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Mini-Review

Cryogenic Hibernation: A Review of Overwintering Mechanisms in the North American Wood Frog (*Rana sylvatica*)

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Abstract

The North American wood frog, Rana sylvatica*, can inhabit extremely cold climates utilizing a variety of adaptations. This review identifies the driving mechanisms behind the overwintering response of* R. sylvatica*. The major response factors instrumental to survival include environmental and behavioral adaptations, internal freezing point depression by increasing blood and tissue concentration of glucose and urea, and reduced metabolic activity by increasing blood and tissue concentration of urea. These factors were contrasted between the Alaskan and Ohioan variants to explore and explain the relationships between their overwintering response factors and their geographical positioning.*

Keywords: Rana sylvatica, Cryoprotectant, Glucose, Hypometabolism, Urea

Of the amphibians known to be native to North America, the wood frog (*Rana sylvatica*) is the only one that has been found to inhabit the Arctic Circle[1]. Amphibians are ectothermic and cannot generate their own heat. Consequentially, they face a serious challenge living in a habitat with temperatures that drop below freezing for extended periods of time[2]. *Rana sylvatica* respond to these extreme conditions by undergoing an overwintering process that allows them to withstand temperatures of -16°C and below[3]. This review highlights the major environmental and physiological response factors of the overwintering process in Alaskan and Ohioan *R. sylvatica*. Specifically, behavioral adaptations, environmental insulation, urea-induced metabolic inhibition, and usage of both urea and glucose as cryoprotectants are explored.

The internal body temperatures of *R. sylvatica* during hibernation must be kept from declining too low to survive through winter. The habitat of wood frogs is generally limited to woody ecosystems bordered by the Appalachians, Pacific Ocean, northern tree-line, and southern tree-line[3]. Alaskan wood frog populations in the north-west of the geographical range are subject to an average annual snowfall of 174 cm and average January lows at -28°C. Meanwhile, Ohioan wood frog populations in the south-east experience milder conditions, with an average annual snowfall of 35 cm and average January lows of -5°C. In the winter, wood frogs utilize leaf litter and snow as an insulated barrier to maintain a temperature range adequate for survival[1]. Additionally, *R. sylvatica* tend to group close together and reside in depressions in the soil, which is hypothesized to be a behavioral adaptation to maximize insulation during hibernation, similar to other overwintering amphibian species found in Michigan[4]. However, the specialized adaptations *R. sylvatica* have for winter survival limits the maximum temperature they can survive and thus in the south, they are limited to temperate forests[1]. Despite the large habitable temperature range, there does not seem to be differences in behavior between the two geographical variants based on current literature, and thus warrants further study.

During winter, *R. sylvatica* accumulate glucose in their bloodstreams and organs to act as a cryoprotectant[5], depressing the freezing point of water in the organism[6]. This specifically leaves the heart, liver, and kidneys unfrozen but inactive as the muscles freeze and the body cavity fills with ice[5]. Under experimental pre-, during, and post-freeze conditions, plasma glucose concentration does not significantly vary between Alaskan and Ohioan wood frogs. However, under the same conditions, the glycogen reserves drop significantly less in the Alaskan variant during freezing conditions[3]. This is likely due to their large geographic and climate differences which require the Alaskan frogs to withstand colder and longer winters, thus needing a larger energy reserve.

The geographic differentiation of liver glycogen levels between Alaskan and Ohioan frogs is expanded when comparing their relative concentrations of GLUT2. GLUT2 is a major glucose transporter that is especially active in liver and pancreatic β-cells[7]. The Alaskan frogs have an overall GLUT2 concentration 1.8 times greater than Ohioan frogs, and to exhibit an increase in GLUT2 protein concentration in response to freezing stress[8].

Increased expression of GLUT2 will only significantly change the glycogen concentration in liver tissue[5]. This indicates that enzymatic catabolism of hepatic glycogen appears to be *R. sylvatica*'s sole source of glucose for the body during their overwintering response to freezing temperatures. Moreover, it seems that the increased transport of glucose from the liver in the Alaskan *R. sylvatica* allows it to exhibit higher reserves of glycogen for longer hibernation while maintaining similar levels of plasma glucose as in the Ohioan variant.

Similar to glucose, urea acts as a cryoprotectant in *R. sylvatica*[3]. Compared to the Ohioan wood frogs, the Alaskan variant has a greater concentration of total cryoprotectants and compensates their overall slightly lower glucose concentration with a much higher overall concentration of urea[3]. Again, this follows with the geographic differences of Alaskan frogs needing to withstand much longer and extreme temperatures than Ohioan frogs.

Secondly, urea is capable of inducing hypometabolism in wood frogs[9, 10]. The sedentary nature of hibernation inhibits the ability of the wood frog to obtain nutrients, and thus a lowered metabolic rate is important to conserve maximal energy for winter survival. This is accomplished by increasing urea concentration in the plasma which induces hypometabolism, but direct ureic metabolism inhibition is only active in frogs that have undergone desiccation indicative of the winter seasons[10, 11]. Tissues with a high urea concentration exhibit a decrease in metabolic activity, but even non-urea-loaded tissues have a decrease in metabolic activity under desiccation, albeit slower[10]. This appears to be an adaptation of *R. sylvatica* allowing them to selectively reduce their metabolism with urea-loading tissues which decreases the amount of time spent active in unfavorable conditions. However, the effect of urea-loading is inconsistent between tissues and season which could result from differences in tissue-specific enzymes and heat or desiccation effects (which varies depending on the season) on enzymes[11]. Tissue-specific hypometabolism selectivity is a possible contributor to ensuring the vital organs survive with enough energy to support life after the thaw.

Conclusions & Future Study

Rana sylvatica is a remarkable amphibian that owes its success as a species to many factors. They have behaviorally adapted to survive harsh winters by burrowing. However, the more significant factor is their physiological shifts which allow them to conserve maximum energy by depressing their metabolism and freezing parts of their bodies while leaving some organs unharmed[1, 10, 3].

A lack of behavior differences between the Alaskan and Ohioan wood frogs requires confirmation to attribute the geographical survival variance mainly to physiological differences. Additionally, there is lacking literature on wood frogs outside of Alaska and Ohio to support climate adaptations and exclude genetic differences. Further research on the mechanisms which allow *R. sylvatica* to seamlessly exit cryogenic hibernation is recommended as the current literature is lacking. This could provide insight on preventing or assisting in the medical recovery of frostbite and hypothermia if we can understand these mechanisms. Alternatively, elective medical cryogenic hibernation is gaining popularity as a possible future aid to treatment. Small scale applications at the tissue or organ organization level would allow for a widening of the human organ transplantation window with a better-preserved tissue. Larger scale applications could allow whole body freezing to suspend animation in terminally ill patients while a cure is developed. However, a major hurdle is that there is currently no known method of recovering tissues from cryogenic hibernation. Mimicry of the wood frog's cryogenic hibernation mechanisms in humans could be one avenue to overcome this challenge.

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