



Osmoregulation in fish Mechanisms and clinical implications

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According to the available fossil record, the earliest vertebrates originated in Pre-Cambrian seas roughly 550 million years ago. Presently, the majority of all living vertebrate species still dwell in aquatic environments. Water in its myriad forms covers approximately 73% of the Earth's surface. Aquatic habitat "osmoversity" ranges from hypersaline, desert pools (128 ppt = quadruple seawater salinity) to the nearly ion-free waters of many rainforest streams and pools. Fish species have adapted to living in both of these osmotic extremes as well as a vast range of salinities in between. Because of these and other extraordinary adaptations, the current global fish fauna is comprised of some 25,000+ described species, representing the most abundant and diverse group of vertebrates on the planet.

Life in an aqueous medium poses many challenges for fishes while simultaneously offering endless opportunities for the exploitation of new niches. Arguably, the greatest challenge to the fishes is the maintenance of water and electrolyte homeostasis in the face of a broad (and sometimes rapidly changing) array of salinities. Furthermore, fish possess comparatively thin, semipermeable gill epithelia designed for the efficient transfer of gases. Living cells, however, require a stable, unchanging osmotic environment for normal function. To maintain solute concentrations within limits compatible with life, fish have developed remarkable strategies for osmoregulation.

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Maintaining water and electrolyte homeostasis often requires the expenditure of considerable energy to overcome the osmotic gradients that span their delicate gill tissues.

Because marine teleosts (=bony fishes) reside in an environment that is hyperosmotic (1000 mOsm/kg) compared to their body fluids (250–500 mOsm/kg), they face the chronic challenges of dehydration (hypovolemia) and salt loading (hypernatremia, hyperchloremia) [1]. On the other hand, freshwater teleosts live in an environment that is hypoosmotic (<1 mM) with respect to their body fluids (150 mM), and must confront the challenges of volume loading (hypervolemia) and salt depletion (hyponatremia, hypochloremia). Marine elasmobranchs (sharks, rays and skates), however, face salt loading and hypervolemia. Their total body fluid concentrations are hyperosmotic to seawater primarily due to the presence of high levels of urea and other nitrogenous compounds. However, their plasma NaCl concentrations alone are only about 50% of that of seawater [2]. In the final analysis, freshwater and marine bony fishes as well as marine elasmobranchs all employ different strategies for the regulation of internal water and solute homeostasis. The hormonal control of fluid and electrolyte balance differs between these three groups of fishes as well. A basic understanding of their respective osmoregulatory physiologies, therefore, is important in guiding the clinician towards safe and effective approaches to fluid and electrolyte support.

Marine teleosts

Extrarenal mechanisms

Morphologically, teleosts are well adapted for ion exchange using active and passive mechanisms across various surface membranes to achieve proper osmotic balance and hydration status. Unlike mammals, the skin of most teleosts is scaly, with a protective mucus coating that essentially makes it impermeable and, thus, a very minor site of water flux. In fact, the gills are the primary organs of water efflux because they are vascular, exposed to the environment, and thin walled to facilitate efficient gas exchange. Absorption and excretion of ions between the environment and body fluid of marine teleosts occurs via the gills and the gastrointestinal and urinary tracts. The primary goal of all osmotic mechanisms in marine teleosts is to gain water in body fluids through the active uptake of NaCl. Because of the three- to four-fold difference between teleost body fluids and seawater, marine bony fish must drink saltwater and perform a series of ion exchanges to keep body fluids more dilute. Drinking rate varies with salinity levels [1]. A “typical” marine teleost will drink on the order of 10–20% of their body weight per day, with the ability to drink up to 35–40% if the salinity is high [1]. For an example, a fish weighing 500 g will consume around 100 mL of saltwater in a day.

The major ions involved in osmoregulation are the monovalent ions, sodium (Na^+), chloride (Cl^-), and potassium (K^+), and the divalent ions, magnesium (Mg^{2+}), and sulfate (SO_4^{2-}) (Fig. 1). Table 1 provides a summary of the movement of these ions in marine teleosts. The esophagus cannot absorb water but is permeable to Na^+ and Cl^- , whereas the intestinal wall is permeable to both water and monovalent ions. Sodium and chloride move from the esophagus and gut lumen into the body fluids by diffusion down a concentration gradient, as well as via Na^+ - K^+ ATPase-driven basal membrane pumps and a Na^+ - K^+ - 2Cl^- cotransport system present in the apical brush border membrane [3]. Sixty to 80% of saltwater components that the fish drinks will be absorbed by the esophagus and intestine [2]. Excess Na^+ , Cl^- , and K^+ that are absorbed by the gut are excreted by specialized, mitochondria-rich cells called chloride cells. Chloride cells are present in the gills of all marine teleosts and along the operculum and the skin of the head of many species. Ion transport mechanisms found in chloride cells include active transport and cotransport as well as passive diffusion [4]. Only about 20% of divalent ions (mostly Mg^{2+} and SO_4^{2-}) ingested in seawater are absorbed and subsequently excreted by the kidneys into the urine; approximately 80% are excreted in the feces [5]. Daily urine flow rates in marine teleosts are minimal at 1–2% of body weight because water is highly conserved and can be reabsorbed by the urinary bladder [6]. Overall,

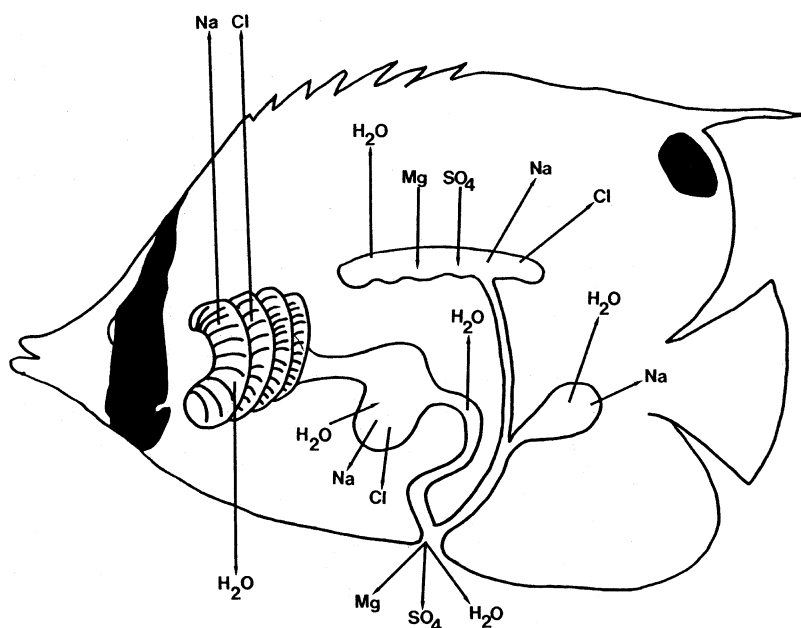


Fig. 1. Electrolyte fluxes in a typical marine fish. (From Stoskopf MK. Clinical physiology. In: Stoskopf MK, editor. Fish medicine. Philadelphia: W.B. Saunders; 1993. p. 48–61.)

Table 1

A summary of where major ions and water are gained or lost to maintain homeostasis and water balance

Major Ion	Absorption	Excretion	Comments
Na ⁺	MT: esophagus, stomach, intestine, kidney, bladder	MT: gills, kidney	MT: Excretion in gills occurs via specialized cells called chloride cells.
	ME: gills, intestine	ME: rectal gland, kidney, gills (minor)	ME: Gills are primary site of uptake; rectal gland is primary site of excretion.
	FT: gills, intestine, kidney, bladder	FT: some loss in feces and urine but generally well conserved	FT: Gill absorption linked to proton pump. Prolactin stimulates Na ⁺ uptake in the urinary bladder.
Cl ⁻	MT: esophagus, stomach, intestine, kidney, bladder	MT: gills, kidney	MT: Cl ⁻ passively follows active absorption/excretion of Na ⁺ .
	ME: gills, intestine	ME: rectal gland, kidney, gills (minor)	ME: Gills are primary site of uptake; rectal gland is primary site of excretion.
	FT: gills, intestine, kidney, bladder	FT: some loss in feces and urine but generally well conserved	FT: Prolactin stimulates co-transport mediated uptake of Cl ⁻ from the urinary bladder.
K ⁺	MT: intestine ME: presumably intestine	MT: gills ???	
	FT: intestine	FT: some loss in feces and urine but generally well conserved	
Mg ²⁺	MT: intestine	MT: intestine, kidney	MT: 20% of divalent ions are absorbed and eliminated in urine; 80% are excreted in the feces.
	ME: intestine FT: intestine	ME: intestine, kidney FT: some loss in feces and urine but generally well conserved	
SO ₄ ²⁻	MT: intestine	MT: intestine, kidney	MT: See MT for Mg ²⁺ above.
	ME: intestine FT: intestine	ME: intestine, kidney FT: some loss in feces and urine but generally well conserved	

Table 1 (continued)

Major Ion	Absorption	Excretion	Comments
H ₂ O	MT: stomach, intestine, kidney, bladder	MT: gills, intestine, kidney	MT: Major site of water loss is gills; additional water loss occurs in feces and urine with an overall net gain of water.
	ME: gills, intestine FT: gills, skin (minor)	ME: kidney FT: kidney	FT: FW teleosts produce copious quantities of dilute urine to counter hypervolemia from passive diffusion of H ₂ O through gills and skin.

Abbreviations: MT, marine teleosts; ME, marine elasmobranchs; FT, freshwater teleosts.

sodium and chloride absorbed by the kidney and intestine are excreted from the gills with a net gain in total body water.

Cortisol likely plays a significant role in natural regulation of ion and water balance by aiding excretion of Na⁺, increasing Na⁺-K⁺ ATPase activity in the gills, and enhancing water absorption in the intestine and urinary bladder. The drinking reflex in marine fishes appears to be controlled primarily by angiotensin II as it is in mammals [7]. Most of the available evidence to date suggests that the renin–angiotensin system is involved in maintaining blood pressure in marine fishes. It is believed that the primary sensory stimulus for the renin–angiotensin system in fishes is hypovolemia [8]. In brief, the current experimental evidence supports the hypothesis that the renin–angiotensin system in saltwater fishes serves to control blood pressure and volume by dipsogenesis, vasoconstriction, and antidiuresis, in the face of the dehydrating effects of the marine environment [9]. All of the mechanisms of ion exchange and fluid balance in marine fishes are still not fully understood and remain a topic of investigation for many researchers. Interested readers are referred to Karnaky for a detailed and current review of the topic [4].

Renallurinary bladder mechanisms

In teleost kidneys, two histologically distinct regions are present that each have different roles: the anterior, cranial, or head kidney and the caudal, tail, or posterior kidney. The anterior kidney is primarily a hematopoietic organ, as fish do not possess bone marrow. The posterior kidney functions in the more familiar roles of filtration and excretion and these roles will be the focus of the present discussion. Unlike the kidneys of mammals, teleost kidneys are not the principal organs of electrolyte balance. Nor are they divided

into distinct cortical and medullary regions like their mammalian analogs. Moreover, filtration by the kidney tissue is not as important in marine teleosts as it is in freshwater teleosts [10]. Histologically, fish kidneys are highly variable, and, overall, the marine fishes have smaller and fewer glomeruli than freshwater fishes [11]. The kidneys of marine fish are specialized for divalent ion excretion and have several unique anatomic features. Interestingly, the urinary bladder is an important component of ion and water balance for marine teleosts. Extensive reviews of renal morphology and function of teleost kidneys are available [4,10,12–14].

The nephron unit consists of a renal corpuscle (glomerulus and Bowman's capsule), the proximal tubule, distal tubule, collecting tubule, and collecting duct [11]. As opposed to freshwater teleosts that have a distinct distal segment of the nephron, marine teleosts lack this feature, and collecting tubules present distally open directly into the collecting duct. In saltwater fishes, the renal tubular system is highly permeable to water throughout its length [11] (Table 2). The glomeruli are concentrated posteriorly in the renal tissue and the tubular portions are positioned anteriorly, each with a separate blood supply. The renal artery leads to afferent arterioles that supply the glomeruli and proximal tubules; however, the distal and collecting tubules receive their blood supply from the renal portal vein. This vein comes from the caudal vein, which carries blood from the posterior portions of the body to the heart. This division of blood flow to either part of the kidneys means that any factors present in the arterial supply can exert effects on the glomeruli and proximal tubules (eg, vasodilation, permeability, ion exchange) without influencing the distal nephron or urinary bladder [14]. Such an arrangement allows for a more complex level of osmoregulatory control.

Although highly variable, glomerular filtration rate (GFR) in marine teleosts is generally about 0.5 mL/kg/h, and can be altered by changes in blood pressure, permeability of the epithelium, or perfusion of the nephron [13,14]. The resulting filtrate is similar to plasma minus the larger blood proteins such as globulins and lipoproteins. There is evidence that glomerular tubules do not just function in filtration but also actively secrete fluid using a NaCl-dependent mechanism [15]. An increase in GFR is the principal mechanism in the osmotic adjustment of euryhaline teleosts when transferred from saltwater to freshwater [13].

Table 2 shows the general pattern of ion absorption and secretion during urine formation in the marine teleost. Renal tubular activity determines urine flow rather than GFR in the marine fishes, and urine flow occurs because the kidneys secrete water with divalent ions and reabsorb water through sodium and chloride uptake [13]. In general, urine production is low at about 0.3 mL/kg/h, accounting for 5–10% of the rate of drinking [14]. Although the osmolality of the glomerular filtrate and of urine are similar (approximately 400 mOsm/kg), the composition is very different. Urine is low in organic compounds and monovalent ions and high in divalent ions compared to glomerular filtrate. Overall, monovalent ions are absorbed and

Table 2

The pattern of solute and water absorption and secretion/excretion during urine formation in fish

Solute	Absorption	Secretion/ Excretion	Comments
Na^+ , Cl^-	MT: proximal tubule, distal tubule, collecting tubule ME: proximal tubule segment I, distal tubule, collecting tubule FT: proximal tubule segment II, distal tubule, and collecting tubule		FT: Reabsorption of Na^+ and Cl^- in distal tubule not accompanied by H_2O .
K^+	MT: collecting tubule FT: proximal tubule II in conjunction with Na^+ and Cl^- absorption		MT: K^+ absorption occurs in conjunction with Na^+ and Cl^- absorption.
Mg^{2+} , SO_4^{2-}	FT: proximal tubule segment II	MT: proximal tubule ME: proximal tubule segments I + II	
H_2O	MT: proximal tubule, collecting duct ME: proximal tubule segments I + II, distal tubule, collecting tubule FT: proximal tubule segments I + II; (small proportion of filtered H_2O is actually absorbed)	MT: proximal tubule ME: proximal tubule segment II	MT: Solute-linked transport occurs in collecting tubules with an overall net gain of H_2O . ME: H_2O fluxes of up to 167% of body H_2O per hour have been reported. FT: Urine is dilute and produced in large volumes compared to MT or ME.
NH_3 , urea, creatinine, TMAO	ME: proximal tubule segment II	MT: proximal tubule	ME: Less than 15% of the filtered urea is excreted.
Glucose, amino acids, macromolecules	MT: proximal tubule ME: proximal tubule FT: proximal tubule		

Abbreviations: MT, marine teleosts; ME, marine elasmobranchs; FT, freshwater teleosts.

bring water with them (solute-linked water transport), and divalent ions are excreted. Residence time of urine in the bladder will affect extent of further absorption of monovalent ions and water by the bladder epithelium [4].

The urinary bladder is an important site of water conservation and active sodium reabsorption in marine teleosts. As an example, the bladder of the marine-adapted toadfish (*Opsanus tau*) reabsorbs 60% of urine produced by the kidneys, and the absorbate is primarily a NaCl solution isosmotic to plasma accounting for 10% of the total fluid absorbed by the gut [16]. Without bladder reabsorption, the fish would need to ingest 10% more seawater and subsequently manage the excess ionic load [16].

Certain species, such as anglerfish, seahorses, pipefish, and goosefish, have aglomerular kidneys that function to produce urine entirely by tubular secretion and reabsorption. In addition, these species receive renal blood supply entirely from the renal portal veins [10]. Other differences in renal physiology and osmoregulation are likely to exist among marine teleost families to provide adaptive strategies in different microenvironments.

Clinical implications

The most significant consequence of an imbalance in ion and water regulation in a marine teleost is that of dehydration [17]. Ironically, dehydration is difficult to assess in teleosts. Clinical signs suggestive of dehydration in fishes include anorexia, lethargy, hiding, darkened coloration, reduced or absent defecation, weight loss, enophthalmia, and tachypnea. Water retention is also possible, and may present as either edema or ascites. Generalized edema is classified as anasarca and is usually an end-stage clinical sign in fish.

Potential situations for osmoregulatory imbalance include suboptimal water quality, which can impede gill ionic exchange; direct trauma to gill tissue; trauma or disease, which lead to large epithelial wounds with resultant water loss; salinity changes or infections, which may effect dipsogenesis, metabolic activity, or absorptive ability. As in terrestrial animals, clinical management of injured or sick marine teleosts should involve appropriate fluid therapy.

Clinically relevant studies to determine choice and rate of fluid administration in saltwater fishes are scarce because of challenges relating to restraint, blood collection, analyses, and economics. Given the physiologic mechanisms of osmoregulation and water balance in marine teleosts, it follows that dehydration must be corrected quickly to prevent irreversible body fluid and organ compromise.

Marine teleosts drink saltwater to maintain body fluids that are more dilute than the environment. Stress, trauma, infections, parasitism, septicemia, suboptimal water quality, overstocking, food aversion, and improper nutrition can all lead to inappetance or anorexia and subsequent dehydra-

tion. Dehydrated saltwater fish are in need of H₂O, but they may not be able to drink enough or carry out the series of ion exchanges efficiently enough to meet their hydration demands. This is especially true if energy stores are low and ATPase pumps in the gills and intestines are impaired or less active.

Marine teleosts suspected of dehydration should be isolated and/or maintained in a system with optimal water quality and low stress. Darkening or covering the tank, reducing population density, and limiting adjacent noise or vibration levels can be helpful. By simply dropping the salinity by 50% (=16 ppt), the fish patient benefits from both decreased energy demands for active transport and cotransport processes and the prevention of infestations of some of the ectoparasitic protozoa, that is, *Cryptocaryon irritans*. Wounds and lacerations should be flushed with a dilute povidone-iodine solution or sterile saline, and either sutured, glued, or covered when indicated to prevent further fluid losses and or potential bacterial invasion [18,19]. A bandage can be applied using either a mixture of a triple antibiotic ointment (neomycin/polymixin/bacitracin) and Orabase Gel or Bio-Dres wound dressing cut to fit, and applied with tissue or ophthalmic polyacrylamide glue (Nexaband).

Thin fish, weaker species, or fish with prolonged inanition should receive alimentation therapy. A blended gruel of fish filets, fish-based cat food high in protein, and/or a high-quality flaked or pelleted fish food can be administered by gastric intubation, and will both provide calories and serve as a vehicle for administration of medications, supplements, and/or fluids. As a general guideline, 1 kcal/kg/day should be administered. Gastric volumes are extremely varied, but 1–3% of the body weight as a volume is a good starting point. Most fish clinicians would agree that clinically abnormal fish are subject to impaired immune function and, therefore, are good candidates for prophylactic broad-spectrum antibiotic therapy [18–21].

Parenteral instead of oral administration of therapeutics may be necessary if fish are critically injured or ill. Topical administration of drugs via short- or long-term immersion baths is possible in fish, and should be the route of choice if either handling or anesthesia represents life-threatening risks. In many cases, light sedation or anesthesia with MS-222 (50–100 mg/L) can improve restraint for injection of antibiotics or other medications with less overall stress [22,23]. Extremely ill fish should not be anesthetized, if possible, due to their compromised ability to fully metabolize anesthetics.

Fluid therapy options for fish are similar to those in other species. Intravenous fluid therapy, however, is difficult to perform in most fishes, especially smaller, more delicate marine tropicals. Indwelling intravenous catheters are not commonplace in fish [22]. In most cases, fluids must be administered either intracoelomically or orally. A rate of 1–3% of body weight is acceptable. As an example, a fish weighing 100 g should get 1.0–2.0 mL intracoelomically or up to 3.0 mL orally per day. The options of plasma or blood transfusion in fish should not be dismissed when indicated,

and can be performed in a manner similar to domestic animals. Oxyglobin may be a useful blood substitute if conspecific donors are either impractical or unavailable (N. Mylniczenko, personal communication).

Most fish can be tube fed quite easily using red rubber catheters or avian feeding tubes of appropriate lengths and sizes. Sedation may be needed for successful tube feeding of active or venomous species, although regurgitation is possible on recovery. Aspiration is not a risk in fish because they do not possess “airways”; nonetheless, care should be taken to keep gills free of regurgitant.

Hypotonic saline (0.45%), normal saline (0.9%), Lactated Ringer’s solution, or other balanced electrolyte solutions are all reasonable choices for body fluid replacement in saltwater fishes. As in any other species, solutions with more than 2.5% dextrose should not be given intracoelomically. A versatile choice would be 2.5% dextrose in 0.45% saline.

Cortisol has known effects on osmoregulation and water balance in marine teleosts. Administration of steroids may increase excretion of Na^+ and enhance water uptake by the intestines and urinary bladder. In this manner, short-acting steroid therapy (eg, methylprednisolone, dexamethasone sodium phosphate) may aid in clinical correction of dehydration and related problems. For example, a 10 mg/mL dexamethasone short-term bath is suggested for alleviating stress or treating shock, improving appetite, and reducing inflammation or edema and swelling in a variety of marine teleosts.

The primary signs of severe and/or long-term fluid overload are edema and ascites. The abdomen may appear distended, and scales may appear scalloped or lifted away from the normal body contour so as to give the fish a “pine cone” appearance. Exophthalmia may develop. Diuretics can be administered by injection or by immersion in a bath at appropriate dosages [24,25]. Glucocorticoids may be indicated if causes of fluid retention are related to inflammatory lesions.

Freshwater teleosts

Introduction

Because the plasma NaCl content of freshwater fishes is hypertonic compared to their environment (~150 mM versus <1 mM), these fishes are faced with potential overhydration (hypervolemia) and salt depletion. Given that their body fluids are hyperosmotic with respect to the surrounding water, there is a tendency for an influx of water across their thin, highly vascularized gill epithelium [26]. These unidirectional water fluxes can approach levels of 50% of the total body water per hour [2]. To compensate for this influx, freshwater teleosts drink very little, yet they excrete large volumes of dilute urine [1]. In contrast to the marine teleost kidney, the freshwater teleost kidney has the task of eliminating large quantities of water while

limiting NaCl loss. To accomplish this, high GFRs are coupled with an almost complete reabsorption of Na^+ and Cl^- ions [10] (Fig. 2).

Extrarenal mechanisms

Whole fish studies and in vitro perfused head and gill preparations have conclusively demonstrated that Cl^- absorption is facilitated by exchange with HCO_3^- . As with saltwater teleosts, this exchange is thought to occur vis-à-vis the mitochondrion-rich chloride cell [4]. Although it was long believed that Na^+ was taken up in exchange for H^+ (or NH_4^+) in an active transport process, recent studies using molecular techniques have shown that protons secreted by a H^+ -ATPase (a proton pump) located in the apical membrane of gill epithelial cells create a negative internal potential across the outer cell membrane [27]. This then serves to drive in Na^+ by a conductive channel. As seen in marine fishes, the chloride cells of freshwater teleosts are found in multicellular complexes. Whereas the tight junctions between chloride cells are quite shallow in marine fishes, these junctions are deeper in freshwater fishes [28]. This ultrastructural detail has significant consequences since the deeper the tight junctions run, the lower the transepithelial conductance. Consequently,

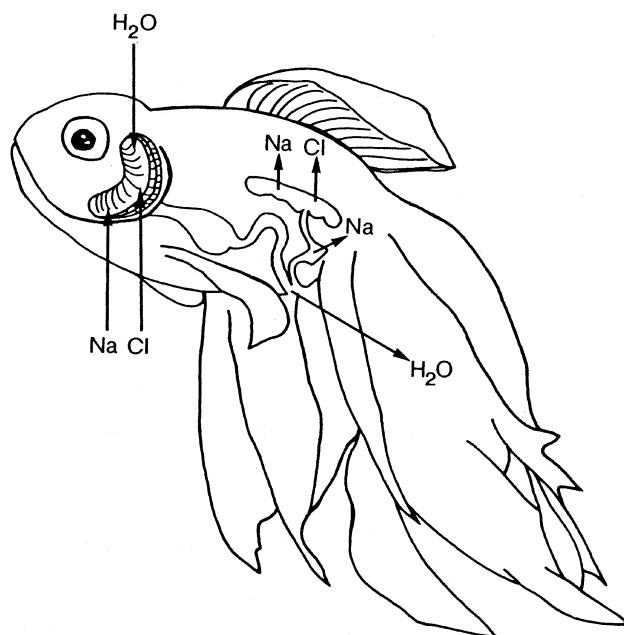


Fig. 2. Electrolyte fluxes in a typical freshwater fish. (From Stoskopf MK. Clinical physiology. In: Stoskopf MK, editor. Fish medicine. Philadelphia: W.B. Saunders; 1993. p. 48–61.)

freshwater teleost gills are much less “ion-permeable” than marine teleost gills [29]. Table 1 provides a summary of the movement of ions in freshwater teleosts.

Kidneys and urinary bladder

Freshwater teleost nephrons are characterized by having two proximal tubule segments, an intermediate segment, a well-developed distal tubule, and a collecting duct [11].

The freshwater teleost nephron is designed to counteract hypervolemia and salt depletion by producing copious volumes (2–6 mL/kg/h) of dilute urine [2]. Table 2 shows the general pattern of ion absorption and secretion during urine formation in a freshwater teleost. Almost all of the NaCl that is filtered by the glomerulus is reabsorbed in the distal tubule, collecting duct and urinary bladder, when present [6].

Arginine vasotocin (AVT) and prolactin are important modulators of osmoregulation in freshwater, where fishes are challenged with hypervolemia and salt depletion [9]. AVT causes vasoconstriction of both branchial and systemic blood vessels. Contrary to the antidiuretic response of tetrapod vertebrates to AVT, AVT typically produces diuresis in teleost fishes [30]. Pang [30] has suggested that the usual diuretic response to AVT in fishes is best explained by differential receptor sensitivity to AVT. In this model, highly sensitive systemic receptors overcome less sensitive, preglomerular receptors that normally produce antidiuretic vasoconstriction. In brief, the diuretic effect of AVT in freshwater teleosts is due to increased systemic pressures.

Prolactin is the dominant osmoregulatory hormone in freshwater fishes [31]. Prolactin reduces branchial salt (and possibly water) permeability, inhibits branchial salt extrusion, intestinal salt uptake, and urinary bladder water permeability, but stimulates urinary bladder Na⁺ uptake [32]. In many cases, the response to prolactin seems to be mediated by gradual, morphologic changes, and is therefore probably more important during chronic, rather than acute, osmotic stress [31].

Clinical implications

Needless to say, “freshwater” is a rather broad category. It encompasses biotopes such as the so-called “blackwater” rain forest streams characterized by low pH, low alkalinity, and low conductivity (=soft or mineral poor) as well as the high pH, high alkalinity, and high hardness characteristic of waters such as those found in the rift lakes of eastern Africa. To complicate matters further, there are numerous species of euryhaline or brackish fishes that inhabit waters with variable salinities such as estuaries, littoral zones, and salt marshes. The diverse nature of osmotic tolerances in freshwater

fishes does make fluid and electrolyte support less straightforward at times. Nonetheless, by researching a particular species' natural history, understanding the basic physiology of freshwater teleost osmoregulation, and following proven techniques of osmoregulatory stress mitigation, informed and effective treatment plans can be developed.

Fish under acute stress from tankmate aggression, injury, disease, handling or transport, produce catecholamines which then increase circulation to the gills to improve oxygen uptake. Simultaneously, the influx of water through the gills rises dramatically in freshwater teleosts, resulting in increased urine production to compensate for this sudden volume loading. This stress-related diuresis can quickly result in serious electrolyte imbalances due to the loss of chloride and other ions in the urine. Concurrently, hyperglycemia needed for a quick energy boost in a fight-or-flight situation is caused by catecholamine-mediated glycogenolysis of liver stores. If the stressful challenge persists, plasma cortisol levels increase and sustain the hyperglycemia via liver gluconeogenesis. The most immediate and life-threatening impact of acute stress, however, is no doubt related to ion depletion [33].

It has long been known that survival rates of handled and/or transported cold- or warmwater fish in aquaculture could be substantially increased by simply adding NaCl at 0.5–1.0% (5–10 g/L) to the tank water [34]. These levels of NaCl are nearly isotonic (0.5%) to hypertonic (1.0%) to blood. Among the ornamental species, levels of 0.5%+ NaCl may be appropriate for African rift lake cichlids; but for species from soft, acidic waters, prolonged NaCl immersion baths should not exceed 0.4% (=4 ppt or 4 g/L). This recommendation is based upon a study in the classic “blackwater” species, the Neon Tetra (*Paracheirodon innesi*), which demonstrated that at salinity levels of 0.5% (5 g/L) using dilute seawater, growth and gonadal maturation were retarded [35]. Interestingly, growth and gonadal maturation were not adversely affected at a salinity of 5 g/L if calcium-deficient seawater was used [35]. Furthermore, at levels of 0.75% (7.5 g/L) using dilute seawater, fatalities were observed in *Paracheirodon innesi*. A study with *Corydoras aeneus* demonstrated that this anecdotally labeled “salt intolerant” genus tolerated long-term immersion in sodium chloride solutions at concentrations of 1–2 g/L. [36]. In water of low hardness and/or conductivity, more complex salt formulations seem to work even better in mitigating stress and reducing fish mortality [34]. Such complex formulations might include NaCl, CaCl₂, Na₂SO₄, NaHCO₃, KCl, MgSO₄, K₃PO₄, or sea salts. An added benefit of prolonged immersion in low salinity (1–4 ppt) salt baths is the prevention of both freshwater “velvet” disease (*Piscinoodinium limneticum*) and freshwater white spot disease or “Ich” (*Ichthyophthirius multifiliis*) [20]. There is additional evidence that suggests that salt formulations can also mitigate other adverse physiologic changes such as metabolic acidosis [37], and the hyperglycemia and hypercortisolemia that occurs in transported salmonid species [34].

Elasmobranchs

Introduction

Elasmobranchs include the sharks, rays, and skates. Although there are several species of freshwater stingrays, the following discussion will focus on the saltwater elasmobranchs. Marine elasmobranchs possess mechanisms for coping with a saltwater environment that are somewhat different from saltwater teleosts. Saltwater elasmobranchs are actually slightly hypertonic to seawater because of their retention of urea and trimethylamine oxides (TMAO) [38]. In fact, urea and TMAO account for approximately 40% of the plasma solutes. Because the body fluids of elasmobranchs have a higher overall solute concentration than seawater, water has a tendency to move across the permeable gill epithelium and into the animal. Like freshwater teleosts, then, marine elasmobranchs are confronted with potential hypervolemia [4]. In fact, water influxes of up to 167% of body water per hour have been reported [2]. To cope with this water loading, GFRs and urine flow rates in saltwater elasmobranchs approach those of freshwater teleosts.

Unlike freshwater teleosts, marine elasmobranchs, like marine teleosts, possess less NaCl in their body fluids than seawater. As stated before, their plasma NaCl concentrations are approximately 50% of those found in the marine environment. Because of this, they face an influx of NaCl of 33 to 100 $\mu\text{M}/100 \text{ g/h}$, and must cope with potential hypernatremia and hyperchloremia [4]. The primary source of Na^+ influx seems to be gill epithelial uptake, but ingested food and consumption via drinking seawater may also contribute [39]. Excess NaCl is excreted at concentrations well above that of the plasma through a specialized structure known as the rectal gland [40]. This rectal (or salt) gland is a finger-like projection off of the caudodorsal aspect of the colorectal region (Fig. 3). The rate of NaCl secretion from the rectal gland is controlled by humoral factors, that is,

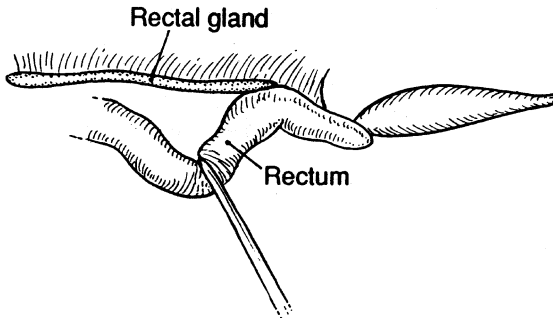


Fig. 3. The rectal gland of sharks. (From Stoskopf MK. Clinical physiology. In: Stoskopf MK, editor. Fish medicine. Philadelphia: W.B. Saunders; 1993. p. 48–61.)

angiotensin II, and volume expansion appears to be a major stimulus for NaCl excretion [38]. Table 1 provides a summary of the movement of ions in marine elasmobranchs.

Urea and trimethylamine oxides

As stated above, marine elasmobranchs maintain plasma that is hyperosmotic to seawater because they maintain very high urea (and other nitrogenous waste products) concentrations in their blood. Urea values have been reported that are greater than 800 mg/dL [41]. Urea is the primary end product of protein metabolism in elasmobranchs [42]. Urea is much more stable in plasma than ammonia, but it does denature proteins [42]. High urea concentrations are usually fatal to vertebrates primarily due to their denaturing effect on enzymes. Marine elasmobranchs are able to tolerate high urea levels due to the protective effects of methylamine compounds such as TMAO, betaine, and sarcosine [43]. It has been demonstrated experimentally that a 2:1 ratio of urea to TMAO effectively minimizes any denaturing of proteins. This is also the ratio found in the blood of all marine elasmobranchs studied to date. Urea concentrations appear to be similar in both plasma and cells [42].

The high level of plasma urea found in marine elasmobranchs appears to be maintained and regulated primarily by the kidney although it is produced in the liver [44]. Urea efflux in elasmobranchs averages 20 to 70 $\mu\text{M}/100 \text{ g/h}$ [45]. The gills seem to be the major site of urea efflux, because renal losses are only 4–5% of the total loss [46] and less than 15% of filtered urea is excreted. In fact, nearly all of the urea filtered by the glomeruli is thought to be reabsorbed via a countercurrent exchange system within the kidney [47]. Morphologic and physiologic evidence supporting the existence of this countercurrent exchange model has been found [48,49].

Gills

Elasmobranch sodium and chloride ion concentrations are greater than those of teleost fishes (as well as terrestrial vertebrates), but are still well below that of seawater [6]. Thus, sodium and chloride can move across a concentration gradient and into the animal via the thin gill epithelium [38]. However, the exact role of gills in sodium and chloride balance is still unclear. Although studies do indicate that the gill epithelium is a source of salt uptake, some reports also indicate albeit indirectly that the gills are also capable of sodium and chloride excretion [6]. There are three lines of evidence to support this hypothesis. First, a $\text{Na}^+\text{-NH}_4^+\text{-2Cl}^-$ transporter-mediated ammonia efflux has been measured in perfused gill preparations [50]. This mechanism could theoretically provide a pathway for Na^+ excretion. Second, when the rectal gland is removed from the animal, the kidney does not secrete a hyperosmotic urine, suggesting the existence of an

additional NaCl extrusion mechanism, perhaps in the gills [51]. Finally, the gill contains two different morphological variants of a mitochondrion-rich cell [52]. However, these cells differ ultrastructurally from the seawater-adapted teleost chloride cell, so their exact functions remain to be elucidated.

Kidney

Elasmobranch nephrons have all of the standard vertebrate components, that is, glomerulus, neck, proximal tubule segments I and II, distal tubule, and collecting duct [4]. Moreover, the arrangement of the nephrons in elasmobranchs is actually more complex [11]. Rather than distinct cortical and medullary regions, the elasmobranch kidney is composed of peritubular sheaths of squamous cells surrounding countercurrent systems of several nephrons bundled together with anastomosing capillary loops [49]. As indicated before, to counteract the water influx across the gills, the GFR is relatively high ($\sim 400 \mu\text{L}/100 \text{ g/h}$) as in freshwater teleosts. Unlike freshwater teleosts, however, marine elasmobranchs can pick up salts from their seawater environment. Na^+ , Cl^- , K^+ , and Ca^{2+} are reabsorbed from the glomerular filtrate with urine-to-plasma ratios near 1.0 for Na^+ and Cl^- [6]. Table 2 shows the general pattern of ion secretion and absorption during the process of urine formation in the marine elasmobranch.

Hormonal influences

Endocrine control of elasmobranch osmoregulation has been poorly studied except for the rectal gland. Salt secretion by the gland appears to be controlled by a variety of hormones and neurotransmitters. The primary stimulant seems to be either a vasoactive intestinal peptide or another peptide from the gut called rectin [53,54]. Adenosine acts as a stimulant to the rectal gland at concentrations above 10^{-5} M [55]. At lower concentrations, adenosine acts as an inhibitor of the rectal gland [56]. Somatostatin inhibits the stimulatory action of both vasoactive intestinal peptide and adenosine [57]. Angiotensin II appears to be a vasoconstrictor in elasmobranchs via facilitating release of catecholamines in the periphery [58,59].

Clinical implications

The veterinary medical literature is still extremely limited with regard to normal baseline data for commonly assessed clinical values, that is, complete blood counts, blood chemistry values, blood gases, and pH. The necessity of obtaining these values to administer appropriate supportive care cannot be overemphasized. Indeed, obtaining baseline information on normal animals handled during routine procedures can give the clinician a good starting point for accurately addressing the fluid and electrolyte needs of the abnormal elasmobranch patient. Accurate feed records are also very helpful for assessing changes in feeding behavior that may be indicative of disease. Sharks and rays

can both be bled from the caudal or “tail” vein, which is easily accessed from the ventral midline in most patients [41]. Annual physical exams are advised, during which blood sampling should be a routine component.

Sick and injured elasmobranchs undoubtedly face fluid, electrolyte, and pH challenges that can, in all likelihood, be mitigated by appropriate veterinary intervention. However, due to the paucity of baseline data, it can be very difficult for the veterinary clinician to know for sure if the results of routine diagnostic blood testing are within normal limits or not. Having a preestablished database will surely help to resolve this conundrum. In general, it is fairly safe to assume that anorectic, diseased, highly stressed, and/or injured animals are at risk for dehydration from a variety of mechanisms because plasma ion concentrations are much less than that of seawater. Metabolic acidosis from increased lactic acid levels is also a probable complication in debilitated elasmobranchs, particularly animals that have recently undergone capture/shipping stress or have been subjected to tank mate aggression. It is unclear if blood urea levels typically go up or down in debilitated animals. Protein-restricted diets that mimic the anorectic state have been shown to decrease urea production by the liver in one study [60]. It is yet unclear as to whether or not this has practical applications in the anorectic animal subject to a normal saltwater environment. Theoretically, however, lower urea levels could mean that the animal is at further risk for hypovolemia due to a decrease in blood osmolarity compared to seawater.

Although largely empirical, fluid and caloric support do seem to help anorectic, injured, or diseased animals. Oral administration of fresh water seems to be a useful remedy; this might be due to its corrective effect on dehydration secondary to shipping stress and/or anorexia (B. Whitaker, personal communication). For caloric support, our hospital routinely utilizes a gruel made from either a high grade canned seafood-based feline diet or a dog/cat canned recovery/caloric support formula mixed 50:50 with a pediatric electrolyte solution for sick or injured elasmobranchs. The gruel mixture is administered by stomach tube at 2–3% of the animal’s body weight while under sedation with MS-222. Subjectively, we feel that starting animals on supplemental oral alimentation is helpful and worth the effort of capture, sedation, and administration. However, the authors are unaware of any controlled studies that demonstrate the efficacy of food or water administration to debilitated elasmobranchs.

Parenteral fluid administration is possible and considered to be valuable in treating sick, stressed, or injured elasmobranchs. Continuous intravenous infusions are nearly impossible to maintain in nonsedated animals, but single bolus infusions of intravenous fluids may be helpful in debilitated specimens (S. Miller, personal communication). Intravenous fluids are also recommended during surgical procedures. Intracoelomic fluids may be more appropriate after short-term sedation for minimally invasive procedures. A variety of fluids commonly utilized in standard veterinary practice have been

employed including Lactated Ringer's solution, 0.9% NaCl, 0.45% NaCl, and 2.5–5% Dextrose solutions [61] (S. Miller, personal communication). For the treatment of metabolic acidosis due to lactate buildup from overexertion after capture and/or shipping, a solution of either 0.45% NaCl + 40 mEq sodium acetate or 0.9% NaCl + 40 mEq sodium acetate seems to be helpful (S. Miller, personal communication). Intravenous fluid rates based upon a daily rate of 60 mL/kg may be used during surgical procedures, and a single dose of intracoelomic fluids at 66 mL/kg may be used after routine handling (S. Miller, personal communication). Solutions may also be adjusted to more closely match the high osmolarity of elasmobranch blood. These are based upon fluids used in physiologic studies and are made in-house and then filter sterilized. Autoclaving is not recommended, as it will lead to precipitation of the material (C. Luer, personal communication). Components may be purchased from chemical supply companies, and should be reagent grade. These components are weighed out (dry weight) and combined in sterile water. An elasmobranch modified Ringer's solution may be made based upon the following formula (C. Luer, personal communication). The numbers are in grams of dry weight per 1 L of pure water.

Sodium Chloride (NaCl) 16.35
Potassium Chloride (KCl) 0.45
Calcium Chloride ($\text{CaCl}_2\text{-}2\text{H}_2\text{O}$) 0.74
Magnesium Chloride ($\text{MgCl}_2\text{-}6\text{H}_2\text{O}$) 0.61
Sodium Sulfate (Na_2SO_4) 0.07
Sodium Monophosphate (NaH_2PO_4) 0.12
Sodium Bicarbonate (NaHCO_3) 0.67
Urea 21.00
Trimethylamine Oxide ($\text{TMAO-}2\text{H}_2$) 7.99
Glucose 0.90

The stock solution can be mixed ahead of time, but the urea and TMAO are not stable and should not be added until the solution is needed. Other formulas are also available [61]. The administration of colloid solutions has not been investigated in elasmobranchs to the authors' knowledge. This would be an interesting area to explore.

It is unclear if decreasing the salinity of the tank water would be an effective method for fluid/electrolyte support for marine elasmobranchs. Theoretically, decreased salinity may help to relieve the fluid drain from sick or injured animals and help them achieve osmotic homeostasis. As far as the authors know, this has never been investigated in any controlled manner. Anecdotally, there have been indications that some marine elasmobranch species are intolerant of lowered salinities. Conversely, freshwater stingrays (*Potamotrygon* sp.) housed in long-term, low-salinity baths (2–4 ppt) as advocated above for freshwater teleosts, have never shown any intolerance for these solutions at the John G. Shedd Aquarium.

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