregulation, the cost will scale as a consequence of increased drinking rate, elevated ion concentrations of imbibed water and higher subsequent ion excretion requirements. The modeling approach described above for toadfish (Little et al., 2023) can be altered to incorporate a 60 % increase in drinking rate (Genz et al., 2008) and an imbibed seawater salinity of 60 ppt to provide the estimated costs of esophageal desalination and intestinal ion absorption under hypersalinity (Table 1). These conditions increase the relative cost to 5.4 and 2.2 % of SMR, respectively. Note that these estimates assume that the active component of esophageal desalination accounts for 50 % of chloride transport (Little et al., 2023). The approach of Kirschner (1993) (i.e. 1 ATP per 6 NaCl equivalents) can also be applied to the gill on the basis of the total absorbed chloride, which results in a relative cost of

**Table 1**
Theoretical energetic modeling for osmoregulation on basis of chloride transport across the esophagus, intestine and branchial epithelium as a percentage of standard metabolic rate (SMR).

| | Cl- Transported (μmol/kg/h) | Fraction (%) | ATP:Cl- | ATP consumed (μmol/kg/h) | O2 consumed (μmol/kg/h) | SMR (%) |
|---|---|---|---|---|---|---|
| Esophageal Desalination | | | | | | |
| Active | 1364 | 50 | 0.33 | 225.1 | 45.01 | 5.4 |
| Passive | 1364 | 50 | 0 | 0 | 0 | 0 |
| Intestinal Absorption | | | | | | |
| NBC mediated | 27.2 | 2.7 | 0.22 | 6.0 | 1.2 | 0.07 |
| NHE mediated | 24.2 | 2.4 | 0.66 | 16.0 | 3.2 | 0.19 |
| VHA mediated | 3.0 | 0.3 | 0.5 | 1.5 | 0.3 | 0.01 |
| NKCC | 954.5 | 94.6 | 0.17 | 132.3 | 32.5 | 1.93 |
| Gill Excretion | | | | | | |
| NKA | 3737 | 100 | 0.17 | 622.8 | 124.6 | 7.4 |
| *Total* | | | | | | 15.0 |

All values based on a drinking rate of 4 ml/kg/h, a seawater chloride concentration of 950 mM, rectal chloride concentration of 63 μmol/kg/h, and a standard metabolic rate of 52 $mgO_2$/kg/h.

7.4 % of SMR. Overall, this would predict that osmoregulation accounts of 15 % of SMR at 60 ppt. While there are not many measures of SMR under hypersaline conditions, those that do exist either tend to show no clear difference from normal seawater (Haney and Nordlie, 1997; Jordan et al., 1993; Nordlie et al., 1991), or trend toward a decline in SMR at hypersalinity (Ern and Esbaugh, 2018; Haney et al., 1999; Sardella and Brauner, 2008; Swanson, 1998). While it has been argued that the variance around SMR determinations would make detecting costs of osmoregulation difficult, a 7.5 % increase in metabolic rate should be easily detectable via intermittent flow respirometry using routine sample sizes (e.g. 10–12) when accounting for published standard deviation values (e.g. Zambie et al., 2024). These findings may suggest a hidden cost to hypersalinity exposure whereby fish may sacrifice other aspects of their basal metabolism. Alternatively, they may improve aerobic efficiency by reducing mitochondrial proton leak, which has been documented following hypoxia acclimation (Ackerly et al., 2023) and implicated in metabolic suppression following warming acclimation (Zambie et al., 2024). In either case, it seems clear that more study is warranted in this area.

## 7. Interactions with other stressors

As mentioned in Section 2, marine hypersalinity often occurs in shallow coastal ecosystems, many of which can be otherwise productive habitats (e.g. seagrass beds). This raises the very real possibility that hypersalinity would coexist with swings in temperature and oxygen on daily and seasonal cycles. The risk of hypoxia would be furthered because high salinity and high temperature water holds less oxygen. Such multi-stressor exposures could act to exacerbate the effects of hypersalinity – or the effects of warming/hypoxia – on exposed animals as outlined by the osmorespiratory compromise. The osmorespiratory compromise describes how the morphological characteristics and physiological roles of the gills creates a conflict between osmoregulation and respiration (Randall et al., 1972). In short, a greater gill surface area, reduced diffusion distance and increased convection (e.g. ventilation rates) would benefit oxygen uptake, but also result in greater diffusive water and ion movement. While this basic pattern holds true for many freshwater fishes, the evidence from seawater fishes is less conclusive with respect to the significance of the compromise (Gonzalez, 2011). Supportive evidence includes work from coho salmon acclimated to 30 ppt that exhibited large increases in plasma osmolality and sodium concentration following acute hypoxia exposure (Damsgaard et al., 2020). Interestingly, lower salinities did not demonstrate such effects. In contrast, no effects on plasma osmolality following acute hypoxia were observed in red drum acclimated to 30 ppt (Ern and Esbaugh, 2018). But it is also important to note that some fish, particularly hypoxia-tolerant species, appear to have the capacity to decouple respiratory gas and water movement by selectively augmenting water permeability (Giacomin et al., 2020; Ruhr et al., 2020; Wood and Eom, 2021). Again, this has mostly been observed in freshwater habitats, including in freshwater killifish and oscar (Wood and Eom, 2021). But work in seawater acclimated killifish has demonstrated a significant reduction in drinking rate during acute hypoxia exposure with no elevation in plasma osmolality (Giacomin et al., 2020). This suggests that hypoxia-induced hyperventilation did not result in significantly elevated branchial water loss, and in fact, there was likely a significant reduction in branchial water loss. Regardless, there has been very little effort to explore the significance of the osmorespiratory compromise in hypersaline habitats that have salinities significantly higher than typical seawater, either with respect to the impacts on osmoregulation or hypoxia tolerance. It seems likely that outcomes from the osmorespiratory compromise observed in normal seawater would be magnified in hypersalinity.

Another possible cumulative stressor is carbon dioxide, which also can vary on daily and seasonal cycles. Even small increases in ambient $CO_2$ can interfere with normal $CO_2$ excretion and result in respiratory acid-base disturbances that must be corrected by excreting protons into the environment (e.g. Esbaugh et al., 2016; Esbaugh et al., 2012). In marine fish, acid excretion is performed by branchial ionocytes via apical sodium hydrogen exchangers (NHE) (Evans et al., 2005; Marshall and Grosell, 2006). These electroneutral transporters allow the ionocyte to leverage the large inward gradient for sodium to excrete protons without directly using ATP. Because hypersalinity would only increase the sodium gradient, there is no concern that hypersalinity would impact the ability of fish to compensate for respiratory acid-base disturbances. However, it is interesting to note that activation of apical NHE could raise the intracellular sodium concentration of ionocytes, and since marine fish have only one ionocyte type that performs both ionoregulatory and acid-base functions (Allmon and Esbaugh, 2017; Lonthair et al., 2020), this could have negative consequences for transcellular chloride excretion. Albeit any constraints could be overcome by increased NKA activity, but this would raise the cost of osmoregulation. While the cumulative effects of hypersalinity and hypercapnia seem unlikely to materially affect most fishes, it is possible that early life stage fishes are an exception. This life stage is characterized by high surface to volume ratios that promote greater osmotic water movement, and during the yolk sac stage are subsisting on endogenous energy reserves. It would not be altogether unsurprising if hypercapnia reduced hypersalinity tolerance in these life stages, both with respect to survival and growth.

## 8. Concluding thoughts

This review has taken a different approach to the topic of hypersalinity, whereas many previous works have emphasized the unique extremophile physiological traits of fish species that can survive at inordinately high salinities (Brauner et al., 2012; Gonzalez, 2012; Gonzalez et al., 2005), I have instead chosen to explore hypersalinity through the lens of an emerging coastal stressor that is being exacerbated by climate change and human activity (i.e. evaporation, riverine diversion, desalination effluent). One surprising finding from this work is that the true physiological limitations that govern upper salinity thresholds of most species remain opaque, although it seems likely that they are species specific. For example, analysis performed here would suggest that branchial and intestinal constraints may play a major role for typical seawater euryhaline species that can survive in hyperosmotic environments up to 60 ppt. The consistent evidence of osmoregulatory plasticity in seawater euryhaline species may also lead to the inference that a lack of osmoregulatory plasticity is itself the root cause of salinity limits in stenohaline species. In these cases, a rigid physiological phenotype programmed to a specific salinity could experience physiological constraints even when challenged with small increases in environmental salinity. Yet, some euryhaline species exhibit upper salinity thresholds of 20 ppt (i.e. "freshwater" euryhalinity). It seems unlikely that serious osmoregulatory constraints in the gills or intestines would be imposed at this salinity level, which raises a question of whether constraints on renal magnesium sulfate clearance impose serious challenges on a subset of species.

Finally, it is important to consider that many of the data reviewed in this paper used hypersalinity as an experimental treatment to help understand foundational physiological processes, and while these studies have no doubt provided enormous value to osmoregulatory physiologists, hypersalinity is also a burgeoning real-world environmental stressor with lethal and sub-lethal consequences. This is particularly noteworthy given the findings from modeling efforts of the cost of osmoregulation in hypersalinity, which give rise to the possibility that hypersaline acclimated fishes are undertaking metabolic suppression. As such, greater attempts to explore the sub-lethal effects of chronic hypersalinity exposure on measures of ecological performance (e.g. metabolism, growth, activity and reproduction) should be undertaken.

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## CRediT authorship contribution statement

**Andrew J. Esbaugh:** Writing – review & editing, Writing – original draft, Visualization, Funding acquisition, Formal analysis, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Data availability

No data was used for the research described in the article.

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