Part I – The Problem

The global decline of amphibians is a complex problem with many contributing factors such as climate change, introduction of pathogens, and exposure to chemicals. Changing land use patterns, including habitat fragmentation and alteration due to road construction and maintenance, are localized contributors to the problem. In addition, runoff water from roads contains toxins and chemicals. Deicing compounds or road salts, used extensively during winter weather, contain primarily sodium chloride (NaCl) and are a main contributor to the runoff issue. Between 20 and 22 million tons of salt are used on U.S. roads annually (Stromberg, 2014; Dupuis, 2015). To put this in perspective, that’s 137 pounds of salt for every American.

But what happens to all of that salt once it’s applied? It dissolves and splits into Na\(^+\) and Cl\(^-\) and gets carried away by runoff and eventually deposited into water sources. Data from long-term scientific studies, which have tracked this issue for decades, present some startling information about what this accumulation of salt has done to water quality and composition of solutes over time. For example, a group of scientists tracked salt levels from 1952 to 1998 in the Mohawk River in Upstate New York. Over that period, the salt concentration of the water in some samples increased by 243% (Stromberg, 2014).

Road salt pollution, while a concern for human health due to the contamination of drinking water, is a bigger, and often lethal, issue for organisms that live in these impacted freshwater environments. Increased salinity levels interfere and overwhelm the osmoregulatory capabilities of the animals. Many studies have documented the negative impact road salt specifically has on freshwater fish, crustaceans, and amphibians such as salamanders and frogs (Novotny, Murphy, & Stephan, 2008; Collins & Russell, 2009; Karraker & Gibbs, 2011; Jones, Snodgrass, & Ownby, 2015).

Questions

1. Generate several hypotheses about how the amount of salt runoff into freshwater habitats could be reduced. Think broadly! Your answers might include changes to the composition of deicing agents, changes to the landscape, etc.

2. Why are freshwater vertebrates impacted more than terrestrial vertebrates?

Figure 1. Salt residue left after deicing treatment; note drains and river. Source: Photo used with permission of Chesapeake Stormwater Network.
Part II – Introduction to the Renal System

In order to fully comprehend the negative impact road salt pollution can have on aquatic animals such as frogs, we need to look at the vertebrate renal system and the process of osmoregulation in general. Examining how the renal system functions under normal conditions will be helpful for understanding the challenges animals face when their environments change rapidly and substantially.

The vertebrate renal system, also known as the urinary system, consists of two kidneys, two ureters, a bladder, and a urethra. The kidneys filter blood and produce urine. During this process, known as osmoregulation, waste products are removed from the blood and excreted in urine while nutrients such as glucose and amino acids are reabsorbed and returned to the body via the bloodstream. Osmoregulation also entails the careful balancing of water and ions. The amount of water and ions excreted or retained depends on many factors such as blood volume and pressure, hormones, diet, and environmental factors.


While the renal systems of almost all vertebrates possess the structures just discussed, the specific characteristics and function of these structures have been altered, shaped over the millennia by the process of natural selection, allowing an animal to osmoregulate as efficiently as possible in its unique environment. For example, the nephrons of the mammalian kidney are adapted for concentrating urine, thus conserving water. This function is primarily accomplished by their long loops of Henle. This particular nephron structure is critical for life on land where freshwater is often hard to find and body water conservation is imperative for survival. On the other hand, freshwater fish have nephrons that conserve ions while simultaneously producing large volumes of dilute urine to help rid their bodies of extra water. Amphibian kidneys function similarly to freshwater fish. For example, truly aquatic amphibians, in order to survive their very hyposmotic environment, have had to adapt to handle a constant influx of water by producing dilute urine while conserving ions like Na⁺ and Cl⁻. One adaptation that permits this is the reduction—or even complete disappearance—of the loops of Henle from their nephrons.

Questions

1. Why is the reabsorption of nutrients within the nephron critical for an animal’s survival?
2. Explain the relationship between increased levels of NaCl in the extracellular fluid surrounding a nephron and the amount of water reabsorbed into the blood.
3. In order to conserve body water, what part of the nephron must be well developed in terrestrial vertebrates living in very dry environments? Describe how that happens within this component of the nephron.
4. In order for amphibians and freshwater fish to produce dilute urine, what part of their nephron is lacking or at least significantly reduced in size?
5. What would happen to the concentration or osmolality of the filtrate within nephron tubules if an animal consumes or takes on a lot of salt as the result of its environment becoming saltier?
6. Suppose the salinity of a freshwater pond increased substantially as the result of receiving a lot of deicing runoff. Would this impact mammals and amphibians relying on this drinking water source equally? Why or why not?
Part III – Renal System Adaptations

Many species of frogs spend a great deal of their life in water. Even those that are not considered to be truly aquatic generally return to the water for mating and laying eggs. However, some species of frogs are almost exclusively aquatic, spending the majority of their life in ponds, swamps, streams, and lakes.

The green frog, *Rana clamitans*, is a species that is almost exclusively aquatic. It is found across the eastern half of the United States and in southeastern parts of Canada. Frogs like the green frog have unique renal system adaptations that allow them to maintain proper water and ion balance despite living in a very dilute environment.

Watch the video at [http://youtu.be/GayYc9ytG2E](http://youtu.be/GayYc9ytG2E), which provides an introduction to these unique adaptations.

The first of these adaptations introduced in the video, a reduction in body fluid osmolality, helps amphibians take on less water. Compared to mammals that have a body fluid osmolality of around 300 mOsm, the lower osmolality of amphibians, which is around 250 mOSm, reduces the amount of work the kidneys must do in order to rid their blood of extra water. This adaptation is key to their survival. The less water that enters the animal via osmosis, the less the kidneys have to remove, reducing the energy required to maintain homeostasis.

The second adaptation, changes to the structure and function of nephrons, is critical for the production of dilute urine. For example, the glomeruli are uniquely designed to maintain a very high glomerular filtration rate, resulting in the production of very dilute filtrate and thus dilute urine. In addition, amphibians do not have loops of Henle in their nephrons. The closest thing they possess to a loop of Henle is a short intermediate tubule that connects the proximal and distal tubules. Together, these adaptations result in amphibians being able to easily excrete more than half of the water that enters the nephrons. In addition, amphibians possess active transport mechanisms in their proximal convoluted tubules, which pump Na⁺ and Cl⁻ out of the filtrate and back into the extracellular fluid for reabsorption into the blood. This pumping occurs even against an electrochemical gradient. Furthermore, amphibians can reabsorb Na⁺ across their bladder wall. As a result of these two adaptations, amphibians can reabsorb 99% of the Na⁺ and Cl⁻ ingested (Shoemaker, 1977). In summary, these adaptations ensure that extra water taken on by the amphibian is efficiently excreted while ions are conserved, resulting in the production of hyposmotic urine. In fact, these adaptations are so efficient that the kidneys of amphibians are unable to produce hyperosmotic urine at all.

The elimination of nitrogenous waste products is a problem all animals face as a result of protein catabolism, and yet this problem is handled quite differently among vertebrates. For example, reptiles and birds eliminate their nitrogenous waste as uric acid. This compound requires very little water to process, but does require energy to produce. Mammals excrete their nitrogenous waste products as urea, which requires more water to process, but less energy. Amphibians, like freshwater fish, excrete their nitrogenous waste products as ammonia. This compound requires a lot of water to process, but very little energy to produce. Thus, ammonia is an ideal waste product for freshwater vertebrates as they need to rid themselves of extra water anyway. However, ammonia is toxic and cannot remain in circulation without causing great harm to body cells and tissues; it must be excreted quickly in the urine.

Questions

1. Explain why aquatic amphibians constantly take on water but lose ions to their environment.
2. List the energy saving adaptations that help aquatic amphibians manage the challenges of living in a dilute environment as efficiently as possible.
3. Why isn’t the production of uric acid an adaptation of aquatic amphibians?
4. Why is ammonia a safe waste product for amphibians to produce, but would be lethal to desert reptiles and mammals?
5. Given the adaptations of aquatic amphibians’ renal systems, hypothesize what would happen to the osmolality of their body fluids over a short period of time if they were removed from their freshwater environment and placed in a desiccating environment such as a dry rock in the hot sun or a salty pond.
Part IV – Deicing Disrupts the Environment and Osmoregulation in Amphibians

While the adaptations discussed thus far allow amphibians to thrive in freshwater environments, they are unfortunately the very characteristics that make them more vulnerable to rapid environmental changes, such as those brought about by the application of deicing compounds.

Deicing compounds can be transported as runoff up to 170 m from the roads on which they are applied (Karraker & Gibbs, 2011). Over a season of heavy deicing application, freshwater habitats that catch runoff experience a significant increase in the amount of salt they contain. Normally freshwater should have a salinity of 1 to 250 mg/L, but some habitats very near roads treated heavily with deicing agents have been discovered to have a salinity of almost 18,000 mg/L immediately after runoff (Collins & Russell, 2009; Hill, Wyse, & Anderson, 2012). Furthermore, salt is conserved in aquatic environments, as it is not subject to rapid loss or biological use. As a result, salt that enters freshwater is more likely to remain and accumulate.

Questions

1. Many scientific studies examining the relationship between increased salinity in freshwater habitats and species richness of amphibians only collect data for one or maybe two years. How might this lead to inaccurate conclusions about the problems caused by the application of deicing agents?

2. It was generally thought that salinity increases in freshwater habitats during early spring months and declines over the rest of the year, but recent studies indicate this might not be the case. In fact, salinity levels may actually increase during the summer months even though no deicing agents are actively applied. Why?

Some aquatic species such as salamanders and frogs are very sensitive to the increased salinity; prolonged exposure to concentrations above 250 mg/L is harmful. Concentrations above 1100 mg/L and 1700 mg/L are lethal for some species of salamanders and frogs, respectively (Collins & Russell, 2009). Watch the video at http://youtu.be/vFM7wfkvhuc, which introduces some of the issues aquatic amphibians have when the salinity of their habitat increases.

3. Considering what you just saw in the video, take a moment to hypothesize how the following renal system adaptations could actually compound the problems amphibians encounter if salinity levels increased to 1000 mOsm:
   a. Adaptation: body fluid concentration of approximately 250 mOsm.
   b. Adaptation: high glomerular filtration rate.
   c. Adaptation: diminished loop of Henle.
   d. Adaptation: production of ammonia from protein catabolism.

In freshwater, amphibians gain body water by simply feeding on insects and via osmosis across the skin. Because these passive forms of water acquisition provide more than enough body water, amphibians do not need to drink free water. However, as the salinity of water increases, amphibians will drink water.

4. If amphibians can gain freshwater by eating insects, what must the osmolality of insects’ body fluid be compared to that of amphibians?

5. Why does freshwater move passively via osmosis into the frog’s body across the skin?

6. If the salinity of the water increases such that the osmolarity is higher than that found within the frog’s body, what happens to the frog’s ability to gain water by eating insects and osmosis across the skin?

7. As the salinity of the water increases, will the drinking of water provide hydration?

8. Some species of amphibians that are adapted to life in saltier water have a naturally elevated osmolality of body fluids. What benefit does this provide?

As salinity continues to increase, aquatic amphibians will begin to lose water rapidly across the skin via osmosis. Water movement via osmosis is bidirectional; water molecules passively flow down their concentration gradient. As
water leaves the frog, the osmolality of its blood begins to increase. This stimulates the release of hormones such as aldosterone and neurohypophyseal hormones, which work in concert to increase active Na+ transport across the skin into the body and increases the reabsorption of Na+ within the proximal convoluted tubules. In dilute freshwater, these hormones would actually help the amphibian remain hydrated, but work against the animal as salinity levels increase.

9. What is the driving force causing water to efflux across the skin of the frog as water salinity levels increase?

10. In freshwater, the release of aldosterone and neurohypophyseal hormones into the blood results in increased hydration of body fluids. Why?

11. As water salinity levels increase, what negative impact would the release of aldosterone and neurohypophyseal hormones have on an amphibian?

Increasing water salinity levels trigger the kidneys to slow the loss of body water by decreasing the rate of glomerular filtration, thus reducing the volume of urine produced. Remember, the nephrons of amphibians are unable to produce hyperosmotic urine so a decrease in urine production is likely their best option. However, as the rate of glomerular filtration slows and the production of urine decreases, ammonia is not removed as quickly as it should be from the blood. If these renal system adjustments are only needed for a very short period of time the animal can survive, but long-term environmental changes can overwhelm the capabilities of the renal system.

12. What happens to the composition of the blood if the glomerular filtration rate is chronically depressed?

13. How does the production of ammonia compound the problem of a decreased glomerular filtration rate?

14. Explain why the inability of amphibians to produce hyperosmotic urine is a detriment in salty water.
Conclusion

Some species of aquatic amphibians such as the green frog are stenohalinc. While they are well adapted to life in very dilute water, they are unable to adjust when the salinity of the water increases. This leads to decreased fitness and eventually a decline of the species. Anthropogenic causes such as the application of deicing agents are just one of many causes leading to the global decline of amphibians.

References


