Alaskan wood frogs stock up on solutes to survive

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Rana sylvatica found in southern Quebec, Canada. Credit: W-van / Wikipedia.

Outwardly, the tiny wood frog, *Rana sylvatica*, does not look like your regular arctic inhabitant. Yet despite their tiny stature, these little frogs are actually quite hardy and can tolerate freezing of up to two-thirds of their body water. For 25 years, Jon Costanzo and Richard Lee, Jr, both

from Miami University, USA, have been studying these wood frogs, which are native to Alaska, Canada and the northern USA, to unravel their secrets. Costanzo explains that one well-known technique in the Ohioan population is to stock up on cryoprotectant solutes: 'When you load your blood with solutes, whether it's glucose, urea, glycerol or whatever, you drive down the [blood's] freezing point. Therefore, at any given temperature, you reduce the amount of ice that forms.' So, even when temperatures drop to -5°C in Ohio, some water remains unfrozen. In Alaska, however, temperatures go down to -30°C during winter and so Costanzo and Lee wondered whether Alaskan frogs have some additional tricks to aid their survival.

To collect the frogs, Costanzo sent two of his graduate students, Clara do Amaral and Andrew Rosendale, out into the Alaskan wilderness during early August. After capturing their amphibian subjects, the duo then made a long 6-hour drive to Anchorage, Alaska, to ship them back to the lab in Ohio. Upon their return the team then set about preparing the frogs for winter; they mimicked the Alaskan fall by gradually shortening day length and lowering the temperature. Once acclimatized, the team then kept the frogs in simulated hibernation in the dark at 4°C for 8 weeks, alongside some hibernating Ohioan frogs.

To begin, the team first tested the Alaskan frogs' freeze tolerance and endurance; some were lowered to an icy ?16°C over 2 weeks, while others were kept at chilly ?4°C for up to 12 weeks. In both cases, the frogs recovered remarkably quickly, but, as Costanzo recalls: 'If you take the Ohio frogs and freeze them to ?4 or ?5°C it would take them days to fully revive. These Alaskan frogs were back on their feet within 2 days.'

So, how exactly were the Alaskan frogs able to prevent total body freezing at temperatures that would freeze stiff an Ohioan frog? To find out, the team turned to frogs that they had euthanized at each stage (after the natural summer, and the mimicked fall and winter periods). The

team were struck by how large the livers in the Alaskan fall and winter frogs were – they accounted for 22% of the total body mass (compared with just 8% in Ohioan frogs). To fuel such a huge change, the team thinks the Alaskan frogs trim down and use their body fat and some of their muscle protein to make glycogen – an essential precursor of the cryoprotectant solute glucose that is mobilised when the tissues begin to freeze.

The team also found that levels of another cryoprotectant solute, urea, had also shot up by 10-fold. This huge surge in urea concentration, along with a modest increase in glucose levels, caused an increase in plasma osmolality (a measurement of solute amount in liquids) by 100 mOsmol $kg^{?1}$. However, the team saw that total plasma osmolality had increased by 173 mOsmol $kg^{?1}$. What solute accounts for this additional 73 mOsmol $kg^{?1}$? The team have ruled out a few contenders, but they don't know exactly what it is yet. One thing's for certain, it's not a solute found in the Ohioan population. So it seems the Alaskan frogs stockpile the same solutes as their southern relatives (albeit to greater levels), but they also have some unique tricks of their own.

More information: Costanzo, J. P., do Amaral, M. C. F., Rosendale, A. J. and Lee, R. E. Jr. (2013). Hibernation physiology, freezing adaptation and extreme freeze tolerance in a northern population of the wood frog, *J. Exp. Biol*, 216, 3461-3473. jeb.biologists.org/content/216/18/3461.abstract

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