

A SIMPLE MODEL FOR ESTIMATING THE ICE CONTENT OF FREEZING ECTOTHERMS

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Abstract—1. Although body ice content is an important variable affecting freeze tolerance, present calorimetric methods for its measurement necessarily require the termination of a freezing protocol.

2. A simple iterative model, based on the colligative properties of solutions and requiring precise measurements of only equilibrium freezing point (of the unfrozen organism) and of core body temperature, allows estimation of the percentage of body water frozen at any time during a freezing episode.

3. This model can also predict the lethal temperature for a freezing ectotherm, assuming that death occurs due to osmotic dehydration when 67% (or any other known lethal fraction) of the body water is frozen.

4. The basic model is easily extended to evaluate the effects of variables such as: body mass, initial body water content, initial osmotic concentration, and test chamber microenvironment.

5. This model is not intended to supplant existing more exact biophysical models of freezing kinetics. Rather it is proposed as a first approximation which is generally supported by published data and which should be of significant practical value for investigators of freeze tolerant organisms.

Key Word Index: Freezing model; freeze tolerance; ice content; supercooling; cold tolerance; calorimetry

INTRODUCTION

Although any degree of tissue freezing is damaging or lethal for most organisms, some species have the remarkable ability to tolerate the freezing of an appreciable portion of their extracellular body water. Freezing tolerance has long been recognized in certain terrestrial arthropods and intertidal molluscs (see Zachariassen, 1985; Loomis, 1987; Cannon and Block, 1988; Storey and Storey, 1988 for recent reviews). More recently, freeze tolerance has been well documented in a number of amphibians and reptiles (e.g. Lotshaw, 1977; Schmid, 1982; Costanzo *et al.*, 1988; Storey *et al.*, 1988; Claussen *et al.*, 1990; Costanzo and Claussen, 1990).

Most ectotherms will remain unfrozen as they are progressively cooled to below their equilibrium freezing point (FP_{eq}) until some limit to supercooling (the crystallization temperature, T_c) is reached (Fig. 1). Their body temperature will subsequently rebound, as a result of ice nucleation, to a temperature (the rebound temperature, T_r) which, at least for large or moderate sized ectotherms, is near to, but slightly below, their FP_{eq} . This abrupt rise in temperature, due to the liberation of the heat of fusion, represents the onset of the exotherm and is a clear indication of the initiation of freezing.

The terminology used to describe freezing processes is rather inconsistent. Some authors have used "melting point" to avoid the apparently anomalous situation in which an organism remains unfrozen below its equilibrium freezing point. This, however, is not accurate for those species showing thermal hysteresis (in which the melting point is not the same as the freezing point). Many authors have used "supercooling limit" or, more commonly, "supercooling

point" to describe that temperature at which ice nucleation occurs. However, supercooling is actually a process and there is no unique "supercooling point" for a given solution. The alternative term "crystallization temperature" is technically more correct and has been adopted for our model.

Cellular osmotic dehydration is believed to be a primary factor contributing to the death of freeze-tolerant animals (Mazur, 1984). As body tissues progressively freeze, the water is removed as ice and the remaining extracellular fluid becomes increasingly concentrated. Intracellular water is removed osmotically and eventually a lethal limit of cellular dehydration is reached. For several species, this occurs when about 67% of the total body water has frozen (Williams, 1970; Lee and Lewis, 1985; review by Storey and Storey, 1988). Body ice content is thus an important variable for predicting the ability of an animal to survive a given freezing episode. Calorimetry can be used to determine ice content with the aid of appropriate equations as developed by Murphy and Pierce (1975) and Lee and Lewis (1985). These procedures, however, necessarily require the termination of the freezing protocol. Also, they subject the specimen to very rapid warming, which could affect survivorship and thus estimates of mortality due to freezing *per se*.

Observations on the freezing time-courses of the garter snake, *Thamnophis sirtalis* (Costanzo *et al.*, 1988) stimulated our interest in the dynamics of the freezing process. This led to the evolution of an iterative model based on the colligative properties of solutions and on the assumption of a dynamic equilibrium among three variables: ice content, core body temperature of the freezing organism, and elapsed time. Although the component physical relationships

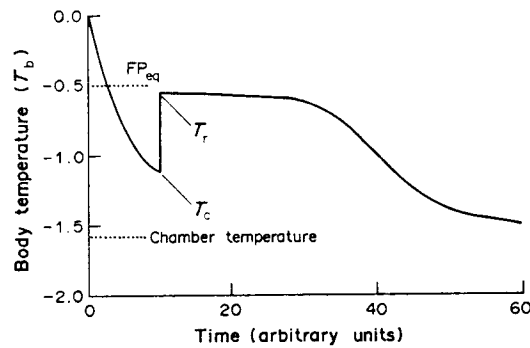


Fig. 1. A typical freezing time-course for an ectotherm. In this hypothetical example, an animal, with an initial body temperature (T_b) of 0°C and an equilibrium freezing point (FP_{eq}) of -0.5°C , is placed in a chamber at -1.6°C . The animal is supercooled from the time T_b drops below FP_{eq} until the crystallization temperature (T_c) is reached at time 10. The abrupt onset of freezing produces the rebound temperature (T_r) which marks the start of the exotherm. Chamber temperature is essentially equivalent to equilibrium body temperature (T_c).

are well known, we have integrated these into a novel model which allows continuous estimation of ice content throughout the time-course of an uninterrupted freezing episode. We develop the model below, and we further extend it to predict the nature of the freezing time-course and the duration of freeze tolerance, and to examine the effects of various parameters on freezing responses. Finally, we test our model with available published data and we consider its advantages and short-comings. Though not intended to replace more exact and complex biophysical descriptions of freezing kinetics, this simple model is revealed as a good and practical estimator of the ice content of freezing ectotherms.

METHODS

The equilibrium freezing point (FP_{eq}) of a one osmolal (Osm) solution is -1.86°C . Inasmuch as freezing point depression is one of the colligative properties of a solution, there is a simple relationship between FP_{eq} and osmotic concentration. A 0.5 Osm solution will thus freeze at -0.93°C , or, more generally,

$$C_i/C_x = FP_{eq}/T_x \quad (1)$$

where C_i = initial osmotic concentration, C_x = osmotic concentration at some later time, x , during a freezing episode, FP_{eq} = equilibrium freezing point of the original solution or organism, and T_x = temperature of the solution (or organism) at time x .

The conversion of water to ice liberates an appreciable amount of heat. At any given time, so long as substantial amounts of unfrozen fluid remain available, there will be an equilibrium between the formation of additional ice with the coincident production of heat, and progressive loss of heat to the cooler environment. The amount of ice that can be formed at any given time is limited by the cooling capacity of the system. Excess ice formation would generate excess heat which would in turn melt existing ice. As freezing commences, we thus observe a

dynamic relationship between time, temperature and ice content. As new ice forms, the osmotic concentration of the remaining extracellular fluid increases. This reduces the equilibrium melting point of the as yet unfrozen fluid, which, so long as new ice is being formed, will approximate the temperature of the solution or the core body temperature of the ectotherm.

At any given time, x , during the formation of new ice, ice content can be estimated as

$$F = 100 - 100(FP_{eq}/T_x) \quad (2)$$

where F = the percentage of the body water frozen, FP_{eq} represents the equilibrium freezing point of the original unfrozen organism, and T_x represents solution or body temperature at time x . As noted above, T_x is equivalent to the equilibrium freezing point of the remaining fluid compartment, but will differ from FP_{eq} . FP_{eq} can be determined empirically (except in rare instances of thermal hysteresis) by determining, with slow warming, the temperature at which the last ice crystal of a previously frozen specimen melts, or, can be more practically estimated from the osmotic concentration of the extracellular fluid of an unfrozen animal. It is again important to note that the rebound temperature (T_r) of a previously supercooled organism does not equal (but rather will always be lower than) FP_{eq} and should not be used as an accurate estimate of this parameter.

Equation (2) alone is sufficient for the estimation of the ice content of a freezing organism. Conversely, if F and FP_{eq} are known, the relationship

$$T_x = 100FP_{eq}/(100 - F) \quad (3)$$

will estimate the equilibrium temperature associated with a given ice content. For example, if one assumes that 67% of body water frozen represents the lethal limit, then the body temperature of a freezing animal yielding this degree of ice formation at equilibrium equals $3FP_{eq}$.

In order to extend the model to incorporate time, both the capacity of the cooling system to extract heat and the actual cooling rate of the organism within that system must be considered. The tendency of an organism to lose heat to the cooling system depends upon the gradient between the core body temperature of the animal and the equilibrium temperature of the cooling bath or chamber. For the sake of simplicity, we assume a linear approximation of heat loss based on the familiar "Newton's Law of Cooling" (e.g. see Bakken *et al.*, 1981), or that

$$CR_x = CR_i(T_c - T_x)/(T_c - FP_{eq}) \quad (4)$$

where CR_x = the cooling rate (e.g. degrees per hour) of the body when at temperature T_x , CR_i = the initial cooling rate (e.g. the rate prior to freezing when body temperature = FP_{eq}), and T_c = equilibrium body temperature (e.g. temperature of the cooling bath or chamber).

The cooling potential for a given organism, expressed as calories removed per unit time, is a function of the cooling rate times the mass of the organism in question. For a freezing animal, one must also consider the ice vs water composition of the organism, since the specific heat of ice (0.5 cal g^{-1}) is half that of water. Fortunately, these values are

almost constant over the temperature ranges of major biological interest (see Spaargaren, 1984). One must in addition consider both the specific heat of the unfrozen water (i.e. 1.0 cal g^{-1}) and of the dry mass of the organism (typically about 0.3 cal g^{-1} ; Layne and Lee, 1987a). Therefore,

$$CP_x = CR_x M((W(F + 100))/10,000 + (100 - W)/30) \quad (5)$$

Where CP_x = the cooling potential for a given freezing animal at a body temperature T_x , W = the water content of the organism (expressed as a %), M = the mass of the animal in grams, and 30 = the specific heat of the dry mass (here assumed to be 0.30) times 100.

As discussed above, the cooling potential dictates the rate of formation of new ice. The tendency to cool (lose heat) over time is balanced by the heat of fusion from the formation of ice. Since the heat of fusion represents about 80 cal g^{-1} , the amount of new ice (in grams) formed per unit time will approximate $CP_x/80$. Specifically, if W = total water content as a percentage of body mass, then

$$N = 10,000(CP_x/80)/(WM) \quad (6)$$

where N = new ice formed as a percentage of original water content. This new ice will combine with already existing ice to generate a new value for F . This, in turn, will determine a new equilibrium temperature [equation (3)]. The new temperature will modify the cooling gradient [equation (4)], and hence the cooling potential [equation (5)], which will in turn influence the rate of formation of still additional ice [equation (6)]. The freezing time-course model thus involves iteration of these inter-related factors. We have developed a microcomputer (BASIC) program to facilitate these computations.

Core body temperature will change over time as described by equation (3), but, if the cooling potential is modest and the increase in ice content is slow, the temperature of the organism may remain on a plateau for a prolonged period of time.

To complete the model, we have incorporated the pre-freezing cooling curve (from FP_{eq} to T_c), which is based on equation (4). The T_c , however, was arbitrarily selected from reported ranges and does not, in our present model, affect the freezing time-course.

For illustrative purposes, we use typical data for freezing garter snakes in much of the discussion to follow: $M = 50 \text{ g}$, water content 75%, $FP_{eq} = -0.55^\circ\text{C}$, $T_r = -0.65^\circ\text{C}$, $T_c = -2.5^\circ\text{C}$, $T_c = -1.4^\circ\text{C}$ (Costanzo *et al.*, 1988).

RESULTS AND DISCUSSION

As noted above, the rebound temperature (T_r), resulting from ice nucleation and the onset of freezing, may approximate the equilibrium freezing point (FP_{eq}) but will always be somewhat lower [equation (3)] due to the formation of some ice. T_r should not be used as an estimate of FP_{eq} .

The term "exotherm" has been used inconsistently in the literature. Some authors consider that the exotherm ends when the now partially frozen animal has cooled back down to its T_c . This may be con-

venient, but it is also a quite arbitrary end point with no biological significance. As our model clearly suggests (see below), ice formation and, hence, continued release of heat of fusion, may continue well past this point. We prefer to view the exotherm as continuing so long as any appreciable amount of heat is being produced due to the formation of ice within the organism. We realize, however, that the termination of the exotherm is accordingly not sharply defined.

The nature of the freezing process is not clearly perceived by many biologists. Some believe that the complete freezing of a supercooled organism is virtually instantaneous upon nucleation. This may be approximately true for very small organisms, supercooled to an appreciable degree, and subjected to a high cooling potential. It is not true, however, for most systems as demonstrated by our model and as clearly shown by the excellent studies of Lee and Lewis (1985) and of Layne and Lee (1987a). These authors, in contrast, suggested that ice formation proceeds gradually over time in a regular curvilinear fashion. This may be a reasonable empirical approximation but, as we will argue below, this view is also inaccurate, in part because it ignores the initial "burst" of ice formation associated with the onset of the exotherm. This initial ice formation results in the rebound temperature (T_r). Salt (1964) argued that T_r has no biological significance. We disagree.

The initial surge of ice formation obviously contributes to total ice content. It is thus important to incorporate this factor into a freezing response model. One way to estimate initial ice production would be to multiply the heat of fusion (calories degree $^{-1}$) by the degree rise in body temperature associated with ice nucleation (i.e. the difference between T_r and T_c). Such a value would probably always be an underestimate, however, since it does not account for the additional heat of fusion dissipated to the cooling environment and thus not contributing to the rise in core body temperature. A better estimate can be derived from the difference between FP_{eq} and T_r [equation (3) where $T_r = T_r$]. This is the method used in our model and is the basis for our contention that T_r indeed has biological significance.

The described freezing responses of organisms vary considerably. Although most organisms, especially small ones, supercool to an appreciable degree, some freeze with little or no supercooling. Some animals exhibit an abrupt rise in temperature upon nucleation but then almost immediately resume cooling. Others show a very prolonged plateau after nucleation which may last for hours or even days. Our model suggests, however, that much of this variation is due simply to the mass of the organism and the cooling capacity of the system utilized.

Cooling potential

Cooling potential markedly affects the shape of the freezing time-course [Fig. 2(A)]. At high cooling rates (e.g. $1.0^\circ/\text{min}$), there is little evidence of a plateau, and the organism will quickly cool to equilibrium chamber temperature. This is very similar to the freezing responses reported for certain invertebrates (e.g. compare with Fig. 1 of Murphy and Johnson, 1980, and of Block *et al.*, 1988). At lower cooling

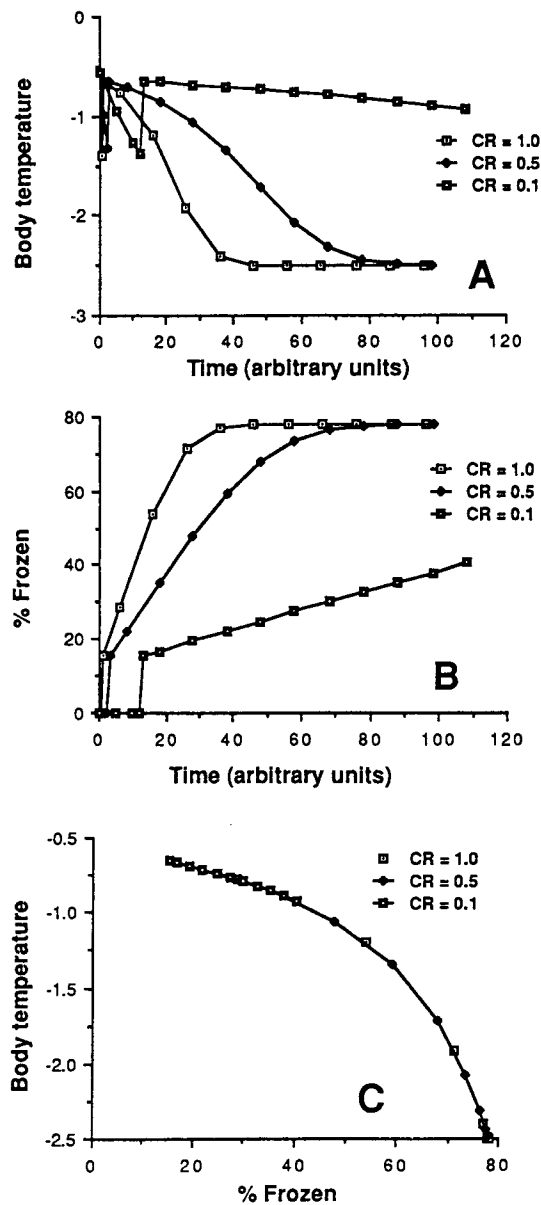


Fig. 2. The effects of cooling rate (CR) on the freezing responses of hypothetical 50 g ectotherms. In this, as in other figures, the units of time, though arbitrary, are consistent within each figure. Changing the units has no effect other than to expand or compress the curves. Water content, FP_{eq} , T_c , T_r , and chamber temperature were set at 75%, -0.55 , -1.4 , -0.65 and -2.5°C respectively in this and in all subsequent figures, except as noted.

rates (e.g. $0.1^\circ/\text{min}$), there is a distinct plateau comparable to that reported for freeze tolerant frogs and reptiles (Schmid, 1982; Storey, 1985; Layne and Lee, 1987a; Costanzo *et al.*, 1988; Claussen *et al.*, 1990; Costanzo and Claussen, 1990).

According to our model [Fig. 2(B)], the rate of new ice formation is approximately linear (after the initial surge at nucleation) at low to moderate ice content, but clearly non-linear as maximal ice content for a given system is approached. The smooth curvilinear increase in ice content over time presumed by Lee and Lewis (1985) and especially by Layne and Lee

(1987a), though a reasonable empirical approximation, is probably not correct.

Our model indicates that the relationship between ice content and body temperature is markedly curvilinear and is independent of cooling rate [Fig. 2(C)]. For a typical garter snake, the model predicts a 67% ice content (the value lethal for many animals) at a body temperature of -1.65°C . This is in excellent agreement with preliminary data on the lower lethal temperature for freezing snakes (Costanzo *et al.*, 1988).

Data appropriate for testing our model are sparse. Values extracted from Williams (1970) are perhaps the best available for testing certain of these predicted relationships. Data for the subtidal clam, *Venus mercenaria*, agree quite well with the ice contents predicted by our model at the specified body temperatures [Fig. 3(A)]. In contrast, the data for the intertidal mussel, *Mytilus edulis*, do not. Williams (1970) attributed this interspecific difference in ice content at a given temperature to the fact that about 20% of the cellular water of *M. edulis* is bound or osmotically inactive (referred to as unfreezable water by Storey and Storey, 1988). We accordingly modified our model to incorporate any desired level of bound water. This essentially involved modifying equations (2) and (3) by substituting for 100 (the percentage of freezable body water) the value $(100 - B)$, where B = the percentage of the water bound. The resulting line for 20% bound water indeed fits the *Mytilus* data reasonably well [Fig. 3(A)]. Our model further predicts [Fig. 3(B)] that early stages of ice formation are relatively independent of the percentage of water bound, but that organisms with more bound water will actually cool faster during a freezing episode [Fig. 3(C)]. This presumably results from the more rapid depletion of water available for freezing (and hence for the generation of heat of fusion to counter body cooling). Empirical data for testing these predictions appear to be lacking at present.

Body mass

Body mass is another factor which can markedly influence the time-course of a freezing response. Using a 50 g garter snake as a "standard", we assume that the instantaneous cooling rate at the FP_{eq} will vary with body mass $^{-0.33}$ (see Claussen and Art, 1981 for derivation). Equation (4) was modified accordingly, and this, of course, also affects equations (5) and (6). Not surprisingly, the data [Fig. 4(A)] indicate that the effects of body mass, for a system of constant cooling capacity, are essentially identical to the effects of varying cooling capacity, for a constant body mass (Fig. 2). It is noteworthy that, for a given cooling system, the relationship between ice content and body temperature [Fig. 4(B)] is independent of body mass. Equation (2) may thus be used for organisms of any size.

An ectotherm exposed to very low ambient temperatures will necessarily experience the effects of a steep gradient between body temperature and chamber equilibrium temperature. This remains true even if the animal is insulated to lessen the cooling rate. Our model reveals [Fig. 4(C)] that bath or chamber temperature, for a fixed initial cooling rate, has only a modest effect upon the initial plateau

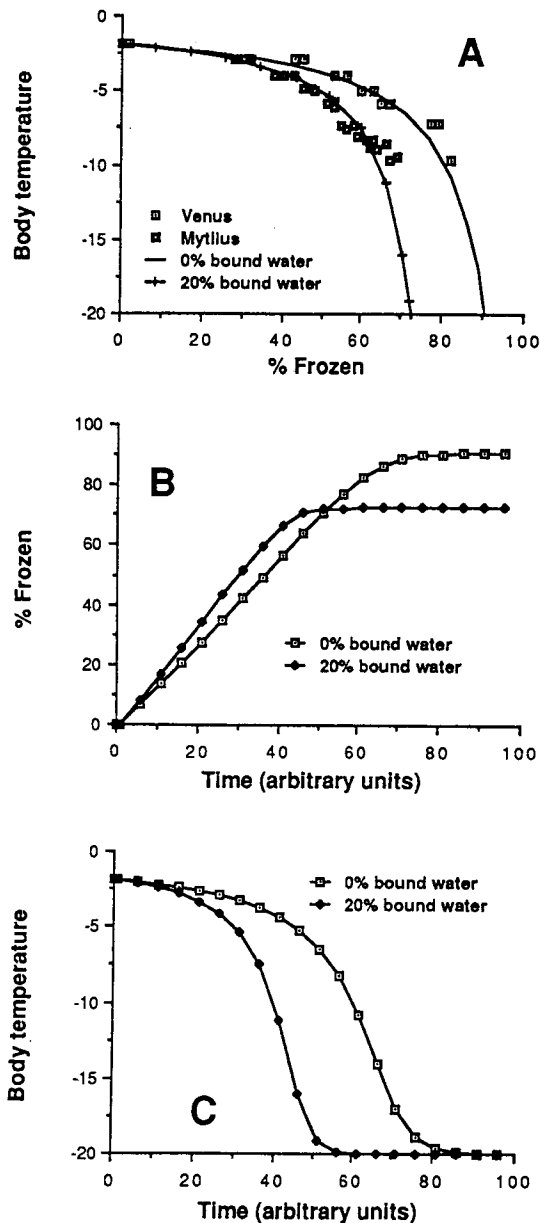


Fig. 3. A test of the model using data from Williams (1970). In A, the body temperatures (T_b) of *Mytilus edulis* and *Venus mercenaria* are plotted in relation to the percentage of their body water frozen. The data for *V. mercenaria* and *M. edulis* agree quite well with the model predicted curves for 0 and 20% bound water respectively. In B, the predicted time-courses of ice formation with and without bound water are compared, and, in C, the cooling curves during the exotherm are compared.

portion of the cooling curve. The plateau can thus be maintained for a period of time, even at very low bath temperatures, so long as adequate amounts of body water remain unfrozen.

Body water content

Up to this point, our modelling has assumed a uniform body water content of 75%. The water contents of ectotherms, however, often vary seasonally in response to changing physiological and

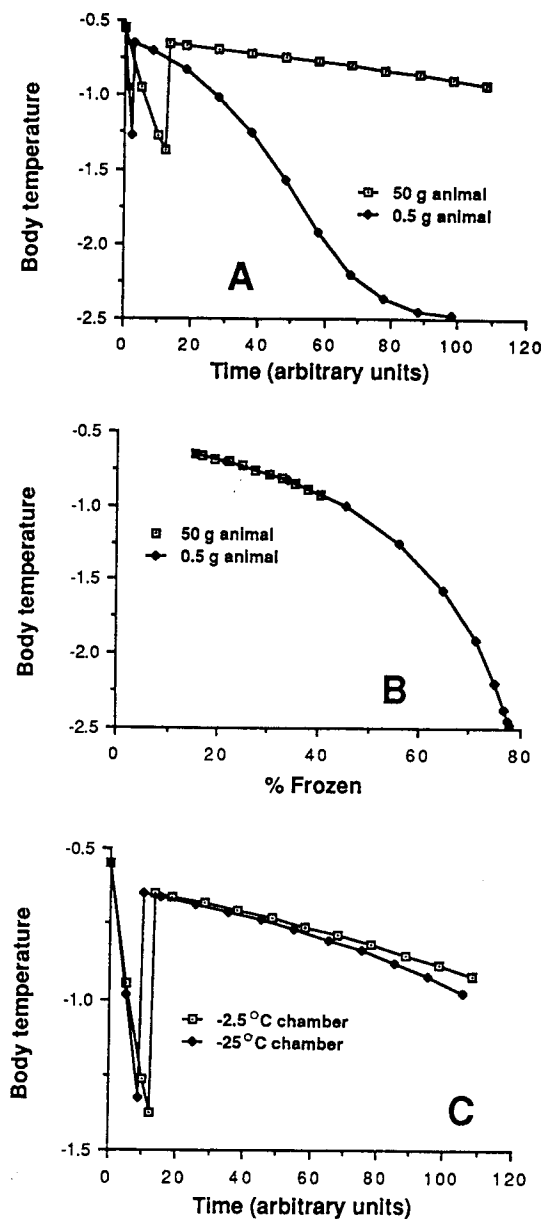


Fig. 4. The effects of body mass (A and B) or chamber temperature (C) on freezing responses of ectotherms.

environmental conditions, and, during winter, may be either greater or lower than before. Water content can easily be manipulated in our model by changing the value for W in equation (6). The results [Fig. 5(A)] reveal that, everything else being equal, dehydrated animals cool more rapidly (due presumably to a quicker depletion of freezable water [Fig. 5(B)] accompanied by a reduction in specific heat) and exhibit less of a freezing plateau as a consequence of their lower water content. Initial water content does not, however, affect the relationship between body temperature and ice content.

We have also assumed, until now, fixed equilibrium freezing point (FP_{eq}) and rebound temperatures (T_r). The difference between these temperatures is indicative of the initial surge of ice formation and thus does affect the post-nucleation cooling rate [Fig. 5(C)].

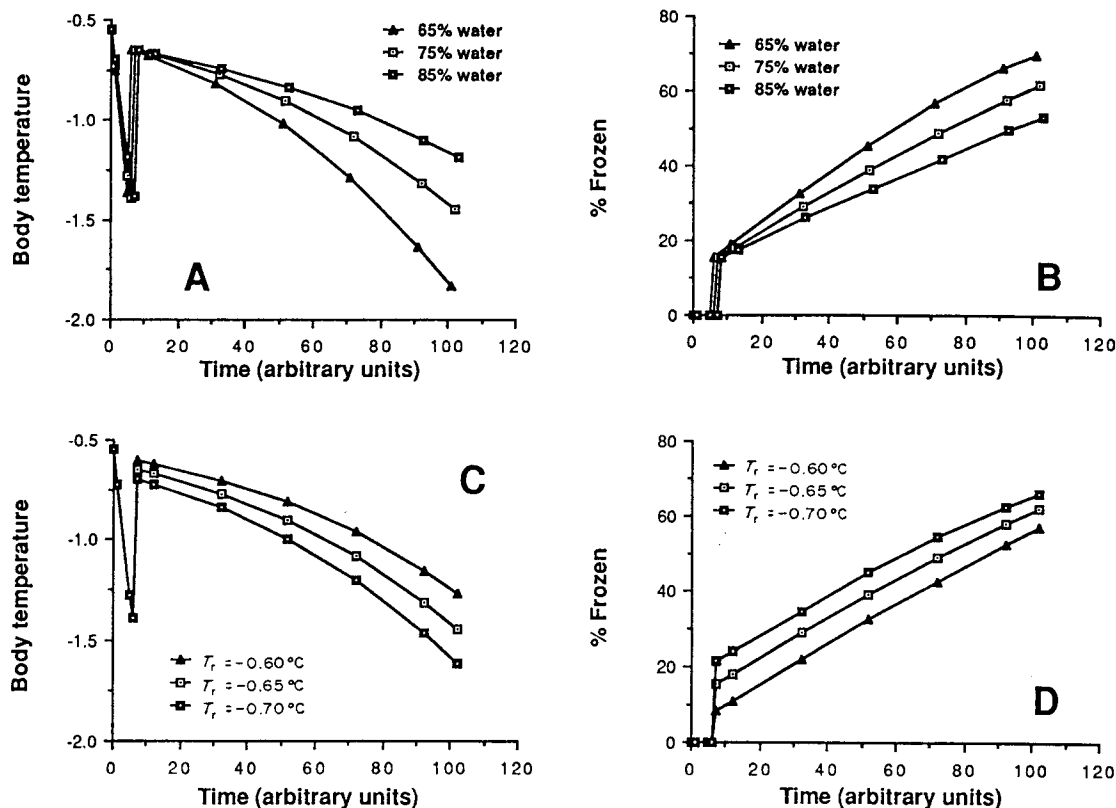


Fig. 5. The effects of variable water content (A and B) or rebound temperature (C and D) on freezing responses of ectotherms.

Both the rate of new ice formation *after* the initial surge [Fig. 5(D)] and the relationship between body temperature and ice content are essentially independent of rebound temperature (and thus of the nucleation surge). The difference between FP_{eq} and T_r is undoubtedly influenced by the degree of supercooling, but the data needed to incorporate fully the T_c into our model are not available at present.

Osmotic concentration

Thus far, we have noted several factors (cooling rate, bath temperature, body mass, water content) which fail to affect the fundamental relationship between body temperature and ice content. One factor which does, however, have a substantial influence is initial osmotic concentration. Osmotic concentration affects both the time-course pattern of freezing [Fig. 6(A)] and the rate of new ice formation [Fig. 6(B)]. More importantly, the body temperature in equilibrium with a given ice content varies with initial solute concentration [Fig. 6(C)]. An animal with an initial concentration of 200 mOsm would reach 67% ice content at -1.12°C , whereas animals with initial concentrations of 300 and 600 mOsm would reach this level of freezing only at body temperatures of -1.67 and -3.35°C respectively. A typical subtidal marine invertebrate, with an initial osmotic concentration of approx. 1000 mOsm, would cool to about -5.6°C before reaching a 67% ice content. Thus, freeze tolerance in some marine ectotherms is achieved at low body temperatures, even without the aid of cryoprotectants. Many terrestrial

arthropods can accumulate remarkable amounts of cryoprotective solutes and can tolerate freezing at very low body temperatures. Salt (1964) has reported glycerol concentrations as high as 5 M in certain overwintering insects. Together with other solutes, their osmotic concentration must thus equal, at least, a remarkable 5300 mOsm, which means they would reach 67% ice content only at a body temperature approaching -30°C ! The accumulation of additional solute will thus, by itself, lower the temperature at which a critical level of ice will form. Some of the action of these cryoprotective substances appears to be primarily colligative, though this is clearly not all that is involved (see Layne and Lee, 1987a; Storey and Storey, 1988).

Although we indicated earlier that initial water content has no effect on the temperature vs ice content relationship, this was based on the unrealistic assumption that osmotic concentration is independent of water content. Actually, pre-freezing dehydration may tend to increase the rate of body cooling [Fig. 5(A)] but the corresponding increase in initial osmotic concentration may also lower the body temperature at which lethal freezing occurs [Fig. 6(C)]. The interaction between pre-freezing dehydration and post-freezing osmotic dehydration needs additional study.

Microenvironment

The microenvironment of a freezing organism can markedly affect the freezing time-course (Layne and Lee, 1987b; Costanzo and Claussen, unpublished

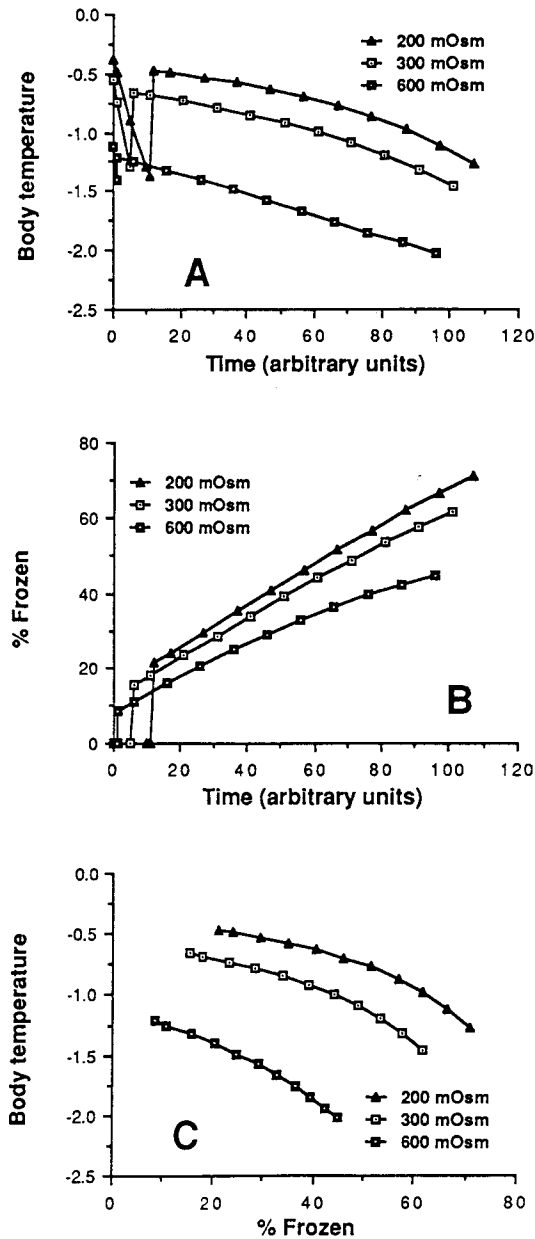


Fig. 6. The effects of osmotic concentration on the freezing responses of ectotherms. T_c was set at -1.4°C , whereas T_r was set at 1.0°C below the computed FP_{eq} for each osmotic concentration. However, T_c and T_r affect neither the curve of post-exotherm ice formation (B) nor the relationship between body temperature and ice content (C).

data). An animal well-insulated by surrounding soil, dead leaves, etc. will cool slower (this factor is already incorporated into the model as it affects cooling rate); however, in addition, this insulative material will tend to retain more of the heat of fusion generated by ice formation and will thus modify the microenvironment of the freezing organism and hence the cooling capacity of the "system". This factor will reduce the rate of new ice formation needed to counteract the cooling capacity. The relationship, however, is quite complex. We have not attempted to incorporate all aspects of this problem into our model, but we have taken a preliminary look at modification of micro-

environment by the heat of fusion. Not surprisingly, increased insulation tends to prolong the plateau and markedly extends the period of ice formation, thereby deferring the attainment of a lethal ice content. Added insulation, however, does not affect the basic relationship between body temperature and ice content.

We are clearly not the first to recognize the interaction between osmotic concentration and temperature for a freezing solution. Many authors (e.g. Williams, 1970; Zachariassen, 1979; Rubinsky and Cravalho, 1984) have evaluated this relationship. Zachariassen (1979) even presented an equation from which, with the incorporation of our equation (1), equations (2) and (3) can be derived. He actually made only an empirical association between ice content and temperature, however. Zachariassen (1979) graphically estimated ice content at -7.7°C for an NaCl solution ($FP_{eq} = -1.1^\circ\text{C}$) to be 84%, whereas equation (2) predicts a value of 85.7%. We believe that our model is the first to incorporate the factors of ice content, specific heat of dry tissue, temperature, and elapsed time into a unified simple model.

Model assumptions and appraisal

The validity of our model is dependent upon certain underlying assumptions. It assumes a somewhat uniform distribution of ice within the body of the organism with no rigid compartmentation and that all solutes initially present are osmotically active throughout the freezing episode. Also, although it does not demand that the body of the freezing organism be entirely isothermal, our model does assume that any existing thermal gradients are uniform or modest enough that a single measurement of core body temperature will accurately reflect the temperature at the ice-solute interface. Heart beat (and perhaps circulation) in freezing vertebrates tends to persist throughout most or all of the period of ice formation (Lotshaw, 1977; Costanzo and Claussen, unpublished data; Layne, personal communication; but see Storey, 1985), and this may help to reduce compartmentation. However, ice nucleation within an organism is heterogenous and the rate of crystal growth is influenced by viscosity and other factors (MacKenzie, 1977) not incorporated into our model. Also, the thermal gradients existing within a freezing animal can actually be quite complex (see Rubinsky and Cravalho, 1984). This complexity, however, is greatly exaggerated by the very rapid cooling rates (e.g. $8^\circ\text{C}/\text{min}$) used in this latter study. At the relatively low cooling rates characteristic of freezing under natural conditions, the thermal gradient problem is probably not severe (Costanzo and Claussen, 1990).

Our model seems to work well for moderate to large sized ectotherms, in which the rebound temperature (T_r) is only slightly lower than the equilibrium freezing point (FP_{eq}). This, however, does not seem to be the case for many small arthropods (see Salt, 1964) which supercool to far below zero and subsequently rebound only a few degrees. This apparent departure from the predictions of our model is likely due, at least in part, to methodology. Cryobiologists working with very small arthropods typically monitor temperature at the surface of the

organism, rather than attempting to measure core temperature. During cooling prior to freezing, body temperature will very closely track surface temperature, so this method provides a perfectly acceptable measure of the rate of cooling and of T_c . However, upon nucleation, a sharp gradient between surface temperature and core temperature will be established due to the internal generation of heat of fusion. A surface temperature probe (which is necessarily also exposed to the cooling environment) can detect this heat, but will probably not give an accurate reflection of the thermal changes occurring within the body of the organism. This is especially likely because of the very low temperatures resulting from the extreme supercooling and because of the very small size of the organisms and, hence, the transient nature of these thermal changes. Further work is needed to separate the effects of methodology from possible departures from model predictions.

The basic model also assumes that the total amount of solute within an organism remains constant throughout the freezing time course. This is not necessarily the case. Freezing wood frogs, for example, synthesize substantial amounts of glucose after the initiation of freezing (Storey and Storey, 1988). The model could easily be modified to incorporate these changes if the rate of synthesis were known. This would provide some interesting insights into the kinetics of ice accumulation.

Among the most powerful features of our model is its ability to predict ice content at any time during the freezing process without interrupting the experiment in progress. Implementation of the model requires a precise determination of FP_{eq} (which can perhaps be best accomplished via osmometry of plasma or haemolymph) and of body temperature, T_x [equations (2) and (3)] during freezing. If possible, T_x should be accurately measured to the nearest 0.01°C. A substantial error in measuring osmotic concentration (and thus in estimating FP_{eq}) or in measuring T_x will introduce a large percentage error in the estimation of the initial ice surge, but fortunately, such errors are not accumulative, but rather become progressively less significant at higher ice contents (Table 1). It is important, however, whenever possible, to check model estimates with empirical estimates of ice content at the termination of an experiment (e.g. using the method of Lee and Lewis,

1985). Such empirical estimates are, of course, also subject to measurement error.

Any model is, at best, an imperfect reflection of the real world. Our model is certainly too simple to fully accommodate all of the factors associated with the freezing of an actual organism, and indeed it is not intended as an exact biophysical representation. We believe, however, that our model provides a remarkably good first approximation of the freezing dynamics of intact animals. The general agreement in time-course patterns and the close fit of published data (Fig. 2) support its general validity. The simplicity of the model provides a clearer picture of freezing responses and allows for easy manipulation of the various parameters. The model can be used, as we have done to some extent above, to develop certain predictions about freezing animals. These can then be empirically tested. Departures from model predictions may, in some instances, provide insights into novel biological adaptations for freeze tolerance.

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Table 1. The sensitivity of the model to an error (indicated by an asterisk) in determination of equilibrium freezing point, FP_{eq} , through measurement of osmotic concentration or in measuring body temperature, T_x . Model estimates for body ice content are presented for the onset of ice nucleation (case 1) and for the attainment of equilibrium body temperature (case 2). Correct values: $FP_{eq} = -0.52$ C, initial $T_x (= T_i) = -0.60$ C, final $T_x (= T_c) = -1.60$ C

Measured osmotic conc. (mOsm)	Estimated FP_{eq} (C)	Measured T_x	Estimated ice content (%)	Error (%)
Case 1:				
280	-0.52	-0.60	13.3	—
280	-0.52	-0.65*	20.0	51.5
260*	-0.48*	-0.60	20.0	51.5
Case 2:				
280	-0.52	-1.60	67.5	—
280	-0.52	-1.65*	68.5	1.5
260*	-0.48*	-1.60	70.0	3.7

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