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Size- and temperature-independence of minimum life-supporting metabolic rates

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Summary

1. Mass-specific metabolic rates of 173 animal species under various conditions of prolonged food deprivation (aestivation, hibernation, sit-and-wait existence) and/or living at temperatures near the freezing point of water were analysed.

2. These minimum life-supporting metabolic rates are independent of body mass over a nearly 80-million-fold body mass range and independent of temperature over a range of –1·7 to 30 °C, with a mean value of 0·1 W kg^{-1} and 95% CI from 0·02 to 0·67 W kg^{-1}.

3. Additionally, 66 measurements of anoxic metabolic rates in 32 species capable of surviving at least 1 h of anoxia were analysed. While similarly mass-independent, anoxic metabolic rates are significantly more widely scattered (1200-fold 95% CI); they are on average one order of magnitude lower than during normoxia and depend on temperature with Q_{10} = 2·8.

4. Energy losses at the time of 50% mortality during anoxia are 30–300 times smaller than the energy losses tolerated by normoxic organisms in the various energy-saving regimes studied.

5. These principal differences form the basis for proposing two alternative strategies by which organisms survive environmental stress: the regime of *abandoned metabolic control* ('slow death'), when, as in anoxic obligate aerobes, measured rates of energy dissipation can predominantly reflect chaotic processes of tissue degradation rather than meaningful biochemical reactions; and the regime of *minimum metabolic control*, when biochemical order is sustained at the expense of ordered metabolic reactions. Death or survival in the regime of abandoned metabolic control is dictated by the amount of accumulated biochemical damage and not by the available energy resources, as it is in the regime of minimum metabolic control.

Key-words: Aestivation, anoxia, arthropods, ectotherms, endotherms, survival

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Introduction

According to the second law of thermodynamics, the highly organized non-equilibrium state of life is subject to spontaneous degradation. Living organisms must invest energy to cope with continually accumulating disorder: for example, to replace degrading enzyme macromolecules or to maintain ion gradients across membranes (Hand & Hardewig 1996). The necessity of preserving existing biochemical order dictates the minimum energy requirements of the organism.

Reduced metabolic rates are characteristic of organisms exposed to environmental stress. Two different strategies of coping with accumulating disorder under extreme conditions can be envisaged. The organism can modify its cellular structures to maximally protect them against degradation. Once this is done, it can switch off virtually all metabolic processes until the adverse environmental conditions pass. The drawback of this strategy, which can be called the strategy of *abandoned metabolic control*, is that, no matter how low, the rate of spontaneous degradation of cell structures is never zero. In the absence of metabolic control from the organism's side, its death will follow as soon as the amount of damage accumulated in its tissues passes the critical threshold.

Alternatively, the organism can attempt to survive environmental stress, such as prolonged food deprivation, by continually sustaining its order and, hence, maintaining a low but non-zero metabolic rate. In this strategy, which can be termed the strategy of *minimum*

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metabolic control, survival time will be determined by the amount of energy available for the organism. The word 'minimum' refers to the idea that, for the sake of energy conservation, the rate of controlled metabolism reaches its minimum possible value sufficient for the maintenance of biochemical homeostasis.

In other words, measurements of what is uniformly called 'metabolic rate' can either reflect organismal energy expenditure on the maintenance of order and on performing other meaningful functions (true metabolic rate), or they can represent the rate at which organismal cellular structures spontaneously degrade. Previously, this conceptual duality in the interpretation of measured rates of energy dissipation by living organisms has not been explicitly recognized.

In the regime of minimum metabolic control, when the organism continually repairs the breakdown of cellular structures at the expense of internal energy resources, it should be able to tolerate much larger energy losses per unit body mass than in the regime of abandoned metabolic control. This is because such energy losses are non-random and occur in tissues specifically designed for this purpose (e.g. lipid reserves in hibernating mammals) without threatening the integrity of the organism. In the case of abandoned metabolic control, when the measured energy loss is due to unordered, chaotic biochemical processes, death of the organism, caused by critical damage, can be unrelated to, and occur well before, the depletion of the internal energy resources.

In the present paper these ideas are explored quantitatively. We compare absolute values of mass-specific metabolic rates of metabolically depressed organisms across different taxa, body mass intervals and environmental conditions. Previous analyses have focused on particular taxa (Storey 1997; Withers, Pedler & Guppy 1998; Geiser 2004) and particular types of metabolic depression (Storey 1996, 2002; Geiser 2004), with interspecific comparisons predominantly made in terms of relative rather than absolute changes in metabolic rate, and on a whole-body rather than a mass-specific basis (Hand & Hardewig 1996; Guppy & Withers 1999).

Methods

In the search for minimum life-supporting metabolic rates we focused on two conditions: prolonged food deprivation and low ambient temperatures. Metabolic rates reported in terms of oxygen consumption were converted to energetic units assuming 20 J per ml O_2. Only those experimental temperatures that fell within the natural temperature range experienced by the species in the physiological state of interest were used. Where several literature sources for the same species were available, the one reporting the lowest value was taken. An annotated list of the data is presented in the Appendix, which can be downloaded from www.biotic-regulation.pl.ru/datasets.htm or obtained from the authors on request. However, an evaluation of the patterns that were found is more readily made with an understanding of the groups of organisms used, and their physiological states.

To facilitate analysis, the 173 species were divided into seven groups (Table 1). Among endothermic animals, the lowest metabolic rates are observed in hibernating species (Geiser 2004). Hibernation can last for several months, when the animals typically do not have access to energy resources other than those stored in their bodies (McNab 2002). Minimum metabolic rates reported for 42 hibernating species (41 mammals and 1 bird) by Geiser (2004) formed Group I 'hibernating endotherms' (Table 1).

Another group of animals known for their ability of surviving for months without food is represented by arthropod sit-and-wait strategists, ticks and scorpions. Lighton & Fielden (1995) measured standard metabolic rates of eight species of North American ticks (body mass 3·2–70·4 mg); Lighton *et al*. (2001) analysed standard metabolic rates of nine species of desert scorpions (0·12–15 g). In both cases, at 25 °C standard metabolic rates of these sit-and-wait strategists are an order of magnitude lower than those of other similar-sized arthropods. These data, together with one additional measurement of standard metabolic rate of the Antarctic Tick *Ixodes uriae* (7 mg, 5 °C) (Lee & Baust 1982), were included in the present analysis.

Prolonged starvation is also common in groundwater ecosystems, where autotrophic production is lacking and nutrient supply is highly sporadic. Metabolic rates of three subterranean aquatic crustaceans (12–93 mg, 11 °C) capable of surviving more than 1 year without food, and experimentally starved for 180 days (Hervant *et al*. 1997), were combined with the 18 tick and scorpion data points and 1 aestivating Australian arid-zone crab, *Holthuisana transversa* (~10 g, 25 °C) (MacMillen & Greenaway 1978), to form Group II called 'arthropod sit-and-wait strategists'. An inherent feature of this group is relatively high ambient temperature (mean 22 °C).

Polar aquatic arthropods not only have to exist at low temperatures, but also often experience seasonal food shortages caused by declining primary productivity during the months of prevailing darkness and extensive sea-ice cover. These species are therefore appropriate for studying minimum life-supporting energetic requirements. Marine copepods in both the Arctic and the Antarctic can survive winter food shortages by reducing their metabolic rates, with complete cessation of feeding and retreat to ocean depths in some species (Auel, Klages & Werner 2003; Ikeda, Sano & Yamaguchi 2004). We included metabolic rate data of three overwintering, metabolically depressed copepods from the Northern hemisphere, *Neocalanus cristatus* (Ikeda *et al*. 2004), *Calanus hyperboreus* (Auel *et al*. 2003) and *C. finmarchicus* (Ingvarsdóttir *et al*. 1999), and minimum metabolic rates recorded for seven species of postoverwintering Antarctic copepods

Table 1. Summary of minimum mass-specific metabolic rates investigated in this study. *n* number of species in the group (number of measurements in the anoxia group); *T* (°C) experimental temperature (body temperature for group I); *M* (g) body mass; *q* (W kg^{-1}) mass-specific metabolic rate (per unit body mass); min, mean and max values refer to minimum, geometric mean and maximum values of the corresponding variable in the group (arithmetic mean for temperature), respectively; 95% CI is the 95% confidence interval of *q*-values in a given group assuming a log-normal distribution. The four rightmost columns show parameters of the ordinary least square log–log regression of *q* on *M*, $\log_{10} q = a + b \log_{10} M$, *a* and *b*-values are shown with standard deviations, *P* is probability level, r^2 is coefficient of determination

Group of organisms	*n*	*T* (°C)			*M* (g)			*q* (W kg^{-1})				$\log_{10} q - \log_{10} M$ regression			
		Min	Mean	Max	Min	Mean	Max	Min	Mean	Max	95% CI	*a* ± 1 SE	*b* ± 1 SE	*P*	r^2
Normoxia															
I Hibernating endotherms	43	1·2	**8·6**	30	6	**100**	80 000	0·061	**0·21**	0·83	0·063 … 0·67	–0·51 ± 0·09	–0·086 ± 0·042	0·047	0·09
II Arthropod sit-and-wait strategists	22	5	**22**	25	0·0032	**0·13**	15	0·10	**0·24**	0·44	0·11 … 0·51	–0·66 ± 0·04	–0·047 ± 0·027	0·099	0·13
III Coldwater arthropods	15	0	**0·3**	3	0·0012	**0·039**	33	0·052	**0·14**	0·41	0·034 … 0·55	–1·01 ± 0·10	–0·10 ± 0·05	0·063	0·24
I + II + III	80	0	**11**	30	0·0012	**3·8**	80 000	0·052	**0·20**	0·83	0·061 … 0·65	–0·69 ± 0·03	–0·015 ± 0·015	0·34	0·01
IV Coldwater ectothermic vertebrates	32	–1·5	**0·9**	12·8	2	**100**	3500	0·018	**0·060**	0·19	0·016 … 0·23	–1·27 ± 0·14	+0·02 ± 0·06	0·71	0·00
V Aestivating ectothermic vertebrates	22	14·5	**23**	30	0·28	**47**	6500	0·028	**0·077**	0·31	0·022 … 0·27	–0·95 ± 0·10	–0·09 ± 0·05	0·088	0·14
VI Coldwater non-arthropod invertebrates	28	–1·7	**–0·3**	1·8	0·05	**1·6**	20	0·0093	**0·081**	0·55	0·011 … 0·69	–1·00 ± 0·08	–0·28 ± 0·10*	0·012	0·22
VII Aestivating non-arthropod invertebrates	11	20	**23**	30	0·1	**1·8**	59	0·0063	**0·064**	0·16	0·009 … 0·47	–1·26 ± 0·13	+0·26 ± 0·16	0·14	0·23
IV + V + VI + VII	93	–1·7	**8·5**	30	0·05	**15**	6500	0·0063	**0·071**	0·55	0·014 … 0·38	–1·07 ± 0·05	–0·06 ± 0·03	0·049	0·04
All normoxic species	173	–1·7	**9·6**	30	0·0012	**8·0**	80 000	0·0063	**0·11**	0·83	0·020 … 0·67	–0·89 ± 0·03	–0·05 ± 0·02	0·003	0·05
Anoxia															
All species	66	3	**17**	30	6×10^{-7}	**0·08**	952·5	$9{\cdot}1 \times 10^{-7}$	**0·013**	5	4×10^{-5} … 3·4	–1·82 ± 0·17	+0·10 ± 0·06	0·13	0·05
Organisms with body mass > 1 mg	55	3	**15**	30	0·0015	**0·55**	952·5	0·00038	**0·018**	0·47	5×10^{-4} … 0·60	–1·77 ± 0·11	0·00 ± 0·06	0·91	0·00

*The steep slope in this group is due to three high *q*-values of 3 species of small coldwater sponges (Witte & Graf 1996); if these species are excluded, the remaining 25 species of this group show no significant dependence of *q* on *M*: $a = -1{\cdot}11 \pm 0{\cdot}09$, $b = -0{\cdot}097 \pm 0{\cdot}12$, $P = 0{\cdot}44$, $r^2 = 0{\cdot}03$.

at 0 °C (Kawall, Torres & Geiger 2001), a total of 10 data points corresponding to a body mass range of 1·2–23 mg. Metabolic rate values taken from the study of Kawall *et al*. (2001) represent mean (averaged across individuals) minimum oxygen consumption rates recorded in 30-min intervals in several individuals of a given species that were monitored for 10–20 h each. These minimum values, presumably approaching standard metabolic rate of the species studied, are several times lower than their routine metabolic rates, similar to the situation found in coldwater fish (Steffensen 2002). Group III 'coldwater arthropods', totalling 15 species, was completed with metabolic rates of a 5-week starved Antarctic isopod *Glyptonotus antarcticus* (33 g) (Robertson *et al*. 2001), the scavenging Antarctic amphipod *Waldeckia obesa* (0·6 g) starved for 64 days (Chapelle, Peck & Clarke 1994), a 1-month starved, Arctic amphipod *Themisto libellula* (50 mg) (Percy 1993) and two Arctic decapods *Sclerocrangon ferox* and *Sabinea septemcarinata* (~10 g) (Schmid 1996). Mean temperature for Group III was 0·3 °C (Table 1).

The second set of minimum metabolic rates comprised ectothermic vertebrates and non-arthropod invertebrates. The extensive compilation of standard and routine fish metabolic rates presented by Clarke & Johnston (1999) includes 25 coldwater species (experimental temperature from –1·7 to 1 °C). Minimum metabolic rates recorded for each species (these correspond to the largest individuals measured, body mass range from 2 g to over 2 kg) formed the bulk of Group IV 'coldwater ectothermic vertebrates'. Additionally, this group comprised several hibernating ectotherms, the turtle *Chelydra serpentina* (3·5 kg, 10 °C), the snake *Thamnopsis sirtalits* (63 g, 2 °C), the frog *Rana temporaria* (25 g, 3 °C) (Gatten 1978; Costanzo 1985; Boutilier *et al*. 1997), three deepwater sit-and-wait fishes (28–100 g, 5 °C) (Cowles & Childress 1995) and one blind cave-dwelling salamander *Proteus anguinus* (17 g, 12·8 °C) adapted to prolonged periods of food deprivation (Hervant, Mathieu & Durand 2001). Group IV totalled 32 species with a mean temperature of 0·9 °C.

Metabolic depression is characteristic of aestivating vertebrates surviving seasonal drought at high temperatures. Metabolic rates of 22 aestivating vertebrates listed by Guppy & Withers (1999) in their Table 6 (3 fish species, 14 amphibians and 5 reptiles, body mass range from 1 g to 6·5 kg, temperature range from 15 to 30 °C), and the lizard *Tupinambis merianae* (90 g, 17 °C) (de Souza *et al*. 2004) were included in the present analysis to form Group V 'aestivating ectothermic vertebrates'.

Group VI 'coldwater non-arthropod invertebrates' comprised 28 polar and subpolar species, including 8 bivalve species, 5 gastropods, 1 brachiopod, 1 polychaete worm, 7 sponges and 6 echinoderms (Houlihan & Allan 1982; Davenport 1988; Peck 1989; Schmidt 1996; Witte & Graf 1996; Peck, Brockington & Brey 1997; Pörtner *et al*. 1999; Kowalke 2000; Brockington & Peck 2001; Gatti *et al*. 2002; Peck, Pörtner & Hardewig 2002; Sommer & Pörtner 2002; Harper & Peck 2003; Stead & Thompson 2003). In this group metabolic rates were almost exclusively reported on a dry mass (DM) or ash-free dry mass (AFDM) basis, apparently because of high variability of water and inorganic matter content in many species. In vertebrates and gastropods the amount of ash (inorganic matter) in dry tissue mass is usually low, so the ratios of dry tissue mass to wet mass and ash-free dry mass to wet mass are roughly similar. In coldwater fish, AFDM amounts to 10–30% of wet mass (Torres & Somero 1988); in gastropods it constitutes about 20–25% of wet tissue mass (Penney 2002), a percentage common for many vertebrates. To allow for meaningful comparisons with vertebrates and aestivating invertebrates (mostly gastropods), metabolic rates reported per unit DM or AFDM in group VI species were standardized to 80% water content by dividing by a factor of five. Conversely, body masses reported as DM or AFDM were converted to 'standard' tissue mass by multiplying by a factor of five.

Among non-arthropod invertebrates, gastropod molluscs are known for their ability to survive long periods of drought in a metabolically depressed state. Metabolic rates of 10 aestivating snail species (shell-free body mass 0·1–60 g, temperature 20–30 °C) and 1 annelid worm (Coles 1968, 1969; Schmidt-Nielsen, Taylor & Shkolnik 1971; Burky, Pacheco & Pereyra 1972; Horne 1973; Herreid 1977; Riddle 1977; Abe & Buck 1985; Rees & Hand 1990; Pedler *et al*. 1996; Withers *et al*. 1998) formed Group VII 'aestivating non-arthropod invertebrates'. In molluscs, metabolic rates were expressed per unit shell-free (i.e. tissue) mass.

Anoxic metabolic rates of 32 species capable of surviving at least 1 h of anoxia (3 crustacean species, 5 insects, 11 bivalves, 2 gastropods, 2 fish, 1 frog, 1 turtle and 7 worms) with body mass ranging from approximately 1 µg to nearly 1 kg (a billion-fold range) were collated. There were 66 measurements in total, corresponding to different developmental stages, temperatures and periods of anoxia. Where not determined by direct calorimetry, anoxic metabolic rates were calculated from the rate of lactate accumulation assuming 120 J per 1 mmol lactate (Herbert & Jackson 1985).

Results

NORMOXIA: ABSOLUTE VALUES OF MASS-SPECIFIC METABOLIC RATE AND MASS EFFECTS

Hibernating endotherms (Group I) at $\bar{M}$ = 100 g and $\bar{T}$ = 8·6 °C (mean body mass and temperature) have essentially the same metabolic rate $\bar{q}$ = 0·21 W kg^{-1} as ticks, scorpions and other arthropod sit-and-wait strategists (Group II), $\bar{q}$ = 0·24 W kg^{-1}, despite the lower body mass and higher temperature of the latter

group, $\bar{M}$ = 0·13 g and $\bar{T}$ = 22 °C (Table 1). Had minimum mass-specific metabolic rate depended on body mass and temperature,

$$q \propto Q_{10}^{(T-T_0)/(10\,^\circ\mathrm{C})} M^b, \qquad \text{eqn 1}$$

typical values of Q_{10} = 2 and $b \approx -0{\cdot}3$ would have produced an almost 20-fold difference in $\bar{q}$ between the two groups. Within the ticks, the Antarctic *Ixodes uriae* (M = 7·4 mg, adult males, non-feeding stage) has nearly the same metabolic rate at 5 °C, q = 0·22 W kg^{-1}, as similarly sized North American species at 25 °C, e.g. *Amblyomma cajenense* with M = 7·5 mg and q = 0·26 W kg^{-1}, in agreement with the proposed metabolic cold adaptation in terrestrial arthropods (Addo-Bediako, Chown & Gaston 2002). Coldwater arthropods (Group III, $\bar{M}$ = 0·039 g, $\bar{T}$ = 0·3 °C) have somewhat lower $\bar{q}$ (= 0·14 W kg^{-1}) than terrestrial arthropods and endotherms. However, the observed 1·7-fold difference between Groups II and III is less than the 3·1-fold difference expected from eqn 1. The 1·5-fold ratio between the mean q-values of Groups I and III differs greatly from the ratio of 0·17 predicted from eqn 1, according to which the much heavier endotherms should have lower q than the small coldwater arthropods. The combined arthropod–endotherm data set (Fig. 1a), containing 80 species with body masses spanning more than seven orders of magnitude, from about 1 mg fresh mass in the overwintering copepod *Calanus finmarchicus* to 80 kg in the hibernating bear *Ursus americanus*, is characterized by $\bar{q}$ = 0·20 W kg^{-1} and 95% CI from 0·061 to 0·65 W kg^{-1}.

Mass-specific metabolic rates in Groups IV–VII of ectothermic vertebrates and non-arthropod invertebrates are consistently lower than in Groups I–III, but remarkably similar to each other at 0·06–0·08 W kg^{-1} (Table 1). The ecological significance of this similarity is readily illustrated by comparison of taxonomically distinct and ecologically differentiated species. Thus, the metabolic rate of the 4-kg Central African lungfish *Protopterus aethiops* (0·028 W kg^{-1} at 30 °C) (Janssens 1964), which survives several months of drought by burrowing into mud, is close to that of a small snail *Sphincterochila boissieri* (shell-free mass 1·8 g, 25 °C, 0·020 W kg^{-1}) aestivating in the Negev desert of Israel (Schmidt-Nielsen *et al*. 1971), to that of the Antarctic gastropod *Trophon longstaffi* (standardized shell-free mass 2·8 g, 0 °C, 0·026 W kg^{-1}), which feeds only a few times a year (Harper & Peck 2003), and to that of the deep-water sit-and-wait fish predator *Melanocetus johnsoni* (100 g, 5 °C, 0·033 W kg^{-1}) (Cowles & Childress 1995).

The frequency distribution of the $\log_{10}$-transformed q-values from the combined Groups IV–VII (non-arthropod invertebrates and ectothermic vertebrates), with $\bar{q}$ = 0·071 W kg^{-1} is statistically different from that of the combined Groups I–III (arthropods and endotherms), with $\bar{q}$ = 0·20 W kg^{-1} at $P < 10^{-7}$ (Fig. 2).

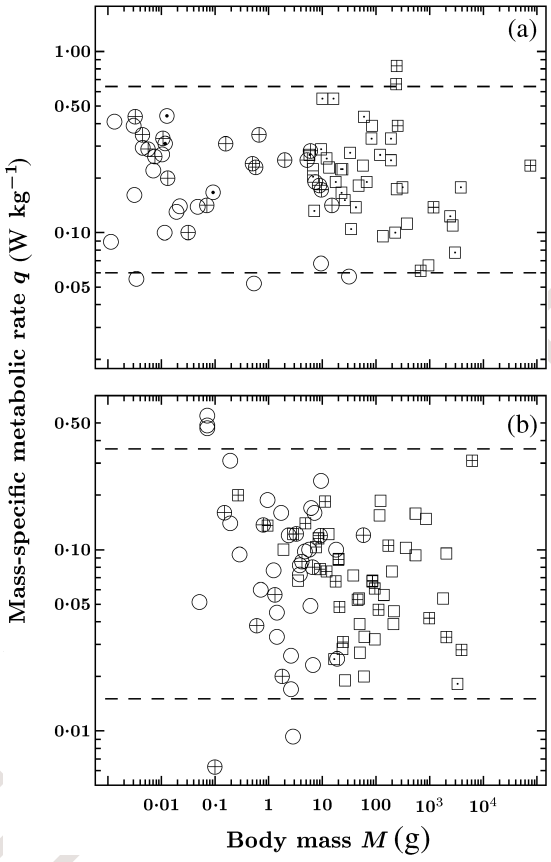

Fig. 1. Minimum life-supporting mass-specific metabolic rates q in various organisms. Boxes and circles denote vertebrates and invertebrates, respectively. Open symbols refer to temperatures ≤ 5 °C, dotted symbols refer to temperatures > 5 °C and < 15 °C, symbols with a + refer to temperatures = 15 °C. Dashed lines denote 95% confidence intervals assuming a log-normal distribution of q-values. (a) Hibernating endotherms, arthropod sit-and-wait strategists, coldwater arthropods, i.e. groups I, II and III from Tables 1, n = 80 species. (b) Coldwater fish and hibernating ectothermic vertebrates, aestivating vertebrates, coldwater non-arthropod invertebrates, aestivating non-arthropod invertebrates, i.e. groups IV, V, VI and VII from Tables 1, n = 93 species.

The difference between arthropod and non-arthropod invertebrates (mostly represented by bivalves, sponges, echinoderms) probably has to do with the largely immobile, inactive lifestyle of the latter group compared with actively moving arthropods. That is, tissues normally accustomed to high levels of physiological activity require more energy for their maintenance even when metabolically depressed.

The three highest values displayed by group VI coldwater invertebrates, 0·47–0·55 W kg^{-1} (Fig. 2b), belong to the three deep-sea sponges from the Greenland-Norwegian Sea (Witte & Graf 1996). These values are more than 2·5 times higher than any of the others in this group, including several other species of polar sponges. Kowalke (2000) suggested that these high values could be an artefact of the unnatural physiological state of deep-sea sponges assessed at atmospheric pressure. The biological significance of these

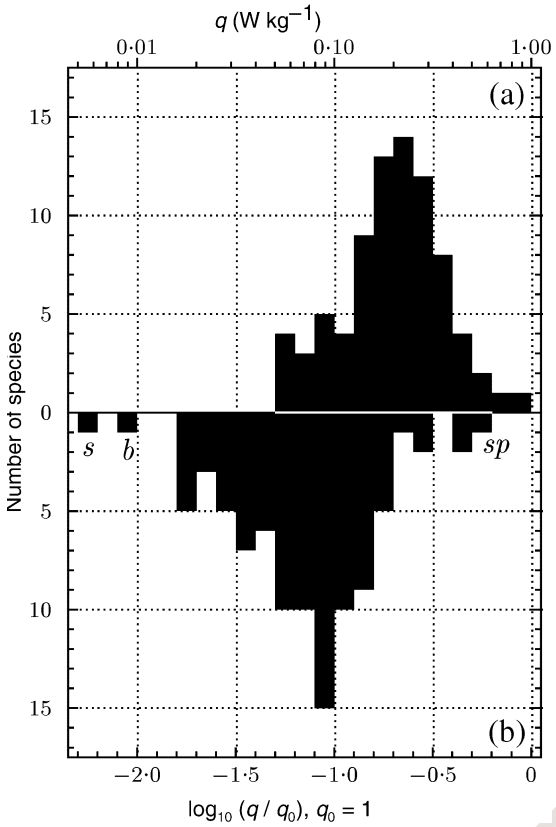

Fig. 2. Frequency distribution of $\log_{10}$-transformed minimum life-supporting mass-specific metabolic rates q in: (a) endotherms and arthropods (Groups I–III from Table 1) and (b) ectothermic vertebrates and non-arthropod invertebrates (Groups IV–VII from Table 1). Outliers: *s* aestivating Tanzanian snail *Bulinus nasutus* (shell-free mass 0·1 g, 24 °C); *b* Arctic bivalve *Astarte montagui* (ash-free dry mass 0·6 g, 0 °C); *sp* coldwater sponges from the Norwegian Sea (ash-free dry mass 10–20 mg, –0·5 °C).

high values remains to be established. Although the sponges studied by Witte & Graf (1996) (10–20 mg AFDM) are among the smallest organisms in group VI species and much smaller than the four sponges studied elsewhere (1–4 g AFDM) (Kowalke 2000; Gatti *et al*. 2002), an even smaller bivalve, *Cyclocardia astartoides* (10·8 mg AFDM), at 0 °C has a metabolic rate of 0·05 W kg^{-1} (Peck & Conway 2000; as cited by Harper & Peck 2003), which is lower than the group average.

The log–log regression of q on M is not significant ($P < 0{\cdot}05$) in any of the seven groups studied, with the exception of Group I, where $P = 0{\cdot}047$ is on the verge of statistical significance, and Group VI, where this dependence is solely due to the three sponges discussed above (Table 1). For the total data set of 173 species, a slight mass dependence at $b = -0{\cdot}05$ ($P = 0{\cdot}003$, $r^2 = 0{\cdot}05$) is found, owing to the fact that Group I–III animals, with higher $\bar{q}$ (= 0·020 W kg^{-1}) are on average somewhat smaller ($\bar{M}$ = 3·8 g) than Group IV–VI animals with lower $\bar{q}$ (= 0·071 W kg^{-1}, $\bar{M}$ = 15 g) (Table 1).

ANOXIA: MASS EFFECTS AND SURVIVAL TIME

Anoxia represents a severe stress to obligate aerobes. Much energy can be stored within the animal body to support normal biochemical processes even in the later absence of an external food supply. However, absence of oxygen, which cannot be stored within the animal body for any prolonged period, prohibits normal biochemical and physiological functioning of obligate aerobes. Many air-breathing species die after several minutes of oxygen deprivation. For organisms tolerating hours and days of anoxia it has been long known, although never emphasized, that metabolic rates measured during anoxia reflect processes of biochemical degradation, such as tissue autolysis (Shick, de Zwaan & de Bont 1983). Only recently has it been explicitly recognized that anoxic existence can represent gradual death rather than sustainable maintenance: the slower the processes of degradation, the longer the survival time (Knickerbocker & Lutz 2001; Milton, Manuel & Lutz 2003). These observations, and the data that are presented below, justify the application of the proposed notion of abandoned metabolic control to describe obligate aerobes under anoxic conditions.

The observed anoxic metabolic rate of 32 species is widely scattered, from 4×10^{-5} W kg^{-1} after 5·6 years of anoxia in embryos of *Artemia fransciscana*, the champion of anoxic survival (Warner & Clegg 2001), to 3 W kg^{-1} in larvae of the bivalve *Crassostrea virginica* with a time to 50% mortality (LT_{50}) of 11 h (Widdows, Newell & Mann 1989) (Fig. 3). Even when these extreme values pertaining to organisms with $M << 1$ mg are excluded, the remaining 55 measurements for 31 species have a 1200-fold 95% CI compared with only 34-fold 95% CI in the 173 normoxic species in the regime of minimum metabolic control (11-fold and 27-fold in Groups I–III and IV–VII, respectively) (Table 1). This wide scatter presumably reflects the chaotic nature of anoxic metabolic rates, which are on average about one order of magnitude lower than minimum metabolic rates in normoxia. Anoxic metabolic rates do not depend on body mass (Table 1).

The second profound difference between normoxic and anoxic metabolic rates is revealed by comparison of energy losses tolerated by anoxic and normoxic organisms. Energy losses of ticks starved for 1 year and hibernating bears amount to 4300–20 000 kJ (kg DM)$^{-1}$ or 16 000–40 000 kJ (kg fat-free DM)$^{-1}$ (data from Lighton & Fielden 1995; Geiser 2004; Tinker, Harlow & Beck 1998; details of calculations are provided in the Appendix). By contrast, mass-specific energy losses, Ψ, tolerated by long-term anoxia survivors at the LT_{50}, are significantly lower. For example, anoxic metabolic rates of the bivalves *Arctica islandica* and *Astarte borealis* with LT_{50} values of 46 and 80 days (Hand & Hardewig 1996) are 3 and 1·5 kJ (kg DM)$^{-1}$ day^{-1}, respectively (Oeschger 1990). This gives Ψ = 138 kJ (kg DM)$^{-1}$ and Ψ = 120 kJ (kg DM)$^{-1}$ for the two species, respectively. These values are 30–300 times

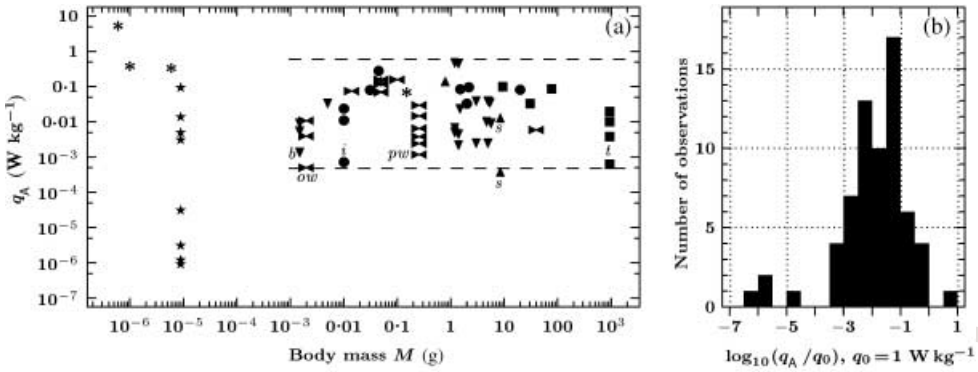

Fig. 3. Mass-specific rates q_A of energy dissipation by animals during various periods of anoxia. (a) Boxes, circles, triangles, up-down triangles and bow-ties denote vertebrates, arthropods (insects and crustaceans), gastropods, bivalves and worms, respectively. Asterisks denote the bivalve *Crassostrea virginica* developmental stages from larvae to adult organism and anoxia periods from 3 to 15 h at 22–25 °C (Widdows *et al*. 1989; Stickle *et al*. 1989). Stars denote embryos of *Artemia franciscana* during the following anoxia periods (counted downwards): 9 h, 6 days (Hand 1990), 1·5 h (Hontoria *et al*. 1993), 12 h, 1 month, 1·4, 2·5 and 5·6 years (Warner & Clegg 2001) at room temperature. Letters referring to groups of vertically arranged symbols denote the following species and anoxia periods (counted downwards): *t* turtle *Chrysemys picta bellii*, 0·5, 3, 10 and 84 days at 20, 15, 10 and 3 °C, respectively (Herbert & Jackson 1985); *s* snail *Pila virens*, 3 days of anoxia in the active hydrated state and 6 months of anoxic aestivation at 27 °C (Meenakshi 1957); *pw* priapulid worm *Halicryptus spinulosus*, 1, 2, 5, 10, 12 and 14 days at 10 °C (Oeschger *et al*. 1992); *b* bivalves *Pisidium* spp., 14, 100 and 140 days; *ow* oligochaete worm *Potamothrix hammoniensis*, 14, 61 and 134 days; *i* aquatic insect larvae *Chironomus anthracinus* (Diptera), 5, 15 and 25 days (Hamburger *et al*. 2000). Dashed lines are 95% CI of *q*-values for $M > 1$ mg assuming log-normal distribution. With one exception (see above), all values are for temperatures from 10 to 30 °C. (b) Frequency distribution of $\log_{10}$-transformed q_A values.

lower than the above estimates of energy losses tolerated by bears and ticks in the regime of minimum metabolic control.

We analysed the dependence of LT_{50} and anoxic mass-specific metabolic rates, q_A, in species capable of surviving more than 12 h of anoxia. Because anoxic metabolic rate declines rapidly at the onset of anoxia, to its characteristic low values (e.g. Herbert & Jackson 1985), only studies where metabolic rate had been estimated during the time period not less than one-fifth of the LT_{50} were used in the analysis. The low estimates for *Artemia fransiscana* were excluded because these are tentative estimates, rather than direct measurements (Warner & Clegg 2001).

In the 17 species studied (20 observations) anoxic metabolic rates are inversely proportional to LT_{50} (Fig. 4a). Thus, to survive $LT_{50} \approx 10^k$ days in anoxia, the organism must depress the mass-specific rate of disorder accumulation to at least $q_A \approx 10^{-k}$ W (kg DM)$^{-1}$. One day, for example, can be survived at q_A as high as 1 W (kg DM)$^{-1}$, while to survive for 100 days q_A cannot be higher than about 0·01 W (kg DM)$^{-1}$. In other words, the better an organism protects its tissues against chaotic biochemical reactions, the longer it will survive. For example, an Indian snail *Pila virens* (8·5 g shell-free mass) can aestivate for nearly a year in dry mud under anoxic conditions at 27 °C with $q_A = 1{\cdot}9 \times 10^{-3}$ W (kg DM)$^{-1}$ (Meenakshi 1957). When placed in anoxic water, the same snail cannot survive more than 4 days. The animal is apparently unable to sufficiently protect its tissues against detrimental biochemical processes, which, in this unprotected hydrated state, occur at a much higher rate of $q_A = 0{\cdot}065$ W (kg DM)$^{-1}$.

The lower limit of mass-specific energy loss during the period of anoxia survival, estimated as:

$$\Psi = q_A \times LT_{50}, \qquad \text{eqn 2}$$

is not dependent on survival time (Fig. 4b). Independent of their metabolic rate, animals die after the cumulative energetic yield of chaotic anoxic biochemical processes has, on average, passed $\Psi = 70$ kJ (kg DM)$^{-1}$.

If q_A represents the lowest metabolic rate observed after prolonged periods of anoxia approaching LT_{50}, then the product $\Psi = q_A \times LT_{50}$ can somewhat underestimate total energy loss during the entire period of anoxia exposure. However, as far as the initial decline in the rate of energy dissipation is typically rapid, total anoxic energy loss will be of the same order of magnitude as Ψ. For example, in the turtle *Chrysemys picta*, total energy loss during 12 weeks of anoxia at 3 °C is approximately twice the value of $q_A \times 12$ weeks, where q_A is the lowest metabolic rate measured during the 12th week (Herbert & Jackson 1985). On the other hand, if q_A is measured before the rate of energy dissipation reaches its minimum value, then Ψ, equation 2, can overestimate the total anoxic energy loss. The wide scatter of Ψ-values around the mean, Fig. 4(b), may reflect these inaccuracies.

Besides quantitative differences between normoxic and anoxic energy losses (Fig. 4b), an important, expected, distinction is the absence of correlation between long-term anoxia survival and the internal store of energy (glycogen) available for the organism. While under normoxic conditions, long-term hibernators, such as bears, are known for exceptional lipid stores. Species with extreme resistance to anoxia, e.g. the bivalves *Astarte borealis* and *Arctica islandica*, and the priapulid worm *Halicryptus spinulosus*,

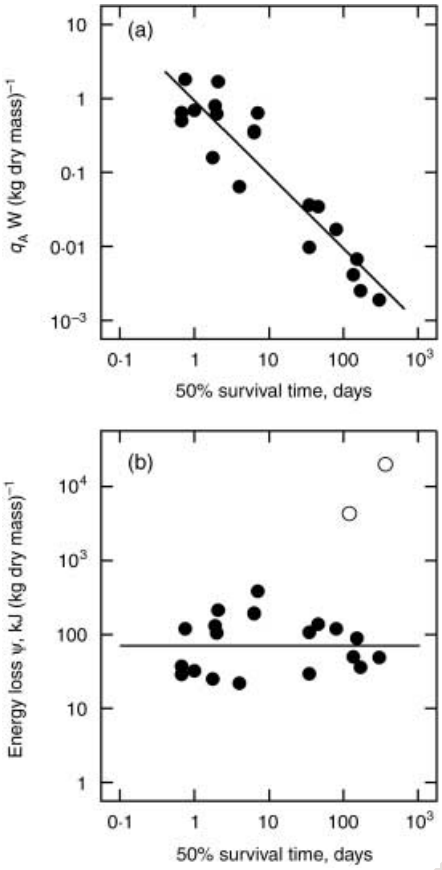

Fig. 4. Metabolic rate, energy loss and survival time in anoxia. (a) Mass-specific rates q_A of energy dissipation by organisms capable of surviving more than half a day of anoxia. Regression line $\log_{10} q_A$ (W kg^{-1}) = –0·041 – 1·0 $\log_{10}$ LT_{50} (days), where LT_{50} is the 50% survival time ($P < 10^{-5}$, $r^2 = 0{\cdot}86$, $n = 20$). (b) Filled circles: energy loss ψ (eqn 2) during anoxia; it is independent of survival time; regression $\log_{10}$ $\Psi = a + b \log_{10} LT_{50}$ gave $a = 1{\cdot}85 \pm 0{\cdot}12$ (± 1 SE), $b = 0{\cdot}02 \pm 0{\cdot}09$, $P = 0{\cdot}85$, $r^2 = 0{\cdot}002$. Open circles: normoxic energy losses of bears during hibernation and ticks during prolonged starvation, see text for details.

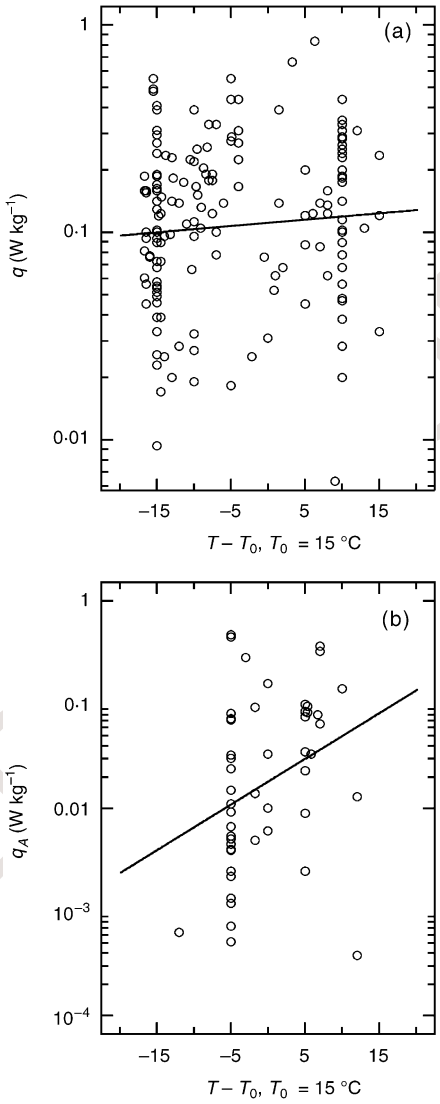

Fig. 5. (a) Temperature-independence of minimum life-supporting metabolic rates q; $\log_{10} q = a + b\,(T - T_0)$, $a = -0{\cdot}91 \pm 0{\cdot}03$ (±1 SE), $b = 0{\cdot}0046 \pm 0{\cdot}0028$ (±1 SE), $P = 0{\cdot}10$, $r^2 = 0{\cdot}02$, $n = 173$. (b) Temperature dependence of anoxic rates of energy dissipation q_A; $\log_{10} q_A = a + b\ T - T_0)$, $a = -1{\cdot}74 \pm 0{\cdot}12$ (±1 SE), $b = 0{\cdot}044 \pm 0{\cdot}019$ (± 1 SE), $P = 0{\cdot}023$, $r^2 = 0{\cdot}097$, $n = 53$.

have only modest glycogen stores: 5–10% of dry tissue mass, as compared with less anoxia-tolerant species, where it can be as high as 40% (Oeschger 1990). Conversely, death by anoxia can occur both at low and high levels of glycogen depletion. For example, the oligochaete *Potamothrix hammoniensis*, with LT_{50} = 170 days, remains viable at glycogen levels of less than 1%, while larvae of the midge *Chironomus anthracinus*, LT_{50} = 35 days, and the bivalves *Pisidium* spp., LT_{50} = 170 days, start dying while still containing 15% and 6% glycogen per unit dry tissue mass, respectively (Hamburger *et al*. 2000). Oeschger (1990) noted that it is not the high glycogen content, but, essentially, the reduction of metabolic rate that is a critical prerequisite for long-term survival of anoxia.

ANOXIA VS NORMOXIA: TEMPERATURE EFFECTS

Finally, an important distinction between anoxic and normoxic metabolic rates lies in their temperature (in)dependence. In the absence of a mass dependence, one can write

$$q = q_0 Q_{10}^{(T-T_0)/(10\,^\circ\mathrm{C})}, \qquad \text{eqn 3}$$

where q_0 is metabolic rate at a reference temperature T_0. Taking logarithms of both sides in eqn 3, one obtains $\log_{10} q = a + b(T - T_0)$, where $a = \log_{10} q_0$ and the slope b of the regression of $\log_{10} q$ on temperature is equal to $b = (\log_{10} Q_{10})/10$, so that $Q_{10} = 10^{10b}$.

Most metabolic rates in both the normoxic and anoxic data sets were measured at temperatures from 0 to 30 °C, so T_0 = 15 °C (global mean surface temperature) was chosen as the reference point. For anoxic data, the four lowest values for *A. franciscana* were excluded, as noted previously. For a few species, where

anoxic metabolic rates were measured at different temperatures, the value at the lowest measured temperature was taken. The estimated slopes b (Fig. 5) allow the estimation of normoxic Q_{10} as Q_{10} = 1·11 (95% CI from 0·98 to 1·26) and anoxic Q_{10} as Q_{10} = 2·75 (95% CI from 1·15 to 6·61). Minimum life-supporting metabolic rates are practically independent of temperature (Q_{10} = 1·11, P = 0·1), while anoxic rates of energy dissipation show a significant temperature dependence (Q_{10} = 2·8, P = 0·02). At Ψ = const (eqn 2), the increase of q_A with temperature brings about a proportional decline in survival time LT_{50}, a phenomenon consistently detected in many species. For example, the turtle *Chrysemis picta* survives 4–5 months in anoxia at 3 °C and only half a day at 20 °C (Herbert & Jackson 1985); the polychaete worms *Marenzelleria viridis* and *Hediste versicolor* (0·07 g) survive more than 10 days of anoxia at 5 °C and no more than 2 days at 20 °C (Fritzsche & von Oertzen 1995).

The observed temperature-independence of minimum life-supporting metabolic rates does not have a straightforward mechanistic explanation. Ion-pumping, which supports *trans*-membrane ion gradients, accounts for a considerable proportion of the basal metabolic rates of many organisms (Hand & Hardewig 1996; Hulbert & Else 2000), and is preserved in many metabolically depressed normoxic species (Guppy & Withers 1999). The physicochemical process of spontaneous leakage of ions along concentration gradients is not exponentially dependent on temperature, so that energy expenditures spent on counteracting such leakage should also be relatively temperature-independent. Singer *et al*. (1993) suggested that this could explain why minimum life-supporting metabolic rates should not depend on temperature. However, the problem appears to be more fundamental when considered within a broader framework, together with such phenomena as the temperature-independence of the catalytic activity of homologous proteins (Somero 1995), and the temperature-independence of the rates of protein and RNA turnover (Marsh, Maxson & Manahan 2001) established in comparisons of species adapted to different ambient temperatures. These findings provide support for the idea that life is able to function at its own preferred optimum rhythm (Makarieva, Gorshkov & Li 2005a,b,c) overriding various physicochemical limitations such as, in this particular example, Boltzmann's temperature law (Clarke & Fraser 2004), with biochemical restructuring during the course of evolution. In the meantime, the typical value of Q_{10} = 2·8 established for anoxia is consistent with the interpretation of anoxic energy dissipation as indicative of uncontrolled processes similar to biochemical reactions occurring *in vitro* or in a dead body.

4 Discussion

We have argued in favour of the existence of two metabolic regimes for survival of unfavourable environmental conditions: the regime of abandoned metabolic control and the regime of minimum metabolic control. In the former regime, metabolic control is abandoned in the sense that the on-going biochemical processes are gradually undermining the initial biochemical and physiological homeostasis of the organism, instead of sustaining it. For example, continuous accumulation of lactate in the blood and internal organs of the turtle *Chrysemis picta*, hibernating under anoxic conditions, ultimately leads to severe acidosis and death (Ultsch, Hanley & Bauman 1985). Energy production in anoxic turtles is proportional to the rate of lactate accumulation, which means that at the organismal level, the rate of heat dissipation by anoxic turtles effectively reflects the rate at which the turtles die rather than their meaningful metabolic rate maintaining biochemical homeostasis. And, indeed, the higher the rate of heat dissipation, the shorter the survival time in anoxic hibernating turtles (Herbert & Jackson 1985; Ultsch *et al*. 1985).

We have avoided using the term down-regulation, although it is widely present in the literature (Hochachka & Lutz 2001; Storey & Storey 2004). There is some ambiguity associated with usage of this term. Regulation *per se* implies a meaningful controlling process initiated by the organism. Importantly, regulation by definition is an optional process, i.e. the organism can either perform it or not, or perform it in different ways. In this sense, metabolic down-regulation would imply the choice of the organism to reduce its metabolic rate. The term 'hypobiosis' introduced by Guppy & Withers (1999) perfectly matches this situation. Guppy & Withers (1999) wrote, for example, that some frogs develop aestivating cocoons, depress metabolism and enter the state of hypobiosis well before the dryness conditions actually set in. That is, the decrease of metabolic rate in these frogs is not dictated by the environmental conditions, but is a consequence of control by the organism itself.

For comparison, when the rates of all oxygen-consuming biochemical processes drop radically at the onset of anoxia, this is not down-regulation. The organism has no option, its metabolic rate inevitably decreases as dictated by the physicochemical oxygen-deprived environment. Nevertheless, the word down-regulation is often used just to indicate the decline of metabolic rates during anoxia or other states of metabolic depression (Hochachka & Lutz 2001). Similarly, there is no option for high metabolic rates in a state of complete desiccation or freezing, owing to absence of liquid water in both cases.

Another caveat for use of the term 'down-regulation' is that it can refer not to the residual low rates of heat dissipation in the metabolically depressed state, but to the processes of biochemical conservation of tissues that precede this state. Recent research has revealed an impressive variety of mechanisms that can be used by the organism to protect its cellular structures from spontaneous degradation. Reversible phosphorylation, suppression of enzyme activity, extension

of enzyme life span and channel 'arrest' preventing the leakage of ions through membranes (Hochachka & Lutz 2001; Storey & Storey 2004) are among the best studied. These biochemical modifications undoubtedly result from meaningful, genetically programmed controlling biochemical processes. Biochemical conservation can take place both in the regime of abandoned metabolic control, where it is a prerequisite of prolonged survival, such as in anoxia-tolerators, and in the regime of minimum metabolic control, such as in hibernating mammals, which have to conserve, in an inactive state, a major part of the physiological machinery responsible for locomotion, feeding and other activities. However, biochemical conservation (which does represent a process of metabolic down-regulation and results in a metabolically depressed state) typically takes a short time before or at the very onset of the adverse conditions. For example, in cells of strong anoxia-tolerators, such as turtles, that survive days and months of anoxia, protective biochemical rearrangements can take approximately 100 min (Hochachka & Lutz 2001).

Thus, the existence of regulated, organism-controlled processes of biochemical conservation at the onset of anoxia does not contradict our proposition that the residual rates of heat dissipation during anoxia in the studied species can be predominantly chaotic. These rates will then reflect the fact that the organism is unable to conserve itself completely. But, if the degree of conservation is high, the organism can suppress the chaotic processes of biochemical degradation to a very low level. The term 'cryptobiosis', used to denote physiological states with practically zero rates of heat dissipation under adverse environmental conditions such as anoxia, desiccation and starvation (in some taxa) (Guppy & Withers 1999; Clegg 2001; Gutiérrez *et al.* 2001) corresponds therefore to the regime of abandoned metabolic control with nearly perfect biochemical conservation. Survival times of organisms in this state can be very long, from years to possibly centuries (Clegg 2001). At the same time, there can be cases of abandoned metabolic control with poor or no biochemical conservation, with short survival times and relatively high rates of heat dissipation. Organisms typically experiencing only very short periods of anoxia in their natural environments do not need an advanced mechanism of biochemical conservation.

REGIME OF MINIMUM METABOLIC CONTROL

This regime is truly life-supporting. So long as the internal energy reserves of the animal persist, no detrimental changes accumulate in the animal body, because this energy is spent on supporting the existing biochemical and physiological order. For example, 4 months of muscle disuse do not produce any significant muscle atrophy in hibernating bears (Tinker, Harlow & Beck 1998). At the cellular level, such fundamental order-supporting mechanisms as ion pumping and basic protein turnover continue to function in this regime (Guppy & Withers 1999).

Based on the analysis of 173 species with body masses ranging from 1·2 mg to 80 kg, and experimental temperatures from –1·7 to 30 °C, it was shown that minimum life-supporting mass-specific metabolic rates are practically independent of body mass and temperature, and constitute on average 0·1 W kg^{-1} (Table 1). This low rate of controlling metabolism appears to be sufficient for living cells to perform important functions at the organismal level even during the period of metabolic depression, such as lactation in hibernating bears (Tinker *et al.* 1998), construction of protective epiphragms in aestivating snails (Withers *et al.* 1998) and oogenesis in aestivating amphibians (Seymour 1973).

Log–log regression of minimum mass-specific metabolic rates q on body mass M yielded a weak dependence, $q \propto M^{-0.05}$ with $P = 0{\cdot}003$ and $r^2 = 0{\cdot}05$ (Table 1). This dependence would have produced a tenfold range of q-values over 20 orders of magnitude range in body mass. Do the very smallest organisms such as bacteria indeed possess significantly higher metabolic rates during periods of metabolic depression than metazoans? According to the available evidence, the answer is no. In a study of endogenous metabolic rates of 56 bacterial species, the 10 lowest values were found to average 0·2 W kg^{-1} (Makarieva *et al.* 2005c), which is directly comparable to the values obtained here for metazoans (Table 1). At the other end of the size scale are the largest ectothermic animals. Carey *et al.* (1982) estimated metabolic rate of the White Shark *Carcharodon carcharias* (body mass about 900 kg) from the data on the rate of body warming to be about 0·2 W kg^{-1}. In endotherms, the largest organisms depress metabolic rate by a smaller percentage than the smallest ones (Geiser 2004). If this rule applies to ectotherms too, then the lowest metabolic rate supporting life of the shark cannot be much smaller than the above value, i.e. it should fit accurately into the established range of minimum life-supporting values (Table 1). Summing up, there is no evidence in favour of the existence of a dependence of minimum mass-specific metabolic rate on body mass. This is consistent with the expectation that, to the degree that the living matter is biochemically universal among organisms of different body sizes, the minimum metabolic rate needed to sustain orderliness of a unit live mass should also be size-independent (Makarieva, Gorshkov & Li 2003, 2005c).

It remains to be ascertained what the most appropriate mass basis is for determining the proposed universality of minimum life-supporting mass-specific metabolic rate: whether it should be wet mass, dry mass, carbon mass, etc. In the present study, Group III coldwater arthropods display metabolic rates that are almost twice lower than in Group II terrestrial arthropods (Table 1). This discrepancy might be a consequence of a higher water content in Group III species. For example, in the seven coldwater copepod species studied by Kawall *et al.* (2001) mean wet mass/dry

mass ratio is 6·1 ± 0·7 (SE), which is higher than the characteristic value of 3·3 corresponding to a typical 70% water content in terrestrial arthropods. If expressed per unit dry mass basis, metabolic rates of Group II and III species can be more similar than per unit wet mass basis.

REGIME OF ABANDONED METABOLIC CONTROL

When normal biochemical functioning is impossible (e.g. in the absence of oxygen for obligate aerobes), the organism cannot sustain its biochemical order. In such cases, living tissues are left to degrade. However, if the adverse period is relatively short and tissue protection is good enough for the amount of accumulating damage not to reach a critical threshold, the animals can survive environmental stress in the regime of abandoned metabolic control, as suggested by us for the 32 anoxic species analysed in the present paper.

It is important to stress that this does not mean that anoxic survival must invariably occur in this regime. Apparently, there are obligate and facultative anaerobes capable of sustainable, metabolically controlled existence under anoxic conditions. At the same time, species under normoxic conditions of minimum metabolic control can also exhibit elements of abandoned metabolic control. For example, an inherent pattern in mammalian hibernation is periodic arousal accompanied by elevation of metabolic rate (McNab 2002). This might be an indication that during torpor, biochemical order in some tissues is not fully sustained and the accumulating disorder has to be repaired by the organism via more vigorous metabolic control during arousals. Periodic bursts of elevated metabolic rate at the time-scale of days and weeks have also been recorded in some aestivating snails (Schmidt-Nielsen *et al*. 1971) and diapausing insects (Denlinger, Willis & Fraenkel 1972).

A critical energy loss threshold corresponding to 50% survival in anoxia was established at around Ψ = 70 kJ (kg DM)$^{-1}$ (Fig. 4b). Anoxic metabolic rates of the cysts of *Artemia franciscana* (LT_{50} is about 2·5 years) estimated from the rate of the decrease in the nucleotide pool (Warner & Clegg 2001), $q_A = 3 \times 10^{-6}$ W (kg DM)$^{-1}$, indicate that the embryos die well before the critical threshold of 70 kJ (kg DM)$^{-1}$ is reached. Energy loss during 2·5 years of anoxia amounts to less than 1 kJ (kg DM)$^{-1}$. This could be an indication that the reported metabolic rates have been significantly underestimated. Warner & Clegg (2001) remark that it is unclear which processes in the *Artemia* embryo could require the expended free energy. In the framework of the present consideration the answer is transparent. No meaningful processes make use of this energy. Instead, this energy is released during spontaneous chaotic biochemical reactions, the studied nucleotide store simply being one of the most reactive pools.

Accumulating cell damage can account for the observed increase in postanoxic time to hatching with increasing anoxia exposure (i.e. embryos exposed to less than a month of anoxia hatch in 23 h, while those exposed to 5·6 years hatch in 388 h only) (Warner & Clegg 2001). Extra time can be used by the animals to heal tissue damage accumulated during prolonged anoxia. Post-anoxic 'overshoot' (i.e. the increase of postanoxic metabolic rates above the normoxic value before anoxia) (e.g. Oeschger *et al*. 1992; Moratzky *et al*. 1993) might also be a consequence of rapid compensation of the detrimental consequences of anoxia or any other environmental stress survived in the regime of abandoned metabolic control (Block, Worland & Bale 1998).

Besides anoxia, survival of higher organisms during freezing and desiccation is also likely to occur in the regime of abandoned metabolic control. In both cases, absence of liquid water in tissues should automatically slow down any biochemical reactions, even if no depression of metabolic rate is performed by the organism itself. Special protecting mechanisms may include, for example, degradation of mitochondria in frozen animals (Danks, Kukal & Ring 1994). In the regime of abandoned metabolic control mitochondria could serve as the undesirable hotspots of spontaneous chaotic biochemical reactions, so their elimination should contribute to biochemical stabilization of the cells.

Generally, any organism capable of conserving its tissues to the degree where the residual chaotic biochemical reactions occur at a rate $q_A << \Psi/t$, where t is the desired survival period and $\Psi \sim 100$ kJ (kg DM)$^{-1}$, should be able to safely abandon metabolic control with no threat to viability. For example, dry spores of the bacterium *Bacillus cereus* having a metabolic rate of less than 6×10^{-4} W (kg DM)$^{-1}$ (Desser & Broda 1965) should be able to survive for at least several years.

If the regime of the abandoned metabolic control effectively represents 'slow death', the rate of biochemical reactions in such animals should be similar to that of dead organisms under similar conditions. To our knowledge, no studies have explicitly examined this problem. It is noteworthy that the range of respiration rates in dead wood tissues (coarse litter) in central Amazon forests, 0·014–1·0 μg C (g wood C)$^{-1}$ min^{-1} (Chambers, Schimel & Nobre 2001), which correspond to energy dissipation rates of 0·0009–0·06 W kg^{-1} (at 10% carbon content in wood tissues), for the most part falls outside the 95% CI of controlled minimum metabolic rates, which is from 0·02 to 0·67 W kg^{-1} and is within the lower part of the 95% CI of anoxic rates (Table 1).

Sinclair, Klok & Chown (2004) studied metabolic rates of the freeze-tolerant Antarctic caterpillar *Pringleophaga marioni* (0·3 g) frozen to –5·8 °C, –6 °C and –18 °C. Metabolic rate of live caterpillars at –5·8 °C was about 0·1 W kg^{-1}, well in the range of minimum life-supporting metabolic rates in arthropods (Table 1). At –6 °C metabolic rate of the caterpillars dropped abruptly by

approximately twofold with $Q_{10} = 2 \times 10^3$. A similar abrupt drop of metabolic rate was observed in frozen as compared to supercooled Goldenrod Gall Fly larvae, *Eurosta solidaginis*, and some other species (Irwin & Lee 2002). These declines in metabolic rate can be readily interpreted as the moment when metabolic control is abandoned (e.g. cessation of transmembrane ion pumping) (Sinclair *et al*. 2004), while the residual rate of heat dissipation can be considered the rate of spontaneous disorder accumulation. In this case, the benefit of energy savings associated with the regime of abandoned metabolic control (e.g. freezing) can be nullified by the detrimental effects of the chaotic biochemical processes occurring in the organism. This possibility has not been mentioned in discussions of the ways in which *Eurosta solidaginis* optimizes winter survival (Irwin & Lee 2002).

In our view, the proposed conceptual distinction between the regimes of minimum and abandoned metabolic control, as well as the established properties of minimum life-supporting metabolic rates, can yield insights into several ecological questions. For example, as far as mass-specific metabolic rate drops with increasing body size within specified taxonomic groups, a temperature-independent lower limit to mass-specific metabolic rate would pose an upper limit to body size that can be attained by organisms of a given taxon at a given temperature. In other words, in a given taxon the largest species living at low ambient temperatures will have to be smaller than the largest species from the same taxon living at high temperatures (see Makarieva, Gorshkov & Li 2005d,e).

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