

Chapter 1

Metabolic Depression: A Historical Perspective

Philip C. Withers and Christine E. Cooper

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Abstract An extended period of inactivity and reduced metabolic rate of many animals and plants, as well as unicellular organisms, has long been recognized by natural historians, e.g., Aristotle and Pliny. Biologists have studied this phenomenon since the 1550s (Gessner) and 1700s (Van Leeuwenhoek, Buffon). The period of inactivity can be less than a day, a few consecutive days or weeks, an entire season, or even many years. It can involve very different physiological states in response to a variety of environmental stimuli, such as extreme temperatures or unavailability of food or water. These periods of inactivity have been described and classified according to the group of organisms in question, extent and duration of the metabolic depression, ambient and body temperatures, state of body water (frozen or hyperosmotic), or availability of oxygen. Cryptobiosis, or “hidden life,” is an extreme form of inactivity, with often complete cessation of metabolism. It was first described in the 1700s, was further characterized in the 1800s, and in the 1900s physiological studies delineated the extent of metabolic depression. Molecular mechanisms for cryptobiosis have been sought since the late 1900s. Cryptobiosis includes three physiological states, anhydrobiosis (desiccation), osmobiosis (high osmotic concentration), and cryobiosis (freezing), where metabolic depression is associated with an altered physical state of cell water and

P.C. Withers (✉) and C.E. Cooper
Department of Zoology, School of Animal Biology M092, University of Western Australia,
Crawley, Western Australia, 6009 and Department of Environmental and Aquatic Science,
Curtin University of Technology, PO Box U1987, Perth, Western Australia, 6845
e-mail: pwithers@cyllene.uwa.edu.au; C.Cooper@curtin.edu.au

often involves accumulation of compatible solutes, and one physiological state, anoxybiosis (anoxia), where metabolic depression occurs at the normal cellular hydration state. Dormancy (torpor) is a less extreme form of inactivity, associated with a moderate reduction in metabolic rate (hypometabolism). Although first described by Aristotle and Pliny, studies in the 1900s delineated the basic physiological changes that accompany dormancy. Dormancy allows avoidance of unfavorable short- or long-term climatic conditions and conservation of energy and water. Hibernation is long-term multiday torpor during winter, whereas aestivation is dormancy during summer. In ectotherms, the metabolic depression that accompanies dormancy is intrinsic, with metabolic rate declining to about 10 to 20% of resting metabolic rate at the same body temperature. The molecular mechanisms for intrinsic metabolic depression are poorly understood. In endotherms, torpor involves a fundamental physiological change in body temperature regulation that markedly reduces metabolic rate and water loss, often to <10% of the normothermic resting metabolic rate at the same ambient temperature. Most of this reduction in metabolic rate reflects the decreased setpoint for thermoregulation resulting in reduced metabolic heat production and a Q_{10} effect; there may be some intrinsic molecular-based metabolic depression in some hibernators. Dormancy allows species to exploit ephemeral environments and colonise habitats that would otherwise be unsuitable for growth or survival at certain times of the year. There are costs to dormancy, but for many species, the energetic and hygric advantages outweigh these costs.

1.1 Introduction

An extended period of inactivity and reduced metabolic rate is a widely recognized behavioral and physiological state in both animals and plants. Aristotle (384–322 BC), and then Pliny (23–79 AD), first described hibernation for mammals, and also erroneously for a variety of birds that disappeared seasonally (they actually migrated elsewhere during winter). More rigorous scientific observation in the 1500s (Gesser) and 1700s (Buffon) confirmed that many mammals hibernate. The invention of glass lenses and the microscope in the late 1500s/early 1600s allowed observations of various small microorganisms and animals, including seemingly lifeless invertebrates (rotifers, tardigrades, etc) that could be rehydrated and would then recommence activity. Such observations led to claims in the 1700s and 1800s of resurrection of dead animals, and spontaneous generation of life, but careful scientific study dispelled the claims of resurrection of life from dead organisms (Commission of the Biological Society of France in 1860) and spontaneous generation of life (Pasteur in 1864). Many subsequent physiological studies in the 1900s and 2000s have confirmed profound behavioural and physiological inactivity in rotifers, tardigrades, and nematodes, as well as larger animals, bacteria, fungi, and plant seeds (Keilin 1959).

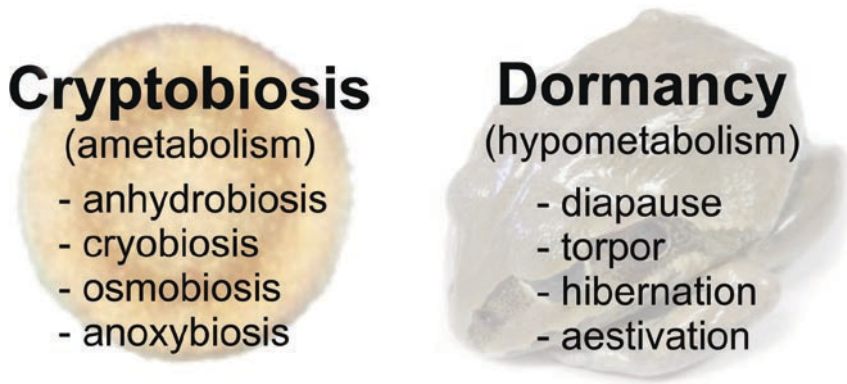


Fig. 1.1 Schematic summary of types of cryptobiosis, which is an ametabolic state (e.g., an anhydrobiotic *Artemia* cyst), and dormancy, which is a hypometabolic state (e.g., an aestivating *Cyclorana* frog). Terminology adapted from Keilin (1959)

A confusing array of different terms has been used to describe these periods of inactivity, depending on the group of animals in question, the extent and duration of the metabolic depression, the body and ambient temperatures (low or high), the physical state of body water (frozen or hyperosmotic), and the availability of oxygen. Two different states of inactivity and metabolic depression can be discerned, cryptobiosis and dormancy (Keilin 1959; Fig. 1.1). Cryptobiosis, which means “hidden life,” is an extreme behavioral and physiological state, with no activity and essentially no metabolism. Dormancy describes a state of reduced (but not complete lack of) metabolism, i.e., hypometabolism. Thus, there would appear to be a continuum in the potential metabolic state of organisms, from complete absence of metabolism, through hypometabolism, to the normal resting metabolic rate.

1.2 Cryptobiosis

The study of cryptobiosis (or abiosis) started with observations by van Leeuwenhoek (1702) of certain “animalcules” (wheel animals, rotifers) that he found in dry sediment in roof gutters, in an apparently lifeless state but which resumed normal activity when rehydrated (Schmidt 1948; Keilin 1959; Tunnacliffe and Lapinski 2003). Cryptobiosis is a state of “suspended animation” associated with complete inactivity and metabolic depression; it is most commonly observed for invertebrate animals (Keilin 1959) and is often a strategy to survive seasonal cold or desiccation. The similar term anabiosis, or “return to life” (Preyer 1891), describes the resurrection of apparently completely lifeless organisms. Van Leeuwenhoek did not, however, suggest that these “lifeless” animals were completely desiccated, nor did he describe this state in terms of latent life or resurrection. His observations were followed about a half century later by descriptions of microscopic nematodes (eelworms,

Anguillulina tritici) in an apparently lifeless state; these “lifeless” white fibres in grain quickly resumed activity when rehydrated, although in their desiccated state they crumbled to powder if disturbed (Needham 1743; Baker 1753).

The extremely depressed metabolic state of cryptobiosis is often related to a dramatic change in the state of cell water through desiccation (anhydrobiosis), osmotic stress (osmobiosis), or freezing (cryobiosis), although metabolic depression due to a lack of oxygen (anoxybiosis) occurs under conditions of normal cell-water. Cryptobiosis has independently evolved several times, within bacteria and protists as well as many multicellular plants (mosses, lichens, liverworts, higher plants) and animals (nematodes, rotifers, tardigrades, crustaceans, insects; Clegg 2001; Rebecchi et al. 2007). A few remarkable organisms are able to survive all of these forms of cryptobiosis, e.g., diapaused cysts of brine shrimp (e.g., Clegg et al. 1996; Clegg 1997) and tardigrades (Nelson 2002).

Anhydrobiosis is a response to desiccation (loss of cell water). It has been observed for a variety of invertebrate animals and plants during extreme desiccating conditions (Van Leeuwenhoek 1702; Spallanzani 1776; see Keilin 1959; Rebecchi et al. 2007), but not vertebrates. Spallanzani (1776) described “resurrection” by cryptobiotic rotifers and tardigrades, which had a remarkable tolerance of high temperatures and could survive in a vacuum. The French biologists Doyère and Porchet resumed studies in the 1850s of resurrection by desiccated rotifers and tardigrades, but came to the different conclusions that these microorganisms could survive complete desiccation and cessation of life processes (Doyère) or that no organism can survive complete desiccation and be resurrected after the cessation of life processes (Porchet). A Commission of the Biological Society of France examined these counter-claims and concluded that the resistance of rotifers and tardigrades to high temperatures increased with the extent of desiccation – rotifers could be resurrected after 82 days of desiccation in a vacuum followed by exposure to 100°C for 30 min, before rehydration (Keilin 1959). Gavaret (1859) extended these observations of extreme tolerance to 50 days in a vacuum over sulphuric acid, then heating to 110°C. The best known example of cryptobiotic animals is probably the desiccated “eggs” (actually cysts of about 3,000 cells in an arrested stage of early development) of brine shrimp (*Artemia*) which can survive extended periods of complete desiccation and are also remarkably resistant to extremes of temperature. Anhydrobiotic organisms can be remarkably long-lived (e.g., 30 years for a nematode (*Tylenchis polyhynchus*; Steiner and Albin 1946), and tolerant of physical and chemical extremes e.g., very low temperatures (<-200°C) and high temperatures (>100°C) and hydrostatic pressure (>500 MPa), and irradiation (see Rebecchi et al. 2007).

Various physiological and biochemical aspects of anhydrobiosis have been reviewed by Womersley (1981) and more recently by Tunnacliffe and Lapinski (2003), Watanabe (2006) and Rebecchi et al. (2007). Many anhydrobiotic organisms must control their evaporative water loss during entry into anhydrobiosis using behavioural, physiological, or morphological adaptations. For example, tardigrades contract into a “tun” when they dehydrate, rotifers have a similar dehydrating behavior, and nematodes coil or aggregate to reduce the rate of water loss. Compatible solutes, such as trehalose, sucrose, or glycerol, are generally accumulated during anhydrobiosis.

These solutes presumably structure cell water and protect against intracellular damage, as well as retarding molecular motion for chemical reactions. Some rotifers, plants, and microorganisms, however, are desiccation tolerant in the absence of accumulated protectant solutes, so further studies are required to determine their molecular mechanisms for anhydrobiosis (Tunnacliffe and Lapinski 2003).

During anhydrobiosis, the metabolic rate is often so low as to be unmeasurable. The ametabolic state and resistance to thermal extremes can be attributed to the altered physical state of cell water. Desiccated *Artemia* cysts have a cell water content less than 0.15 ml g⁻¹ of dry mass, and the remaining water is tightly bound to cell proteins (Clegg 1975). The lack of cell water as a solvent in these cysts presumably precludes molecular movement, and hence cell metabolism. In contrast, at a water content of 0.15–0.6 ml g⁻¹ of dry mass, there is not only bound water but also water loosely associated with intracellular structures; this vicinal water provides limited hydration and mobility of cell metabolites, and, hence, supports limited metabolism. At higher cell water contents, the presence of bulk water allows free solute movement and maintenance of the normal metabolic rate. For example, the metabolic rate of prediapause cysts (approx. 4 μg dry mass) of 0.036 μl O₂ h⁻¹ is reduced to approximately 0.007 μl O₂ h⁻¹, then is further reduced for dormant encysted embryos to 0.0001 μl O₂ h⁻¹ or even less by 5 days after deposition (Clegg 1976, 1978, 1997; Glasheen and Hand 1989; Clegg et al. 1996).

Some plant seeds, particularly of annual species, are also spectacularly resistant to desiccation; they may survive with as little as 5–10% water content and remain viable despite desiccation for very long periods, e.g., 50–200 years (Becquerel 1907; Ewart 1908; Turner 1933). Indian Lotus seeds, some perhaps more than 1,000 years old retrieved from an ancient lake bed in China, can still germinate (Ohga 1923; Keilin 1959). Many desert plants survive extended periods of high temperature and low rainfall. These “resurrection” plants, such as the Rose of Jericho (*Selaginella* spp) and pincushion lilies (*Boryia* spp) can desiccate to about 5% water content during dry periods, but survive and “come back to life” after rain.

Two other forms of cryptobiosis that also involve an altered state of cell water are freezing (cryobiosis, e.g., frozen insects and frogs) and high osmotic concentration (osmobiosis, e.g., brine shrimp cysts in a salt lake). Both involve perturbation of the physical state of water in the intracellular environment, which presumably results in the extreme metabolic depression. Both freezing and high osmotic concentrations require special adaptations for survival.

Many animals (and plants) can survive freezing temperatures (see Storey and Storey 1996). Anecdotal reports of animals surviving freezing date back to Pliny (frozen fishes; see Claussen et al. 1990). Some organisms supercool or have antifreeze solutes to avoid freezing. A super-cooled state would be associated with a substantial reduction in metabolic rate due to the Q₁₀ effect. However, some remarkable animals and plants tolerate actual freezing of their extracellular fluids. Henry Power, in 1663, observed that vinegar eelworms (probably the nematode *Turbatrix aceti*) survived freezing (Wharton 2002) while Réaumur (1737) first reported freeze-tolerance for an insect. The first convincing report of freeze-tolerance for a vertebrate was the European wall lizard *Podarcis muralis* (Weigmann 1929;

Claussen et al. 1990). Insects (e.g., Arctic woolly bear caterpillars, *Gynaephora groenlandica*) and the New Zealand alpine weta (*Hemideina maori*) survive freezing for up to 10 months of the year (Ring 1981; Duman et al. 1991; Zachariassen 1985). Intertidal invertebrates such as gastropods, mussels, and barnacles routinely freeze when exposed at low tide (Aarset 1982; Loomis 1987). Some nematodes, slugs, and centipedes also tolerate freezing. Amongst vertebrates, some amphibians (*Rana*, *Pseudacris*, *Hyla* spp) and reptiles (turtles, lizards and snakes) can survive freezing (Schmid 1982; Costanzo et al. 1988; Storey et al. 1988; Claussen et al. 1990; Churchill and Storey 1992; Costanzo et al. 1985; Dinkelacker et al. 2005).

Freeze-tolerant animals can only withstand freezing of their extracellular fluids as any ice formed within cells disrupts cell membranes and destroys intracellular integrity (Storey and Storey 1996). As ice forms, solutes are excluded from the ice-crystal structure and this increases the osmotic concentration of the unfrozen extracellular fluids, thereby lowering its freezing point. Intracellular fluid remains in osmotic equilibrium with the extracellular fluid, which consequently also becomes osmo-concentrated. Some freeze-tolerant animals have specific ice-nucleating agents in their extracellular fluids, to promote freezing there rather than inside cells. Extracellular freezing is promoted by ice-nucleating proteins in the tardigrade *Adorybiotus coronifer* (Wright 2001). The increased osmotic concentration of the extracellular fluids withdraws water from inside cells by osmosis, lowering its freezing point but also decreasing the cell volume. For most freeze-tolerant animals, the critical minimum cell volume is when about 65% of the total body water is frozen. Compatible cryoprotectant solutes can be used as “antifreeze” to decrease the freezing point of the body fluids and to facilitate tolerance of tissue freezing by preserving macromolecular structures within the cells (whereas high concentrations of many solutes would perturb the structure and function of the intracellular macromolecules). For example, the spring peeper frog (*Pseudacris crucifer*; Churchill and Storey 1996) and wood frog (*Rana sylvatica*; Storey and Storey 1988) release glucose from their liver in response to ice forming on its skin; the 450 times increase in blood glucose provides a compatible cryoprotectant function (it also provides locally available nutrients during thawing before the circulatory system becomes functional again). Some *Hyla* accumulate glycerol, as do many insects. Insects can also accumulate other compatible solutes such as polyols (mannitol, sorbitol) and sugars (e.g., trehalose; Storey and Storey 1989, 1990, 1991). There is likely a role of intracellular cryoprotectants (but not trehalose) in freeze-tolerance of the tardigrade *Adoribiotus coronifer*. Many plants also accumulate compatible solutes in their fluids to prevent freezing during winter, or tolerate freezing of water in their xylem and other extracellular water pools.

Metabolic rate is reduced for frozen insects and frogs; tissue ATP declines during freezing, and anaerobic end-products accumulate (Storey and Storey 1986, 1988). Freeze tolerance by hatchling turtles is associated with, but apparently is not limited by, the anoxic tolerance of their tissues (Dinkelacker et al. 2005). These freeze-tolerant animals have essentially complete metabolic depression, not just because of the low ambient temperature and its depressing Q_{10} effect, but also because of the impairment of circulatory exchange of O_2 and CO_2 as well as other nutrients and waste products, and the hyperosmotic state of the intracellular fluids.

Osmobiosis requires that animals are tolerant of a high ambient osmotic concentration (Keilin 1959). For example, the metabolic rate of hydrated *Artemia* cysts during osmobiosis (exposed to 5 mol l⁻¹ NaCl) is only 0.00009 $\mu\text{l O}_2 \text{ h}^{-1}$ (Glasheen and Hand 1989). The obligate parasitic nematode *Steinernema carpocapsae* has similar tolerance of osmotic stress, at least for nonionic solutes such as glycerol and polyethylene glycol (Glazer and Salame 2000). Soil nematodes tolerate osmotic concentrations of up to 1 M (e.g., Van Gundy 1965). Many freshwater and terrestrial tardigrades form a resistant tun when exposed to concentrated salt solutions (Wright et al. 1992). The hyperosmotic tolerance of osmobiotic animals is similar to that of cryobiotic animals, and extracellular and intracellular compatible cryoprotectants are also important in osmobiosis.

Anoxybiosis (Keilin 1959) involves survival of a lack of oxygen, but at a normal hydration level (in contrast to the other forms of cryptobiosis). It occurs in some invertebrate and vertebrate animals. For example, cysts of brine shrimp (*Artemia*) can survive extended periods of anoxia. During anoxybiosis, the metabolic rate of hydrated *Artemia* cysts is so low as to be unmeasurable, but it returns to prediapause levels (approximately 0.007 $\mu\text{l O}_2 \text{ h}^{-1}$) when the cyst is in oxygenated water (Clegg 1976, 1997; Glasheen and Hand 1989; Clegg et al. 1996). Anoxybiosis is rare amongst vertebrate animals. Hatchling turtles survive in nitrogen at 4°C for 17 (*Malaclemys terrapin*) to 50 days (*Graptemys geographica*; Dinkelacker et al. 2005). Some adult turtles are extremely tolerant of anoxia, especially at reduced temperatures, e.g., painted turtles *Chrysemys picta* survive up to 5 months of anoxia at 3°C (Jackson 1968, 2000); they have a coordinated reduction of both ATP-consuming and ATP-producing pathways involving various metabolic adaptations (Storey 1996; Hochachka and Lutz 2001).

All of these forms of cryptobiosis involve complete inactivity and a very low rate of metabolism that reflects extreme changes to the physical state of water in cells, or lack of oxygen. The ratio of depressed metabolic rate to normal resting metabolic rate (D/R) is less than one-twentieth, approaching zero for some species (Fig. 1.2; see Guppy and Withers 1999). Osmobiotic *Artemia* cysts have a low D/R, of about 0.0028, which is similar to hydrated diapaused cysts (0.0031). Hydrated prediapause cysts have a D/R of about 0.18, which is similar to that of other dormant animals (see below). Anoxic or anhydrobiotic *Artemia* cysts have an extremely low D/R, and anhydrobiotic nematodes and tardigrades presumably also have an unmeasurably low D/R. Not unexpectedly, those forms of cryptobiosis that are associated with marked perturbation of the state of intracellular water (anhydrobiosis, osmobiosis, cryobiosis) have the most extreme metabolic depression, i.e., to less than one-thousandth of normal metabolic rate. The mechanisms whereby these animals and plants survive extreme conditions at very low metabolic rates would seem to be more related to molecular tolerance of the compromised state of their cell water than molecular or physiological adaptations of the cascade of metabolic enzymes in normally hydrated tissues. In contrast, anoxybiosis is especially intriguing because it does not involve an altered state of intracellular water, but the metabolic depression is similarly extreme (like anhydro-, cryo-, and osmo-biosis) rather than moderate (as for dormancy).

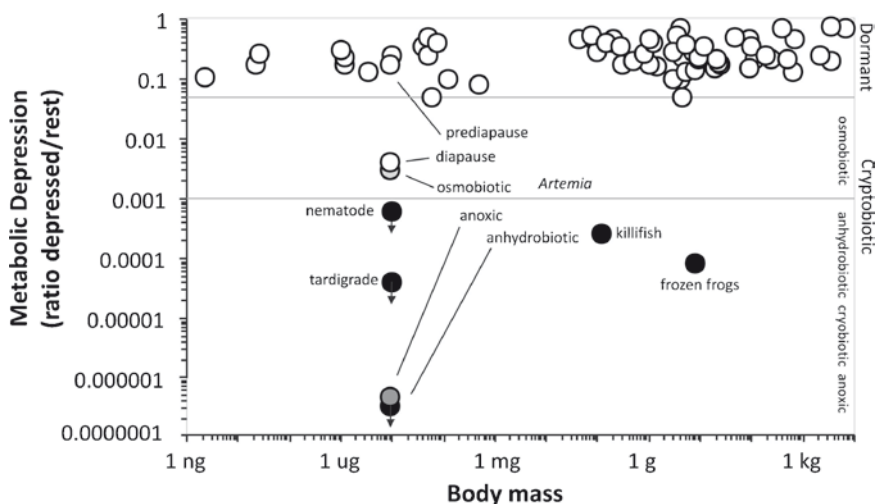


Fig. 1.2 Summary of the extent of metabolic depression (ratio of depressed to normal resting metabolic rate) for protists and animals when dormant (open symbols) or cryptobiotic (osmobiotic, light grey; anoxic, medium grey; anhydrobiotic, solid symbols); arrows indicate that lower ratios would be measured for animals in complete anhydrobiosis. Data from Guppy and Withers (1999)

1.3 Dormancy

Dormancy is a widely used term meaning inactivity or lethargy. It includes a variety of behavioural and physiological states involving inactivity and depressed metabolism. During dormancy, activity is minimal, although animals can move and respond to stimuli, and metabolic rate is reduced to a low level (hypometabolism). Dormancy can be a response to a circannual rhythm, or a variety of environmental factors, including temperature or the availability of food or water. It can be a short-term event (<24 h) or it can occur for a few consecutive days, an entire season, or even many years. Dormancy can also involve seasonal arrest (diapause) or opportunistic inactivity (quiescence) of a developmental stage.

Unlike cryptobiosis, dormancy does not involve marked changes in the physiological state of the animal (e.g., osmotic or hydration state). While cryptobiosis is a reactive response to an environmental stress (e.g., freezing at low ambient temperature; desiccation at low ambient humidity; hypoxia at low ambient pO_2), dormancy is preemptive and anticipates stressful physiological conditions to avoid dramatic changes in the internal environment. Dormant animals are inactive but can usually be aroused by disturbance without requiring any major change in their physiological state (e.g., level of hydration or oxygen level). Mechanisms for dormancy are more likely to be intrinsic metabolic controls rather than molecular adaptations to an altered state of intracellular water or oxygen levels. There are many different forms of dormancy, including developmental diapause and adult torpor.

Diapause involves the cessation of development of a subadult life stage, often for avoidance of harsh environmental conditions. It is often a programmed and obligate part of the life cycle, in response to regular and predictable environmental extremes, and synchronises the next stages of the life cycle with appropriate environmental conditions. Diapause is especially common in insects but is also observed in a variety of other invertebrate animals, as well as many plants (e.g., buds, bulbs, rhizomes, and seeds), but is rare amongst vertebrate animals. The embryonic diapause of a variety of mammals (e.g., macropod marsupials, mustelids, deer) is a reproductive strategy for delayed implantation and development of embryos rather than a strategy for metabolic depression, but presumably the diapaused embryo metabolically depresses during this period of arrested development. Diapaused eggs of annual killifish (*Astrofundulus limnaeus*) form a vitreous-like egg envelope and have a high resistance to desiccation; the embryonic tissues do not become dehydrated during diapause (Podrabsky et al. 2001). Quiescence is similar to diapause, but is a period of facultative inactivity in response to an immediate change in environmental conditions rather than a programmed and obligate response. It is terminated by the return of more favorable environmental conditions. It may be a response to harsh environmental conditions such as low or high temperature, or drought. Many invertebrates and plants (particularly seeds) become quiescent.

Torpor is inactivity and a reduction in metabolic rate below the normal resting value, often in response to extreme ambient conditions. It characteristically occurs in adults and is usually a response to daily or seasonal environmental changes (hibernation in winter or aestivation in summer). Torpor by endothermic mammals and birds involves daily or seasonal changes in their thermoregulation setpoint and use of thermogenic metabolism so it is physiologically different from ectothermic torpor, which can occur at constant ambient and body temperatures (see below).

1.4 Ectothermic Animals

Torpor by ectothermic animals is typically a seasonal or longer-term dormancy in response to environmental extremes (low temperatures and scarcity of food in winter, or high temperatures and scarcity of water in summer). Dormant animals and plants can survive long periods, e.g., over 23 years for a pulmonate snail *Oxystyla pulchella* (Baker 1934) and about 5 years for the Australian water-holding frog *Cyclorana platycephala* (Van Beurden 1980). Most dormant animals maintain normal hydration, but the earthworm *Allolobophora chlorotica* and the leech *Placobdella parasitica* can survive up to 70% loss of body water (Keilen 1959).

Early natural history observations suggested that many animals enter dormancy during unfavorable periods, and return to activity months or years later. Spencer (1896) observed aestivation of some Australian desert amphibians and Smith (1930) pioneered studies of aestivating lungfish (*Protopterus aethiopicus*). Some lizards had been inferred to hibernate over winter (e.g., Mayhew 1965) and various crocodylians

had been suggested to aestivate (Taplin 1988). Dormancy by reptiles was first considered to be more of a behavioural and thermal inactivation in comparison to the marked physiological changes that accompany dormancy in endotherms (see below). Mayhew (1965) coined the term brumation to describe hibernation and aestivation in ectothermic vertebrates, recognizing their possibly lesser physiological sequelae compared with dormant endotherms. However, it is now clear that there are physiological adjustments and intrinsic metabolic depression in many (but not all) dormant reptiles.

Physiological confirmation of metabolic depression was generally not achieved for dormant ectothermic animals until technical advances of respirometric techniques in the late 1900s. These pioneering studies of metabolic depression in aestivating invertebrates (e.g., Rakshpal 1962; Keister and Buck 1964; Coles 1968; MacMillen and Greenaway 1978; Abe and Buck 1985) and vertebrates (Moberley 1963; Seymour 1973; Delaney et al. 1974), and subsequent studies (see Guppy and Withers 1999), indicate that the extent of metabolic depression is to as little as one-fifth of the resting metabolic rate (Fig. 1.2; similar to hydrated diapaused *Artemia* cysts). The onset of inactivity can be quite immediate in response to impending environmental stress (e.g., aestivating frogs quickly seek refuge and become inactive in dry conditions), but the onset of metabolic depression requires days to weeks (e.g., Seymour 1973; Etheridge 1990; McClanahan et al. 1983; Withers 1993; Pedler et al. 1996). Consequently, daily cycles of metabolic depression are impractical for ectotherms, although temperature-induced daily torpor is energetically advantageous.

Daily thermal torpor has important metabolic savings for ectotherms since their body temperature (T_b) is essentially equal to ambient temperature (T_a), particularly in the absence of solar radiation for behavioural thermoregulation. This means that any decrease in T_a hence T_b induces an exponential decline in metabolic rate (MR) as described by the Q_{10} relationship (e.g., Withers 1992)

$$MR_{T_{b_2}} = MR_{T_{b_1}} Q_{10}^{(T_{b_2} - T_{b_1})/10}$$

where $MR_{T_{b_1}}$ is metabolic rate at T_{b_1} and $MR_{T_{b_2}}$ is the metabolic rate at T_{b_2} . For most physiological variables, Q_{10} is generally about 2.5 (varying from about 2 at high temperatures to 3–4 at low temperatures; Withers 1992; Guppy and Withers 1999). T_a -induced decreases in metabolic rate can thus provide substantial energy savings for an ectotherm.

Hibernation (winter dormancy) is long-term multiday torpor in response to cold and scarcity of food. The above-ground structures of plants may die during unfavorable winter conditions, but some develop protective scales around the stem tips so that the buds survive. Some plants have dormant underground bulbs, rhizomes, tubers, or corms, which are buffered from environmental extremes by the soil. For ectothermic animals, hibernation is primarily an inactive state accompanying a low T_b , with many physiological sequelae including a lowered metabolic rate. Metabolic rate is lowered during hibernation by the thermal Q_{10} effect, but some hibernating ectotherms also have an additional intrinsic metabolic depression. For example, the metabolic savings accruing to a hibernating frog from decreased

temperature of 22–2°C due to a Q_{10} effect is considerable (e.g., about 90% of the 22°C rate). This hibernation metabolic reduction from decreased T_a and a Q_{10} effect results in about 100 days survival time for even a 1-g frog, >200 days for a 10-g frog, and even longer survival times for larger frogs (Hillman et al. 2008). These periods are more than adequate for survival of a cold winter period (e.g., 3 months), so further energy reduction in energy consumption by intrinsic metabolic depression would seem unnecessary (and would result in relatively small absolute energy savings). Nevertheless, the decrease in metabolic demand for O_2 during hibernation is often augmented by further metabolic depression beyond the Q_{10} effect. For example, the metabolic rate of *Rana temporaria* declined by 40% after 3 months under water compared with prior to submersion at 3°C, and this intrinsic hypometabolism was intensified by a 75% reduction from standard metabolic rate if frogs were submerged in water made progressively hypoxic (Donohoe and Boutilier 1998; Donohoe et al. 1998). These 40 and 75% reductions in aerobic metabolism further increase survival time by 2–4 fold. Reptiles can also have an intrinsic metabolic depression during hibernation, independent of body temperature change, e.g., the horned lizard (*Phrynosoma m'calli*; Mayhew 1965) and the desert iguana (*Dipsosaurus dorsalis*; Moberley 1963).

Aestivation (summer dormancy) is long-term multiday torpor in response to hot and dry periods. It usually involves an inactive state often with a water-resistant covering since desiccation is usually a danger for aestivating animals. For example, aestivating earthworms form a mucous cocoon to resist desiccation (Laverack 1963; Righi 1972), and many insect pupae are remarkably resistant to water loss (Keister and Buck 1964; Lees 1955, 1956). Amongst vertebrates, fishes, amphibians, and reptiles enter a similar aestivation state. Eggs of annual killifish (*A. limnaeus*) enter diapause when their ephemeral ponds dry over summer; the eggs develop a vitreous-like egg envelope and have a high resistance to desiccation, so that the embryonic tissues do not become dehydrated during diapause (Podrabsky et al. 2001). Some adult fishes and amphibians aestivate (Richards, Chapter 6, this volume; Carvalho et al., Chapter 7, this volume), often in a protective cocoon. Aestivating fishes form a cocoon of dried mucus (e.g., lungfishes, Delaney et al. 1974; salamander fish, Pusey 1990) whereas many anuran amphibians and a few salamanders form a cocoon of shed skin (first described by Lee and Mercer 1967), to reduce cutaneous water loss; the cocoon covers the entire body surface except for the nostrils (e.g., Fig. 1.3). Some reptiles also aestivate (e.g., Gregory 1982; Abe 1995), but they do not need to form a cocoon to reduce evaporative water loss as their epidermis is already relatively water-impermeable.

There is an unequivocal metabolic depression for some ectotherms (e.g., snails, fishes, amphibians, and reptiles) to about 0.2 (20%) of normal metabolic rate during aestivation or hibernation. The earthworm *Glossoscolex paulistus* has a metabolic depression of about 22% of normal metabolic rate at 15°C, but metabolic depression is only about 64% of normal metabolic rate at 35°C (Abe and Buck 1985). The “side-walker” malacostracan land crab (*Holthuisana transversa*) has a metabolic depression of about 28% (MacMillen and Greenaway 1978). Metabolic depression by insects (eggs, pupae, and adults) varies from about 10 to 60% of normal metabolic rate (see Guppy and Withers 1999). Diapaused eggs of annual killifish (*A. limnaeus*) have a low aerobic metabolic rate (10% of normal) with a minor



Fig. 1.3 Aestivating hydrid frog (*Cyclorana australis*) with a thin cocoon of shed skin that reduces evaporative water loss

anaerobic contribution (Podrabsky and Hand 1999). Fish and amphibians generally have a metabolic depression of about 20% of normal metabolic rate (e.g., McClanahan 1967; Seymour 1973; Delaney et al. 1974; van Beurden 1980; Withers 1993). Evidence for metabolic depression is more equivocal for reptiles, in part, because of their less extreme changes in morphology and behaviour, but it is often about 20 to 50% of normal metabolic rate (e.g., Seidel 1978; Kennett and Christian 1994; Hailey and Loveridge 1997; Abe 1995). Moberley (1963) measured an intrinsic metabolic reduction of up to 50% of normal metabolic rate for hibernating desert iguanas (*Dipsosaurus dorsalis*). Metabolic depression to 20–50% of normal metabolic rate has been recorded for some reptiles in the field (e.g., Nagy and Medica 1986; Nagy and Shoemaker 1975; Christian et al. 1995), but these measurements would include behavioural and thermal effects as well as intrinsic depression. Christian et al. (1996) suggested that the slight decrease in metabolism of aestivating fresh-water crocodiles (*Crocodylus johnstoni*) reflected a starvation effect rather than an intrinsic metabolic depression.

Intrinsic metabolic depression in these ectotherms is independent of any obvious change in T_b or perturbation of internal homeostasis. It is essentially a preemptive metabolic depression in response to the impending environmental stress rather than being a consequence of a change in the temperature, ionic, osmotic, or any other discernable physiological aspect of internal environment. The molecular and biochemical mechanisms for this intrinsic metabolic depression are not well understood (e.g., Storey 2001; Storey and Storey, Chapter 2, this volume), although its physiological significance is a considerable extension of the hibernation/aestivation period that can be survived by conserving endogenous energy and/or water stores.

1.5 Endothermic Animals

Endothermic animals are able to maintain a constant T_b over a relatively wide range of T_a s by thermoregulation achieved through proportional modulation of endogenous metabolic heat production. As such, endotherms can remain active over a wide T_a range. However, maintenance of a high and stable T_b is energetically expensive, especially, for small species with a high surface area to volume ratio, and when there is a large thermal gradient between the animal and its environment. Availability of food (or water) may, therefore, place limitations on the animals' ability to effectively thermoregulate. Thus, many endothermic species are heterothermic, and use torpor (Fig. 1.4) to reduce their energy requirements when environmental conditions are extreme or food and/or water are not available (Geiser, Chapter 5, this volume).

The characteristics used to define torpor for endotherms vary, resulting from attempts to define a phenomenon which may be more correctly viewed as a continuum of metabolic and thermoregulatory states including normothermia, facultative hypothermia, and torpor. Often, torpor is defined as a reduction in T_b to below some arbitrary level, most often to <30 or $<31^\circ\text{C}$ but sometimes $26\text{--}36^\circ\text{C}$ (Hudson 1978; Barclay et al. 2001). However, an arbitrary T_b definition does not consider variation in normothermic body temperature between taxa, i.e., a bird with a normothermic T_b of 42°C requires a 12°C drop in T_b to be considered torpid, defined as $T_b < 30^\circ\text{C}$, but a marsupial with a normothermic T_b of 34°C only requires a 4°C drop in T_b . Some investigators use the more stringent definition of torpor

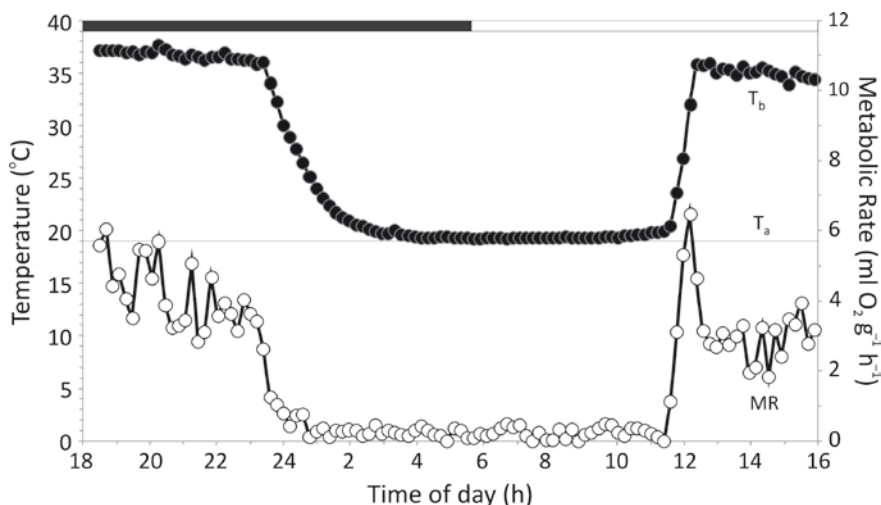


Fig. 1.4 Metabolic rate (MR) and body temperature (T_b) during a torpor cycle of a stripe-faced dunnart (*Sminthopsis macroura*) at an ambient temperature (T_a) of 18.5°C . Figure courtesy of Fritz Geiser (Zoology, University of New England)

that T_b approximates T_a , but many torpid endotherms defend a substantial T_b/T_a differential at low T_a , and at high T_a their T_b may approximate T_a even though the animals clearly are not torpid. Better definitions of torpor use a relative measure; for example, a 5°C reduction in T_b below normothermic values (e.g., Cooper and Geiser 2008) or a reduction in metabolic rate below that of basal metabolic rate (e.g., Geiser et al. 1996). Torpor may be distinguished from pathological hypothermia by the ability to arouse from torpor to normothermia using endogenous heat production.

For normothermic endotherms, metabolic rate increases as T_a decreases below the thermoneutral zone, to balance the heat lost to the environment with metabolic heat production, thus maintaining T_b constant at a species' specific set-point. Historically, heterothermic endotherms were believed to be poor thermoregulators, with torpor resulting from an inability to maintain thermal homeostasis and keep T_b at the set point when exposed to low T_a s (Lyman 1978). However, by the 1970s, it was generally accepted that torpor is a controlled physiological process resulting from a readjustment of the animal's T_b set-point to a new lower level (Mrosovsky 1990). Early evidence to support this came from studies indicating that there is often a $T_b - T_a$ differential of several degrees during torpor, vasomotor activity, and muscle tone continue to have a regulatory function during torpor, and drugs known to inhibit thermoregulation in normothermic animals also reduce the $T_a - T_b$ differential in torpid animals (Mrosovsky 1971, 1990). Additionally, thermoregulatory responses to artificial hypothalamic cooling or warming (Hartner et al. 1971; Mills and South 1972), and evidence of proportional control of metabolic heat production below species-specific T_b set-points by torpid animals (e.g., Heller and Colliver 1974; Florant and Heller 1977) clearly indicated that torpor was a precisely controlled state, rather than an inability to thermoregulate. During torpor, an animal does not thermoregulate if T_a is above the torpor T_b set-point. However, if T_a falls below the torpor T_b set-point, then T_b is regulated at that setpoint by thermogenesis in the same manner as for the normothermic thermoregulatory response.

From the early 1800s, torpor was considered to be an extension of sleep (Rasmussen 1916). Subsequent experiments have indicated that entry into torpor occurs during periods of short-wave sleep (SWS), as first described by South et al. (1969). It is during SWS that an animal actively thermoregulates; thermoregulation is temporarily abandoned during periods of rapid eye movement (REM) sleep. The proportion of time spent in REM sleep decreases as the depth of torpor increases, with animals spending the majority of time in SWS during shallow torpor, and all of the time during deep torpor (Heller et al. 1978). This provides further evidence that torpor is a tightly regulated state.

The terminology used to describe patterns of heterothermy displayed by endotherms is historically variable. The term "deep hibernation" was first used by Lyman (1948) to describe the pattern of deep torpor demonstrated by the golden hamster (*Mesocricetus auratus*), whereas Hudson (1978) used the term "shallow daily torpor" to describe torpor lasting only a few hours at a relatively high T_b as occurs in dasyurid marsupials and many small rodents. Today, torpor is generally

accepted to be of two main types, multiday deep torpor or shallow daily torpor (Geiser and Ruf 1995; Geiser 2004a). Species undergoing multiday torpor may do so for several days to weeks or months. Body temperatures are usually less than 10°C, commonly between 0 and 5°C, and metabolic rates during torpor are reduced to approximately 5% of basal metabolic rate (Geiser 2004a). Species that undergo shallow daily torpor do so for periods less than 24 h and minimal body temperatures are usually >11°C. Metabolic rate during torpor usually drops to around 30% of basal. There is an overall negative linear relationship between torpor duration and minimal T_b (Geiser 1994). Hibernation is the commonly accepted term for torpor occurring in response to winter conditions (e.g., marmots, *Marmota flaviventris*; Davis 1976; Anonymous 2003), while the term aestivation applies to summer torpor (e.g., cactus mouse, *Peromyscus eremicus*, MacMillen 1965; Anonymous 2003).

Observations of torpor by mammals date back to Aristotle and Pliny, but Gessner in the 1550s and Buffon in the 1750s began the early experimental work (Rasmussen 1916; Kayser 1961). From that period to the present, a vast literature has been amassed on the subject. Much of this information is for rodents due to the prevalence of heterothermy in this group and their abundance in the temperate regions of the northern hemisphere (Kayser 1961). In particular, much knowledge has been gleaned from studies of the families Gliridae (Aristotle first wrote of hibernation for *Glis glis*), Sciruidae, and Cricetidae. Hibernation by the insectivores Tenrecidae (tenrecs) and Erinaceidae (hedgehogs) is well known and has been studied since the 1930s (Kayser 1961). Bats are famous for their propensity to enter torpor (Davis 1970). Early work on heterothermy by bats indicated that although small temperate microchiropterans regularly used both seasonal and daily torpor, large tropical megachiropterans were essentially homeothermic (Eisentraut 1934; Reeder 1949; Lyman 1970). More recently, this distinction has become blurred (Lyman 1970), and now strong phylogenetic signals in the pattern of torpor use have been identified amongst bats (Cooper and Geiser 2008). Although suspected since the early 1900s, Dausmann et al. (2004, 2005) provided the first physiological data on hibernation by a primate, the Madagascan fat-tailed dwarf lemur (*Cheirogaleus medius*), which also was the first tropical mammal shown to undergo multiday torpor. The monotreme *Tachyglossus aculeatus* has been known since the early 1900s to hibernate, and amongst marsupials torpor has been recognized since the mid 1950s. The work of Geiser and coworkers since the early 1980s has added substantially to our knowledge of heterothermy amongst marsupials.

Aestivation was described for mammals by MacMillen (1965) in his classical study of cactus mice (*P. eremicus*), which ceased activity during dry summer periods. Although torpor was historically associated with cold climates, it is now known to occur in species inhabiting a range of habitats including arid environments, e.g., marsupials, bats, and rodents (Geiser 2004b) and the tropics, e.g., primates (Dausmann et al. 2004, 2005) and marsupials (Cooper et al. 2009). Presumably, the energy and water savings associated with torpor are of adaptive advantage for surviving periods of energy and water shortage in a wide range of habitats (Geiser 2004a).

There is a strong phylogenetic pattern in the type of torpor used by marsupials and bats (Cooper and Geiser 2008). Didelphid, dasyurid, myrmecobiid, and petaurid marsupials, and small pteropodid bats use daily torpor, whereas burramyid, acrobatid and microbiotheriid marsupials, and vespertilionid bats undergo multiday torpor. The pattern of heterothermy for rodents is complex and shows no phylogenetic relationship in this speciose group, presumably being related more to environmental and biological factors than to phylogenetic history (Cooper and Geiser 2008).

Heterothermy is not as well studied in birds as in mammals. Anecdotal reports of torpid birds date back to the 1760s, but it was not until the 1950s that the use of torpor by birds was widely accepted (McAtee 1947; Lyman et al. 1982). Early reports of torpidity amongst birds referred to swifts, swallows, hummingbirds, ptarmigans, and parakeets (McAtee 1947). Hummingbirds have long been the classical avian heterotherms (e.g., Ruschi 1949), undergoing regular daily torpor which may be either spontaneous (occurring despite an abundance of food) or induced (occurring in response to limited food) to balance their considerable energy budgets during periods of nocturnal rest (Pearson 1960). Jaeger (1948, 1949) described avian hibernation in the poorwill (*Phaenoptilus nuttali*), and this species remains the only bird for which there is physiological evidence of multiday torpor. A number of birds use shallow daily torpor, and recent data suggest that torpor use may be more common amongst birds than first appreciated. Heterothermia has now been reported for the coraciiform, colliiform, apodiform, trochiliform, strigiform, columbiform, and passeriform families (McKechnie and Lovegrove 2002), although separating true torpor from the more commonly occurring nocturnal hypothermia is a definitional issue. It also appears that wild, free-living birds are more likely to undergo torpor bouts than birds studied in captivity (Cooper et al. 2008), contributing to the difficulties of describing avian torpor.

Torpor has important energetic consequences for endotherms, as it substantially reduces the energetic costs of endothermy at T_a s below the thermoneutral zone (Fig. 1.4). The thermoregulatory increment above basal metabolic rate is reduced if the animal defends a smaller thermal differential between T_a and T_b , or is eliminated if the animal simply thermoconforms (if $T_a > \text{torpor } T_b$ set point). The reduction in T_b resulting from the decrease in heat production leads to a further, exponential, decline in metabolic rate as a consequence of the Q_{10} effect. Some authors (e.g., Geiser 1988) have suggested that intrinsic metabolic depression may also play a role in reducing energy expenditure during torpor in species such as bats, based on reductions in metabolic rate further below basal values than expected by the Q_{10} effect (i.e., $Q_{10} > 3$). However, the contribution of any intrinsic metabolic depression to the overall energy savings during torpor would be so small compared with that of the reduction of a thermoregulatory increment and the Q_{10} effect that the adaptive value to the animal is likely to also be small (Withers 1992; Guppy and Withers 1999). The overall energy saving resulting from torpor depends on the length of the torpor bout and the depth of torpor. For example, the daily energy saving for a dunnart using shallow daily torpor is about 36% for 13 h of torpor but for a hibernating ground squirrel, is about 85% over 6 months.

Torpor may also play an important role in maintaining water balance. First noted for placental mammals and birds (e.g., Carpenter 1969; Lasiewski 1964; MacMillen 1965; Buffenstein 1985) and more recently for marsupials (Cooper et al. 2005; Cooper et al. 2009), a combination of a decrease in respiratory water loss and a drop in body temperature reduces total evaporative water loss to as little as 14–50% of normothermic values. Respiratory water loss is decreased by a reduction in respiratory minute volume (due to a reduced demand for gas exchange resulting from a lower metabolic rate) and a drop in T_b reduces water vapour pressure at the lungs and skin surface. Interestingly, relative water economy, or ratio between metabolic water production and evaporative water loss, becomes less favorable during torpor, presumably because the reduction in metabolic rate, and thus water production during torpor is greater than the reduction in evaporative water loss (Cooper et al. 2005, 2009).

Torpor is distinguished from pathological hypothermia by a torpid animal's ability to arouse to normothermic T_b using endogenous metabolic heat production. This thermogenesis may occur by shivering (unsynchronized contractions of skeletal muscle) or by nonshivering thermogenesis such as the metabolism of brown adipose tissue (BAT) as occurs in some placental mammals, or more general tissue thermogenesis. Arousal from torpor is energetically expensive, and the high cost of rewarming tissues is one reason that precludes animals with large body masses from using torpor. Recent evidence (Geiser et al. 2002) has indicated that passive rewarming, using external heat sources (e.g., sun basking) may dramatically reduce the energetic costs of arousal and enhance the overall energetic benefits of torpor.

For animals undergoing long-term hibernation, periodic arousals to normothermia during the hibernation period are common. Although the reason for these periods of arousal and subsequent reentry into torpor is not clear, it is apparent that there is some physiological “need” to periodically arouse, perhaps, to reestablish homeostatic conditions perturbed by the reduction of metabolic substrates or accumulation of metabolites, to overcome temperature-dependant effects on circadian cycle, neural sensitivity, or rapid eye movement sleep, or to regain free water lost by evaporation during hibernation (Thomas and Geiser 1997). Dausmann et al. (2004, 2005) found for hibernating mouse lemurs that periodic arousals to normothermia occurred in individuals with well insulated hibernacula, which remained at low T_a . However, for individuals with poorly insulated hibernaculae, which experienced large ambient temperature cycles, passive rewarming to a T_b of 30°C was sufficient to substitute for arousal and these animals remained torpid.

Torpor, hibernation, and aestivation have important ecological consequences as they allow endotherms to avoid unfavorable environmental conditions by undertaking a period of inactivity, during which they dramatically reduce their energy expenditure. By avoiding unfavorable conditions, species can inhabit environments that would otherwise be inhospitable on a daily, intermittent, or seasonal temporal scale. The dramatic energy reductions associated with periods of dormancy obviously extend the period of time during which animals can remain inactive, but may also be an important mechanism for species to balance their daily energy budgets, and spontaneous torpor is common in many species. Despite these advantages, there also appear to be significant costs associated with torpor. Many species can not use or

survive torpor, and species physiologically capable of torpor do not necessarily use it routinely. Many physiological processes (e.g., digestion, growth, muscle function) are optimal at a relatively high and narrow range of T_b s, in part due to the Q_{10} effect on chemical processes and because of optimal temperatures for enzyme-mediated biochemical processes. Thus, maintenance of thermal homeostasis has fundamental physiological advantages over heterothermy (but this occurs at the cost of high energy expenditure). Use of torpor is associated with some physiological risk, such as having insufficient energy reserves or being at too low a T_b for arousal, or thermal death (e.g., freezing). In addition to physiological costs, there are also potential ecological drawbacks of torpor, including vulnerability to predation, competition for resources from individuals remaining active, reduced or delayed reproduction or development, and lower rates of essential activities such as cell division and digestion. The periodic arousals characteristic of multiday hibernators indicates that there is a necessity for at least periodic periods of high T_b (e.g., fat-tailed lemurs; Dausmann et al. 2004, 2005), and the use of torpor is restricted to relatively short-term periods.

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