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THE SCIENTIFIC WONDERS OF WETLANDS

Frozen Frogs May Help Doctors Improve Human Transplants

By: *Christine Baptista, Wetland Biodiversity Technician*

Wood frogs, a common species in Ontario, demonstrate a very rare capability – they can freeze their bodies into what appears to be solid ice – a trait known as freeze tolerance. Doctors and other medical researchers are trying to utilize this trait to improve current methods for freezing human organs intended for transplant.

When a wood frog freezes approximately 65% of the water in its body turns to ice, and its brain and heart

preventing the cells from dehydrating, and the frog from dying.



During winter hibernation, the common wood frog stops its heart and brain and freezes into what looks and feels like a frog-shaped ice cube. Photo: Kenneth & Janet Storey

Glucose limits ice formation in the body and binds water molecules within the frog's cells. This curbs the damage caused by cell shrinkage, which is common with freezing.

Wood frogs freeze from the inside out, so the internal organs are not crushed by the expansion of the ice. They also thaw from the inside out. This ensures the crucial internal organs are able to function first.

In nature and in the lab, the thawing process takes place over a period of several hours.

Researchers have determined that the temperatures at which a wood frog can remain frozen, and alive, are between -6 degrees Celsius and -1 degrees Celsius. Scientists also believe that wood frogs often use snow as insulation in order to maintain these temperatures. Canadian biochemists Ken and Janet Storey of Carleton University believe this trait evolved during an ice age approximately 15,000 years ago.

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completely stop functioning. In essence, the frog enters what in humans would be considered total cardiac arrest. The wood frog's liver contains glucose which lowers the freezing point of the body tissue, acting as natural antifreeze for the frog,

This trait may have allowed wood frogs to survive over other species that became extinct during that time.

The ability to freeze and thaw gives modern day wood frogs an upper hand over other frogs in Ontario because they are the only amphibian species that can survive north of the Arctic Circle. This ability gives them a much larger home range.

Wood frogs are also the first species to “wake-up” in the spring, which is a huge reproductive benefit. Wood frogs generally lay their eggs in vernal pools – temporary wetlands that tend to dry up by the summer. Laying their eggs early in the season ensures the young are able to metamorphose into froglets before the vernal pool dries up.

Though wood frogs are the only Ontario species known to be freeze tolerant, some other species of amphibians and reptiles display this trait, as do many species of insects and invertebrates.

So, fascinating as it all is, what practical use does this have for humans?

When it comes to organ transplants, one of the biggest problems is that organs do not last very long from the time that they are harvested from the donor to the time that they are transplanted into the receiver. For example, a heart only lasts about six hours outside of the human body. This greatly restricts the physical distance the heart can travel to a recipient.

Once scientists are armed with the knowledge of how wood frogs freeze their organs for an entire winter and then hop away in the spring without a problem, they are hoping to apply the same principles to learn how they can freeze organs to ensure they can be stored for longer periods of time. This will guarantee that the organs can be utilized by an organ recipient who is not in close proximity to the donor. Frogs, being vertebrates, have

similar internal structures to those of mammals, making the comparison reasonable, though not exact.

In 1999, Boris Rubinsky, an engineer at the University of California at Berkely, successfully preserved rat livers in a partially frozen state, then thawed and implanted the organs into recipient rats. One of these rats was able to survive for five days.

More recently Rubinsky and colleagues at Sheba Medical Center in Israel have done similar preservation procedures using rat hearts.

Although this is a step in the right direction, rats are still a far cry from larger, more complex mammals, such as humans.

Using knowledge about the wood frog's ability to freeze its whole body as a template for humans to someday do the same is an exciting possibility, but one that scientists believe will take more than a few years. In the meantime, the scientific community will continue to study the wood frog and attempt to mimic one of nature's greatest accomplishments – the survival and revival of a “frozen” animal year after year.

Editor's Note – If you are interested in learning more about

Canadian researchers Kenneth and Janet Storey and their involvement in the study of wood frogs, check out the following website:

http://www.pbs.org/safarchive/3_ask/archive/qna/3274_j-n-kstorey.html



Salamanders May Have the Answer to the Possibility of Limb Regeneration in Humans

By: Lisa Sealock

In several laboratories across North America salamanders are involved in experiments that could change modern medicine as we know it. Many of the salamanders in these labs have, or will have, a leg cut off. But they recover, with perfect new limbs growing back in weeks.

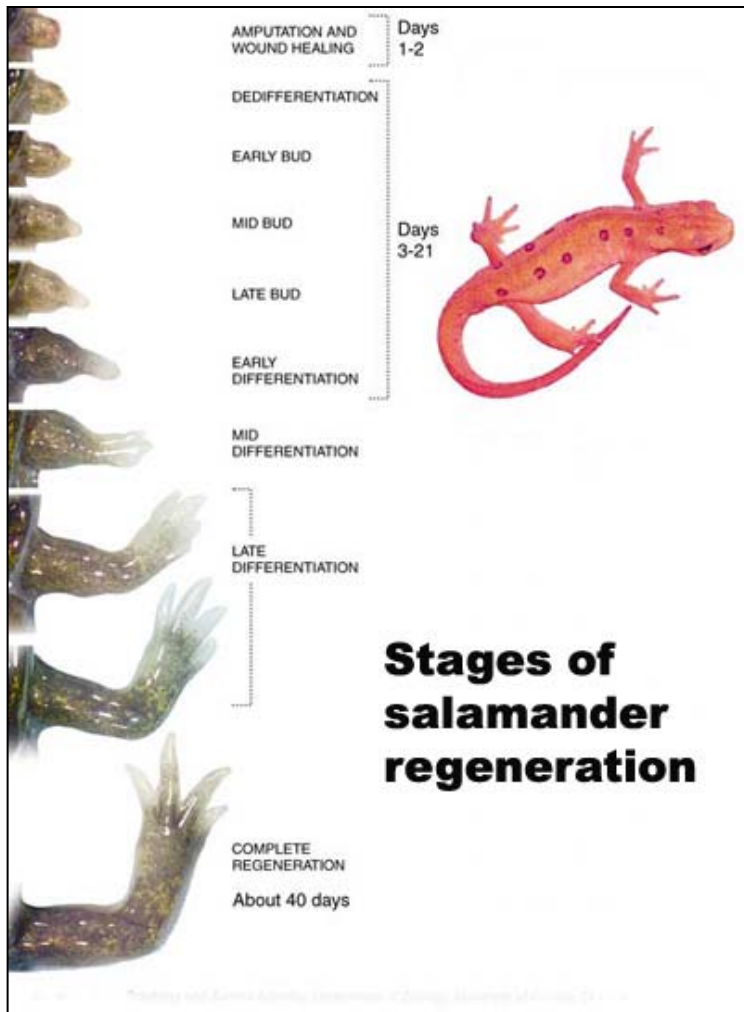


Image from cellscience.com/reviews2/salamander

Salamanders are the subjects of numerous research experiments because of their incredible regenerative capabilities. They can grow back not only limbs but also tails, parts of their hearts and the retinas and lenses in their eyes. Scientists are researching salamanders in the hope that one day the salamander's regenerative abilities may be applied to people.

Many scientists believe it's just a matter of time before they will be able to regenerate an arm or at least a finger. One such scientist is Dr. David M. Gardiner, a biologist who runs the laboratory at the University of California at Irvine with Dr. Susan V. Bryant, the dean of biological sciences and his wife.

Regenerative medicine is a field that involves the regrowing or repairing damaged organs. This area of science has recently become a hot topic. The majority of the attention is focused on stem cell research. In the case of stem cells, the cells are grown outside of the body and turned into particular types of tissue and transplanted into patients.

Some scientists believe that inducing the same regeneration in people that occurs in salamanders and some other animals is a better approach than stem cell research. They argue that natural regeneration, which might be accomplished with drugs or genes, would be easier than transplanting, especially because the tissue would be the patient's own, doing away with the problem of rejection. These scientists also believe that even if salamanders' regenerative abilities cannot be reproduced in humans, studying regenerating animals will at least provide clues for stem cell scientists.

Scientists have been studying natural regeneration for 200 years and have yet to make much progress in understanding it. As a result, this area of research garners little attention.

Now, however, recent breakthroughs in genetic techniques are allowing scientists to better understand the mechanisms of regeneration. That and the excitement surrounding regenerative medicine are creating new scientific and even commercial interest in regeneration.

Venture capitalists recently invested \$9 million in starting the company Hydra Biosciences, a company that could be the first of its kind in attempting to replicate natural regeneration in people. They are supporting the research of scientists who are working to identify the genes that help amphibians regenerate.

The company is largely based on the work of Dr. Mark T. Keating, a company founder and a biologist at Harvard who studies regeneration in zebrafish.

Dr. Keating feels that there is little evidence to suggest that human cells are fundamentally different from those of zebrafish. Armed with this belief he is trying to stimulate the human body's natural ability to regenerate.

Humans do have the ability to regenerate some parts like livers, muscles and bones, however this regeneration is generally limited to single types of tissues. Salamanders can regrow multiple types of tissue to complete structures like limbs, and scientists have demonstrated that the planarian, a small flatworm, can be cut into pieces as small as one-279th of the whole, and entire new worms will grow from the segments.

According to a 2002 New York Times article, "there is only one possible example of multi-tissue regeneration in people, and that is that young children are capable of regrowing fingertips above the top joint, including the bone, skin and nail. This was discovered by accident in England in 1974, when a child who lost a fingertip in a farm accident was taken to a hospital. The doctor, too busy to provide the standard treatment, sewed the skin closed. When she got around to it over a day later, she discovered that the finger was regrowing."

Still, some experts argue that this is not an example of true regeneration, but rather an extension of the fast growth that normally occurs in children's fingers.

Professor Jeremy Brockes of University College London believes that he can explain how animals are able to regenerate in the face of injury. He presumes that cells near the site of the injury, already specialized like muscle cells, lose their specialized properties and go

back to a primordial state in a process called de-differentiation. The animal, in effect, creates its own stem cells when they are needed.

Those stem cells form a mass called a blastema and proliferate rapidly. They then redifferentiate to form the tissue needed to rebuild the limb or organ.



From the top: Red-backed salamander, Spotted salamander and Blue-spotted salamander

Dr. Tsilfidis is one of many scientists who wonder why the ability to regenerate has not spread more widely through evolution. She speculates one possible reason is that higher animals spend more time on specialized cells in order to perform sophisticated functions, and these cells are more difficult to de-differentiate. Another theory is that the advanced immune systems of higher life forms mistakes rapidly proliferating cells for cancer and kills them.

According to a 2002 article in the New York Post, there are a few examples of this type of regeneration occurring in higher animals. Dr. Thomas A. Reh, a professor of biological structure at the University of Washington, found that neurons in a chicken retina could be replenished because other nerve cells called glial cells de-differentiated. In the future, this information may be used to help heal blindness caused by retinal diseases like macular degeneration.

Regeneration has also been found in mammals, in a strain of mice with an altered immune system.

A few years ago, Dr. Ellen Heber-Katz, an immunologist at the Wistar Institute in Philadelphia, was conducting an experiment with mice that had developed a disease similar to lupus. Dr. Heber-Katz punched a pattern of holes in each mouse's ear, a common practice that allows researchers to identify which mouse was which.

When she checked on the experimental mice three weeks later, there were no ear holes. Dr. Heber-Katz could not tell the mice apart. That ruined her experiment but sent her into a whole new field of study.

She has since found that those mice, unlike other mice, can also regrow parts of their hearts, tails and spinal cords. The fact that the "healer mice" do not form scars appears to be important, as scars block the ability of cells to divide and block signals from the epidermis that appear necessary to begin regeneration.

The fascination surrounding regeneration has been around since the 1700's, when a French scientist, René-Antoine Ferchault de Réaumur noticed at the fish market that some crayfish had legs that were not the same size. He theorized that some legs had grown back after having been cut off. Other scientists around the same time found that hydra, snails and other creatures could regenerate, which set off a frenzy of experiments.

But understanding the mechanisms of regeneration has proven to be quite difficult. Dr. Thomas Hunt Morgan, a scientist studying regeneration at the beginning of the 20th century, basically declared the subject resistant to control. Dr. Morgan moved on to other areas, studying genes and chromosomes in the fruit fly, work that won him a Nobel Prize as the father of modern genetics.

Currently, scientists hope to understand regeneration by finding the genes involved. Dr. Heber-Katz has found at least seven locations on the chromosomes of her "healer mice" that appear to contain genes that correlate with the ability to regenerate.

Dr. Sánchez Alvarado is methodically turning off genes in planaria in hope of discovering which are necessary for regeneration. Dr. David Stocum, a biology professor and the dean of the School of Science at Indiana University-Purdue University Indianapolis, is studying which genes are active in tadpoles, allowing them to regrow limbs, but are absent in adult frogs, which cannot.

Dr. Keating has identified a gene that keeps cells in an embryo from dividing. When the gene was turned on in mouse muscle cells that were growing in culture, the cells de-differentiated into stem cells. According to Dr. Keating the same gene is present, but turned off, in people, except in the fingertips, the one part of the body where regeneration has been seen.

Dr. Keating has also made mouse muscle cells de-differentiate by using an uncharacterized mixture of proteins extracted from a newt.

According to the scientists focusing on natural regeneration, it will take some time before they fully understand the complex mechanisms involved. Only time will tell what new breakthroughs scientists can learn by studying the over 300 species of secretive salamanders.

Wetlands: Are They Nature's Medicine Cabinet?

By: Lisa Sealock

Wetlands provide many benefits including water quality improvement, floodwater storage, wildlife habitat, aesthetics and biological productivity. But did you know that wetlands also have a rich history of providing plants with medicinal values?



Think about all the plant life that can be found in a wetland. With such tremendous diversity it's no wonder that some of these species serve medicinal purposes.

Below are some examples of wetland plants found in Ontario and their current and traditional uses:

Bog bean (*Menyanthes trifoliata*)

Found in bogs, marshes and shallow water, this plant was once held to be of great medicinal value as a remedy against scurvy. The chief constituents are a small quantity of volatile oil and a bitter glucoside called Menyanthin. An extract is made from the leaves, which possesses strong tonic properties, and

which renders great service in rheumatism, scurvy and skin diseases.

Boneset (*Eupatorium perforliatum*)

Easily recognizable by paired leaves that unite at the base of the stem, boneset was a favourite medicine of North American First Nations communities and has always been a popular remedy in the United States. The leaves and tops are gathered after flowering has commenced as they contain a volatile oil, some tannic acid, and Eupatorin. Boneset acts slowly and persistently, and its greatest power is upon the stomach, liver, bowels and uterus.

Boneset has been used to treat muscular rheumatism, the common cold, fever, indigestion and influenza.

Golden Rod (*Solidago spp.*)

European herbalists and physicians have long recognized goldenrods as important plants. Even their generic name, *solidago*, means "to make whole," referring to their supposed medicinal benefits. The Chippewa had an even better name for the goldenrod family in general, calling it *gizisomukiki*, which translates as "sun medicine." Goldenrod is a safe and gentle remedy for a number of disorders. It is a valuable astringent remedy treating wounds and bleeding. The plant has been used to calm stomachs, allay nausea, pass stones, cure wounds, and to treat diphtheria, bronchitis, and tuberculosis.

Hawthorn (*Crataegus spp.*)

This tree can attain a height of 30 feet and lives to a great age. Hawthorn is used to cure insomnia, prevent miscarriage, heart ailments, strengthening muscles, prevent arteriosclerosis, angina, poor heart action and for treating nervousness.

Slippery Elm (*Veronica officinalis*)

The inner bark of this small tree has important medicinal value. The bark, which is the only part used, is collected in the spring and dried. The abundant mucilage it contains has strengthening and healing qualities. It not only has a soothing and healing action on all parts it comes in contact with, but also possesses as much nutrition as is contained in oatmeal!

Slippery elm has been used to treat gastritis, colitis, coughs, typhoid fever, pleurisy, heart conditions, dysentery, cystitis, irritation of the urinary tract, diarrhea, constipation, wounds, boils, ulcers and burns.

St. John's Wort (*Hypericum perforatum*)

There are many ancient superstitions regarding this herb. Its family name *Hypericum* is derived from Greek and means 'over an apparition,' a reference to

the belief that the herb was so obnoxious to evil spirits that a whiff of it would cause them to fly.

St. John's wort has been used for centuries to treat mental disorders as well as nerve pain. In ancient times, doctors and herbalists wrote about its use as a sedative and treatment for malaria as well as a balm for wounds, burns, and insect bites. Today, St. John's wort is used by some people to treat mild to moderate depression, anxiety, or sleep disorders.

Cattails (*Typha latifolia*)



One plant that is easy to recognize is the cattail. In the spring, the stalks can be peeled or eaten raw or boiled. In summer, the pollen heads can be eaten raw or dried and ground into flour. As for medicinal uses, the fresh-pounded root

can be used as a poultice on infections and stings. The sticky substance at the base of the green leaves is an antiseptic, a coagulant, and has a numbing quality that can be used for toothaches. Rashes can also be treated by placing boiled leaves over the affected area.

These are just a few examples of medicinal plants found in wetlands, plants that have been used throughout the ages by people around the world. So the next time you take a walk near a swamp, marsh or bog, think about all the wonderful medicinal applications that are contained in the vegetation surrounding you.

Editors Note: We do not recommend experimenting with the medicinal properties of plants without first conducting thorough research and consulting the advice of a professional.

For a great list of some of the books and guides available on the subject of medicinal and herbal plants, try visiting the following website:

www.forestshop.com/MEDICINE.html#medicinal

Another great website to check out for more information:

www.botanical.com

2005 SPRING TOAD FESTIVAL: Toaddally Chilly, but Still a Toaddally Fun Event

By: Lisa Sealock

On Saturday, April 30th and Sunday, May 1st Toronto Zoo, along with the help of Adopt-A-Pond and Banrock Station Wetlands Foundation Canada, hosted the sixth annual Spring Toad festival. This event is meant to celebrate the arrival of spring, but with the chilly temperatures it seemed more like we were celebrating the arrival of winter!

Past festivals have seen the America's wetland ponds filled with American toads and the air filled with their amorous calls. Unfortunately, the temperature was too cold, and not a single American toad was brave enough to test the waters. Luckily we did hear the calls of a few Leopard frogs and upon closer inspection were able to see them floating in the water. There were also lots of cool invertebrates that visitors got a chance to learn about and check out under microscopes at the Wetland kiosk.

The Giant toad was on hand to greet visitors and give out stickers and fun activity sheets. Mike the magician wowed festival goers with his great tricks and the face painters were kept very busy decorating the faces of delighted children with frogs, toads and tadpoles. Enthusiastic Zoo volunteers worked hard to teach visitors how to use radio-telemetry equipment to track a mystery toad and allow them to become official Toronto Zoo Toad Detectives. Participants also got the chance to learn how to take part in Frogwatch-Ontario and were trained in the art of making origami toads (which by the way is a little tricky when your hands are really cold).

We can't forget about the premier screening of the World of Toad Patrol either. Children got a chance to watch the animated series that stars eight young toadlets on a mystical quest. Cut outs of several characters were available for kids to get their photograph taken with, and a toad crossing sign was put up just in case any American toads decided to join the party.

Despite the chilly temperatures, the festival was a great success and those that did make it had a great time! The festival is on again next year, and planned for the first week of May. Let's all cross our fingers that next year the weather is a bit more agreeable and that the American toads are eager to join in all the wonderful festivities. "Hop" to see you all next year!



Hmmm..... is this Zoo visitor happy to see the Giant Toad or trying to stay warm?



Visitors got the chance to visit the Wetland kiosk to learn all about the wonderful creatures you can find in wetland water.



There may not have been any toads, but we did get a chance to see a few leopard frogs



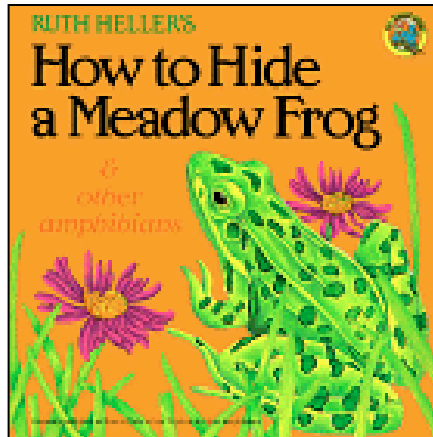
Toad Patrol characters taking a break from getting their pictures taken with kids.

By: Christine Baptista, Wetland Biodiversity Technician

How to Hide a Meadow Frog & Other Amphibians

Author: Ruth Heller

Published by Grosset & Dunlop (2002)
ISBN 0448409658



This book is perfect for children of all ages as it helps them learn about various amphibian species, and how their colours may camouflage them from predators. But in keeping with lessons about the natural life cycle, the last page gently explains the importance of the predator-prey cycle.

The language is suitable for those who can read on their own. Only the common names of species are used, so as not to confuse children with strange and complex Latin names. All the species common names are capitalized so they stand out, and the largest and probably the most difficult words in the book are *camouflaged* and *salamander*.

The illustrations are bright and vibrant, and the amphibians are well camouflaged so children get the full understanding of their colour changing abilities. They are still obvious enough for younger eyes to find them. In illustrating the various amphibians in hiding, children are also learning about the natural and critical habitats used by these animals, habitats that must be protected and left undisturbed.

Only one out of the seven species mentioned (the Leopard frog) can be found here in Ontario. Nonetheless, the book is still a beneficial read for children and adults alike who are always looking to learn a thing or two about amphibians, or wildlife in general.

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Amphibian Voice is distributed to schools and communities participating in the Adopt-A-Pond programme. The purpose of this newsletter is to provide information on amphibian, turtle and wetland conservation issues and efforts in Ontario.

Send in your stories, drawings and photographs to the address below and we will "hoppily" include them in future issues.

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We welcome support of our programme! Please make cheques payable to "Toronto Zoo" and send them to the following address. Thank you!

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