Crossing the Salt Barrier

Samir Bhattacharya

Salinity of water is a stumbling block for the movement of aquatic animals. There are thousands of fish species whose localization in water bodies is strictly restricted to either the sea or freshwater (rivers, lakes, ponds, etc.), or brackish water (where river or lake water mixes with sea water). Only three species known so far have conquered this deadly salt barrier and made crossing it a regular life process. This article gives an indepth account of the processes and strategies that enable them to do so.

Samir Bhattacharya, whose teaching and research area is comparative studies of mechanism of hormone action in different vertebrates, is at the School of Life Science, Department of Zoology, Visva-Bharati, Santiniketan. By setting up different models of hormone action, he is now attempting to explain various signal transduction processes involved in seasonal reproduction

Take any organism from sea water (SW) and place it in freshwater (FW, land-locked water). It will immediately die. The reverse is equally true – FW organism placed in SW will also die immediately. Why does such an instantaneous death occur? Such deaths occur mainly because animals living in SW or FW have developed opposite adaptation strategies. Let us discuss this by taking the teleostean (bony) fish as an example. The reason for selecting fish, as a model organism of aquatic adaptation is their diverse distribution in various qualities of water bodies and the maximum amount of adaptation their various species demonstrate. One can broadly divide the waters of this world into three main categories – seawater, brackish-water and freshwater, depending on the variation in salinity. Salinity or salt concentration is mainly judged by the NaCl concentration, although variation in other salts and minerals do occur. The salinity of seawater is approximately between 3.0–3.5%, brackish water 0.9–1.5% and fresh water 0.3–0.5%. These large differences in salinity show why marine of FW fish die when transferred. However, tolerance to salinity may vary widely among fish. If we take salinity tolerance as a marker of evolution of fish species it can be clearly seen that they have evolved from no tolerance to minimal tolerance and ultimately to complete tolerance. Fish which cannot tolerate any change in salinity and die

Tolerance to salinity may vary widely among fish. If we take salinity tolerance as a marker of evolution of fish species it can be clearly seen that they have evolved from no tolerance to minimal tolerance and ultimately to complete tolerance.

instantaneously, when transferred to water having a very small change in salinity are termed stenohaline and are found strictly in SW or FW. The majority of SW and FW fish are stenohaline. Fish which can tolerate a small change in salinity are called euryhaline and are found in brackish water. These are strikingly few in number as compared to the stenohaline species. Only a very few species, specifically three in number, which can tolerate a large change in salinity, i.e. SW or FW, are known as amphihaline. Amphihaline fish are migratory fish like hilsa in our country and salmon in the Atlantic and Pacific Oceans. Why is it that not even ten different species of teleostean fish are successful in crossing the salt barrier among more than twenty thousand species of teleosts? The reasons are very interesting and are explored below.

The Real Barrier

Let us try to understand the situation. The osmotic pressure (OP) of the internal tissue fluid (known as the internal milieu, IM), in most aquatic vertebrates and lower vertebrates (e.g. Myxine or Hagfish), follows fairly closely the variations in the OP of the external environmental (EE) water. But teleostean fish have evolved further. In teleosts, both marine and fresh water, serum osmolality varies between 230 and 330 milliosmols (mOsm)[1]. You may now start appreciating the problem. The amount of NaCl in the IM of a SW or FW teleost is about 223mOsm or 0.7%, while in SW, salinity is approximately 957mOsm or 3%. The salinity of SW is more than four times that of the concentration in the IM of marine fish. In FW, the situation is just the reverse. The salinity of FW varies widely but is on an average 96mOsm or 0.3%, which means that the salinity of the EE is less than half of the salinity of the fish's IM. Thus, a marine fish lives in an environment where its IM is much lower than EE and a fresh water fish lives in an environment where just the converse is true. That is why a marine fish's IM is hyposaline in comparison to the EE whereas a fresh water fish's IM is hypersaline when compared to the EE (see *Figure 1*). This difference poses the real hurdle in crossing the salt barrier,

[1] Relationship between molar concentration and osmotic activity is conveniently expressed by use of the term osmol (Osm) where, for example, 1mM or 0.0058% NaCl is equivalent to 1.863mOsm.

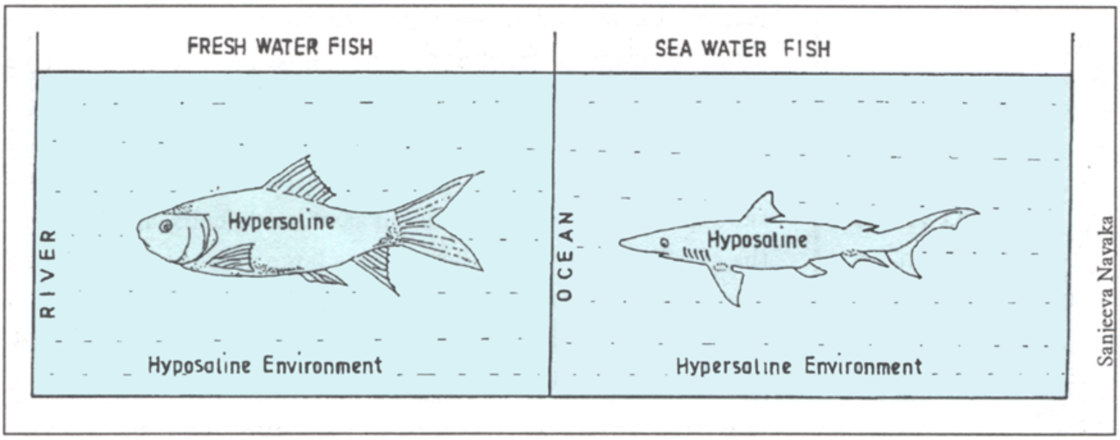


Figure 1. FW and SW fish live in completely different environments.

but why? Let us discuss.

Irreversible Adaptation

In the marine fish, the OP of the IM being much lower than that of the EE, there is continual loss of body water by osmosis which follows the concentration gradient rule and consequent penetration of ions by passive diffusion following the ion concentration gradient rule. A marine fish, therefore, faces two major problems – (i) threat of loss of water, and (ii) danger of excess penetration of ions (ionized salts). To solve the first problem, a marine fish drinks abundant quantities of water. As it drinks mostly to make up for the loss of water, diuresis is slight. Conditions are exactly opposite in FW fish. OP of the IM is higher than the EE, so that there is a continual loss of ions while water invades concomitantly. Hence, it has two completely opposite problems – (i) threat of excess water penetration, and (ii) danger of losing ions.

Glossary

FW = Fresh water
SW = Sea Water
OP = Osmotic pressure
EE = External environment.
IM = Internal milieu

By means of a simple experiment you can visualise the truth of the above facts. Take a marine fish and place a simple knot around the oesophagus with the help of a thread so that it cannot drink water. This will lead to rapid loss of water followed by death within a short time. On the other hand, a FW fish will be able to survive for a long time with an obstructed oesophagus, as water will invade through the gills and mucous membranes of the mouth and skin.

Structural and physiological requirements for iono-osmoregulation between FW and SW fish are opposite in nature.

Hence marine and fresh-water fish have developed opposite kinds of adaptive features to survive against their respective threats. To minimise the loss of water, marine fish kidneys contain few or no glomeruli and the tubules absorb water and excrete salts, so that the urine is concentrated. To extrude excess salts special type of cells, known as chloride cells, are found in the gill filament. Since they drink a large amount of water, the intestine absorbs water but selectively excludes salts. In fresh-water fish, the kidney has a large number of glomeruli. Kidney tubules absorb salts and eliminate water, therefore excreting a large amount of very dilute urine. However, despite reabsorption of the salts in the tubules, the urine is richer in salts than the EE. There is a continual loss of ions through the urine in addition to the losses through gills and skin. A fresh-water fish therefore covers its body and gill surface with large amounts of mucous secretion to minimise this loss. This is the reason why a fresh-water fish is slippery whereas a marine fish is rough!

Hence structural and physiological requirements for iono-osmoregulation between FW and SW fish are opposite in nature. It is now conceivable why a marine fish dies instantaneously when transferred to fresh water or the other way around. Majority of the marine and fresh water fish is stenohaline which cannot survive if transferred to another environment. Euryhaline fish also die when transferred to seawater. Only amphihaline fish are able to overcome this strong barrier.

Amphihaline Fish can Overcome this Barrier

To complete their lifecycle, amphihaline fish show remarkable adaptation in being able to migrate from the sea to fresh water bodies or vice-versa. They have to survive in two different environments for the sake of their progeny. There are two distinct types of amphihaline fish. One lives in seawater, grows there and after becoming reproductively mature starts migrating to breed (spawn) in riverine or land locked fresh water. The young, born in FW migrate to SW and live there until they are, in turn, ready to spawn. These fish are known as amphihaline potamotocous e.g. salmon and hilsa. Another type of amphihaline fish which live in riverine or lake water (FW), when reproductively mature, migrate to the sea (SW) to breed there. These kinds of fish are known as amphihaline thalassotocous e.g. eel. To cross the salt barrier is, therefore, an obligatory part of every amphihaline fish cycle. But this is not an easy process. Reproductively mature salmon migrate from the sea to the river with great difficulty (more than 90% of the migratory population die in this process), but they don't stop until they reach their breeding ground. This urge to breed is such a strong motive, that it necessitates a drastic modification of their osmoregulatory mechanism.

To cross the salt barrier is, therefore, an obligatory part of every amphihaline fish cycle.

Figures 2a,b describe the situation. Migration from river to sea or from the sea to the river involves crossing a transitional zone known as estuary, where sea and river waters meet each other and mix. Thus, the salinity of the estuarine water is higher than FW but lower than SW and this water is called brackish water. It is an important water body for migratory fish as here they adapt to their new environment. Fish, like mullet and bhetki, living

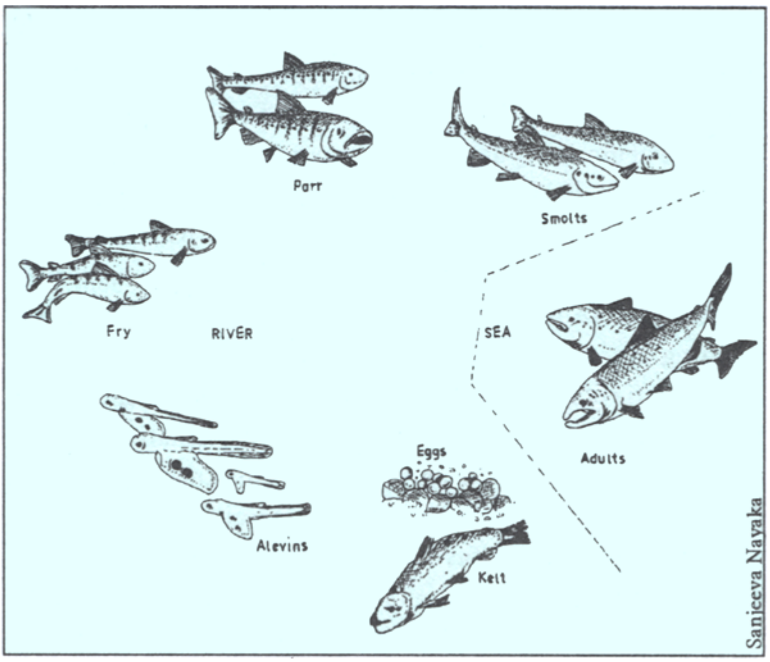


Figure 2a. Life Cycle of Salmon. Adult salmon migrate from sea towards the river. After reaching their hatching ground, the eggs are laid in the gravel. The spawned fishes are called kelts. Alevin is a stage from hatching to fry (nutrition requirement during this transformation comes from yolk sac) and fry grows to a specific stage called parr. The parr undergo internal and external transformation to reach a stage called smolt. The smolts are ready to adapt to marine environment and go down to the sea.

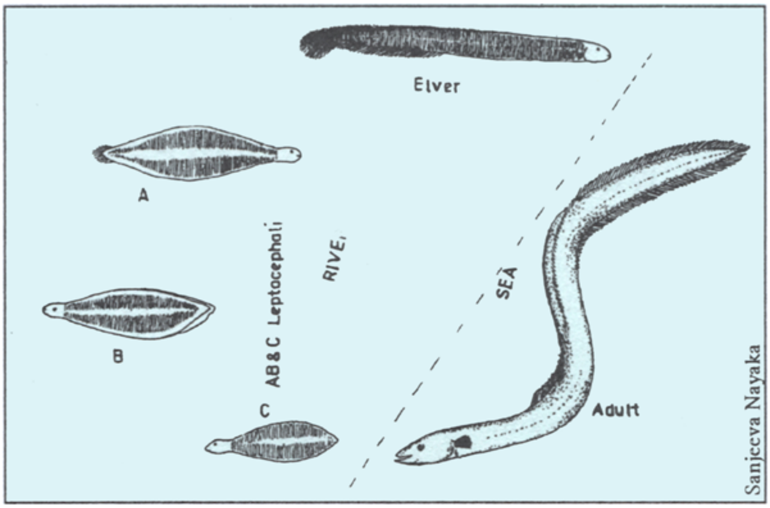


Figure 2b. Life cycle of eel. The adult eel migrates to sea during low tide, lays eggs and dies. The larval stage of the eel is called leptocephalous and they appear in different forms. The leptocephalous transforms into young eel, known as elvers who start upstream migration.

in the estuary are euryhaline in nature and are brackish water fish. These fish do not migrate. During their upstream journey (the anadromous journey) or downstream movement (the catadromous journey), amphihaline fish use the estuarine waters to adapt to the environment they are approaching.

All salmon (species belonging to the family salmonidae) breed in FW. Adult mature salmon migrate from the sea towards the river in shoal, cross the estuarine region, enter into the river, and continue their anadromous movement till they reach their hatching ground, which is very specific for a particular shoal. A high rate of mortality during this strenuous upstream migration ensues. This mortality is possibly due to the loss of stored energy for coping with the complete shift in osmoregulation along with the exertion involved in this anadromous movement especially since they do not feed during this migration. On reaching the spawning ground, male and female salmon spawn. At a specific stage of development the young salmonid, called parr, undergo external and internal transformations which preadapt them for survival and growth in a marine environment. At this point, they are called smolt, a short but critical FW phase bringing them to the status of SW fish. This parr to smolt transformation process is known as smoltification, a physiologically intriguing phenomenon which allows smolt to migrate towards the sea. Many others from the same group of young fish remain as parr, which are strictly FW species and never migrate to the sea. It remains unknown why from the same batch of breed, or the same batch of parr, many attain the smolt stage (after smoltification) and acquire the capability of SW tolerance, while many others remain as parr, having no tolerance to SW and therefore not showing any sign of migration. Smolts are stimulated by internal and external conditions to begin the downstream catadromous migration to the estuary. They remain for a while in brackish water to adapt to the changing salinity before migrating to the sea where growth is very rapid. Interestingly, external identification of parr and smolt is rather easy. Parr is greyish while smolt is strikingly silver, shining under water.

The eel presents a converse case. It's purpose in downstream or catadromous migration is not to find good food in the ocean as with the salmon, but to breed there. FW eel, which are reproductively mature, are faintly yellowish in colour, but prior to their journey turn silvery as in the case of the smolt. Eel on their catadromous migration remain completely inactive and float passively in estuarine waters. They wait for the ebb (low tide) and are almost inert bodies drifting towards the ocean with the movement of the water. When estuarine waters enter the ocean mouth during the ebb tide, eel, being very efficient swimmers, move a long distance into the deep sea to spawn. On their return journey from the sea to the river, the young eel approach the coast following their metamorphosis from the leptocephalous larval stage to the elver (young) stage. Only now can they start their fluvial anadromous migration, which is not reproductive, but trophic for food. Both salmon and eel, although they have opposite directions of movement for specific purposes during amphihaline migration, successfully cross the salt barrier during their anadromous and catadromous migration.

Preparation for Life in a Changed Environment

Silvery eel migrating downstream to the sea develop thickening of the layers of skin which help them adapt to the strongly hypertonic surroundings once they enter the ocean. There is an appreciable increase in the number of gill tissue chloride cells that secrete chlorides. These cells enable the eel to resist the salt invasion that threatens them in the marine environment. Similar changes occur in smolt–increase in the number of chloride cells and their ultrastructural modification effect the abundance and form of the mitochondria resulting in stimulation of Krebs cycle enzymes. Since they require extrusion of salt against the concentration gradient, detectable increase in salt linked ATPase activity occurs. ATP is a substrate of this enzyme and is supplied by the Krebs cycle. Such an active state of chloride cells is also marked with rapid increase in protein synthesis supported by the proliferation of ribosomes. Now these cells are capable of pumping out salts to the surrounding medium whatever the concentration of ions in the EE may be. Not surprisingly, in parr there are very few chloride cells and these are poorly developed.

An experiment performed by a group of Japanese scientists, Utida and his associates, clearly shows the internal physiological difference between parr and smolt in relation to their ability to adapt to salinity changes (*Figure 3*). Both parr and smolt were collected from the same area of a body of FW and transferred to SW. An immediate increase in serum osmolality above the normal range (230–330mOsm) was detected in both, and this rise continued till 8h. In comparison to parr, a striking decrease in osmolality followed in smolt, a trend that ultimately reached normal at 96h. This experiment demonstrates that smolt transferred into the hypertonic surroundings behave like marine fish although still in FW, while parr has no such capability. This is a preadaptive phenomenon in smolt, where a distinct ability to change the osmoregulatory mechanism necessary for marine life was built up during the smolt to parr transformation.

The silvery colour that appears in smolt and eel prior to their downstream migration, is due to the deposition of crystalline purines, guanine and hypoxanthine, both beneath the scales and

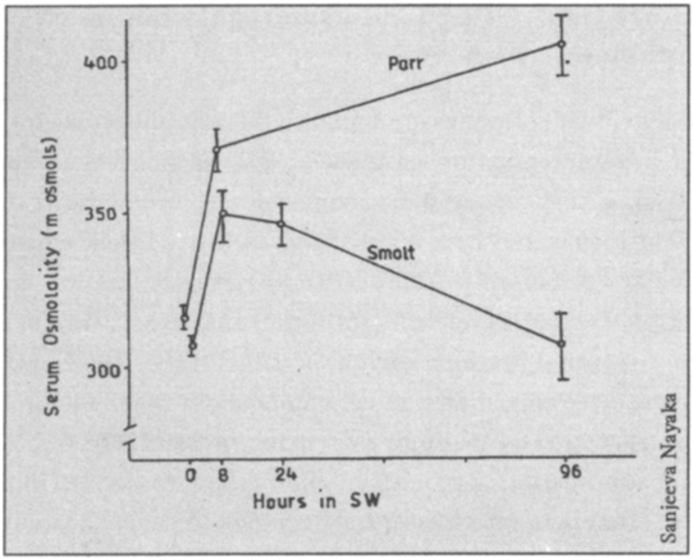


Figure 3. Results of the experiment by Utida and his associates showing variations in serum osmolality of parr and smolt stages of salmon, Oncorhynchus masu, when transferred from FW to SW [1]

in the second deep dermal layer. This provides a solid support to block the entry of salts through the skin in the new hypersaline environment. Another characteristic feature of the silver eel that appears prior to its sojourn to the deep sea is the change in the colour of the retina. In the yellow eel (FW form), the retina is crimson in colour due to a mixture of the pigments, prophyropsin and rhodopsin. When a yellow eel changes into a silver eel, the colour of the retina changes to golden with the appearance of a pigment called chrysopsin. This has a genuine functional advantage. This change favours vision in deep oceanic waters where maximum light penetration is at a wavelength of only about 480nm, far less than that in river waters. Since chrysopsin can absorb this shorter wavelength more efficiently than prophyropsin, vision is greatly improved.

About the preparation for anadromous migration (to FW), very little is known. Adult mature salmon and elver (young eel) both develop an enormous capacity to secrete mucous. This covers their gill filaments and skin once they enter into the riverine water helping them in retaining the IM osmolality in the new hyposaline environment. There is a remarkable decrease in the number of chloride cells, which indicates a clear modification of the osmoregulatory apparatus suitable for riverine life.

Cortisol facilitates adaptation to seawater by increasing absorption of water in the intestine with concomitant selective exclusion of ions and by stimulating reabsorption of water from the urinary bladder.

Factors Controlling Iono-osmoregulation in Amphihaline Fish

Till date, two hormones are found to be very important for the shift in osmoregulation from SW to FW or viceversa. One is cortisol, a corticosteroid hormone released from the adrenal cortex. Fish do not have adrenal glands, instead their corticoid tissue are dispersed in the head kidney region known as the interrenal which secrete cortisol. Interrenalectomy (removal of the interrenal organ) seriously inhibits smoltification. Interrenalectomized mature eel do not show silver colour, and their transfer even to dilute SW results in death. On the other hand, administration of cortisol allows them to survive in SW. Cortisol therefore is an essential hormone to enable adaptation to marine life. Cortisol also has a role in facilitating adaptation to seawater by increasing absorption of water in the intestine with concomitant selective exclusion of ions and by stimulating reabsorption of water from the urinary bladder. Injection of cortisol into the yellow eel induces the rise in Na^+ -K^+ ATPase activity in chloride cell. There is an enormous increase in the plasma cortisol levels during smolting of salmon. Parr plasma has very low levels of cortisol while smolt plasma contains remarkably high levels of cortisol.

The other important hormone is prolactin, but its requirement is for fresh water life. Prolactin induces the mucous secretion that covers gill filaments and the body surface, inhibiting salts from going out and water from entering. These are the two essential requirements for SW fish as they live in a hyposmotic surrounding. Prolactin also regulates water and ion movement through the intestine and urinary tract. Prolactin, therefore, is very important in maintaining ion balance in FW. Besides cortisol and prolactin, thyroid hormone, growth hormone and insulin have also been shown to be involved in iono-osmoregulation of amphihaline fish.

Concluding Remarks

Adaptation to SW and FW require totally different osmoregulatory mechanisms. For this reason a large number of fish living in the sea, river or estuary are confined to the kind of water that they are adapted to living in. Migration never occurs due to the extremely strong salt barrier. Only a few species of fish have conquered this barrier, and made it a part of their regular life cycle. This is possible due to the successful shift of osmoregulatory mechanisms that are, in turn, regulated by certain specific hormones.

Prolactin induces the mucous secretion that covers gill filaments and the body surface, inhibiting salts from going out and water from entering.

Acknowledgement

The author wishes to thank S Guha, A Bandyopadhyay, C Majumdar, A Chatterjee and I Bandyopadhyaya for helping with preparation of the manuscript.

Suggested Reading

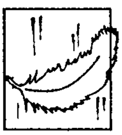
[1] M Fontaine. Physiological mechanisms in the migration of marine and amphihaline fish. *Advances in Marine Biology*. Academic Press, New York. 13. 241–355, 1975

[2] G Boeuf and Y Haracha. Criteria for adaptation of salmonids to high salinity seawater in France. *Aquaculture*. 28. 163–176, 1982.

[3] T Sakomoto, M Twaata and T Hirando. Kinetic studies of growth hormone and prolactin during adaptation of coho salmon, *Onychorhynchus kisuutch* to different salinities. *General and Comparative Endocrinology*. 82. 184–191, 1991.

[4] G Boeuf. Salmonodio smolting: a pre-adaptation to the oceanic environment. *Fish Ecophysiology*. (eds.) J C Rankin and F B Jensen, Chapman and Hall. London. 105–135, 1993.

Address for Correspondence
Samir Bhattacharya
Department of Zoology
Visva-Bharati University
Santiniketan 731 235
West Bengal



1994

Images made by positron emission tomography and magnetic resonance imaging literally show the human mind at work.

Scientific American
September 1995.