

THE IMPORTANCE OF BODY TISSUE COMPOSITION TO CALORIMETRIC ASSESSMENTS OF ICE CONTENT IN WHOLE ANIMALS

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Abstract—1. Calorimetry was used to estimate ice content of frozen wood frogs (*Rana sylvatica*). Values for the composition and thermal constants of their tissues were manipulated mathematically to ascertain the impact of each parameter on estimates of ice content.

2. Small variations in body water content, melting point and specific heat of the dry mass had slight to moderate effects on estimated ice content.

3. Large changes in estimates were noted if the properties of the dry mass were totally discounted. Calculations that do not include the relatively low specific heat of dry tissues significantly decrease estimated ice content.

4. Thus, accurate measurements of ice content require the inclusion of both body water and dry mass.

Key Word Index: Calorimetry; cryobiology, freeze tolerance; frogs; ice content; tissue composition

INTRODUCTION

Accurate measurement of ice content is important for understanding the nature of freeze-tolerance in animals. This is because the formation of large amounts of extracellular ice dehydrates body cells to the point that their functional integrity may be compromised (Mazur, 1984). Not surprisingly, mortality rises among freeze-tolerant vertebrates when they are frozen at lower temperatures that produce higher ice contents (Layne and Lee, 1987, 1989; Schmid, 1982; Storey *et al.*, 1988).

Calorimetry can be used to estimate ice content by monitoring temperature change when an animal is placed into a closed system. Temperature change upon thawing is dependent on tissue ice content and the thermal properties of the wet mass and dry mass of an organism (Murphy and Pierce, 1975; Crowe *et al.*, 1981; Lee and Lewis, 1985; Layne and Lee, 1987, 1989). Indeed, Layne and Lee (1987) reported that inclusion of the dry mass properties in calculations of ice content gave an estimate of 65.3% in the freeze-tolerant frog *Rana sylvatica*, whereas, calorimetry based on equivalent masses of ice gave an estimate of 56.0%. Conversely, Storey *et al.* (1988) reported that inclusion of dry mass had little difference on estimates of ice content in the freeze-tolerant turtle *Chrysemys picta*. Therefore, further study is merited to clarify this discrepancy.

We compared estimates of ice content for *R. sylvatic* using different calorimetric constants for the body water content and specific heat of the dry mass. Additional considerations are discussed relative to integrating body tissue thermal properties into calculations of ice content.

MATERIALS AND METHODS

We collected adult *R. sylvatica* from Ontario Co., New York during October 1988. Frogs were subjected to a 10 week conditioning routine that simulated field conditions in autumn and was comparable to that used by Layne and Lee (1989) to induce freeze tolerance.

Our freezing protocol followed procedures used by Layne and Lee (1987). Frogs were placed in plastic centrifuge tubes (50 ml capacity) and frozen at -2.5°C in a Neslab refrigerated bath. A thermocouple passed through the cap of each tube and was placed against the abdomen of the frog. We compiled temperatures on an Omega RD-106 multichannel recorder. It thus was possible for us to determine the time that freezing commenced as well as the duration of the exotherm produced by the freezing of body water. All frogs were frozen for 24-30 h which is sufficient time for each frog to reach an equilibrium ice content (Layne and Lee, 1987).

Calorimetry was done in a glass vacuum thermos containing 100 ml of distilled water. The introduction of a frozen frog into the calorimeter caused a decline in water temperature which we measured to the nearest 0.1°C using an Omega HH-22 digital thermometer.

Properties of both wet and dry mass were included in calculations of body ice using the following equation (see also Murphy and Pierce, 1975; Crowe *et al.*, 1981; Lee and Lewis, 1985; Layne and Lee, 1987, 1989):

$$W_i = \frac{F(W_w)(S_w)(T_i - T_f) + (T_s - T_f)[(W_d)(S_d) + (W_s)(S_w)]}{(T_s - T_f)(S_w) + Q + S_i(mp - T_i) + S_w(T_f - mp)}$$

Table 1. Ice content estimates for *R. sylvatica* ($N = 4$) frozen for 24–30 h

Varied parameter	Estimate of ice content				
(1) Specific heat of dry mass	53.5%	51.7%	51.4%	51.1%	46.2%
	(0.00)	(0.25)	(0.29)	(0.33)	(1.00)
(2) Melting point (°C)	51.6%	51.4%	51.4%	51.2%	50.9%
	(0.0)	(-0.4)	(-0.5)	(-1.0)	(-2.0)

Variations were modelled for the specific heat of the dry mass and melting point for each frog. Adjusted values for each varied parameter are given in parentheses. The standard values for body water content, specific heat of the dry mass and melting point were 78.1%, 0.29 cal/g°C and -0.5°C, respectively.

where

- F = calorimeter constant (1.003)
 mp = melting point of body fluids (°C)
 Q = heat of fusion of water (79.7 cal/g°C)
 S_d = specific heat of the dry tissue (cal/g°C)
 S_i = specific heat of ice (0.5 cal/g°C)
 S_w = specific heat of water at 20°C (0.9988 cal/g°C)
 T_f = final temperature of the water in calorimeter (°C)
 T_i = initial temperature of the water in calorimeter (°C)
 T_s = temperature of the body tissues (°C)
 W_d = weight of dry mass in body tissues (g)
 W_i = weight of ice in body tissues (g)
 W_s = weight of water in body tissues (g)
 W_w = weight of water in the calorimeter (g).

We expressed ice content as a percentage of the total water content of a frog. For *R. sylvatica* standard values for the body water content, melting point and specific heat content of the dry mass were 78.1%, 0.5°C and 0.29 cal/g°C, respectively (Layne and Lee, 1987).

We made mathematical manipulations of the specific heat of the dry mass (S_d), melting point and body water content and calculated their effect on estimates of ice content. In manipulations of body water content, the values for W_i and W_d were varied simultaneously so that the total body mass was

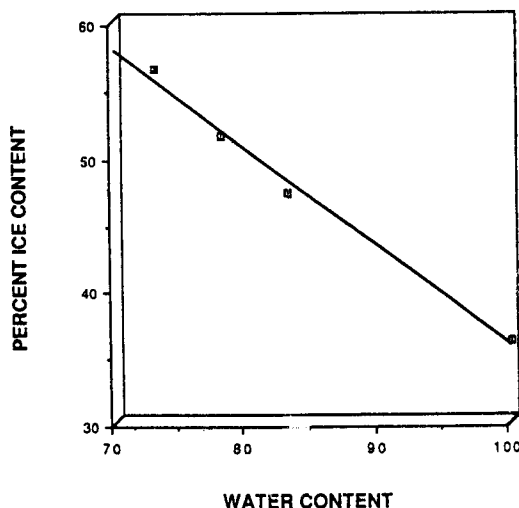


Fig. 1. The relationship between predicted ice content and body water content values that were modelled into the equation. Other calorimetry constants were kept at standard values.

unchanged. Only one parameter was varied at a time in these calculations. Our adjusted values for each parameter are given in Table 1. Physical constants for water and ice were not varied.

RESULTS

All frogs completed their exotherms several hours prior to the ice determinations and should have reached an equilibrium ice content (Layne and Lee, 1987). Our earlier studies (Layne and Lee, 1987, 1989; Layne *et al.* 1989) found that the freezing protocol was not lethal to frogs if they were permitted to thaw slowly at 5°C. The ice content of frogs averaged 51.4% (+4.1%, 1 SD) when standard values for body water content (78.1%) and specific heat of the dry mass (0.29 cal/g°C) were used.

Our calorimetric estimates of ice content were very sensitive to manipulation of the total water content of the frogs (Fig. 1). For example, a reduction in the value of water content by 5% generated nearly as great an increase in the estimated ice content. Conversely, if we raised the water content by the same amount to 83.1%, then the estimated ice content decline by a slightly smaller percentage (4.3%).

Our ice content estimates were not as greatly influenced by changes in the specific heat of the dry mass (Table 1). Elevating this value by 0.04 cal/g°C (or nearly 14%) gave only a slight reduction in the estimate of ice content. Lowering the specific heat by the same value caused a slight elevation in the ice content estimate. Likewise, small increases in the melting point to 0.0°C and decreases to -2.0°C only had nominal impact on the ice content estimate. Extreme deviations in the specific heat of the dry mass to 0.00 or 1.00 cal/g°C generated moderate and potentially significant changes in the ice content estimates.

DISCUSSION

Our frogs had ice contents similar to values reported by Storey (1984) for winter-conditioned *R. sylvatica* and considerably lower than the ice contents of conspecifics collected by us in the spring (Layne and Lee, 1987). These interstudy differences parallel seasonal variations in equilibrium ice content of the treefrog *Hyla versicolor* (Layne and Lee, 1989). Causality for these differences in ice contents are not certain but may be related to seasonal differences in cryoprotectant levels that have had been reported among freeze-tolerant frogs (Layne and Lee, 1989; Schmid, 1982; Storey and Storey, 1987).

If experimental data are to be integrated with models of freezing injury, then accurate estimate of the ice content of frogs is critical. Ice formation in tissues and its evaluation are highly dependent on several physical properties of biological materials. This is related in part to large differences in the specific heat of water and dry matter in an organism (see Materials and Methods). Over 20% of a frog's body mass is dry matter which has a significantly lower heat capacity than water.

In this study we found that manipulating the body water content had a pervasive effect on ice estimates. We attribute this to the different heat capacities of the wet mass (water) and dry mass and the relative proportion of each in a living frog. The frogs are assumed to have a higher heat capacity than they actually do when the dry mass is ignored. Therefore, a lesser component of the temperature change recorded during calorimetry is attributed to the heat of fusion released during the melting of ice in body tissues. In turn, this leads to underestimation of ice content.

Modest manipulation of specific heat of the dry mass had a small effect on the estimates of ice content. Greater variation in this constant generated more substantial changes in the ice content estimates. Indeed, if specific heat for the dry mass approaches 1.0 cal/g°C, then the net effect would be to model the system along the calorimetric properties of water or equivalent masses of ice.

When we adjusted the calorimetry constant for the dry mass to the same value as water we did not obtain as great an effect on reducing the ice content estimate as did assuming the entire mass of a frog is water. This is because the total water content is still assumed to be 78.1% in the first situation and a smaller pool of water means that the proportion of ice will be greater. Therefore, the mass of ice is divided by a larger value for the mass of tissue water which further reduced the estimate for ice content.

Accordingly, we reported in an earlier study (Layne and Lee, 1987) that ice content estimates, based on calorimetry for equivalent masses of ice, lead to lower values than those derived using calculations which include the thermal properties of the dry mass. The thermal assumptions of ice based calorimetry are that the whole frog has a specific heat equal to water, which are shown here to produce lower estimates of ice content than values that include the specific heat of the dry mass (0.29 cal/g°C).

Alternatively, Storey *et al.* (1988) reported that the ice contents of the turtle *C. picta* were unaffected by the basis of calorimetric calculations. This is despite their low value for the specific heat of the dry mass in the turtles (0.06 cal/g°C). This may have been obscured by variability among individuals and experimental variations. The body mass of their turtles was generally under 4 g and this inherently will generate smaller temperature changes than we obtained with our frogs which were often three to four times heavier. Finally, their one specimen frozen at -10.9°C did seem to confirm our arguments. Its projected ice content using ice based calorimetry was 59%; whereas inclusion of the thermal properties of the dry mass gave an estimate of 66%.

Inherent inaccuracies exist in calorimetry especially prior to equilibrium. First, ice formation appears to progress in a regionalized fashion throughout the body of a frog (Storey, 1985, 1986). Moreover, heat loss from body regions may differ due to surface/volume relationship (e.g. limbs vs body trunk). Temperature differences consequently exist throughout the body of a frog, and it would not be possible accurately to integrate a precise body temperature into the equation for the frog. However, these temperature differences would be relatively small ranging from near the melting point of body fluids (-0.6°C) to perhaps 1°C lower. Second, during freezing, body fluids change their osmotic concentration as cryoprotectant is being mobilized from stores in the liver. The dynamics of adding this solute cause the melting point progressively to increase until a steady-state cryoprotectant concentration is reached. Nevertheless these differences in even the most extreme circumstances would translate into inaccuracy only 1 or 2% of the total body water content and a fairly negligible contribution to the total magnitude of ice forming during even the earliest stages of a time course.

In summary, we provide evidence that estimation of ice contents is highly dependent on the use of appropriate thermal constants for the body tissues of animals. These factors should be included whenever ice contents are measured.

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