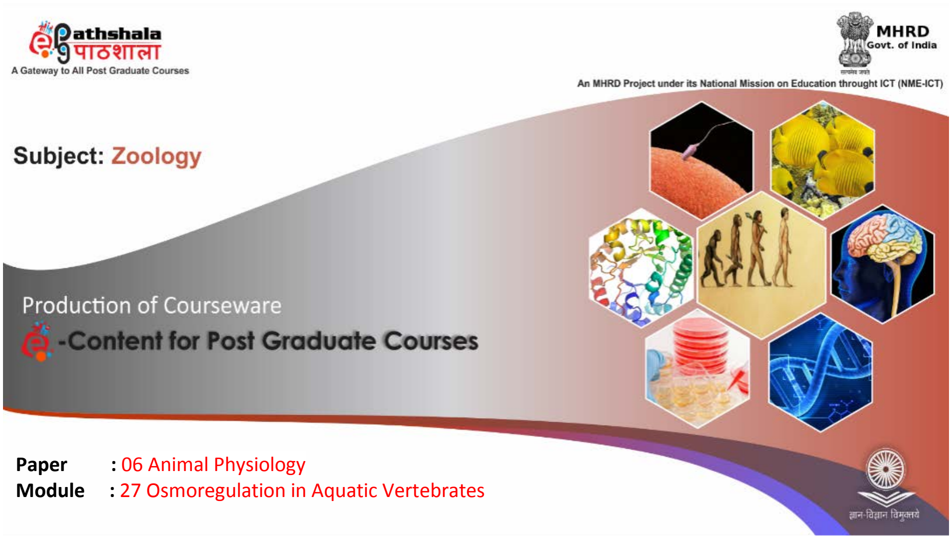

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Description of Module	
Subject Name	ZOOLOGY
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Module Name/Title	Osmoregulation
Module Id	M27:Osmoregulation in Aquatic Vertebrates
Keywords	Osmoregulation, Active ionic regulation, Osmoconformers, Osmoregulators, stenohaline, Hyperosmotic, hyposmotic, catadromic, anadromic, teleost fish

Contents

1. Learning Outcomes

After studying this module, you shall be able to

- Learn about the major strategies adopted by different aquatic vertebrates.
- Understand the osmoregulation in cyclostomes: Lamprey and Hagfish
- Understand the mechanisms adopted by sharks and rays for osmotic regulation
- Learn about the strategies to overcome water loss and excess salt concentration in teleosts (marine and freshwater)
- Analyse the mechanisms for osmoregulation in catadromic and anadromic fish
- Understand the osmotic regulation in amphibians (fresh-water and in crab-eating frog, a salt water frog).

2. Introduction

Table 1 represents the major strategies adopted by aquatic vertebrates. Both freshwater and marine vertebrates are enlisted with examples in Table 1.

S. No.			Habitat	Osmotic concentration (mOsm L^{-1})
1	**Sea Water**			~1000
2	Cyclostomes	Hagfish (*Myxine*)	Marine	1152
3		Lamprey (*Petromyzon*)	Marine	317
4		Lamprey (*Lampetra*)	Fresh-water	271
5	Elasmobranch	Ray (*Raja*)	Marine	1050
6		Dogfish (*Squalus*)	Marine	1000
7		Fresh-water ray (*Potamotrygon*)	Fresh-water	308
8	Coelacanth (*Latimeria*)		Marine	954
9	Teleosts	Goldfish (*Carassius*)	Fresh-water	259
10		Toadfish (*Opsanus*)	Marine	392

11		Eel (*Anguilla*)	Fresh-water	323
12			Marine	371
13	Salmon (*salmo*)		Fresh-water	340
14			Marine	400
15	Amphibians	Frog (*Rana*)	Fresh-water	200
16		Crab-eating frog (*R. cancrivora*)	Marine	830

1. There are two distinct groups of marine vertebrates:

a. The organisms whose osmotic concentrations are either slightly above or equal to the osmotic concentration of sea water such as crab-eating frogs, elasmobranchs, *Latimeria* and hagfish. This group of marine representatives encounter no major issues of water balance because there is no osmotic water flow due to same osmotic concentration inside and outside environment.

b. The other group of organisms are those whose inside concentrations (osmotic) are about 1/3rd that of outside environment i.e. sea water such as in teleosts and lampreys. This group lives in osmotically more concentrated medium and thus remain hyposmotic and invariably live in danger of osmotic water flow to the medium (water loss). Thus marine vertebrates have different osmotic problems and they differ in their means of solving them.

2. In contrast, fresh-water vertebrates which in principle similar to fresh-water invertebrates, are hyperosmotic to the external environment i.e. those organisms whose osmotic concentration are one-third to one-fourth that of sea water.

3. Cyclostomes

Among all living vertebrates, the cyclostomes are the most primitive eel-shaped fishes, characterized as jawless and devoid of paired fins and bony skeleton. These fishes are jawless vertebrates and thus grouped in Class Agnatha. Lampreys and hagfish are the two distinct groups of cyclostomes.

3.1. Lampreys

Lamprey thrives in both the conditions: sea water and the freshwater. In this group of cyclostomes the sea lamprey is anadromic, for example *Petromyzon marinus*, these are those fishes which ascends from rivers to spawn or breed in fresh-water. In contrast, catadromic are those which live and grow in fresh-water and descends to the sea water to breed such as common eel.

Whether marine or fresh-water, the inside osmotic concentrations are nearly one-third to one-fourth (always infractions) that the concentration of sea water. They share a common solution to the problem of life in sea water with the teleost fish (discussed later in this chapter).

3.2. Hagfish

Hagfish are exclusively marine and stenohaline. Hagfish is the only group of true vertebrates whose salt concentrations of body fluids are the same or slightly above the sea water. In hagfish blood the normal sodium concentration is higher than that in the medium.

Hagfish being isosmotic and having in blood high salt concentrations behave osmotically in principle similar to invertebrates, however, they exhibit pronounced ionic regulation.

In all marine vertebrates, except hagfish, their body fluids have salt concentrations at a fraction of the level of the sea water. This situation favours the argument that the vertebrates invaded the sea later only after originally being evolved in fresh-water.

4. Elasmobranchs

a. **Marine elasmobranch**

Sharks and rays (elasmobranchs) are exclusively marine. They have adopted a very interesting solution against the osmotic problem of living in marine habitat i.e. sea.

They behave like most vertebrates in maintaining salt concentrations in their body fluids at a fraction of one-third (roughly) to that of the level of concentration in sea water but equivalent osmotic concentration to sea water i.e. they maintain osmotic equilibrium where their blood osmotic concentration either slightly exceeds or equivalent to that of sea water. This is achieved by retaining large amount of organic compounds in their body fluids, primarily urea.

In marine elasmobranch, the concentration of urea is 100 times more than that in mammals and no other vertebrates could tolerate this much high concentration of urea.

In the elasmobranch, the tissues and all the body fluids requires high urea concentration for proper functioning. Urea is a normal component essential for many biological processes in elasmobranch. If the heart of shark is isolated and perfuse with high urea concentration containing saline solution of ionic composition equal to that of blood, the heart can pump for hours. But when urea has been removed it was observed that the heart stop beating preceded rapid deterioration.

In elasmobranch, besides urea which is a primarily organic compound to maintain osmotic concentration of blood, TMAO (Trimethylamine oxide) is another osmotically important compound.

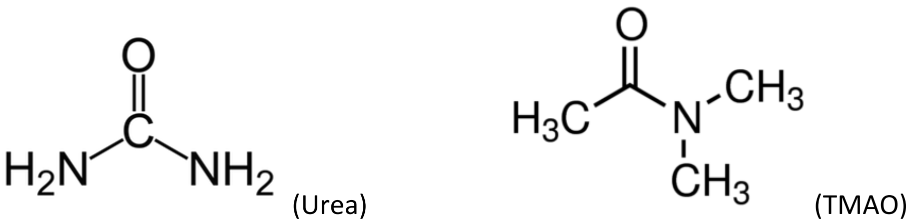


Figure: 1: Structure of urea and TMAO

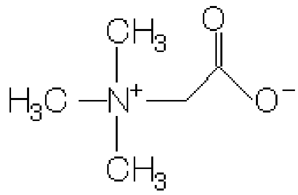
In some vertebrates and mammals the end product of protein metabolism produces a nitrogenous waste called urea, excreted out by excretory organ to the exterior such as kidney in mammals.

However, in sharks, urea is actively reabsorbed by the kidney so that it's retained in blood.

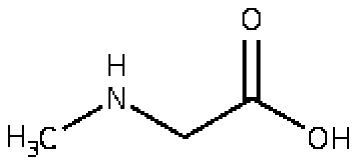
The origin and metabolism of TMAO, organic compound, is poorly understood but it's mainly found in many marine organisms.

Thus, in marine organisms, the elegant solution to obtain osmotic equilibrium or/and to establish low salt concentration in body fluids to the level in sea water is achieved by urea retention.

Although, urea is known to be a disturbing agent for enzymes and many proteins but it was discovered that many compounds could counteract the inhibitory effects of urea including TMAO. Other compounds are betaine, methylated amine and sarcosine.



(Beatiane)



(Sarcosine)

Figure 2: Beatiane and sarcosine structure

When the ratio of urea to methylated compound is 2:1, the counteracting effects on urea are maximum. This ratio of urea/methylamine is found in those marine vertebrates which maintain their osmotic equilibrium by retaining organic nitrogen compounds in their body fluids.

Although the elasmobranchs obtain an isosmotic equilibrium they still have pronounced ionic regulation. The sodium concentration is maintained at half the level in sea water by eliminating the excess sodium which is either ingested with food or diffused through thin gill epithelium into the elasmobranch (shark).

In sharks, the sodium concentration is kept down by its excretion through kidney and a special gland which opens into intestine, the small gland called **rectal gland**.

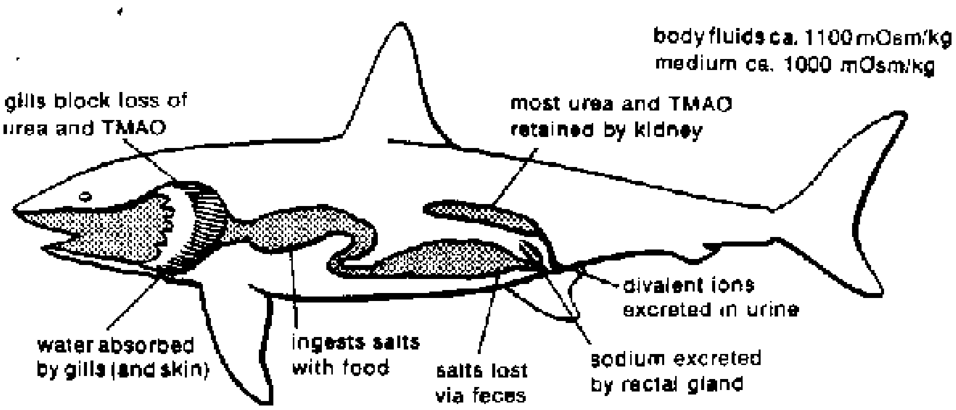


Figure 3: Osmoregulation in marine elasmobranch

The elasmobranchs are osmotically in equilibrium to the level in sea water and thus severe osmotic loss is not a problem by now, also they do not need to drink sea water thereby avoids the associated intake of sodium.

Interestingly the blood is slightly more concentrated in elasmobranch than sea water causing slight osmotic gain of water through gills which in turn used for the excretion from renal glands as well as formation of urine.

b. Fresh-water elasmobranchs

Majority of elasmobranchs are marine but a few of them enter fresh-water and some permanently thrives in fresh-water bodies like rivers and lakes. Even the most typical marine elasmobranch includes some species with tolerance to low salt concentration in medium. There are some examples where marine elasmobranch (sharks and rays) enter rivers and apparently thrives there such as *Carcharhinus leucas* is shark existing in Lake Nicaragua. This shark was supposed to be exclusively marine but the shark in Lake Nicaragua is able to move freely to sea and morphologically resemble the marine forms.

Potamotrygon, commonly known as the Amazon sting ray, is an elasmobranch permanently thriving in fresh-water. It's commonly found at a distance of 400km away from sea in Orinoco and Amazon drainage systems. It can adapt to half sea water concentration but cannot survive in sea water. The average blood composition resembles that of fresh-water teleosts with low blood urea concentration indicating complete adaptation to fresh-water.

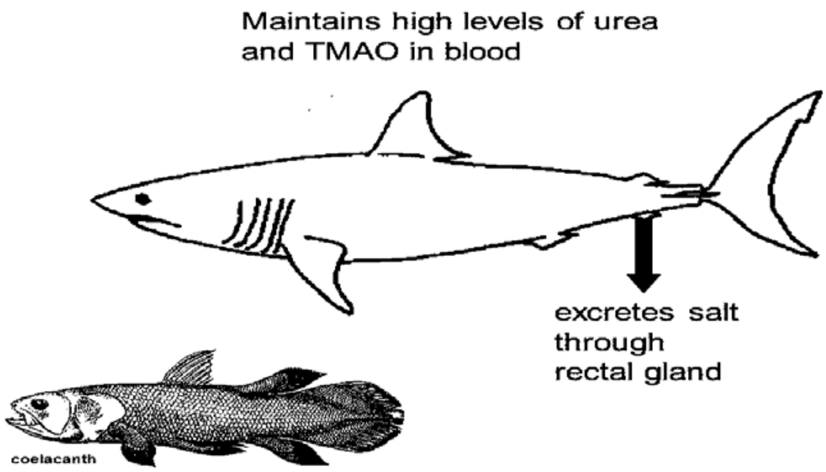


Figure 4: Ureosmotic Shark and Coelacanth

5. The Coelacanth

The Crossopterygii is a group of fishes which is believed to be extinct until 1938 for more than 45 million years. In 1938, a large specimen of more than 50 kg and 15 m long

Coelacanth *Latimeria* (fig 4) was captured from the coast of S.E. Africa. Several species of *Latimeria* caught off the Madagascar and based on possible physiological experimentations, it was suggested that they dealt with the problem of osmoregulation in same way as elasmobranchs.

6. Teleost Fish

Both, marine and fresh-water teleost fishes maintain their osmotic concentration within the same range at about one-third or one-fourth the level in sea water, although marine fish could have higher blood concentrations.

Some teleost fish can thrive in a broad range of salinities, migrating between sea water, fresh-water and brackish water.

Such moves between different salinities are linked with the life cycle of fish. For instance, the salmon is a marine fish which moves to fresh-water for reproduction after reaching maturity; the spawning is followed by migration to the sea.

However, the common eel demonstrate reverse life cycle, it thrives in fresh-water, migrates to sea water for spawning, the larvae drift with the currents to reach coastal region from where they further migrate to fresh-water.

After reaching maturity, eel returns to sea water to spawn, there are profound changes in the osmoregulatory process to tolerate such changes and thrive from one environment to the other.

6.1. Marine Teleosts

Marine fishes are under constant danger of losing water from their body as they are hyposmotic in comparison to more concentrated medium. The water is lost from body surface, particularly gill surface, which is permeable to water. This inevitable osmotic loss of water must be compensated in such marine fishes, performed by them by drinking sea water.

They restore the osmotic loss of water by drinking lots of sea water which comes along with large content of salts. The ingested salts and water are absorbed from intestinal tubes, thereby increasing the salt concentration in body.

But now, the principle problem becomes the elimination of this absorbed excess salt in the body. To achieve a net gain in water, the salts must be expelled out of the body in higher concentration than the salt content ingested with the drinking water, this is achieved by producing more concentrated urine then blood but this purpose cannot be served by the teleosts excretory organ, kidney.

Therefore, to eliminate the excess salt, some other organs perform this function such as gills. Thus, teleosts gills serve dual function of participating in gas exchange and osmotic regulation. As the excretion of salt occurs from lower concentration in blood to the surrounding medium of higher concentration, thus the process of elimination of excess salt across gill epithelium is an active process of transportation.

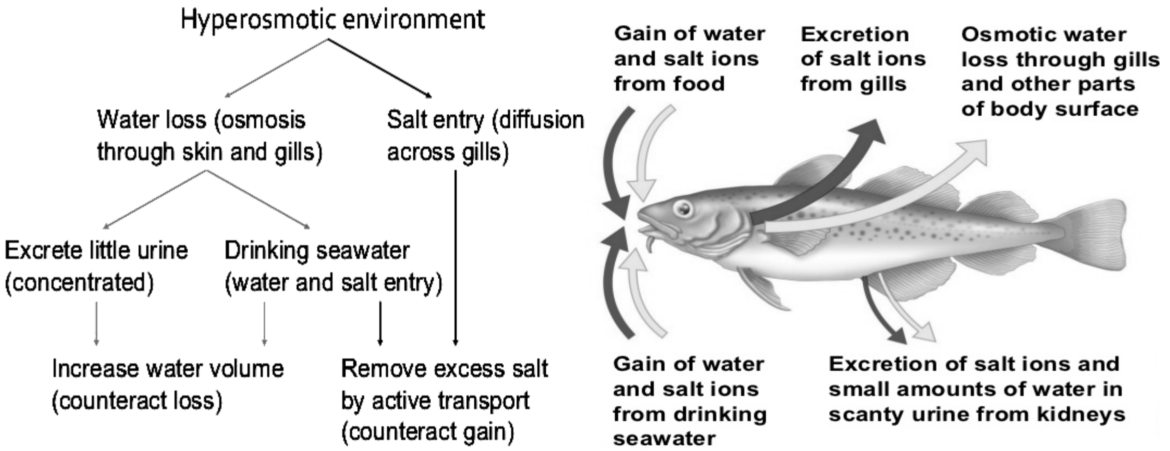


Figure 5: Osmoregulatory mechanisms in marine teleosts

Figure 5 summarizes the chief aspects of osmotic regulation in teleosts living in marine environment.

In figure 5, the movement of water is demonstrated, the osmotic loss of water occurs in the urine and across the gill membrane. To restore the osmotic loss of water content, the fish drinks sea water, gaining both salts and water from the intestine.

In figure 5, this gain of salt content through drinking of salt water *via* mouth has been shown. The movement of salt occurs from external sea water to the body. This excess salt content is eliminated by the secretion of sodium and chloride *via* active transport across the gills. The elimination of sodium and chloride through gills are of major importance in comparison to its loss in the urine as the urine of teleosts is comparatively more dilute than body fluids.

Major excretion occurs through kidney which eliminates sulphate and magnesium divalent ions in urine, constituting one tenth of sea water salt content. Gills only transport sodium and chloride rather than eliminating these divalent ions.

6.2. Fresh-water teleosts

The fresh-water teleosts fish resemble fresh-water invertebrates in their osmotic conditions. The osmotic concentration in body fluids is higher than surrounding medium, usually in the range of 300mOsmL-1.

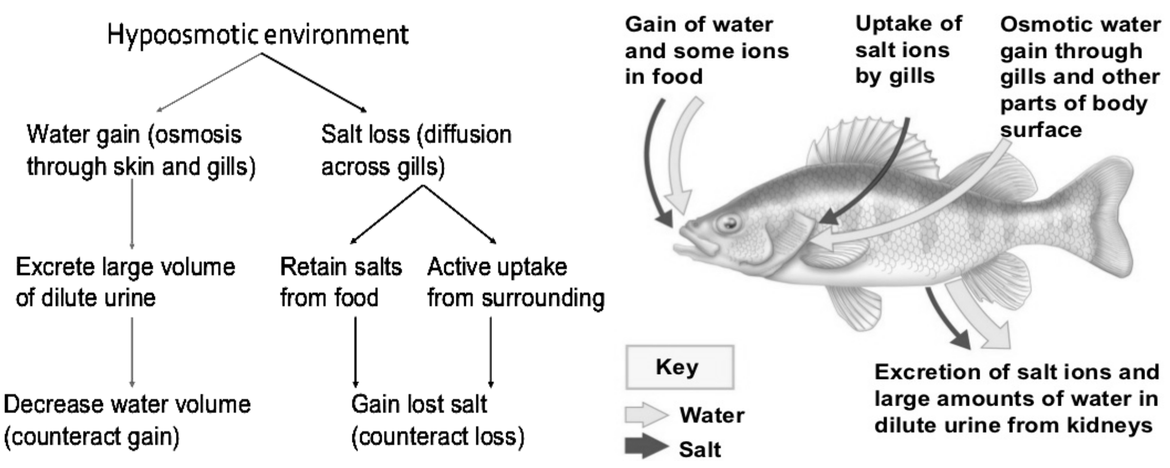


Figure 6: Osmoregulatory mechanisms in fresh-water teleosts

Figure 6 demonstrate the main steps involved in the osmoregulation of fresh-water teleosts. The major problem faced by fresh-water teleosts is the osmotic gain of water *via* gill. The skin play less important role than gills in the inflow of osmotic content, as they are relatively high permeable and facilitate large surface area for osmotic flow.

To overcome the osmotic problem and maintain the water content level excess water is eliminated as dilute urine in quantities ranging to one-third of body weight/day. This may cause significant loss of solute in urine which is covered by the ion uptake through gills as they are slightly permeable to ions.

Thus, gills act as primary site for the main gain of solutes by active transport and only a slight fraction is covered by ingesting with food.

7. Catadromic and anadromic fish

In Greek, *kata* means down, catadromic fish are those which live in fresh-water and migrate to sea. For example, the common eel is catadromic, it thrives and matures in fresh-water and descends to sea water for spawning.

On the contrary, anadromic refers to living in marine water and ascending from sea to fresh-water to breed such as salmon and shad.

(In Greek, "*ana*" mean duo and "*dramcin*" means to run)

Most teleosts are relatively stenohaline (least tolerance to changes the salt concentration of surrounding medium) with restricted ability to migrate between the sea and fresh-water. However, salmon, lampreys (cyclostomes) and common eel migrate between fresh-water and marine water and this movement is associated with their life cycle.

Such moves demands on their mechanisms for osmoregulation and expose the fish to cataclysmic changes.

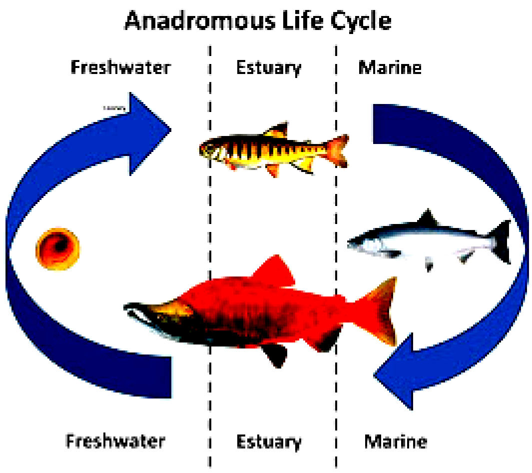


Figure 7: Life cycle showing migratory steps of anadromous fish

If the common eel is restricted from drinking sea water it dies within a few days due to continuous osmotic water loss and dehydration. However, if eel is allowed to drink sea water, it soon begins to subside the weight loss and achieve a steady state within 24-48 hours. If eel is migrated from fresh-water to marine medium, it has been observed that within next 10 hours the osmotic water loss is roughly 4% of body weight.

It was observed that when the direction of migration is reversed, from sea water to fresh-water, it soon begins to gain body weight but a steady state is reached within 24-48 hours as soon as the urine formation increases.

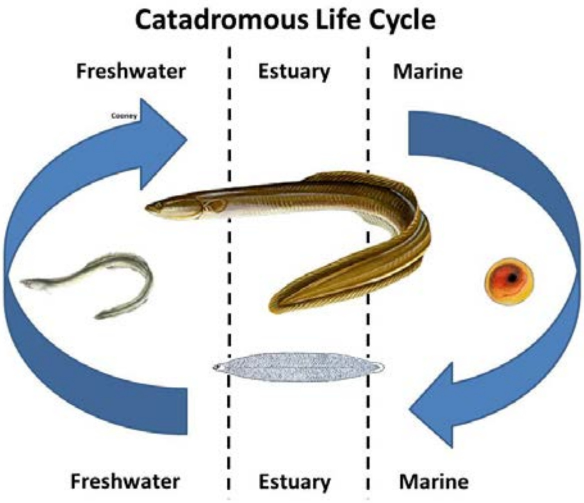


Figure 8: Life cycle showing migratory steps of catadromous fish (Eel)

Initially, when eel descends to sea water from fresh-water, to achieve a steady state the direction of osmotic water flow changes which is followed by the change in the direction of ion transport across the gills to cover up the loss or gain of solutes.

Although, the exact mechanism is not known to us which regulate this change but we assumed that may be endocrine mechanisms are responsible for the change.

8. Amphibians

The unique characteristic feature of amphibians is their dual mode of life on land and water. Mostly they are aquatic or semi-aquatic lives at or near the fresh-water. The indirect development involves laying eggs in water which produce gill breathing aquatic larvae on hatching. Most amphibians (not all) change their mode of respiration at the time of metamorphosis from gill breathing to lung breathing.

Most frogs become more terrestrial however, remain in vicinity of moist habitats or water; on the contrary, some adult salamanders remain completely aquatic and retain their gills for respiration.

Recent studies in South America and Africa suggested the unusual physiological characteristics of some atypical frogs which are highly resistant to evaporation mediated water loss in very dry habitat.

8.1. Fresh-water Amphibians

Amphibians resemble teleosts fish with regards to osmotic regulation. Nearly all amphibians are aquatic (fresh-water) organisms and the main organs of osmoregulation, in adults are skin i.e. cutaneous osmoregulation. In fresh-water, the animal gain water *via* osmotic inflow and this increased water content is reversed by its elimination in form of highly dilute urine. However, there is loss of solute occurring through the skin as well as in the urine which is balanced by the active transport of solute from fresh-water medium (dilute medium). The frog skin, in adult stage, serves as a site for the transport mechanism and become a well known model for active ion uptake investigations.

8.2. Salt-water frog

Amphibians like salamanders and frogs are usually restricted to fresh-water and when placed in sea water they die within few hours. *Rana cancrivora*, commonly known as crab-eating frog of Southeast Asia is the only exceptional case. The crab-eating frog is an ordinary looking, small frog, at home in coastal mangrove swamps, seeks for its food and swims in full-strength sea water.

Frogs could solve the problems associated with living in sea water and maintaining relatively (to vertebrates) low salt concentrations, in two possible ways.

First solution resemble the mechanism adopted by marine teleosts which includes skin as a site to counteract osmotic water loss and covers for the inflow of salt via diffusion across skin.

Second solution is the strategy adopted by marine elasmobranch to eradicate the problem of osmotic loss of water by retaining urea in their body fluids and maintaining osmotic equilibrium with surrounding water. The elasmobranch strategy is adopted by the salt-water frog to maintain osmotic equilibrium by adding large amount of urea as much as 480mmol/L in their body fluids. It is acceptable to follow that salt water frogs employed elasmobranch strategy as it is easy to restrict the osmotic loss of water from their relatively permeable skin by maintain equal osmotic concentration to that of surrounding medium. Unique among vertebrates is the salt tolerance (except hagfish) and the frog would need this feature in order to cover-up water loss solely by adding large amount of solutes and their increasing salt concentration in body fluids.

However, if teleosts strategies were used and they remain hyposmotic, the gain of solute from drinking water would impair the salt balance in the body.

A crab-eating frog remains slightly hyperosmotic like shark when placed in sea water and thus not completely isosmotic to the surrounding medium. This leads to gradual inflow of osmotic water which is requisite to provide water for urine formation. This is a better solution in comparison to drinking from medium for obtaining water and unnecessarily disturbing the salt balance.

In sea frogs (crab-eating) urea is not simply an excretory product but like elasmobranch it's an important osmotic organic component. Additionally, urea is required for normal muscle contraction. Urea is an essential component and should be retained by the animal for its normal life. When the crab-eating frog is present in sea water, urea is retained by decreasing the urine volume. The retention of urea by reabsortion is not an active transport as the urea concentration remains slightly high in urine than in plasma. In sharks, active transport occurs for the reabsorption of urea in the kidney tubules.

In comparison to adult crab-eating frog, its tadpole or larval stages have a greater tolerance for high salinities. However, they differ in their pattern of osmotic regulation where the adults have adopted the elasmobranch pattern of osmotic regulation but teleosts fish pattern occurs in tadpoles.

The crab-eating frog is not independent of fresh-water, although both the adults and the tadpole of crab-eating frogs are highly tolerant to high salinities; a relatively low salt concentration in water is required for the fertilization of eggs and for the metamorphosis to mature form. The spawning in these frogs occurs in dilute water temporary formed near the shore due to frequent torrential rains in the tropics. Thus, the heavy rain dilutes the water and form temporary fresh-water polls essential for the metamorphosis of tadpoles which otherwise remain immature tadpoles at high salinities.

Although, the crab-eating frog is dependent on fresh-water for reproduction and to attain maturity but due to its tolerance to high salt content, it allows the exploitation of rich tropical coastal region which is otherwise restricted to all other amphibians.

9. Summary

There are two distinct groups of marine vertebrates: The organisms whose osmotic concentrations are either slightly above or equal to the osmotic concentration of sea water such as crab-eating frogs, elasmobranchs, *Latimeria* and hagfish. This group of marine

representatives encounter no major issues of water balance because there is no osmotic water flow due to same osmotic concentration inside and outside environment. The other group of organisms are those whose inside concentrations (osmotic) are about 1/3rd that of outside environment i.e. sea water such as in teleosts and lampreys. This group lives in osmotically more concentrated medium and thus remain hyposmotic and invariably live in danger of osmotic water flow to the medium (water loss). Fresh-water vertebrates are in principle similar to fresh-water invertebrates, and hyperosmotic to the external environment i.e. those organisms whose osmotic concentration are one-third to one-fourth that of sea water.

Lampreys, for example *Petromyzon marinus*, and hagfish are the two distinct groups of cyclostomes. Lamprey thrives in both the conditions: sea water and the freshwater. Sharks and rays (elasmobranchs) are exclusively marine. Marine elasmobranchs maintain osmotic equilibrium where their blood osmotic concentration either slightly exceeds or equivalent to that of sea water, achieved by retaining large amount of organic compounds in their body fluids, primarily urea. In marine elasmobranch, besides urea which is a primarily organic compound to maintain osmotic concentration of blood, TMAO (Trimethylamine oxide) is another osmotically important compound (betaine, methylated amine and sarcosine). In sharks, the sodium concentration is kept down by its excretion through kidney and a special gland which opens into intestine, the small gland called **rectal gland**.

Potamotrygon, commonly known as the Amazon sting ray, is an elasmobranch permanently thriving in fresh-water. Coelacanth *Latimeria* dealt with the problem of osmoregulation in same way as elasmobranchs.

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Marine Teleost fishes are under constant danger of losing water from their body as they are hyposmotic in comparison to more concentrated medium. The water is lost from body surface, particularly gill surface compensated by drinking sea water which comes along with large content of salts, thereby increasing the salt concentration in body. The principle problem becomes the elimination of this absorbed excess salt, solved by producing more concentrated urine then blood. This excess salt content is eliminated by the secretion of sodium and chloride *via* active transport across the gills. Besides, major excretion occurs through kidney which eliminates sulphate and magnesium divalent ions in urine.

The fresh-water teleosts fish resemble fresh-water invertebrates in their osmotic conditions. The major problem faced by fresh-water teleosts is the osmotic gain of water *via* gill. To maintain the water content level excess water is eliminated as dilute urine, this may cause significant loss of solute in urine which is covered by the ion uptake through gills as they are slightly permeable to ions. Catadromic fish are those which live in fresh-water and migrate to sea for example the common eel whereas anadromic refers to living in marine water and ascending from sea to fresh-water to breed such as salmon and shad.

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