

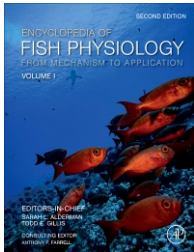
Ion regulation in anadromous fishes

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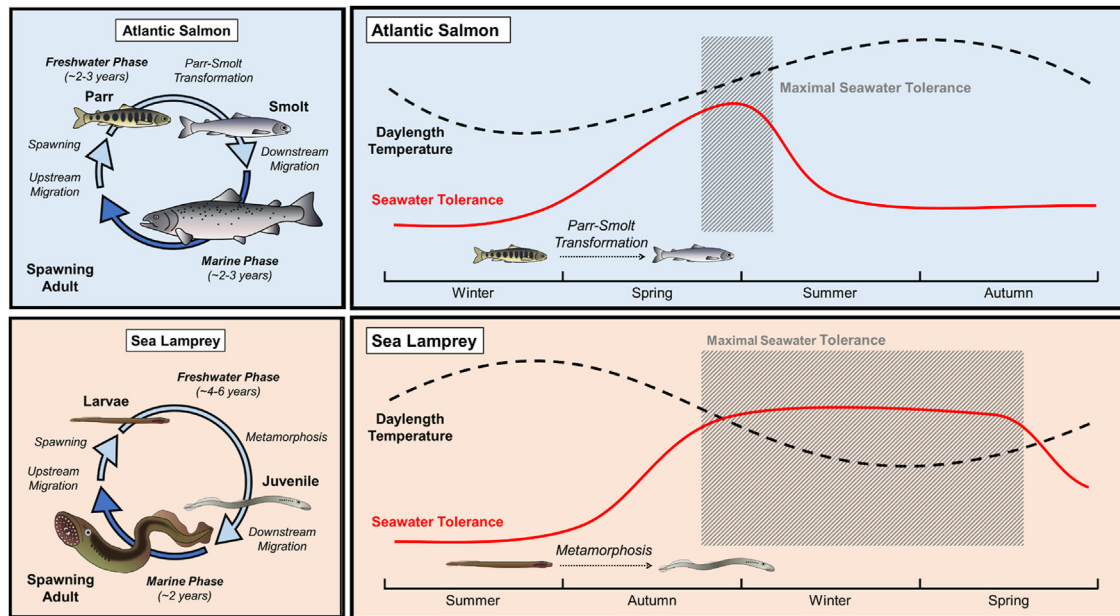
Key points

- Diadromy refers to the predictable migration of fishes between freshwater and marine habitats. Anadromous fishes are those that hatch in freshwater and migrate from freshwater to seawater as juveniles, and catadromous fishes are those that hatch in seawater and migrate from seawater to freshwater as juveniles.
- How diadromy evolved as a way of life in some groups of fishes is still unresolved. Leading hypotheses attribute the evolution of diadromy to selective pressures optimizing a balance of inhabiting more protected nursery habitats during vulnerable developmental life stages and inhabiting resource-rich habitats during life stages which prioritize growth and maturation.
- Freshwater and seawater present opposing osmoregulatory challenges to fishes, as fishes in freshwater are hyper-osmotic to their environment and fishes in seawater are hypo-osmotic to their environment.
- In freshwater, fishes counteract passive loss of salts and absorption of water by actively absorbing salts across their gills, and releasing excess water as large volumes of dilute urine. In seawater, fishes counteract passive incorporation of salts and dehydration by drinking large volumes of seawater, absorbing the salts and water from their intestine, secreting the excess salts across their gills, and retaining water by releasing only very low volumes of isosmotic urine.
- Downstream migrating juvenile anadromous fishes entering seawater must make dramatic osmoregulatory adjustments to survive this freshwater-to-seawater transition. Some anadromous fishes prepare for marine life by making these osmoregulatory changes prior to entering seawater.

Abstract

Diadromous fishes, such as lampreys, sturgeons, eels, herrings, and salmons, migrate between freshwater and seawater environments, which present drastically different osmoregulatory challenges to which these fishes must be adapted. This article briefly introduces the various forms of diadromy and current hypotheses on how diadromous life histories may have evolved. This article describes in greater detail the life cycle of anadromous fishes, and the physiological adaptations that anadromous fishes exhibit to survive transitions between freshwater and marine environments.

Teaching slide



Introduction

Among the over 32,000 species of fishes, approximately half live in freshwater (i.e., streams, rivers, lakes) and half live in seawater (i.e., oceans). These two different environments present starkly different osmotic concentrations to which fish must be adapted. The vast majority of fishes are stenohaline, in that they are exclusively adapted to live either in freshwater or seawater but are not capable of surviving in both environments. A small minority of euryhaline fishes, approximately 5% of all fish species, are capable of surviving in either freshwater or seawater. Despite being capable of surviving in both freshwater and seawater environments, most euryhaline fishes reside only in one or the other. Some euryhaline fishes live in intertidal environments and are regularly exposed to a range of salinities. Approximately 250 species of euryhaline fishes predictably migrate between freshwater and seawater environments once or multiple times throughout their life. These fishes are referred to as diadromous fishes. This article explores the various forms of diadromy and the osmoregulatory physiology that allows diadromous fishes to survive transitions between freshwater and seawater.

Despite accounting for less than 1% of all fish species, diadromous fishes are quite familiar, as they migrate along waterways that are often heavily populated by humans. Most diadromous fishes are anadromous, hatching in freshwater and migrating to seawater to live out juvenile and adult phases before returning upstream to freshwater to spawn (Fig. 1). These include many species of lampreys, sturgeons, herrings, and salmon. Fewer diadromous fishes, primarily eels and also some herrings, are catadromous, hatching in seawater and migrating to freshwater as juveniles and eventually returning to the sea to spawn.

The osmotic concentrations of freshwater and seawater are remarkably different. Freshwater is less than 1‰ salt (approximately 10 mOsm kg⁻¹) whereas seawater is approximately 35‰ salt (over 1000 mOsm kg⁻¹). Most fishes maintain homeostasis of their internal osmotic concentration at approximately 350 mOsm kg⁻¹, one-third that of seawater. Thus, freshwater fishes passively gain water and lose salts to the dilute environment, and seawater fishes passively dehydrate and gain salts from the relatively salt-concentrated environment. To maintain osmotic homeostasis and survive in these disparate environments, freshwater and marine fishes exhibit qualitatively opposite physiological adaptations (Fig. 2). To survive in freshwater, fishes exhibit exceptionally low drinking rates, release high volumes of dilute urine, and actively absorb salts via ion uptake mechanisms in the gills. To stay hydrated and survive in seawater, fishes drink large volumes of seawater for its water content (which they absorb from their intestine), release only very low volumes of isosmotic urine, and actively secrete salts via ion secretion mechanisms in the gills.

During migration between freshwater and seawater, diadromous fishes must therefore considerably adjust their drinking behavior and substantially transform the ion and water permeability and ionoregulatory mechanisms in their gastrointestinal tract, kidneys, and gills. The exceptionally challenging nature of this requirement to transform physiological systems from net ion absorption to net ion secretion (or vice versa) is likely the reason why so few species of fishes exhibit diadromy.

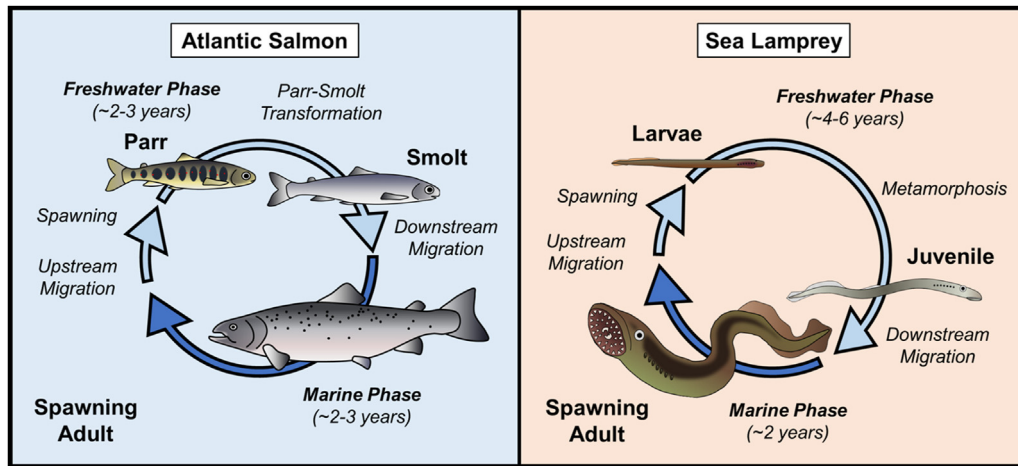


Fig. 1 Generalized life cycle of anadromous fishes. Anadromous fishes hatch in freshwater, migrate out to sea as juveniles, and return upstream again as mature adults to spawn. Atlantic salmon and sea lamprey are two anadromous fishes that undergo dramatic developmental changes prior to their seaward migration which prepare them to survive the transition from freshwater to seawater. In Atlantic salmon, this developmental event is called the “Parr-Smolt Transformation,” in which a freshwater-resident parr becomes a downstream-migrating, seawater-tolerant smolt. In sea lamprey, this developmental event is a true metamorphosis, in which a freshwater-resident larvae becomes a downstream-migrating, seawater-tolerant juvenile. Colors of the arrows indicate approximate proportions of freshwater (light blue) and seawater (dark blue) residency times: Atlantic salmon spend approximately half of their life in the ocean before returning upstream to spawn, and sea lamprey spend only one-third to one-quarter of their life in the ocean before returning upstream to spawn.

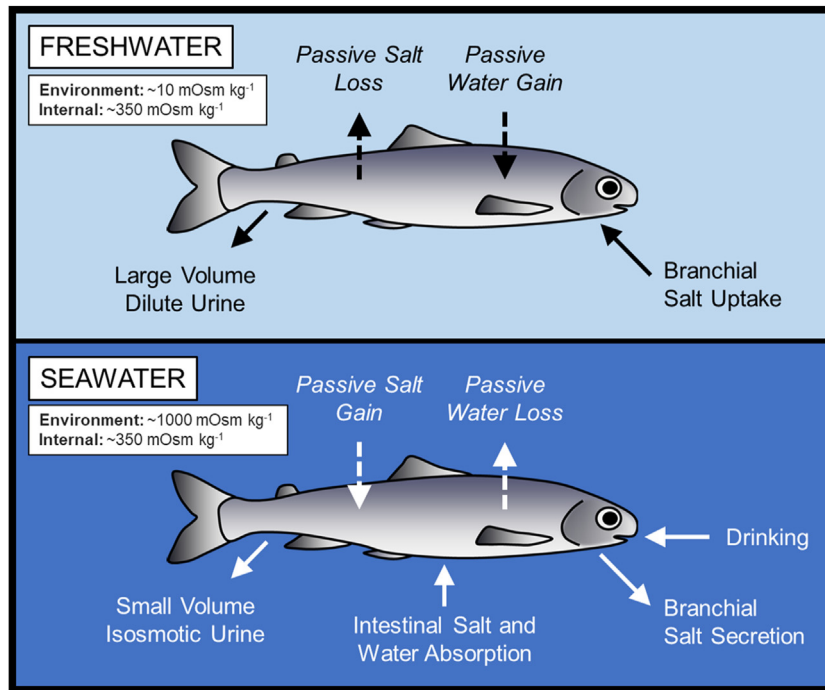


Fig. 2 Osmoregulatory strategies of fishes in freshwater and seawater. Freshwater and seawater present opposing osmoregulatory challenges to fishes. Fishes in freshwater are hyper-osmotic to their environment whereas fishes in seawater are hypo-osmotic to their environment. Downstream migrating juvenile anadromous fishes entering seawater must make dramatic osmoregulatory adjustments to survive this freshwater-to-seawater transition. In freshwater, fishes counteract passive loss of salts and absorption of water by avoiding drinking, actively absorbing salts across their gills, and releasing excess water as large volumes of dilute urine. In seawater, fishes counteract passive incorporation of salts and dehydration by drinking large volumes of seawater, absorbing salts and water in their intestine, secreting the excess salts across their gills, and retaining water by releasing only very low volumes of isosmotic urine.

Forms of diadromy and diadromous fishes

The term diadromy was coined by [Myers \(1949\)](#) to describe the migratory behavior of fishes that move between freshwater and marine habitats. The ability to migrate between these environments is based on a physiological capacity to acclimate to changes in salinity. For most species these movements occur at predictable times of year and during specific life history phases. It should be noted that even though a species may be classified as diadromous, the capacity to migrate between salinities and acclimate to a change in salinity may vary greatly throughout the life cycle. For many species, euryhalinity does not develop immediately. Thus, the larval and early juvenile phases of many anadromous fishes may exhibit stenohalinity rather than euryhalinity. The various life histories exhibited by diadromous fishes present broad variability within families and even genera. Even within species, a high degree of plasticity in life histories can be present. In some cases, a loss of diadromy results in individuals or populations remaining in their habitat of origin throughout their lifetime.

Diadromy is commonly divided into three subcategories, depending on the habitats used during different developmental stages of different species. Anadromous species use freshwater habitats for reproduction and early development. They then migrate to sea for the majority of their feeding and growth phase before returning to freshwater to spawn. Their reproductive habitat is freshwater and most feeding, growth, and maturation occur in seawater. Anadromous species account for approximately 50% of all diadromous species and include members of the Salmonidae (salmon, trout, char), Osmeridae (smelts), Gasterosteidae (sticklebacks), Retropinnidae (southern hemisphere smelts and grayling), Petromyzontidae (lampreys), and Acipenseridae (sturgeons). Some Clupeidae (herring and shads) species are anadromous, but a few are catadromous or considered amphidromous ([McDowall, 1997](#)). Catadromous species reside in freshwater for most of their feeding and growth phase then migrate as adults to the sea to reproduce. Their major feeding, growth, and maturation habitat is freshwater, but the site for reproduction and early development is the marine habitat. About 25% of diadromous species are considered catadromous and include some Mugilidae (mullets) and the Anguillidae (eels) ([McDowall, 1997](#)). Amphidromous species migrate to the sea early in development, undergo growth and development to a post larval/juvenile phase and then migrate back to freshwater to complete the majority of their growth and maturation to the sexually mature adult. Spawning by amphidromous fishes occurs in freshwater. Thus, in this group, the reproductive habitat is freshwater, which is the same as the habitat for principle growth and feeding. About 25% of diadromous species are considered amphidromous, including Eleotridae (sleepers), Prototroctidae, and some members of the Galaxiidae and Gobiidae (gobies) ([McDowall, 1997](#)).

Evolution of diadromy

Diadromy is a trait that has evolved only very rarely, being exhibited in only 36 out of 482 families of fishes ([McDowall, 1988](#)). Various diadromous life histories are seen in a diverse set of fishes and are thought to have had multiple independent origins. The evolution of diadromy has been a popular and contentious topic of discussion for many decades. It is clear that such a complicated life history, requiring such a drastic and impressive suite of behavioral, morphological, and physiological adaptations would only arise if the migration to new salinity habitats afforded some significant improvement in fitness (e.g., growth rate, survival, fecundity). Most hypotheses that have been purposed to explain the evolution of diadromy focus on energetic tradeoffs between food availability and the costs of osmoregulatory acclimation and migration, as well as accounting for changes in competition and predation that may come along with the new environment.

The two most debated hypotheses to explain the evolution of diadromy are the “Productivity Hypothesis” ([Gross, 1987](#); [Gross et al., 1988](#)) and the “Safe-Site Hypothesis” ([Dodson et al., 2009](#)). The Productivity Hypothesis focuses on the selective advantage of migrating to areas with increased food availability, allowing individuals to achieve higher growth rates and, presumably, greater fitness. Primary productivity, and therefore overall food availability, varies greatly from place to place and between freshwater and marine environments. Interestingly, freshwater habitats tend to have higher productivity in tropical regions than in temperate and polar regions whereas the opposite relationship is seen in marine habitats. Therefore, in the tropics, there would be an advantage to migrating from food-poor marine habitats to more productive freshwater habitats (i.e., catadromy). Likewise, in temperate and polar regions, a migration from low-productivity freshwater habitats to food-rich marine habitats (i.e., anadromy) would be predicted. Catadromous species are indeed more common in the tropics and less common in temperate/polar regions while anadromous species are much more common in temperate/polar regions than in the tropics. This alignment of forms of diadromy and latitudes support the Productivity Hypothesis as a possible explanation for the evolution of diadromy.

Other studies have suggested that predation, competition, and even geological history may be at least as important as nutrient availability, which is central to the Productivity Hypothesis ([Bloom and Lovejoy, 2014](#)). The Safe-Site Hypothesis ([Dodson et al., 2009](#)) argues that freshwater habitats are safer for the deposition of eggs after spawning and the early development of fishes due to a reduction in predation risk—freshwater, in this view, is a sanctuary from marine predators. This hypothesis may explain the evolution of anadromy in some ancestral marine species. In many northern latitudes, repeated glacial advances and retreats (as recent as 10,000 years ago) have led to the development of novel freshwater habitats. These recent glacial-derived freshwater systems started with little to no competition or predation allowing for the successful invasion of euryhaline species from the sea. Today, these glacial-derived freshwater systems have a relatively high proportion of anadromous species. Similarly, there is a high proportion of diadromous fish lineages in freshwater habitats of oceanic islands. When these island habitats were newly formed, competition was low, which led to their colonization by euryhaline marine species.

Osmoregulatory physiology of the anadromous fish life cycle

Egg and early development in freshwater

Gametes of fishes, as well as larval and juvenile life stages, appear to be most sensitive to changes in environmental salinity. Therefore, early developmental life stages are generally the least likely to be capable of migrating between freshwater and saltwater environments. In anadromous fishes, spawning and early development occur in freshwater. There are very few examples of anadromous species being capable of spawning in elevated salinities. Helle et al. (1964) observed pink salmon, which are often considered the “most anadromous” salmonid species, spawning in estuaries in Alaska. Similar reports exist for specific populations of European whitefish (Himberg and Lehtonen, 1995) and chum salmon (Scott and Crossman, 1973). Quite surprisingly, there have been observations in the Northwest Territories of Canada of lake char, which is often considered a stenohaline freshwater salmonid, completing their entire lifecycle in salinities of up to 5‰ (Kissinger et al., 2016). Kissinger et al. (2017) experimentally fertilized eggs of lake char at 5‰ salinity and successfully reared developing embryos and larvae for more than a year at that salinity. Although these types of observations are very rare, they do suggest that some populations of anadromous species may show local adaptations that allow for spawning and early development in slightly elevated salinities.

Freshwater residency

Osmoregulation in freshwater

For freshwater fishes, the internal osmotic concentration is hyperosmotic compared to the external environment. Freshwater fishes passively lose salts to their environment and gain excess water. To combat this, freshwater fishes drink very little (Bath and Eddy, 1979) and have high kidney glomerular filtration rates and ion reabsorption capacity to maintain ions (Jobling, 1995). Reabsorption of salts by the nephron is driven by the action of a basolateral Na^+/K^+ ATPase (Nka) which creates a Na^+ gradient that drives apical Na^+ and Cl^- uptake as well as reabsorption of divalent ions and other solutes back into the blood. This high glomerular filtration rate produces a hypoosmotic urine, which allows for the excretion of excess water.

Some salts are replenished from the diet, but most are actively absorbed by specialized freshwater ionocytes. Early in development, prior to the development of full gill function, ionocytes on the yolk sac epithelia and the integument act to actively absorb salts into the body of the fish. Functionally and morphologically, these integumental ionocytes are quite similar to the branchial ionocytes which have been extensively studied in both freshwater and marine fishes. Once functional, the gill takes over as the primary site for active ion uptake in freshwater fishes and drives whole body ionoregulation (Evans et al., 2005). The active ion uptake mechanisms for freshwater fishes can vary greatly from species to species (Hwang and Lin, 2013), utilizing different freshwater ionocyte subtypes and a variety of different patterns of ion transporters (Shaughnessy and Breves, 2021).

Generally, the active uptake of Na^+ and Cl^- is linked to the excretion of H^+ and HCO_3^- . Avella and Bornancin (1989) elucidated a model for active ion uptake by the gills of freshwater rainbow trout where a V-type H^+ ATPase on the apical surface pumps H^+ out of the cell creating an electrochemical gradient favoring the passive uptake of Na^+ into the cell via an apical Na^+ channel. The identity of the apical Na^+ channel is still unclear (Wichmann and Althaus, 2020). Intracellular Na^+ levels are maintained at low levels by the active transport of Na^+ across the basolateral membrane into the plasma by Nka. This aids the apical uptake of Na^+ by diminishing the uphill Na^+ gradient from the water to the cell. Similarly, in some species, an apical Na^+/H^+ exchanger (Nhe) achieves electroneutral Na^+ uptake by directly coupling Na^+ uptake with H^+ excretion. Branchial Cl^- uptake appears to be linked to the passive movement of HCO_3^- down its concentration gradient via an apical $\text{Cl}^-/\text{HCO}_3^-$ anion exchanger (Slc26 members).

Active branchial uptake of Na^+ and Cl^- in freshwater can also occur through electroneutral mechanisms independent of H^+ and HCO_3^- . For example, electroneutral uptake of Na^+ and Cl^- can occur via Na^+-Cl^- cotransporters (Ncc) on the apical surface freshwater of branchial ionocytes (Shaughnessy and Breves, 2021). In these cells, Nka transports Na^+ from the cell into the extracellular fluid and blood plasma. A mechanism for Cl^- transport across the basolateral membrane has been more recently proposed and is still not fully understood. However, it seems likely that chloride channel 2 (Clc-2) located on the basolateral membrane of Ncc-expressing ionocytes may be involved in the final incorporation of Cl^- (Wang et al., 2015; Shaughnessy and Breves, 2021).

Freshwater-to-seawater migration

The migration of juveniles from protected freshwater nurseries to resource-rich estuarine and ocean habitats is the defining life history event of anadromous fishes. Although the timing of and environmental cues promoting the seaward migration varies by species, all anadromous fishes prepare for marine life to some extent before ever starting their seawater journey. This section will highlight the behavioral, morphological, and physiological preparations made by anadromous fishes before and during their seaward migration.

Preparation for marine life: The salmon parr-smolt transformation and the lamprey metamorphosis

The most prominent and well-understood example of the preparations for downstream migration and marine life are made by some of the anadromous salmonids, referred to as the parr-smolt transformation (or more colloquially as “smolting” or “smoltification”), most famously exhibited by Atlantic salmon (Fig. 3). Although the salmonid parr-smolt transformation is not, by strict definition, a true metamorphosis, it shares many qualitative similarities to a metamorphosis, including dramatic behavioral, morphological, and physiological changes and occupation of a new habit and way of life. Salmon parr are characterized by

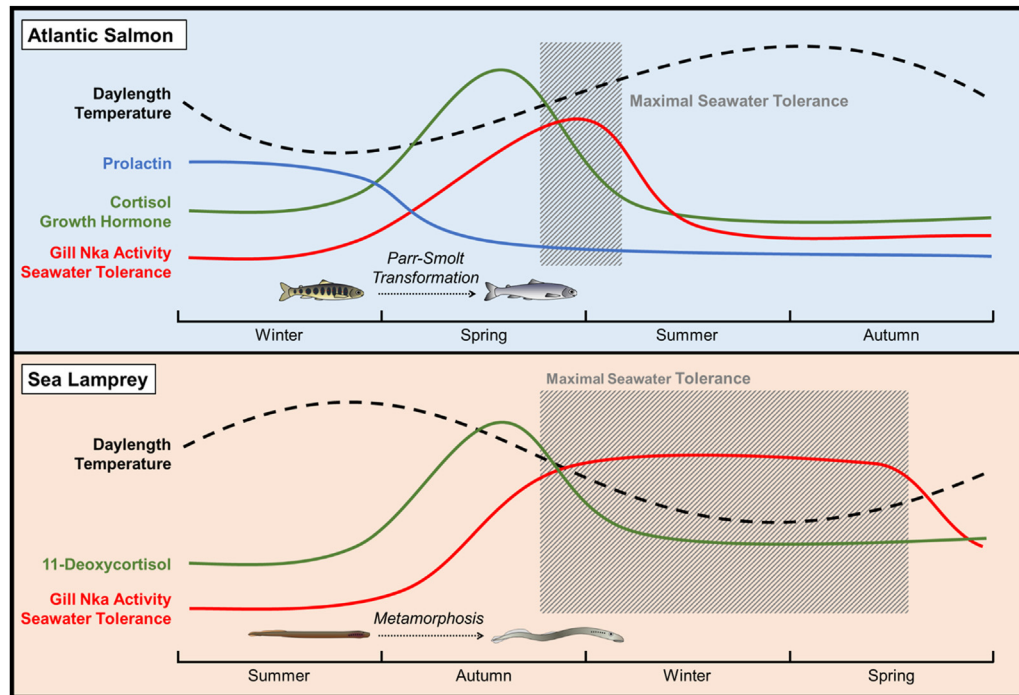


Fig. 3 Illustration of key physiological changes in preparation for seawater entry. The Atlantic salmon parr-smolt transformation and the sea lamprey metamorphosis share many similarities in the physiological adjustments that are made in preparation for marine life. Generally, seasonal changes in daylength and temperature stimulate neuroendocrine signaling pathways which elevate circulating concentrations of seawater-adaptive hormones and reduce circulating concentrations of freshwater-adaptive hormones. These hormonal changes drive the upregulation of expression and activities of salt-secretory processes in the gills, which results in enhanced seawater tolerance before the fish ever enters the ocean. In Atlantic salmon, seawater preparation occurs during spring and is heavily dependent on increasing daylength and temperature. In sea lamprey, seawater preparation occurs during the autumn, but the impacts of decreasing daylength and temperature in driving these preparations, as well as the freshwater- and seawater-adaptive neuroendocrine pathways, are not well-understood. The window of sustained branchial salt-secretory processes and maximal seawater tolerance is much shorter in Atlantic salmon than in sea lamprey. Thus, Atlantic salmon exclusively enter seawater during the spring months, whereas sea lamprey enter seawater throughout winter and spring.

prominently displayed spotting patterns that can vary by species, but which usually include a series of large, dark-colored oval spots called “parr marks.” To undergo the parr-smolt transformation, Atlantic salmon parr must have reached a certain size threshold by mid-winter (usually around 12 cm before February), which typically is achieved in their second year after hatching (McCormick, 2013). Atlantic salmon that have reached this size threshold are called “pre-smolts” and will undergo a parr-smolt transformation from late winter to late spring. During this time, increases in daylength and water temperatures, working through neuroendocrine axes, promote morphological, behavioral, and physiological changes that prepare the fish for migration and seawater entry (McCormick, 2013). The morphological transformation from parr to smolt typically includes a loss of the spotted patterning (which is replaced by a more uniform silver color), a darkening of the fins, and a generally slimmer and more elongate body shape. This change in body shape leaves smolts more suited for a pelagic life and promotes increases in downstream migratory behavior. Physiological changes stimulated by increasing temperature and daylength include activation of neuroendocrine axes resulting in elevated circulating concentrations of seawater-adaptive hormones (such as cortisol and growth hormone) and reduced circulating concentrations of freshwater-adaptive hormones (such as prolactin). These hormonal changes ultimately promote seawater-adaptive physiological changes in the gills, intestine, and kidney resulting in increased seawater tolerance in the salmon smolts (McCormick, 2013). During the parr-smolt transformation, salmonid fishes change the dominant isoform of Nka expressed in the gills and greatly increase its activity. In freshwater, prior to the parr-smolt transformation, salmonids primarily express the $\alpha 1a$ isoform of Nka in the gills. During transformation, the dominant gill Nka isoform switches to $\alpha 1b$. The functional difference in these two isoforms and the reasons they are reciprocally expressed in freshwater and marine habitats are still poorly understood, but may relate to kinetic differences in the function of Nka or due to the changing expression of freshwater and seawater ionocytes that differentially express each isoform. This reciprocal expression of Nka α -subunit isoforms between halohabitats (called “isoform switching”) has now been observed in all salmonid species that have been examined and occurs not only during the parr-smolt transformation but also during the return migration to freshwater, suggesting it has important adaptive function for living in environments with different salinities (Richards et al., 2003; Bystriansky and Schulte, 2011; Bystriansky et al., 2006, 2007).

Less well understood than the parr-smolt transformation are the preparations made by anadromous lampreys. Before entering seawater, lamprey develop from freshwater-resident larvae to seaward-migrating juveniles. This seawater-adaptive developmental

event is even more dramatic than the parr-smolt transformation and categorically defined as a true metamorphosis (Youson, 1979). Lamprey larvae—sometimes still referred to as “ammocoetes” (their historical name from when they were thought to be a different species entirely)—live burrowed in silty sediment and are morphologically characterized by a dark maroon color, a toothless oral hood at their mouth for filter feeding from the water column, and the notable lack of eyes (Youson, 1979). Like smolting salmonids, it appears that lamprey must also achieve a size threshold prior to undergoing metamorphosis in any given year, which has been suggested to be 12 cm and 3 g (Henson et al., 2003). Larval lamprey that have achieved this size threshold by late spring will undergo metamorphosis, which begins in mid-summer and ends in late fall. The lamprey metamorphosis results in juveniles which are silver in color, possess a toothy oral disc and rasping tongue used for penetrating the integument of their prey and extracting blood meals, and the appearance of prominent and fully-developed eyes (Youson, 1979). In contrast to the well documented influence of increasing temperature and daylength during the salmonid parr-smolt transformation occurring in the spring, temperature and daylength are decreasing during the months-long lamprey metamorphosis, and their influence on the progress of the lamprey metamorphosis are not well understood. Like in smolting salmonids, elevated circulation of at least one seawater-adaptive hormone—11-deoxycortisol, a biosynthetic precursor to cortisol which has been shown to be the predominant corticosteroid hormone in lampreys (Close et al., 2010)—during metamorphosis promotes seawater-adaptive physiological changes in the gills and intestine resulting in increased seawater tolerance in the newly metamorphosed juvenile lamprey (Shaughnessy and McCormick, 2020; Shaughnessy et al., 2020; Barany et al., 2021). However, much remains unresolved regarding other endocrine programs driving the salt-secretory preparations during metamorphosis, such as roles for growth hormone and/or prolactin, both of which have recently been identified in lampreys (Gong et al., 2022; Ferreira-Martins et al., 2023).

Preparations made by other anadromous species such as herring and sturgeons are less apparent and less well-understood than those made by salmonids and lampreys, and many of the seawater-adaptive changes in these non-preparing anadromous fishes occur upon seawater entry.

Development of salinity tolerance

All anadromous fishes develop increased seawater tolerance at some point in their life, which is often set by some size threshold. However, the timing of development of salinity tolerance and the degree of anadromy can vary greatly across anadromous fishes (Zydlewski and Wilkie, 2012). For instance, within the salmonids, some species are “strongly anadromous” (e.g., pink salmon, chum salmon) and undergo an early and rapid transformation, developing salinity tolerance and beginning their seaward migration within only a few weeks after hatching. Many herrings (e.g., blueback herring, alewife, American shad) also exhibit a high degree of anadromy, beginning their migration and entering seawater within the first few months after hatching. American shad are one species that stand out among the “strongly anadromous” fishes because they appear to exhibit obligate anadromy, in that exposure to seawater is necessary for their survival (Zydlewski and McCormick, 1997). More “moderately anadromous” salmonids (e.g., coho salmon, Atlantic salmon) spend one to several years in freshwater before undergoing the months-long parr-smolt transformation and developing salinity tolerance. Some species of anadromous sturgeon (e.g., green sturgeon, Atlantic sturgeon), although lacking a dramatic seawater-preparatory transformation, display a similarly moderate degree of anadromy, entering estuaries within the first few years. For anadromous lampreys, which also display a moderate degree of anadromy, the developmental timeline can be even longer, with larvae occupying freshwater for up to 8 years before the onset of metamorphosis and development of salinity tolerance. It should be noted that although most of these “strongly anadromous” and “moderately anadromous” species do enter the ocean at some point in their life, they technically exhibit facultative anadromy, whereby seawater entry is not a strict requirement for their survival. Natural and anthropogenic obstructions to the sea or displacement have resulted in many land-locked populations of these “strongly anadromous” and “moderately anadromous” species that would otherwise migrate to the ocean. The most “weakly anadromous” salmonids (e.g., brook trout) optimize facultative anadromy, in that they may never undergo preparations for a seaward migration or enter seawater at all, even if access to the ocean is easily available, which is a degree of anadromy also shared by many anadromous sturgeons (e.g., white sturgeon, shortnose sturgeon).

Physiological consequences of staying in freshwater

For most anadromous fishes, the consequences for remaining in freshwater are minor. Forgoing marine life could carry the disadvantage of lower availability of food resources for growth in freshwater habitats, especially in higher latitudes, but it also may carry the advantage of a lower-risk of predation. There are few, if any, osmoregulatory consequences of staying in freshwater. Indeed, most anadromous fishes, including species that had previously been thought to require a marine phase, have been shown to possess the capacity to remain in freshwater for the entire duration of their life. Landlocking events, whether natural (e.g., glacial recession) or anthropogenic (i.e., damming and transport), have resulted in many landlocked populations of anadromous species. Landlocked populations of salmonids still undergo the parr-smolt transformation and landlocked populations of lampreys still undergo metamorphosis, and these developmental events in these landlocked populations still include dramatic increases in salt-secretory physiological processes and enhanced seawater tolerance. For salmonids, if seawater entry does not occur in the few weeks around the peak of smolting, seawater tolerance rapidly diminishes back to parr-like levels (McCormick, 2013). These animals, which have lost their seawater tolerance, but retained the morphological characteristics of a smolt, are called “post-smolts” and can carry out a fully freshwater existence. In lamprey, enhanced seawater capacity remains for many months after metamorphosis despite remaining in freshwater (Shaughnessy and McCormick, 2022).

Seawater residency

Once in the marine environment, anadromous fishes face the challenge of staying hydrated. Without making the necessary seawater-adaptive physiological adjustments, fishes would passively and lethally lose water to the more solute-concentrated environment via osmosis. However, anadromous fishes are capable of transforming their physiology, whether by preparatory adjustments made prior to seawater entry or by adjustments made upon seawater entry, to maintain osmoregulatory homeostasis during transition from freshwater to seawater (Bath and Eddy, 1979). These adjustments include changes in drinking behavior, the mechanisms by which the intestine processes ingested water, and the osmoregulatory functions of the gills and the kidney. The result of these physiological adjustments transforms the fish from an animal that was able to absorb and retain salts from a heavily solute-poor freshwater environment and release large volumes of excess water to an animal that is now able to secrete large quantities of excess salt against highly unfavorable ionic gradients and absorb and retain water despite the strong dehydrating osmotic forces of seawater.

Drinking and intestinal processing of seawater

In order to absorb water from the highly concentrated seawater environment, marine fishes drink seawater. The high drinking rates of marine fishes draws stark contrast to their behavior in freshwater of drinking very little or not at all—often only incidentally during feeding. The reflex to drink seawater is stimulated by sensing of elevated Cl^- concentrations and occurs almost instantly upon entry into a hypertonic environment, before even any change in plasma ion concentrations would be detected (Jobling, 1995). The ingested seawater must then be quickly processed by the intestinal tract to avoid osmotically losing additional water to intestinal lumen and further dehydrating. When distended, the intestinal tract provides negative feedback on the drinking reflex, resulting in rapid and repeating rhythm of filling the intestine then processing the imbibed fluids to promote water absorption.

Processing of the imbibed seawater is regionalized within the intestine and can vary among species. Generally, the role of proximal regions of the tract (e.g., esophagus and anterior intestine) is to absorb large quantities of monovalent ions (i.e., Na^+ and Cl^-) from the intestinal lumen into the intestinal blood capillaries and internal compartments of the animal, thus rapidly desalinizing the ingested seawater (Jobling, 1995). By doing so, the dehydrating osmotic gradient across the intestinal epithelium is quickly reversed—what was a dehydrating osmotic force quickly becomes a hydrating osmotic force—and water osmotically follows the absorbed salts into the body of the fish.

The cellular and molecular mechanisms for Na^+ and Cl^- absorption in the proximal intestinal regions varies among species. However, regardless of the particular arrangement of Na^+ and Cl^- transporters on the apical surface, ion and water absorption across the intestinal epithelium is an active process, relying on the electrochemical gradient produced by high rates of activity of the basolateral Nka (Fig. 4). Activity of Nka on the basolateral membrane results in the export of Na^+ and import of K^+ at a 3:2 ratio, thus maintaining low intracellular Na^+ levels and a net inside-negative membrane potential. The low intracellular concentrations of Na^+ provide a favorable gradient for Na^+ absorption from the lumen across the apical surface of the intestinal epithelium. Absorption of Cl^- from the lumen is then coupled to Na^+ absorption, typically by Ncc or $\text{Na}^+ - \text{K}^+ - 2\text{Cl}^-$ cotransporter (Nkcc) expressed on the apical surface of the intestinal epithelium (Shaughnessy and Breves, 2021). Luminal Cl^- is also largely absorbed through a $\text{Cl}^-/\text{HCO}_3^-$ anion exchanger (Slc26 member) that results in large deposits of HCO_3^- into the intestinal lumen.

Once ions are absorbed and the salinity of the luminal fluid is reduced, water moves via osmosis from the lumen to be distributed throughout the animal. Thus, the marine fish has achieved net water absorption (via active ion uptake processes), despite being in a passively dehydrating marine environment. The molecular mechanism(s) by which water moves from the lumen, either by paracellular and/or transcellular transport, is less well-understood, but may include, at least partially, the involvement of aquaporins.

Less critical to the bulk osmoregulatory role of the seawater-adapted intestine are the distal regions, such as the posterior intestine and rectum. These regions are generally more involved in regulation of divalent ions (e.g., Mg^{2+} and SO_4^{2-}), bicarbonate handling, and regulation of luminal pH regulation.

Ion secretion at the gills

Ion absorption in the intestine is critical for achieving net water absorption from the imbibed seawater, however it also results in a continuous supply of ions to the blood that must be secreted to maintain osmotic and ionic homeostasis. Secretion of the absorbed ions occurs at the gills via a specialized, Nka-rich, salt-secretory cell type referred as the "SW-type ionocyte" (Evans et al., 2005). This cell type has also been referred to as a "mitochondria rich cell (MRC)" due to the abundance of mitochondria within these cells to power the high activity of Nka and as a "chloride-cell" due to its primary function of secreting Cl^- .

The SW-type ionocyte is responsible for the active secretion of both Na^+ and Cl^- against a steep concentration gradient, from within the body where they are at physiological (relative low) concentrations to the seawater environment where they are at supra-physiological (relatively high) concentrations. In these cells, Nka is expressed in high abundance along the basolateral membrane, maintaining low intracellular Na^+ concentrations and an inside-negative membrane potential. Also expressed throughout the basolateral membrane are Nkcc and K^+ channels. The low intracellular concentrations of Na^+ provide a favorable gradient for Cl^- (and K^+) to be transported out of the blood and into the ionocyte by electroneutral cotransport with Na^+ via Nkcc. The Na^+ and K^+ that are transported into the cell via Nkcc are actively recycled out of the cell via the basolateral transporters, Nka and K^+ channel. However, Cl^- has no basolateral route to exit. Instead, Cl^- that accumulates within the ionocyte is secreted via a Cl^- channel expressed on the apical surface. A Cl^- channel called the cystic fibrosis transmembrane conductance regulator (Cftr), appears to

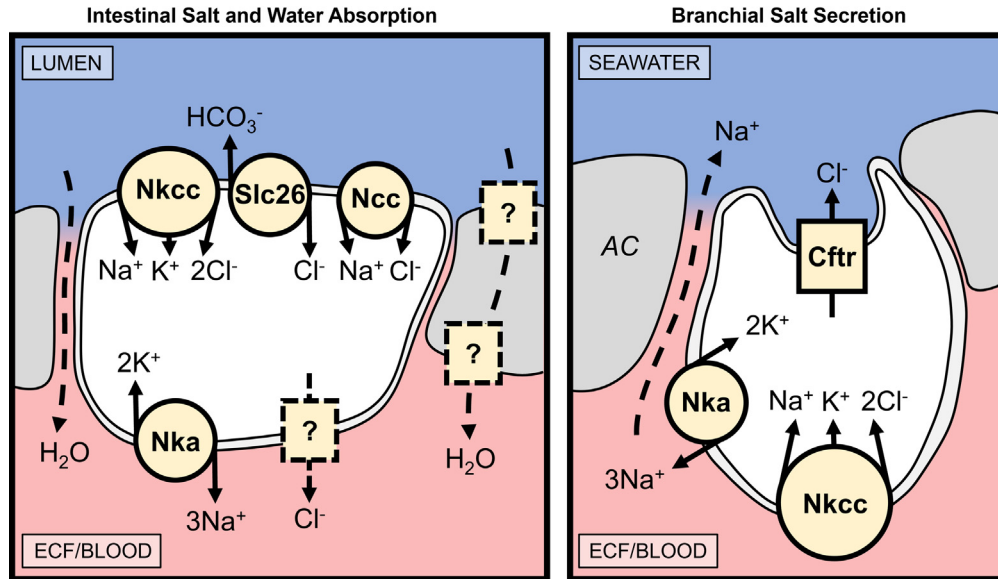


Fig. 4 Simplified diagram of seawater-adaptive osmoregulatory mechanisms in the fish intestine and gill. In the intestine of marine fishes, active absorption of ions from the intestinal lumen into the extracellular fluid (ECF) and blood compartments drives the osmotic absorption of water from the imbibed seawater. On the intestinal epithelium, Na^+ and Cl^- are absorbed transcellularly via ion transporters on the apical and basolateral membranes. The particular mechanism for water absorption, whether paracellular and/or transcellular, remains unresolved. In the gills of marine fishes, excess ions are secreted via a “seawater-type ionocyte”. On the branchial epithelium, Cl^- is secreted transcellularly via Nkcc on the basolateral surface and Cftr on the apical surface, and Na^+ is secreted paracellularly via regulated “leaky junctions” between the ionocyte and an accessory cell (AC). Note: the extracellular fluid (ECF) and blood capillary compartments are simplified. Abbreviations for ion transporters: Cftr, cystic fibrosis transmembrane conductance regulator; Ncc, $\text{Na}^+\text{-Cl}^-$ cotransporter; Nka, Na^+/K^+ ATPase; Nkcc, $\text{Na}^+\text{-K}^+\text{-Cl}^-$ cotransporter.

serve this role as the apical Cl^- channel in SW-type ionocytes in the gills of all teleost marine fishes examined to date. However, it stands unresolved whether the SW-type branchial ionocytes of non-teleost anadromous fishes (e.g., sturgeons and lampreys), which have been shown to express Nka and Nkcc1 in their gills, also express Cftr as the apical Cl^- channel facilitating Cl^- secretion in seawater (Shaughnessy and Breves, 2021).

The mechanism for the branchial secretion of Na^+ is not as clearly defined as the mechanism for Cl^- secretion. It appears that Na^+ is secreted via a paracellular route between the ionocyte and an accessory cell (Evans et al., 2005). This proposed mechanism for Na^+ secretion requires that a concentration gradient exists and is potent enough to drive secretion of Na^+ from the extracellular space into the heavily concentrated seawater environment. The process by which Na^+ is concentrated in the junctional space between the ionocyte and the accessory cell is still unresolved but may be due to the concentrated expression of Nka in the lateral portion of the basolateral membrane. The role, if any, of junction proteins regulating the final secretion of Na^+ also remains unresolved.

Seawater-to-freshwater migration

Finally, to complete their life cycle, anadromous fishes must return to freshwater to spawn. The spawning migration, and the preparatory period leading up to it has garnered far less attention than the seaward migration for most species. This may be due to the challenging logistics of studying maturing adults in the open ocean—studying larval and juvenile life stages in confined riverbeds or experimentally in the laboratory is far more reasonable a proposition. In addition, much of what we know about anadromy is based on commercially important species, especially the salmonids. For example, considerable effort has gone into understanding the parr-smolt transformation and how to improve smolt survival, maturation, and growth for improved aquaculture yield. Since commercial fishing for most anadromous species occurs in marine habitats, and due to the fact that many salmonid species are semelparous and die following spawning, the interest in understanding the biology of anadromous species in preparation for their spawning migration and reproductive success is important mainly for conservation related questions, which unfortunately have not attracted as much research funding as questions related to aquaculture and food production. For these reasons, much of what is known about the freshwater-migratory life stage of anadromous fishes relates to the periodicity and seasonal timing of migration and spawning, including factors affecting reproductive success and, for some species, homing to their natal spawning grounds. There has been very little focus on the physiological acclimation of anadromous fishes back to freshwater.

For many species, there is a relatively well-defined periodicity to the marine phase of life. This varies greatly from species to species, ranging from approximately 18 months in pink salmon to many years in some anadromous sturgeons. The timing of freshwater entry during their migratory year is generally thought to be determined by water temperature and gonadal maturity. Most

anadromous fishes are found in temperate regions, and spawning and early development are most successful in cool freshwater conditions. Recently, climate change has led to increased water temperatures in many river systems used by species of salmon, sturgeon, and lamprey leading to instances of advanced timing for freshwater migration and reduced reproductive success. Topics related to migration, homing, and reproduction will be the focus of other reviews in this series.

For many anadromous species (e.g. salmonids and lampreys), individuals stop feeding during this period of their life cycle and therefore must persist for potentially months on accumulated energy reserves. Most studies that have examined energetic requirements for migration and spawning of anadromous fishes indicate that lipid stores serve as the primary fuel source. When lipid stores become exhausted, upstream-migrating fish begin utilizing protein sources for energy, which eventually leads to the breakdown of their own muscle for fuel. Considering the high energetic cost of changing ionoregulatory modes to re-establish life in freshwater, as well as the potentially intense metabolic costs of sustained upstream swimming, it is fascinating to consider the challenge of balancing energy supply and demand in anadromous species during their spawning migration.

One question that requires attention is whether anadromous species undergo a preparatory phase leading up to freshwater re-entry akin to the parr-smolt transformation. The challenge of reverting back to a system of hyper-osmoregulation in freshwater is likely just as difficult as the acclimation of juveniles to seawater. Unfortunately, few studies have examined ionoregulatory changes leading up to and during the spawning migration. Bystriansky et al. (2007) observed that wild, naturally migrating Arctic char increased gill Nka activity more than three-fold following freshwater entry compared to individuals collected in seawater. A similar increase in gill Nka activity was also reported for seawater acclimated Atlantic salmon experimentally transferred to freshwater (Bystriansky and Schulte, 2011). Most studies examining seawater acclimation of salmonids report an increase in gill Nka, so observations of increased gill Nka activity during freshwater acclimation were surprising. However, a more broad survey of other euryhaline fishes (non-salmonids) showed that freshwater acclimation often involves the up regulation of gill Nka activity, described as a “U-shaped” pattern of regulation, where transfer of euryhaline fish to both higher and lower salinities involves increased gill Nka activity (Jensen et al., 1998). The observation of increased gill Nka activity in freshwater-acclimating Arctic char and Atlantic salmon were also accompanied by a switch in the dominant gill Nka α -isoform from $\alpha 1b$ to $\alpha 1a$, the opposite to what is seen during the parr-smolt transformation in preparation for seawater entry. Interestingly, mRNA levels of the gill $\alpha 1b$ isoform decreased in Arctic char prior to the movement into freshwater suggesting these fish were changing their dominant gill Nka isoform in preparation for freshwater entry. Similar results have since been observed in pink salmon held in seawater during their spawning migration period (Bystriansky, unpublished). The idea that salmonids undergo some kind of preparatory phase before migrating into freshwater is also supported by the work of Hirano et al. (1990) and Uchida et al. (1997) on chum salmon. In these studies, freshwater-type gill ionocytes were observed in seawater prior to river entry, suggesting a preparation for freshwater acclimation. Interestingly, when these salmon were held in seawater, they lost their osmoregulatory capacity and died due to increased plasma osmolality, while salmon allowed to enter freshwater were able to regulate their plasma osmolality and survived.

Conclusions

Anadromous fishes are capable of migrating between freshwater and saltwater environments during their life history. The ability to osmoregulate in both ion-poor freshwater and salt-rich marine environments required a myriad of physiological, morphological, and behavioral adaptations, which allowed these species to take advantage of the benefits of each environment, maximizing growth, reducing competition and predation, and thereby increasing their overall fitness and reproductive success. Due to their abilities, many anadromous species have become important model species for studying questions related to maintaining homeostasis in changing environments.

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