Functional Ecology 2006
20, 471–477

Hibernation patterns in mammals: a role for bacterial growth?

A. D. LUIS† and P. J. HUDSON

Center for Infectious Disease Dynamics, The Pennsylvania State University, University Park, PA, USA

Summary

1. To examine the hypothesis that stimulation of immune function plays a role in periodic arousal from hibernation, bacterial growth during hibernation was estimated using a simple mathematical model of the general dynamics of bacterial abundance at body temperatures experienced during hibernation.

2. In the model, periodic arousals were important for animals infected with *Salmonella* at body temperatures above 7 °C, but not below. In contrast, periodic arousals appeared to be important at all temperatures examined when infected with several species of coliform bacteria and *Pseudomonas*, species that grow well at low temperatures.

3. The modelled outputs were compared with torpor patterns seen in captive European Ground Squirrels, *Spermophilus citellus*, under natural light and temperature conditions. We used maximum likelihood to estimate model parameters and show that the six bacterial species examined are consistent with the immune stimulation hypothesis.

4. Our analyses suggest that bacterial infection could be a selective force on torpor behaviour and warrants further experimental investigation.

Key-words: European Ground Squirrel, periodic arousal, torpor, torpor bout length

Functional Ecology (2006) **20**, 471–477
doi: 10.1111/j.1365-2435.2006.01119.x

Introduction

Hibernation is an adaptive strategy used by endotherms when periods of reduced food availability coincide with low winter temperatures and result in increased demand for metabolic energy to maintain body temperature (Karmanova 1995). Torpid hibernators are thermo-conforming over a wide range of ambient temperatures and allow their body temperature to drop to 1–2 °C above the ambient temperature and drastically decrease their cardiac, respiratory and metabolic rates (Geiser & Kenagy 1988). Indeed, every physiological system seems to be affected (Lyman 1958; Nedergaard & Cannon 1990; Karmanova 1995). The adaptive value of hibernation is to conserve energy, although hibernating animals periodically emerge and their body temperature and cardiac, respiratory and metabolic rates return to normal for a period of usually less than 24 h (Pengelley & Fisher 1961; Willis 1982; Geiser, Hiebert & Kenagy 1990). These periodic arousals can cost animals up to 80% of their stored energy resources (Kayser 1953; Wang 1978), but the true function remains unclear and indeed may be multifactorial. Several hypotheses have been considered to explain these arousals, including restoration of electrolyte balance, regeneration of gonads, prevention of muscular atrophy, elimination of metabolic wastes, evaporative water loss, replenishment of blood glucose and elimination of sleep debt (Fisher 1964; Fisher & Manery 1967; Galster & Morrison 1970; Barnes *et al*. 1986; Wickler, Horwitz & Kott 1987; Daan, Barnes & Strijkstra 1991; Thomas & Geiser 1997). However, several of these hypotheses have been challenged (Willis 1982), and the true function of periodic arousals remains uncertain.

One additional hypothesis is that periodic arousals are necessary for mounting an immune response to tackle pathogens that invade the host prior to or during torpor (Prendergast *et al*. 2002). Energetically the immune system is very costly to maintain (Lochmiller & Deerenberg 2000). Furuse & Yokota (1984) showed that germ-free birds fed a diet with limited protein grew 78% faster than birds with normal gut bacteria. Since hibernation is primarily an adaptation for conserving energy, and the immune system is energetically costly, then we would expect hibernators to reduce their response during torpor but to reactivate the dormant immune system by exhibiting periodic arousals that would test the infection status of the host (Prendergast *et al*. 2002). Although the effect of hibernation on the immune system has received little attention, a few studies have demonstrated that the activation of

†Author to whom correspondence should be addressed.
E-mail: ADL12@psu.edu

lymphocytes, production of antibodies and the acute phase response to lipopolysaccharide (LPS) are arrested during torpor (McKenna & Musacchia 1968; Maniero 2000; Prendergast *et al*. 2002), so it appears that arousals may be needed to mount an effective immune response.

Hibernating animals may not be able to determine their infection status while in torpor (Prendergast *et al*. 2002), consequently they would need to arouse periodically to check for infections. Given the high energetic cost of arousal we should ask how often animals should arouse from torpor. The natural torpor patterns expressed by hibernating animals vary among species and even among individuals of the same species, but some general patterns are observed. For instance, torpor bouts are shortest at the onset of hibernation and become longer as the hibernation season progresses, until prior to emergence when they once again shorten (Geiser & Brigham 2000; Henning *et al*. 2002; Hut, Barnes & Daan 2002; Zervanos & Salsbury 2003). There is good evidence that torpor bout varies with ambient temperature (Ransome 1971; Geiser & Kenagy 1988; Geiser *et al*. 1990; Park, Jones & Ransome 2000). In this study we ask if these patterns could be explained by bacterial dynamics. Presumably, hibernating animals expressing the optimal torpor bout length will be selected for, since if the torpor bout is too long, rampant infection could cause host damage or mortality. On the other hand, if the torpor bouts are too short, the animal would waste critical energy reserves, which may lead to reduced condition in the spring or even mortality.

To examine the hypothesis that bacterial infection could account for host torpor patterns, we designed a mathematical model of the optimal torpor patterns that incorporates bacterial growth rates at different host temperatures and then compared outputs with observed torpor arousal data. We asked two specific questions: What torpor patterns could we expect if the function of arousals is to control bacterial infection? Can we explain the torpor patterns observed in European Ground Squirrels, *Spermophilus citellus*, with our simple mechanistic model? Modelling predicted torpor patterns in relation to bacterial growth could provide insights to direct further experimentation needed to address this hypothesis.

Methods

MODEL AND DATA

Bacterial growth rates in relation to temperature were estimated using the equation from Ratkowsky *et al*. (1982):

$$\sqrt{r} = b(T - T_0) \qquad \text{eqn 1}$$

where r is the bacterial growth rate and T is the temperature. When the square root of the growth rate ($\sqrt{r}$) is plotted against temperature (T), b is the slope of the regression line and T_0 is the x-intercept.

Initially, two representative bacterial species were selected, based on availability of growth rate data in relation to temperature. *Salmonella enterica* data were obtained from Mackey & Kerridge (1988), and a coliform species (C1) from Baig & Hopton (1969) (Fig. 1). Bacterial population growth inside a hibernating animal was examined assuming that bacteria continue to divide at a constant rate with no density-dependent food or space constraint. This was described using the doubling equation:

$$N_{t+1} = N_t * 2^r \qquad \text{eqn 2}$$

where N is the bacterial population size and r is the growth rate of the bacteria (≈1/generation time).

When the host is infected during torpor we assume that the bacteria grow exponentially at a rate determined by the host body temperature, until they either damage or kill the host. The immune stimulation hypothesis postulates that there would be a selective advantage among those individuals that terminate a torpor bout to let the immune system check and combat any itinerant infection before it causes damage. For this model, we assumed arousal and immune function should occur when the bacteria population reaches 10^9. Although this number is essentially arbitrary, we know that for at least some bacterial pathogens (i.e. *Salmonella enterica*), when numbers increase above 10^9, the host becomes septicaemic and may die (Vazquez-Torres *et al*. 2004). We used this figure as a starting point and then undertook a sensitivity analysis to determine the sensitivity of any conclusions to this amount. We also assumed that the bacteria infect the host at the beginning of hibernation and that the initial inoculum size is 100 bacteria.

Antibody production during hibernation is insignificant and even during periods of arousal is significantly less than in non-hibernating animals, and takes much longer to reach detectable levels such that it takes

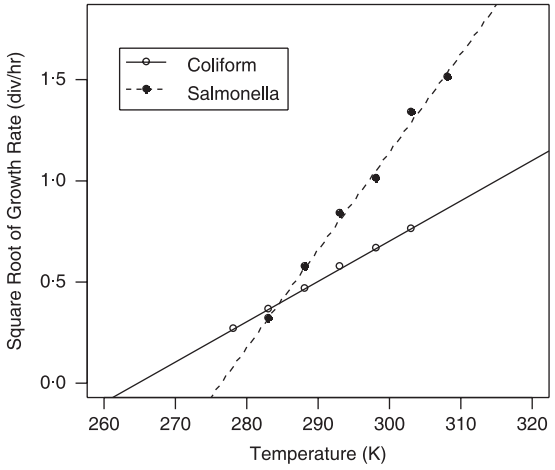

Fig. 1. Relationship between square root of bacterial growth rate and temperature for *Salmonella* (Mackey & Kerridge 1988) and coliform C1 (Baig & Hopton 1969).

several periods of arousal for the acquired immune response to rise (Dempster, Grodums & Spencer 1966; McKenna & Musacchia 1968; Burton & Reichman 1999). In the model we therefore assumed the animals produce no antibodies, and consequently there is no clearance of the bacteria, so the innate immune response is the principal mechanism for controlling infection. The innate response acts to reduce the bacterial population to 10^5, an arbitrary level, but one based on the innate control of bacterial numbers in wild-type mice recorded in both *Salmonella* and *Bordetella bronchiseptica* (Vazquez-Torres *et al*. 2004; Mann 2005; Mann *et al*. 2005). We then explored the significance of this level on our final conclusions using a sensitivity analysis. In the model, the animal remains euthermic for 18 h, which is a typical length of an arousal episode, including re-warming, euthermic period and cooling below 30 °C (Hut *et al*. 2002). After 18 h, the animal returns to torpor, allowing the bacterial numbers to increase again from 10^5.

Next, how the ambient temperature in the hibernaculum and minimum torpor body temperature changed during a hibernation season were considered. A representative profile of minimum body temperature throughout hibernation was recorded in the European Ground Squirrel by Henning *et al*. (2002; Fig. 2c). This temperature profile can be represented over 4320 h or a 6 month hibernation period by the equation:

$$y = 2 * 10^{-6} * x^2 - 0{\cdot}0105 * x + 15{\cdot}011 \qquad \text{eqn 3}$$

where y is the temperature (°C) and x is the time (hours). Since the rate of bacterial growth changes with temperature, torpor bout length becomes dynamic using this changing temperature profile in the model. Torpor patterns over an entire hibernation season were predicted with respect to *Salmonella* and coliform C1 bacterial growth, and subsequently other psychrophilic bacteria (after Baig & Hopton 1969), including coliform species designated C4 and EBT and *Pseudomonas* species P11, P14 and P26.

The model outputs were compared to observed torpor patterns and corresponding minimum body temperatures collected for six European Ground Squirrels held in captivity in outdoor enclosures under natural light and ambient temperature conditions from Hut *et al*. (2002) and one European Ground Squirrel in the laboratory under simulated natural conditions from Henning *et al*. (2002).

ANALYSIS

In the model, torpor bout length is determined by body temperature throughout the bout. The relationship between minimum body temperature during a torpor bout and torpor bout length for European Ground Squirrels was explored and compared to the modelled outputs for the six psychrophilic bacteria from Baig & Hopton (1969). Given the European Ground Squirrel data (Henning *et al*. 2002; Hut *et al*. 2002) and our model (equations 1 and 2), we used maximum likelihood

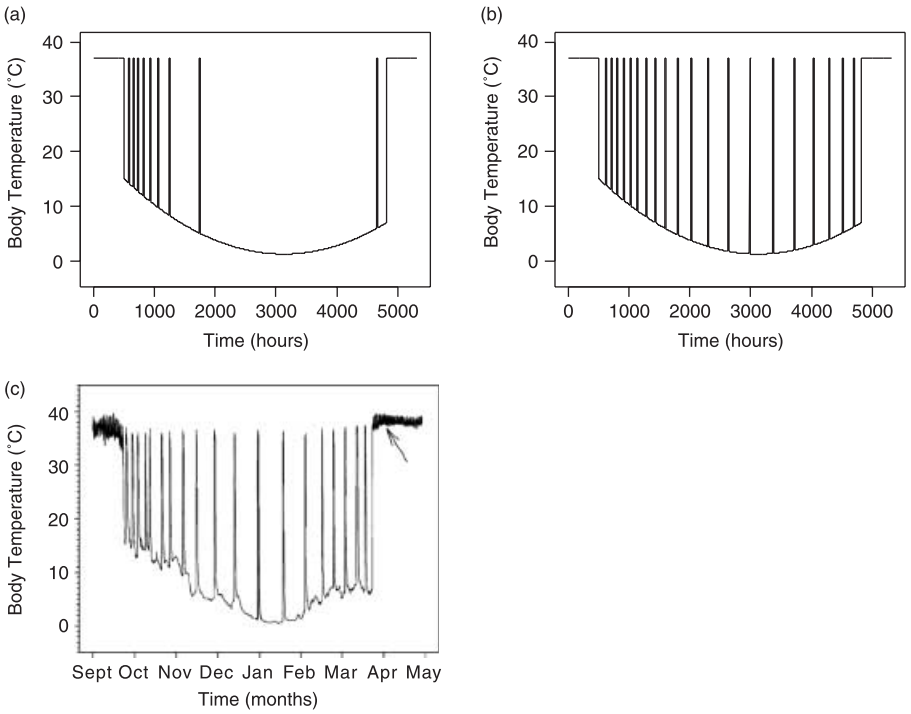

Fig. 2. Predicted and observed torpor patterns throughout a hibernation season. (a) Predicted expression for a hibernating animal exposed to bacterial pathogens with growth properties similar to *Salmonella* and (b) coliform C1. (c) Observed torpor patterns in a European Ground Squirrel in captivity under simulated natural light and ambient temperature conditions (Fig. 1 from Henning *et al*. 2002).

techniques to estimate what parameters for bacterial growth (b and T_0) would be consistent with the immune stimulation hypothesis. We assumed Gaussian likelihoods for the torpor lengths, such that the negative log likelihood is equal to $(1/n) * \log SS$ (e.g. McCullagh & Nelder 1989), where SS is the squared difference between the observed and model predicted torpor lengths. Unique maximum likelihood estimates for b and T_0 were found using the Nelder-Mead algorithm as implemented in the 'optim' function in R (R Development Core Team 2004). However, because of the strong colinearity between b and T_0, there is a range of parameter combinations with almost identical likelihoods. We therefore used two-dimensional profile likelihood to map the set of near optimal parameter combinations. We used likelihood ratios assuming chi-square distributions with one degree of freedom (e.g. McCullagh & Nelder 1989) to compare the observed bacterial growth data with those expected based on the maximum likelihood estimates. All calculations were done using R version 2·0·1 (R Development Core Team 2004).

Results

From known temperature-dependent growth rates of bacteria, we extrapolated growth rates using equation 1 from Ratkowsky *et al*. (1982) (Table 1). Initially, we examined the growth rates of *Salmonella enterica* (Mackey & Kerridge 1988), and a coliform species (C1 from Baig & Hopton 1969). At temperatures above 11·5 °C, *Salmonella* grows better than coliform, but below 11·5 °C, coliform grows better than *Salmonella* (Fig. 1).

We examined bacterial growth rates in a hibernating animal with a body temperature of 10 °C. For *Salmonella*, which has a growth rate of 0·1074 divisions per hour at 10 °C, the initial torpor bout lasted approximately 9 days, and the subsequent torpor bouts were approximately 5·3 days until bacterial numbers reached 10^9. Coliform bacteria have a higher growth rate (r = 0·1354) at 10 °C than *Salmonella*, and, subsequently, this species reached 10^9 faster than *Salmonella*. The model predicted an initial torpor bout of approximately 7·2 days and subsequent torpor bouts of 4·2 days, for this bacterial species.

Running the model using the minimum body temperatures during hibernation described by equation 3 gives two distinct sets of torpor patterns for the two bacterial species, *Salmonella* and coliform (Fig. 2a,b, respectively). Since *Salmonella* does not grow well at temperatures below 7 °C, the optimal torpor bouts lengthen since it now takes much longer for the bacteria to reach 10^9. However, since coliform bacteria grow well at these low temperatures, the torpor bout durations only moderately lengthen (Fig. 2b). Running the model with other psychrophilic bacteria, coliform species C4 and EBT and *Pseudomonas* species P11, P14 and P26 (Baig & Hopton 1969) gives similar patterns as C1 (data not shown). Data from a representative European Ground Squirrel (Fig. 1 from Henning *et al*. 2002) are presented for comparison (Fig. 2c).

Using maximum likelihood, bacterial parameters (b and T_0) that give the best fit of the model to the data were calculated as: b = 0·0119 and T_0 = 256·700. Using these optimal bacterial parameters, torpor bout length was predicted for the body temperatures from European Ground Squirrels and compared to the corresponding torpor bout lengths recorded. Linear regression gives an R^2 of 0·57. Owing to the colinearity of the model parameters, the result is a ridge in likelihood space (Fig. 3). All six bacterial species from Baig & Hopton (1969) (C1, C4, EBT, P11, P14 and P26) are within the 0·01 likelihood ratio statistic, which corresponds to a P-value of 0·92 (1 df).

A sensitivity analysis on the threshold values of 10^5 and 10^9 revealed that changing these parameters by 10-fold changed the likelihood ratio by just 0·01, which is still highly significant ($P > 0·85$) and makes no substantive differences to the findings of this model.

Table 1. Estimated slope, b, and x-intercept, T_0, for the psychrophilic bacteria in Baig & Hopton (1969) when the square root of the growth rate (divisions per hour) is plotted against temperature (K)

Bacterium	b	T_0
Coliform species		
C1	0·020	264·77
C4	0·019	265·17
EBT	0·019	264·74
Pseudomonad species		
P11	0·017	263·70
P14	0·022	265·50
P26	0·019	264·55

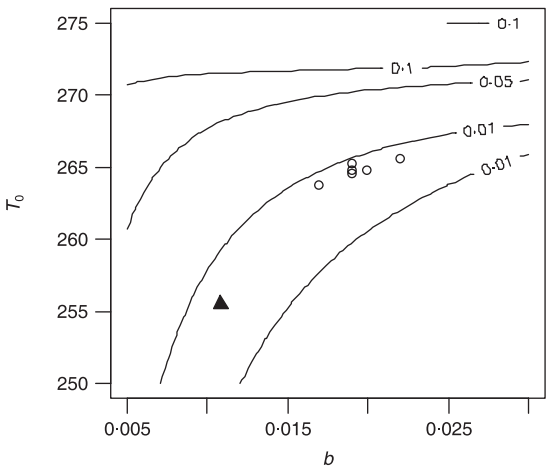

Fig. 3. Bacterial parameters (b and T_0) that give the best fit of the model to the data, using maximum likelihood estimates. Contours show likelihood test statistics. The six psychrophilic bacteria described by Baig & Hopton (1969) are given by circles, and the filled triangle represents the optimal parameter combination.

Discussion

We explored the hypothesis proposed by Prendergast *et al*. (2002) that bacterial infection is an important selective force on torpor patterns because the immune system is impaired during torpor, and periodic arousals are necessary to initiate control of any itinerant infections. Specifically, we asked: what torpor patterns would we expect to see if the function of arousals is to control bacterial infection? How do these predicted torpor patterns compare with observed patterns? Through modelling we found that the predicted torpor patterns are dependent on the growth properties of the bacterium in relation to temperature. Although bacterial growth rates are much slower at temperatures experienced during mammalian hibernation, some bacteria are able to replicate at temperatures as low as –20 °C (Deming 2002). If animals are exposed to pathogenic bacteria with growth properties similar to *Salmonella*, periodic arousal to control infection would be an important adaptation at temperatures above 7 °C, but not below 7 °C because pathogenic bacteria with these growth properties do not grow well at low temperatures. However, if animals are exposed to pathogenic bacteria that are able to grow well at low temperatures, like the psychrophilic coliform and *Pseudomonas* species described by Baig & Hopton (1969), periodic arousals would be important throughout hibernation at all the temperatures examined.

Fitting optimal bacterial parameters to the model results in a ridge in likelihood space in which the six bacteria described from Baig & Hopton (1969) fit within the 0·01 likelihood test statistic. Therefore, 92% of the time we would see data this extreme if the model fit the data. From this, we conclude that several species of psychrophilic bacteria could produce the torpor patterns observed. Furthermore, this simple model, which considers only bacterial growth rates at different temperatures, offers a parsimonious explanation for the general torpor patterns observed in some hibernating animals. These findings do not suggest that all animals arouse because they are infected, but that the animals that express these torpor patterns may have been selected for because they were able to deal with bacteria that are able to grow well at low temperatures, such as some coliform and *Pseudomonas* species. This approach provides support for the hypothesis because the data are consistent with the model but experimental studies are needed to test the hypothesis.

An interesting discrepancy between the model and the data is that in nature, hibernating animals generally do not have a longer first torpor bout than subsequent bouts, whereas the model shows this to be the case. However, in the model the length of the first bout is determined by the bacterial inoculum size, whereas subsequent bouts are not, so the length of the first bout is a consequence of the initial infection size while subsequent ones are not. In nature, some hibernators display a shorter first torpor bout in which they experience shallow decreases in body temperature, called 'test drops'. These animals may be resetting their hypothalamic thermostat which may help to acclimate cellular functions to the onset of low body temperature (Hammel 1967; Zervanos & Salsbury 2003).

Hibernators thermo-conform over a wide range of ambient temperatures; however, when ambient temperature (T_a), drops below a threshold, body temperature (T_b) remains constant. This threshold is the body temperature set point (T_{bset}). As T_a decreases below T_{bset}, the animal thermoregulates and maintains T_b at T_{bset} to avoid a lethal decline in T_b (Geiser & Kenagy 1988; Buck & Barnes 2000). If considered at all temperatures, our model would predict that torpor bout length always increases as T_b decreases. However, in hibernating animals this is observed only when T_b is above T_{bset}. When T_b drops below T_{bset}, the torpor bout lengths decrease (Geiser & Kenagy 1988; Buck & Barnes 2000).

Other hypotheses have been proposed to explain the function of arousals and may explain this phenomenon. Several of these can be grouped under metabolism effects, such as reduction of energy substrates and accumulation of metabolic wastes. Metabolic rate decreases with T_a until it drops below T_{bset}. Therefore these metabolic effects would occur at a slower rate as ambient temperature declines to T_{bset}, allowing for longer torpor bouts (Geiser & Kenagy 1988; Geiser & Brigham 2000), also producing the inverse relationship of torpor bout lengths and T_a as predicted from other temperature-dependent processes such as bacterial growth. However, regression analyses by Geiser *et al*. (1990) suggest that torpor bout lengths at T_a values above T_{bset} are more strongly correlated with T_b than metabolism, as measured by consumed volume of O_2, lending some credence to the immune stimulation hypothesis. However, at T_a values below T_{bset}, metabolic rate increases as the animal thermoregulates. Therefore, the relationship between torpor bout length and temperature at values below T_{bset} is consistent with metabolic rate hypotheses for the function of arousals (Buck & Barnes 2000). However, since at T_a values above T_{bset}, torpor bout length is more strongly correlated with T_b than metabolic rate (Geiser *et al*. 1990; Buck & Barnes 2000), it appears there may be two different arousal triggers, one for T_a values below T_{bset} and another for values above T_{bset}. Buck & Barnes (2000) speculated that animals may need to arouse more often at T_a values below T_{bset} owing to an increase in metabolism, to replenish glycogen stores, whereas above T_{bset}, a temperature-sensitive timing mechanism may operate. This hypothesis is consistent with our model. Nevertheless,these hypotheses for periodic arousals are not mutually exclusive and these processes may, in fact, act in concert.

Burton & Reichman (1999) argued that most pathogens are not able to replicate at low temperatures. Mitosis at low temperatures has been shown to lead to damage caused by disrupted microtubulin polymerization in

some organisms (Roth 1967; Nagasawa & Dewey 1972). However, each bacterial species has different growth properties. Some are better at growth at low temperatures than others and may be able to grow at temperatures lower than 5 °C, although at slower rates (Baig & Hopton 1969). So these bacteria could be an important selective pressure that influences torpor patterns.

Different species of hibernating animals show large variations in their maximum torpor bout lengths. For example, the Edible Dormouse has a reported maximum torpor bout length of 792 h, and the Turkish Hamster has a reported maximum torpor bout length of only 130 h. In terms of the immune stimulation hypothesis, this could be explained by differences in bacteria that these different species have been exposed to. Furthermore, other microorganisms follow the growth relationship described by equation 3 including fungi (Ratkowsky *et al*. 1982). In fact, fungal pathogens often grow better at low temperatures (such as those T_b values experienced by hibernating animals) compared with bacteria (Ratkowsky *et al*. 1982). Differences in maximum torpor bout length may result from exposure to different bacterial or fungal pathogens. Furthermore, as mentioned earlier, the hypotheses for the function of periodic arousal are not mutually exclusive and torpor patterns may result from a combination of factors.

Key simplifying assumptions were made in the model. One assumption is that bacterial growth is not density dependent. We believe this is a reasonable assumption since we are considering time points early in the growth process. We also assume that bacterial growth and immunity rates are immediate and rise to maximum, and the animal enters and arouses from torpor such that its body temperature rises quickly to 37 °C. Although we know that animals may take less than an hour to a few hours to arouse from torpor and longer to enter torpor, increasing or decreasing their body temperatures between euthermic and ambient temperatures (Park *et al.* 2000; Hut *et al*. 2002), no studies that we are aware of have addressed the question of when during this rewarming process the immune system begins to function effectively and when during cooling it ceases to function effectively. Therefore, adding cooling and rewarming phases does nothing to improve the model. To date, no experiments have characterized bacterial growth rates in a hibernating animal, so the data for bacterial growth used in this model came from experiments on minced beef (Mackey & Kerridge 1988) and in culture (Baig & Hopton 1969).

The model indicates that hibernators exposed to bacterial pathogens would benefit from periodic arousals; however, several studies suggest that hibernators exposed to viruses may not. Viral studies performed on hibernating animals indicate that viral replication is undetectable during torpor, yet may occur during periods of arousal (Sulkin *et al*. 1960, 1966; Dempster *et al*. 1966; Main 1979; Herbold *et al*. 1983). Dempster *et al*. (1966) also found that Coxsackie B-3 virus was able to reach higher titres faster in animals recently aroused from torpor, whether infected during torpor or 48 h after arousal, and caused more severe pathology than seen in non-hibernating animals. This would imply that there are immunological trade-offs involved in periodic arousals, not only between conserving resources and immunological defence, but also between the optimal immune responses for viruses and bacteria.

Understanding host–pathogen interactions in wildlife reservoirs is important if we are to obtain insight into the emergence and spread of zoonotic diseases. Some wildlife disease reservoirs are hibernating animals, such as bats, but relatively little is known about disease dynamics in hibernators. Furthermore, with the increased attention to hibernation for medical applications, such as preservation of tissue at low temperature and reduction of physiological damage caused from trauma and ischaemia (Carey, Andrews & Martin 2003), understanding bacterial and immune dynamics at these decreased temperatures could have important implications for human health.

Acknowledgements

We would like to thank Ottar Bjørnstad for his valuable help with the analysis and Bryan Grenfell for his constructive comments on the manuscript. A.L. would like to thank Kat Shea for her guidance during the development of the model and Tom Raffel and Isa Cattadori for their useful input.

References

Baig, I.A. & Hopton, J.W. (1969) Psychrophilic properties and the temperature characteristic of growth of bacteria. *Journal of Bacteriology* **100**, 552–553.

Barnes, B.M., Kretzmann, M., Licht, P. & Zucker, I. (1986) The influence of hibernation on testis growth and spermatogenesis in the golden-mantled ground squirrel, *Spermophilus lateralis*. *Biology of Reproduction* **35**, 1289–1297.

Buck, C.L. & Barnes, B.M. (2000) Effect of ambient temperature on metabolic rate, respiratory quotient, and torpor in an arctic hibernator. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* **279**, R255–R262.

Burton, R.S. & Reichman, O.J. (1999) Does immune challenge affect torpor duration? *Functional Ecology* **13**, 232–237.

Carey, H.V., Andrews, M.T. & Martin, S. (2003) Mammalian hibernation: cellular and molecular responses to depressed metabolism and low temperature. *Physiological Reviews* **83**, 1153–1181.

Daan, S., Barnes, B.M. & Strijkstra, A.M. (1991) Warming up for sleep? Ground squirrels sleep during arousals from hibernation. *Neuroscience Letters* **128**, 265–268.

Deming, J.W. (2002) Psychrophiles and polar regions. *Current Opinion in Microbiology* **5**, 301–309.

Dempster, G., Grodums, E.I. & Spencer, W.A. (1966) Experimental Coxsackie B-3 virus infection in *Citellus lateralis*. *Journal of Cellular Physiology* **67**, 443–454.

Fisher, K.C. (1964) On the mechanisms of periodic arousal in the hibernating ground squirrel. *Annales Academiae Scientiarum Fennicae-Biologia* **71**, 141–156.

Fisher, K.C. & Manery, J.F. (1967) Water and electrolyte metabolism in heterotherms. *Mammalian Hibernation III* (eds K.C. Fisher, A.R. Dawe, C. P. Lyman, E. Schonbaum & F.E. South), pp. 235–279. Oliver and Boyd, London.

Furuse, M. & Yokota, H. (1984) Protein and energy utilization in germ-free and conventional chicks given diets containing different levels of dietary protein. *British Journal of Nutrition* **51**, 255–264.

Galster, W.A. & Morrison, P. (1970) Cyclic changes in carbohydrate concentrations during hibernation in the Arctic ground squirrel. *American Journal of Physiology* **218**, 1228–1232.

Geiser, F. & Brigham, R.M. (2000) Torpor, thermal biology, and energetics in Australian long-eared bats (*Nyctophilus*). *Journal of Comparative Physiology B Biochemical Systemic and Environmental* **170**, 153–162.

Geiser, F. & Kenagy, G.J. (1988) Torpor duration in relation to temperature and metabolism in hibernating ground squirrels. *Physiological Zoology* **61**, 442–449.

Geiser, F., Hiebert, S. & Kenagy, G.J. (1990) Torpor bout duration during the hibernation season of two sciurid rodents: interrelations with temperature and metabolism. *Physiological Zoology* **63**, 489–503.

Hammel, H.T. (1967) Temperature regulation and hibernation. *Mammalian Hibernation III* (eds K.C. Fisher, A.R. Dawe, C.P. Lyman, E. Schonbaum & F.E. South), pp. 86–96. Oliver and Boyd, London.

Henning, R.H., Deelman, L.E., Hut, R.A., van der Zee, E.A., Buikema, H., Nelemans, S.A., Lip, H., De Zeeuw, D., Daan, S. & Epema, A.H. (2002) Normalization of aortic function during arousal episodes in the hibernating ground squirrel. *Life Sciences* **70**, 2071–2083.

Herbold, J.R., Heuschele, W.P., Berry, R.L. & Parsons, M.A. (1983) Reservoir of St. Louis encephalitis virus in Ohio bats. *American Journal of Veterinary Research* **44**, 1889–1893.

Hut, R.A., Barnes, B.M. & Daan, S. (2002) Body temperature patterns before, during, and after semi-natural hibernation in the European ground squirrel. *Journal of Comparative Physiology B Biochemical Systemic and Environmental* **172**, 47–58.

Karmanova, I.G. (1995) The physiology and genesis of hibernation. *Journal of Evolutionary Biochemistry and Physiology* **31**, 116–122.

Kayser, C. (1953) L'hibernation des mammiferes. *Annales de Biologie* **29**, 109–150.

Lochmiller, R.L. & Deerenberg, C. (2000) Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* **88**, 87–98.

Lyman, C.P. (1958) Metabolic adaptations of hibernators. *Federation Proceedings* **17**, 1057–1060.

Mackey, B.M. & Kerridge, A.L. (1988) The effect of incubation and inoculum size on growth of salmonellae in minced beef. *International Journal of Food Microbiology* **6**, 57–65.

Main, A.J. (1979) Eastern equine encephalomyelitis virus in experimentally infected bats. *Journal of Wildlife Diseases* **15**, 467–477.

Maniero, G.D. (2000) The influence of temperature and season on mitogen-induced proliferation of ground squirrel lymphocytes. *Life in the Cold* (eds G. Heldmaier & M. Klingenspor), pp. 493–503. Springer, Berlin.

Mann, P.B. (2005) *Toll like receptor 4 mediated host defense during* Bordetella *infection*. PhD Thesis, The Pennsylvania State University, Pennsylvania.

Mann, P.B., Wolfe, D., Latz, E., Golenbock, D., Preston, A. & Harvill, E.T. (2005) Comparative toll like receptor 4-mediated innate host defense to *Bordetella* infection. *Infection and Immunity* **73**, 8144–8152.

McCullagh, P. & Nelder, J.A. (1989) *Generalized Linear Models*. Chapman & Hall/CRC, Boca Raton, FL.

McKenna, J.M. & Musacchia, X.J. (1968) Antibody formation in hibernating ground squirrels (*Citellus tridecemlineatus*). *Proceedings of the Society for Experimental Biology and Medicine* **129**, 720–724.

Nagasawa, H. & Dewey, W.C. (1972) Effects of cold treatment on synchronous Chinese hamster cells treated in mitosis. *Journal of Cellular Physiology* **80**, 89–106.

Nedergaard, J. & Cannon, B. (1990) Mammalian hibernation. *Philosophical Transactions of the Royal Society of London B* **326**, 669–686.

Park, K.J., Jones, G. & Ransome, R.D. (2000) Torpor, arousal and activity of hibernating Greater Horseshoe Bats (*Rhinolophus ferrumequinum*). *Functional Ecology* **14**, 580–588.

Pengelley, E.T. & Fisher, K.C. (1961) Rhythmical arousal from hibernation in the golden-mantled ground squirrel, *Citellus lateralis tescorum*. *Canadian Journal of Zoology* **39**, 105–120.

Prendergast, B.J., Freeman, D.A., Zucker, I. & Nelson, R.J. (2002) Periodic arousal from hibernation is necessary for initiation of immune responses in ground squirrels. *American Journal of Physiology*. *Regulatory Integrative and Comparative Physiology* **282**, R1054–R1062.

Ransome, R.D. (1971) The effect of ambient temperature on the arousal frequency of the hibernating greater horseshoe bat, *Rhinolophus ferrumequinum* in relation to site selection and the hibernation state. *Journal of Zoology* **164**, 353–371.

Ratkowsky, D.A., Olley, J., McMeekin, T.A. & Ball, A. (1982) Relationship between temperature and growth rate of bacterial cultures. *Journal of Bacteriology* **149**, 1–5.

R Development Core Team (2004) Royal: A language environment for statistical computing. Royal Foundation for Statistical Computing, Vienna Austria.

Roth, L.E. (1967) Electron microscopy of mitosis in amoebae III. Cold and urea treatments: a basis for tests of direct effects of mitotic inhibitors on microtubule formation. *Journal of Cell Biology* **34**, 48–59.

Sulkin, S.E., Allen, R., Sims, R., Krutzsch, P.H. & Kim, C. (1960) Studies on the pathogenesis of rabies in insectivorous bats. II. Influence of environmental temperature. *Journal of Experimental Medicine* **112**, 595–617.

Sulkin, S.E., Allen, R., Sims, R. & Singh, K.V. (1966) Studies of arthropod-borne virus infections in *Chiroptera* III. Influence of environmental temperature on experimental infection with Japanese B and St. Louis encephalitis viruses. *American Journal of Tropical Medicine and Hygiene* **15**, 406–417.

Thomas, D.W. & Geiser, F. (1997) Periodic arousals in hibernating mammals: is evaporative water loss involved? *Functional Ecology* **11**, 585–591.

Vazquez-Torres, A., Vallance, B.A., Bergman, M.A., Finlay, B.B., Cookson, B.T., Jones-Carson, J. & Fang, F.C. (2004) Toll-like receptor 4 dependence of innate and adaptive immunity to *Salmonella*: Importance of the Kupffer cell network. *Journal of Immunology* **172**, 6202–6208.

Wang, L.C.H. (1978) Time patterns and metabolic rates of natural torpor in the Richardson's ground squirrel. *Canadian Journal of Zoology* **57**, 149–155.

Wickler, S.J., Horwitz, R.A. & Kott, K.S. (1987) Muscles and function in hibernating hamsters – a natural analog to bed rest. *Journal of Thermal Biology* **12**, 163–166.

Willis, J.S. (1982) The mystery of the periodic arousal. *Hibernation and Torpor in Mammals and Birds* (eds C.P. Lyman, J.S. Willis, A. Malan & L.C.H. Wang), pp. 92–103. Academic, New York.

Zervanos, S.M. & Salsbury, C.M. (2003) Seasonal body temperature fluctuations and energetic strategies in free-ranging eastern woodchucks (*Marmota monax*). *Journal of Mammalogy* **84**, 299–310.

Received 22 November 2005; revised 2 March 2006; accepted 9 March 2006
Editor: Prof. Brian McNab