

Commentary

Can boreal common frog (*Rana temporaria* L.) survive in frost?

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The first observations concerning the tolerance of vertebrate species to freezing were reported about fifteen years ago in America (Schmid 1982, Storey 1985) where four amphibian species were found to be able to endure freezing (even -6°C – -10°C). Since that discovery, many articles have been published on this topic (e.g. Franks 1985, Marchand 1987, Pegg & Karow 1988, Storey & Storey 1988, 1990, Storey 1990, Storey *et al.* 1996).

One of the species that tolerates freezing is the wood frog (*Rana sylvatica*), whose relative in Finland is the common frog (*Rana temporaria* L.). Previously we suggested that in Finland, under severe winter conditions, the common frog generally survives if wintering in aquatic sites, and terrestrial wintering is successful only occasionally (Koskela & Pasanen 1974, Pasanen & Koskela 1980). However, at the end of the 1980s we observed that part of the frog population overwinter on land (Pasanen *et al.* 1993, 1994), and our observations during the winter of 1992–1993 suggested that the Finnish common frog manages to survive the winter on the ground (Pasanen & Sorjonen 1994). Nevertheless, we cannot say on the basis of these experiments if the frogs placed in the wintering boxes were in the frozen state or not.

The aim of this study was to establish whether the common frog (*Rana temporaria* L.) can endure freezing and survive in frost. In the experiments, all frogs survived 24 hours at -2°C , but a

longer period at temperatures below 0°C caused death in three days. The glucose concentration in the liver after three days was about six times higher than the control level. Glycerol content in the liver was very low the whole time. The common frog is not as well adapted to freezing as the wood frog (*Rana sylvatica*) but can tolerate short periods of freezing temperature (less than three days).

Survival experiments

Altogether 78 mature frogs (35 ♀♀ and 40 ♂♂), 31 juveniles (length > 3 cm) and 41 small frogs (< 1 -year-old, length < 3 cm) were used in the study. The frogs were caught in September near Joensuu, Finland, and prior to the experiment were kept in a basin of water in a refrigerator room ($+6$ – $+8^{\circ}\text{C}$) without food. Experiments were carried out in October and November.

In the survival experiments we used 36 mature frogs (19 ♀♀ and 17 ♂♂), three juvenile frogs and six small frogs. Sixteen mature and six small frogs were used twice. The interval between the two experiments was three weeks. The frogs were put into plastic containers (0.3 l) filled with moss (36 mature frogs) or water (14 mature and all immature frogs). There was one mature or juvenile frog per container, but three small frogs per container. In the first part of the experiments, the frogs were transferred directly from $+6$ – $+8^{\circ}\text{C}$ to frost, and in the second part the frogs were acclimated before the transfer for 2–7 days at $+2$ – $+4^{\circ}\text{C}$. Plastic containers were shut with perforated covers and placed in a refrigerator where the temperature was kept at -2°C (temperature range -1.5°C – -2.5°C). The experiments lasted 24,

41, 48 or 72 hours. After each experiment containers were transferred to + 6–8°C, and the survival of the frogs was observed for 1–4 days. Frogs were weighed before and after the experiments.

In addition to the survival experiment, 42 mature frogs (17 ♀♀ and 25 ♂♂), 28 juvenile frogs and 35 small frogs were used for determinations of glucose and glycerol. They were placed in similar plastic containers and kept below freezing for 0, 12, 24, 48 or 72 hours. The livers of the frogs were weighed, and a sample of liver was taken for determination of glucose and glycerol. Small frogs were weighed and after thawing (in practise 1–2 hours after frost handling) the whole frog was homogenized instantly for glucose and glycerol determinations. Glucose and glycerol were determined by the Boehringer Mannheim UV-method (Anon. 1989) and are expressed as $\mu\text{mol g}^{-1}$ (wet weight).

Results and discussion

In the survival experiments, all frogs endured freezing for at least 24 hours (Table 1), but a longer period resulted in death; and no frogs survived

freezing for three days. There was no difference between sexes, but juvenile frogs were more sensitive to freezing than mature ones. In the containers with moss there were 36 mature frogs, of which 25 survived (69.4%), and in the containers with water there were 16 mature frogs, of which nine (56.3%) survived. Survival did not differ statistically significantly between moss and water (McNemari's test, $Q_{\text{adj}} = 1.976$, $p > 0.1$).

At first, the glucose content in the liver decreased in the freezing treatment (Table 2). The values were slightly smaller after 12 and 24 hours, but then they increased significantly. After three days, the glucose content was about six times higher than the control values. There were, however, no significant differences between sexes ($t = 0.11$ – 1.67 and $df = 3$ – 13). In juvenile frogs, the pattern of glucose content in the liver was similar to that found in mature frogs. In small frogs, the glucose content was determined in the whole animal, but changes were similar to those in the older

Table 1. Experiments on tolerance to freezing (– 1.5– – 2.5°C).

Time (h)	Σ	N ♀	♂	Frogs alive		Attention
				N	%	
Mature						
24	6	3	3	6	100	Non-acclimated
24	11	4	7	11	100	Acclimated 2 days
41	6	4	2	11	66.7	Non-acclimated; alive 3 ♀ and 1 ♂
48	10	5	5	3	33.3	Non-acclimated; alive 2 ♀ and 1 ♂
48	13	5	8	10	76.9	Acclimated 2 days; alive 3 ♀ and 7 ♂
72	6	3	3	0	0	Acclimated 2 days
Immature						
24	6			6	100	< 1-year-old frogs; acclimated 7 days
48	3			0	0	> 1-year-old frogs; acclimated 7 days
72	6			0	0	< 1-year-old frogs; acclimated 3 days

Table 2. Glucose content ($\mu\text{mol/g}$) in the liver (in mature and juvenile frogs) and in the whole body (in the < 1-year-old frogs) after freezing-tolerance experiments. (Superscript indicates statistical differences between vertical values; the results were analysed by one-way ANOVA and the means were compared by Duncan test.) Means with no common letter in vertical column differ at $p < 0.05$ level.

Time (h)	Mature			Juvenile			< 1-year-old		
	N	$\bar{x}$	$\pm$ S.E.	N	$\bar{x}$	$\pm$ S.E.	N	$\bar{x}$	$\pm$ S.E.
0	10	3.8 ^a	0.3	10	1.7 ^{ab}	0.3	10	0.27 ^{ab}	0.04
12	6	1.3 ^a	0.2	7	0.3 ^a	0.1	7	0.21 ^a	0.02
24	7	2.1 ^a	0.2	4	3.1 ^{bc}	1.2	6	0.58 ^c	0.12
48	15	14.4 ^b	1.2	3	4.6 ^{cd}	0.3	12	0.40 ^b	0.13
72	4	24.0 ^c	4.4	4	5.3 ^d	1.3	–	–	–

frogs. After three days of the treatment, however, the glucose content was twice as high as in the controls.

Both glycerol content and glucose content were determined from the same frogs, but the values for glycerol remained very low ($0\text{--}0.5\ \mu\text{mol g}^{-1}$) and were not affected by the treatment.

The weights of the frogs kept in the moss containers decreased slightly: the mean decrease in the weight of the mature frogs was 5.7% (maximum value 20.2%), in juvenile frogs 15.1% (maximum 17.9%) and in small frogs 10.8% (maximum 28.3%).

The Finnish common frog does not survive a long period of frost compared with the American wood frog (*Rana sylvatica*), which tolerates $-8\text{--}-10^\circ\text{C}$ for at least two weeks (Storey *et al.* 1996). The wood frog can be in a frozen state with no breathing, no heart beat or blood circulation, and with up to 65% of their total body water in the form of ice. The common frog is not as well adapted but it can survive short periods of frost (less than three days). This type of situation occurs, for instance, in autumn when frogs are migrating to their wintering places, at which time night frosts are frequent. It is possible that frogs can freeze many times for short periods and after thawing continue to migrate. The present study also indicates that the common frog can survive repeated freezing.

At temperatures below 0°C , the glucose content in the liver of the mature common frog increased significantly in three days; but the increase was dramatically smaller than that ($177\text{--}248\ \mu\text{mol g}^{-1}$) reported for the three American species of amphibian (*Hyla crucifer*, *Pseudacris triseriata*, *Rana sylvatica*) (Storey & Storey 1986ab). In these species, synthesis of cryoprotectants utilizes the large reserves of liver glycogen. At -2.5°C , the rates of glucose production by the liver of *Rana sylvatica* can exceed $20\ \mu\text{mol g}^{-1}\text{h}^{-1}$ (Storey & Storey 1988), and maximal levels of cryoprotectant are reached in 24–48 h. According to these results, the biochemical mechanisms of natural tolerance to freezing in the wood frog are much more effective than in the common frog.

In the present study, the glucose content in the liver after freezing was lower in juvenile than in mature frogs. In the liver of the juvenile wood frog, the corresponding glucose content was $188\text{--}388\ \mu\text{mol g}^{-1}$ (Storey & Storey 1986a). Obviously, juvenile wood frogs

are even better adapted to freezing than mature frogs; but in the case of the common frog, the opposite is true.

The glycerol content in the liver of the common frog was very low throughout the experiments. In three American frog species, the glycerol contents also remained at the same low level ($0\text{--}3.4\ \mu\text{mol g}^{-1}$) after freezing (Storey & Storey 1986b). When another American species, *Hyla versicolor*, was kept for two weeks at freezing below 0°C , the glycerol content increased to over $300\ \mu\text{mol g}^{-1}$ (Storey & Storey 1985). This species uses glycerol as a cryoprotectant. From this, we may conclude that the common frog has no effective cryoprotectant and therefore does not survive freezing for a longer time.

During experimental dehydration, wood frogs tolerate the loss of 50–60% of their total body water (Churchill & Storey 1993). The rate of water loss for unprotected frogs is the same whether the animals are frozen or not but is greatly reduced when frogs are frozen under a protective layer of moss. According to Churchill and Storey (1993), for unprotected animals dehydration death could occur in 7–9 days. Both for the wood frog and also for our common frog, this indicates the importance of selecting well-protected and damp wintering sites for terrestrial overwintering.

The American leopard frog (*Rana pipiens*) endures the loss of up to 50% of its total content of body water (Churchill & Storey 1995). However, the water content of internal organs was found to drop by only 3–8%. It is interesting that dehydration led to a rapid increase in glucose concentration in the liver (to the level $20\ \mu\text{mol g}^{-1}$ wet weight). In *Rana sylvatica*, the activities of some antioxidants (e.g. glutathione oxidase) increase during freezing and are constitutively higher in freeze-tolerant species than in *Rana pipiens* (Joanisse & Storey 1996). This suggests that antioxidant defences play a key role in amphibian freezing tolerance.

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