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As I've written about **before**, some North American frogs have the remarkable ability to freeze solid during winter. Wood frogs (formerly *Rana sylvatica*, apparently now *Lithobates sylvaticus*) achieve this by pumping cells full of glucose, which lowers the freezing point of cytoplasm, thus preventing ice crystals forming inside cells (whilst allowing the rest of the animal to freeze). Although this has been well studied in the lab, the extent to which wild wood frogs can survive freezing is little understood. Indeed, most studies have only looked at frogs from the Midwest United States or Canada- the southern part of the large wood frog range. In a new study in *The Journal of Experimental Biology*, Don Larson and colleagues achieve this and more.

Neatly, Larson et al. (2014) study Alaskan wood frogs in both the lab and the field. In the field, the authors placed temperature loggers just underneath wild frogs as they settled down for winter in the leaf-litter of natural forests. Another group of frogs were put through freezing with the clinical precision of previous lab studies- carefully cooled to -2.5C in plastic boxes for several days. Frogs from both the lab and the field were sacrificed to allow samples of liver, heart and leg muscle for the all-important glucose concentration to be measured.

Before last year, the record lowest temperature for survival in wood frogs was -7C, in frogs from the southern part of their range, although Costanzo et al. (2013) recently pushed this back to -16C whilst studying Alaskan frogs in lab conditions. Larson et al. modestly extend this limit to -18C, which was the lowest temperature recorded by the probes in the field. However, in the area, temperatures are known to drop below -20C, which the frogs can presumably survive.

More strikingly, based on the temperature loggers, the frogs stayed frozen for up to 7 months at a time. *All* of them survived. This substantially exceeds the results of a recent lab study, in which only half of the frogs studied could survive freezing for 2 months (and none could survive for 3 months- Costanzo et al., 2013). Based on these results, it seems wild frogs are better equipped to survive long winters than those rushed through the process in the lab. Interestingly, it is not clear whether the Alaskan frogs perform better than more southern animals because they have acclimated to the colder environment, or because of genetic differences (i.e. true evolutionary adaptation).

The authors further show that glucose concentration rose much, much higher in the wild frogs. In the heart, for example, there was ten times more glucose in frogs caught in the field than frozen in the lab. The authors predict this could be because they store more glycogen (which is broken down to produce glucose) or because they undergo repeated cycles of freezing and thawing at the start of winter, allowing them to better prepare. Either way, it is clear wild wood frogs can really push the limits further than previously acknowledged.

The authors also provide new evidence for a further survival tool in wood frogs, although it certainly isn't unique. It has previously been shown that other freeze-tolerant organisms, from plants to beetles to other species of frogs (Walters et al. 2011), use large antifreeze glycolipids that sit in cell membranes and may prevent ice from crossing into the cytoplasm. Larson et al. demonstrate thermal hysteresis (differences in melting point and freezing point) in extracts from muscle and internal organs (but not skin) that wasn't affected by enzymes that broke down protein. Instead, using NMR spectroscopy, they could show the frogs had membrane glycolipids that were similar to the freeze tolerant beetles.

Incidentally, a completely independent paper, also in *JEB*, has just demonstrated a further important component in the wood frog defence repertoire. The delicate skin of amphibians is prone to infection, and many frogs produce antimicrobial peptides (AMPs) as a first line of defence. Wood frogs have only one AMP so far identified- brevinin-1SY. During stress, such as anoxia, dehydration and freezing, the main immune system often weakens. Katzenback and colleagues (2014) demonstrate that during these periods of stress, brevinin-1SY expression is upregulated and skin extracts show greater a greater capacity to kill microbes, such as *E. coli*. Although the results hold for frozen frogs, this regulation must be more important during other stressors (e.g. dehydration), as freezing temperatures alone would probably limit microbial infection. Nevertheless, this study still highlights the impressive defences of these small frogs.

Wood frogs clearly show remarkable adaptations to freezing, many of which are only just being discovered (Katzenback et al., 2014; Larson et al., 2014). However, by restricting ourselves to the lab, we may be underestimating their capabilities. Larson et al. (2014) provide novel, interesting results, and it would be exciting to see future studies also consider wood frogs in their natural environment.

Refs-

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