Frozen Frogs

by Jan and Ken Storey

In the woodlands, winter loosens its grip only reluctantly. Average temperatures hover well below freezing, snow gathers in drifts that may not melt for five months, and even in April a blizzard can dash one's foolish hopes for milder weather. And yet every year there comes a morning when one steps outside and knows in one's bones that spring is around the corner. The birds chatter, water drips off from the roof and the air has a scent of dust, damp earth and decaying plants. Finally, as if to prove that the season has truly turned, the wood frogs join in a raucous chorus, calling to potential mates with a raspy, duck-like "brack, brack".

Those bracks are our call to action. Donning headlamps and waders, and armed with long-handled nets and sacks, the two of us go off in search of the singers. Known to herpetologists as *Rana sylvatica*, wood frogs are small and brown, with Zorro-like black masks across their faces.

In the winter they hibernate on the forest floor under a blanket of leaf litter. As soon as the disappearing snow releases them, they head straight for temporary pools of meltwater—ditches, gravel pits, hollows on the forest floor—for a few frenzied nights of breeding.

In the dead of night, when we arrive, some of the noisy males float surreptitiously among the dead stalks of last year's reeds. We sometimes catch the glimmer of our headlamps in their eyes, but more often than not, that is all we catch. In a fraction of a second, as our nets swoop down, the frogs dive beneath the surface and are gone. It takes several hours of cold, wet, exhausting work to bag a few dozen frogs.

Are we crazy? Well, no more than any other investigators who are trying to understand an odd, unexplained phenomenon. For wood frogs are amazing. They not only go without eating for an entire winter, they spend those months frozen solid—without a

breath, without a heartbeat, without measurable brain activity. One day they are no more than frogsicles; the next they are too nimble and squirmy for us to catch. How do they do it?

We have puzzled over that question for the past fifteen years. The frogs' ability to endure freezing is not only inherently fascinating, it may point to important biological insights, as well as to valuable medical applications. If biologists can learn how a frog liver or a frog heart withstands freezing, medical scientists may also one day learn to freeze human organs—or even human bodies—without killing them. Along the way, other insights gleaned from wood frogs could contribute to treatments for diabetes and other conditions. Even on the chilliest spring evenings, we draw comfort from such hopes, as we head back to the laboratory with our catch.

How frozen are frozen wood frogs?

To survive, wood frogs must control exactly where and how fast ice crystals form in their bodies: the higher the temperature at which ice begins to form, the more slowly it propagates through the body and the better it is distributed. The better an animal controls those factors, the more time it will have to prepare for its metabolism to be shut down when frozen, and the less it will be damaged when it thaws. As a result, most freeze-tolerant animals begin to freeze at a temperature just below 0 degrees Celsius.

Like the water pipes in a house with a broken furnace, an animal's blood vessels are especially vulnerable to damage from freezing. Cryomedical investigators have tried to freeze human organs to store them for later use. But the capillaries rupture, causing severe internal bleeding when the organs thaw. One way wood frogs address that problem is by beefing up their blood-clotting capacity. When an injury occurs, a biochemical cascade of clotting agents is triggered to stop the bleeding.

Frogs employ an even more important first line of defense. When we dissect frozen frogs in our lab, we invariably find lots of flat ice crystals sandwiched between the skin and muscle layers of their legs. A huge mass of ice—relatively speaking—also fills the abdominal cavity and encases all the internal organs. The ice contains not only the water that was in those spaces originally, but also water that was sucked out of the frog's organs. Indeed, the liver may be dehydrated by as much as 25 percent, and it is visibly wizened. The reason for the disproportion is clear: by sucking some of the water out of its organs—though not too much, otherwise its cells will collapse—the frog keeps the blood vessels in the organs from bursting as they fill with ice.

How do they manage it? They tolerate freezing by flooding their systems with carbohydrate antifreeze. But the antifreeze does not prevent the entire wood frog from freezing; it simply enables the frog to freeze selectively. The antifreeze protects only the water inside the frog's cells; everywhere else, ice crystals spread.

Photo: Jan Storey

Ice crystals form only from pure water; they cannot incorporate foreign matter in their structures. As crystals grow, they expel any other dissolved compounds into the surrounding liquid, gradually raising the concentration of those compounds. You can see the same principle at work when you make Popsicles from scratch: the liquid you pour into the mold may be mildly sweet, but when the Popsicles freeze, the ice separates from the sugar, forming pockets of intensely sweet syrup you can suck out.

By the same token, as water freezes inside a wood frog's abdomen and around its organs, it expels carbohydrate antifreeze that then builds to higher concentrations in the surrounding cells. The higher the concentration of antifreeze inside the cells, the less water can be drawn out of them at a given temperature, and the more liquid remains in the system when it finally reaches equilibrium.

Most freeze-tolerant animals can allow as much as 65 percent of their total body water to turn to ice and still survive. Every animal, however, has to adjust its antifreeze concentrations to its environment. The wood frog, which spends the winter on the forest floor under the insulating snow, generates just enough antifreeze so that 65 percent of its body will turn to ice at between -3 and - 5 degrees Celsius.

Diabetes and Antifreeze

Wood frogs use a kind of antifreeze with some valuable potential medical applications—namely, glucose. Just a few minutes after a frog's skin begins to freeze, its liver begins to pour glucose into its blood. Soon the glucose levels in the animal's organs rise between fifty and a hundredfold, and as ice forms, the concentrations within the cells soar even higher.

Diabetics, of course, are well aware of the dangers of unchecked glucose. If their blood-sugar levels rise between two and tenfold they may suffer from weakness, weight loss and, eventually, coma and death. How do wood frogs endure increases so many times greater? In human diabetics, high glucose causes structural damage to many of the body's proteins; for example, diabetic cataract is caused by glucose binding to proteins in the eye lens to make the lens cloudy. We are finding that wood frogs do not suffer these injuries to cell proteins from high glucose and it appears that the frogs may have evolved natural mechanisms for inhibiting glucose damage. If so, understanding this process in frogs could lead to treatments that minimize that damage in diabetics.

Cryopreservation

Though thin human tissues such as skin and corneas have been successfully stored in liquid nitrogen, no whole organ has ever been frozen without losing its viability once thawed. As a result, an organ transplantation is always a race against the clock: a heart, for instance, stays viable for only four to eight hours after it is removed from its donor. Every day in Canada, many people die for want of transplant organs, and the demand for organs worldwide grows by 15 percent annually.

Before organs can be frozen and stored indefinitely, a host of problems must be overcome. Investigators need to find ways to rapidly and evenly infuse the organs with protective solutions; they need to determine optimum freezing and thawing rates; they need to learn to control ice growth and freeze fractures, and to keep the cells' energy metabolism from failing - and those are just a few of the obstacles. Nevertheless, a good deal of progress has been made, and with any luck, studies like ours will lead to more advances. If we can show which genes are turned on and what metabolic adjustments are made in frog organs as they freeze, investigators can study the same systems in human organs.

What amazing secrets we can learn from these little frogs: what incredible discoveries they may give us in the future. In the meantime, we will pack up our nets and headlamps again this spring to hunt the elusive wood frogs. And we will marvel at what they have already shown us about life in the freezer. How many more incredible adaptations, we wonder, are yet to be uncovered?Λ

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IT'S A FROGGY WORLD OUT THERE!

Here are just a few web sites with frog crafts, games, themes and information for great programming ideas.

www.enchantedlearning.com - Enchanted Learning: a major resource for all sorts of themes - check out the Crafts and the K-3 themes sections. A very cool origami jumping frog provides a craft and then a game and the frog pop-up card would be an excellent challenge for older kids; all with full instructions.

http://familycrafts.about.com/cs/frogcrafts - Family Crafts: check out the frog hat that could be a great camp craft made with a visor or a baseball cap; build a toad house; try the pony bead frog; snack on the Harry Potter chocolate toads.

www.dltk-kids.com
DLTK's Printable Crafts for Kids: many crafts & ideas, mostly for children 6-7 years old.

www.daniellesplace.com/html/frogcrafts.html
Danielle's Place: bean bag frogs and painted stone frogs. For camp, you could expand the painted stone frog idea to create a "stone" ecosystem.

www.perpetualpreschool.com/preschool_themes/frogs/frog_games.htm
Perpetual Preschool: lots of frog games, songs and snacks ideas including a game idea that makes frog tongues from paper party blowers with velcro on the end and then catch flies (either plastic flies with velcro glued on or even just bits of black velcro).

General frog facts:

http://allaboutfrogs.org - facts, stories, games, jokes and much more.
www.naturenorth.com - how to raise wood frog tadpoles at home or school.
www.exploratorium.edu/frogs - simple science about frogs from the San Francisco Exploratorium.
www.cnf.ca/frog/index.html - get involved with the Canadian Nature Federation's Frog Watch
http://collections.ic.gc.ca/amphibians and **www.canadianbiodiversity.mcgill.ca**