ELSEVIER

Thermochimica Acta 349 (2000) 31–42

thermochimica acta

www.elsevier.com/locate/tca

Bioenergetics of diapause and quiescence in aquatic animals

Steven C. Hand*, Jason E. Podrabsky[1]

Department of EPO Biology, Section of Integrative Physiology and Neurobiology, University of Colorado, Boulder, CO 80309-0334, USA

Received 7 June 1999; received in revised form 23 September 1999; accepted 23 September 1999

Abstract

With one exception, all major animal phyla contain species that possess dormant states, a feature that affords tolerance to extreme environmental conditions. Diapause is an obligate, developmentally-programmed form of dormancy that precedes the onset of environmental insult. Calorimetric and respirometric studies reveal that a major metabolic depression accompanies entry into diapause in vertebrates and invertebrates. Under fully aerobic conditions, embryos of the annual fish *Austrofundulus limnaeus* depress metabolism by approximately 90% over a period of several days as they enter diapause. This metabolic shift is achieved without alteration in ATP:ADP ratio. Breakage of diapause involves a lengthened photoperiod and is accompanied by a 200-fold increase in metabolic rate. Similarly, gemmules of the freshwater sponge *Eunapius fragilis* display developmental arrest and exhibit low heat dissipation and respiration when in diapause. Upon diapause breakage, germination occurs within 48–72 h. Across this period metabolic rate increases 12-fold, while adenylate status is unchanged; constant adenylates are also noted during diapause in embryos of the brine shrimp, *Artemia franciscana*. In contrast to diapause, quiescence is a form of dormancy that is a direct response to environmental stress. During anoxia-induced quiescence in *A. franciscana* embryos, heat dissipation rate drops below 0.2% of the aerobic value within hours of anoxic exposure, and ATP declines by 80% or more during this period. Transcription and translation are markedly depressed in the nucleo-cytoplasmic compartment and within the mitochondrion, and these represent two events in a suite of energy-saving measures. Coordinated depression of catabolic and anabolic processes is a hallmark feature of both diapause and quiescence. However, the adenylate energy status and time interval required for achieving metabolic depression are markedly different between the two conditions.

Keywords: Metabolic depression; Dormancy; *Artemia franciscana*; *Austrofundulus limnaeus*; *Eunapius fragilis*

1. Introduction

The most profound bioenergetic transitions known to occur in animals are observed during entry and exit from dormancy. In certain cases involving invertebrates, metabolic rate can be downregulated across a range of 500-fold or more as these organisms approach ametabolic states. Evaluating the mechanisms by

* Corresponding author. Tel.: +1-303-492-8981; fax: +1-303-492-8699.
E-mail address: hands@spot.colorado.edu (S.C. Hand)
[1] Present address: Hopkins Marine Station, Stanford University, Ocean view Blvd., Pacific Grove, CA 93950-3094, USA.

0040-6031/00/$ – see front matter
PII: S0040-6031(99)00511-0

which these modulations are actively controlled, and the physiological requirements for surviving such states, can provide a perspective on the capacities and metabolic repertoires of eucaryotic cells that can be gained from few other naturally-occurring events. In this short review the coverage will not be comprehensive, if for no other reason because of the sheer phylogenetic breadth of this phenomenon; all major animal phyla, save one, contain species that display dormancy at some point in their life cycle [1,2]. Rather, we will use selected examples from both vertebrates and invertebrates to illustrate some of the key characteristics underpinning these metabolic changes.

A second goal of this analysis will be to compare and contrast features of the two major categories of dormancy as defined for animals — diapause and quiescence [3,4]. Diapause is an obligate, developmentally-programmed state of metabolic and/or developmental arrest that is controlled by some type of endogenous physiological factor. An organism enters the state even under optimal environmental conditions that would otherwise promote normal metabolism and development; typically diapause precedes the onset of environmental insult (e.g., the winter season, dry season, etc.). Release from diapause (activation, diapause breakage) requires exposure to a specific stimulus or cue, or combination of cues. On the other hand, quiescence is a metabolic and/or developmental arrest directly promoted by an unfavorable environmental condition like desiccation, anoxia, or temperature extremes. It is controlled simply by exposure to, or removal of, the relevant environmental factor.

Both diapause and quiescence can serve as latent stages in life cycles, during which the organism is refractory to stresses that normally would threaten survivorship. Remarkably, these dormant states can last for weeks, months or decades, and across these intervals of time the animal does not feed. It is, therefore, not surprising that a key requirement of this protracted tolerance is the capacity for metabolic depression (e.g. [5–7]). Length of survivorship in these states is directly correlated with the degree of metabolic downregulation [8]. From an experimental standpoint, microcalorimetry has proven quite useful in evaluating the energy flow of animals during dormancy.

2. Experimental

2.1. Diapause in freshwater sponge gemmules

Among invertebrates, one useful model for the study of diapause is the gemmule of the freshwater sponge *Eunapius fragilis*. Gemmules are asexually produced reproductive bodies composed of undifferentiated cells surrounded by a collagenous capsule, and such structures serve as the overwintering stage in the life cycle of sponges [9]. *E. fragilis* is well suited for the study of entry and exit from diapause, because unlike the case with many sponge species, diapause and quiescence are not intertwined [10]. Gemmules newly formed by the parental tissues are in an obligate state of diapause, and if diapausing gemmules are held in water at room temperature they will not develop. However, vernalization in the cold for 2–3 months releases gemmules from diapause and allows them to resume development when warmed to 20–23°C.

Recently, Loomis et al. [9] characterized metabolic changes during germination of *E. fragilis* gemmules and compared the results with diapausing gemmules. Upon warming post-diapause gemmules to 21°C, heat dissipation (Fig. 1) and oxygen consumption increased 600% during the ensuing 48–72 h, during

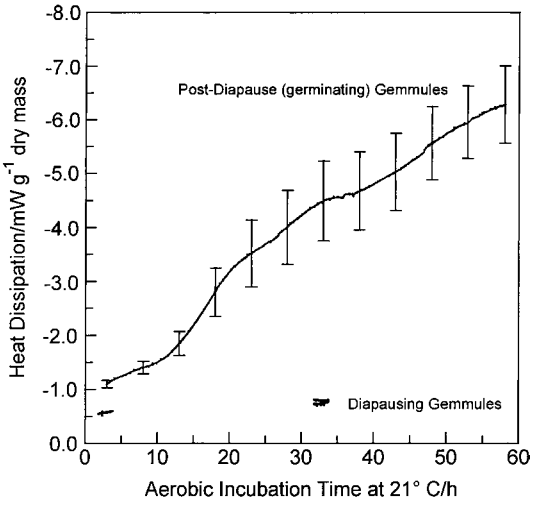

Fig. 1. Heat flux of germinating and diapausing gemmules of *E. fragilis* under normoxia. Data for germinating gemmules are expressed as means of three independent experiments. Error bars represent±one SEM at the indicated time points. Values for diapausing embryos (short line segments) are presented for two independent experiments (redrawn from [9]).

Table 1
Adenylate status in gemmules of *E. fragilis* during germination (after [9])

Time at room temperature (h)	ATP:ADP ratio	Energy charge
0	6.91±0.96[a]	0.81±0.02
6	15.12±1.31	0.86±0.01
18	12.15±1.89	0.83±0.01
30	10.28±0.05	0.80±0.01
42	17.55±0.92[b]	0.79±0.01
54	9.86±2.57	0.77±0.05

[a] Values represent means±one SE (n=3).
[b] Significantly different from Hour 0 value (p=0.008), based on ANOVA with Bonferroni adjustments for multiple comparisons. No significant differences were found for the energy charge values.

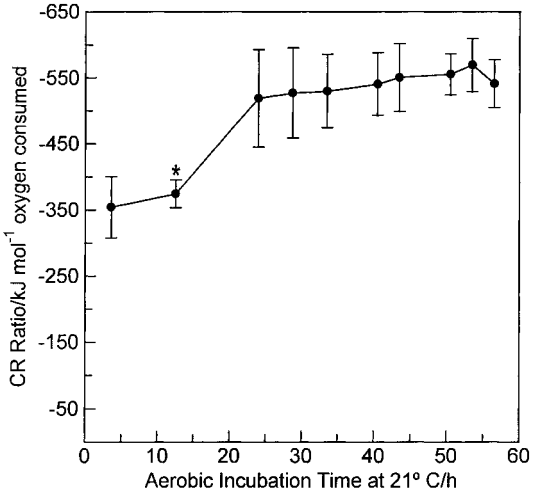

Fig. 2. CR ratios measured for post-diapause (germinating) gemmules of *E. fragilis*. Values are means for 3 h intervals (except for a 1.25 h interval at the first time point). Error bars represent±one SEM (N=3), except at Hours 50.5 and 56.5 where N=4. The * denotes a significant difference from the theoretical oxycaloric equivalent for carbohydrate (-477 kJ mol^{-1} O_2) (redrawn from [9]).

which time germination culminated in the emergence of a new sponge from the gemmule capsule. Energy flow was two-fold lower in diapausing gemmules that had not experienced cold vernalization, and heat dissipation did not change over the same period of incubation (Fig. 1). The diapausing gemmules did not develop or germinate during this incubation at 21°C. The adenylate pools were well charged and relatively constant throughout the germination period for post-diapause embryos (Table 1); even the time zero values (representing gemmules not yet warmed to room temperature) showed substantial ATP content, which suggests that very little ATP depletion occurred during the preceding diapause period.

Empirically determined calorimetric–respirometric ratios (CR ratio; kJ mol^{-1} O_2) can provide insight into the qualitative nature of energy metabolism when compared to the theoretical oxycaloric equivalent for aerobic metabolism of fuels (cf. [11–13]). For example, if the CR ratio is larger than the oxycaloric equivalent, then excess heat is being produced by metabolism that cannot be explained by aerobic processes alone. The CR ratio of post-diapause gemmules increased significantly from -354 kJ mol^{-1} O_2 during the first hours of germination to -541 kJ mol^{-1} O_2 after 57 h (Fig. 2) [9]. The average across the entire germination period was approximately -495 kJ mol^{-1} O_2. The CR ratio at 12.5 h was statistically different from the oxycaloric equivalent for aerobic carbohydrate metabolism (-477 kJ mol^{-1} O_2), which was chosen as a basis of comparison because sorbitol is the primary energy source during germination [14]. The lower CR ratio near the beginning of the incubation might be attributable to the fact that these gemmules were stored at 4°C without aeration for months prior to the start of the incubation. Thus, they may have experienced hypoxia at the beginning of the experiment and were undergoing aerobic recovery. This speculation was supported by the low CR ratio (-363 kJ mol^{-1} O_2) measured 2.5–3.5 h after the anoxic bout shown in Fig. 3. For comparison, immediately prior to the onset of anoxia the CR ratio was -438 kJ mol^{-1} O_2. After 3.5–6.5 h of aerobic recovery, the value had risen to -457 kJ mol^{-1} O_2 and to -527 kJ mol^{-1} O_2 after 10.5–13.5 h of recovery. Low CR ratios are characteristic of aerobic recovery periods in numerous animals (cf. [13]). The CR ratio for diapausing gemmules was approximately -490 kJ mol^{-1} O_2 at the end of the experiment in Fig. 1.

Post-diapause gemmules tolerated severe hypoxia for at least 7.5 h (Fig. 3), during which time heat dissipation was reduced to 6% of the aerobic value. Upon return to aerobic conditions, the heat dissipation climbed rapidly, and gemmules resumed development and emerged. Anoxia tolerance in gemmules may be quite advantageous, because the natural habitat of

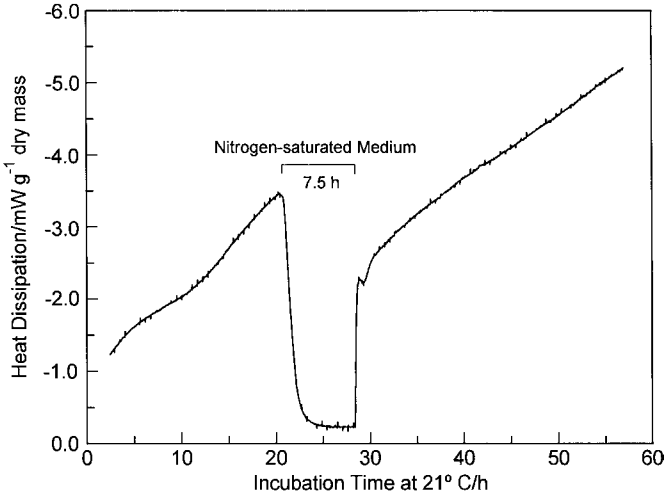

Fig. 3. Response of heat dissipation from post-diapause gemmules of *E. fragilis* in response to perfusion with nitrogen-saturated water (redrawn from [9]).

these gemmules during winter can include burial in pond sediments of low oxygen tension. A recent report [15] has shown that gemmules can survive 4 months of anoxia at 20°C. However, virtually nothing is known about metabolic processes in anoxic gemmules.

Breakage of the diapause state in gemmules may be related to an increase in sorbitol catabolism seen during the transition to active development [14]. The catalytic activity of sorbitol dehydrogenase increases dramatically at this point in *E. fragilis* gemmules, and this activity is associated with a rapid decline in sorbitol levels. Glycogen is most likely being synthesized at the expense of sorbitol. cAMP did not change significantly during the germination of *E. fragilis* gemmules, in contrast to the situation reported for those of *Spongilla lacustris* [16]. Thus, other mechanisms that do not involve cAMP must be responsible for regulating these changes in carbohydrate metabolism.

2.2. Diapause in annual killifish embryos

Annual killifish (Cyprinodontiformes) inhabit ephemeral pools in savanna and desert regions in Africa and South America [17–19]. These ponds dry out on a seasonal basis, which kills the adult and juvenile fish, but the population persists due to the occurrence of diapause embryos that survive the dry season embedded in the pond sediments. Upon the return of the rainy season, the ponds refill, diapause is broken, and the larvae hatch and continue the life cycle. There are a maximum of three diapause stages in annual killifish. Diapause I occurs early in development during the dispersion and reaggregation of blastomeres [18]; this arrested state is promoted by unfavorable environmental conditions and thus is probably not true diapause, but rather a form of quiescence. Diapause II occurs in embryos possessing 38–40 somites, and diapause III is entered right before hatching when the embryo is fully developed [17,19].

Austrofundulus limnaeus is an annual killifish endemic to the northern South America. Rates of oxygen consumption and heat dissipation peak early in development of *A. limnaeus* embryos (8 days post-fertilization, dpf), prior to formation of the embryonic axis (Fig. 4). Energy flow then declines more or less continuously as the embryos continue to develop. At the onset of diapause II (24 dpf) oxygen consumption and heat dissipation are already depressed by 70 and 67%, respectively, as compared to the maximum values at Day 8. Thus, both indicators of metabolic rate show a major depression of energy flow *prior* to entry into diapause II as defined by the onset of developmental arrest. This decline in oxygen consumption and heat dissipation beginning 12 d prior to the onset of diapause II was an unexpected result. In general, respiration and heat dissipation tend to increase continually during early development in fish

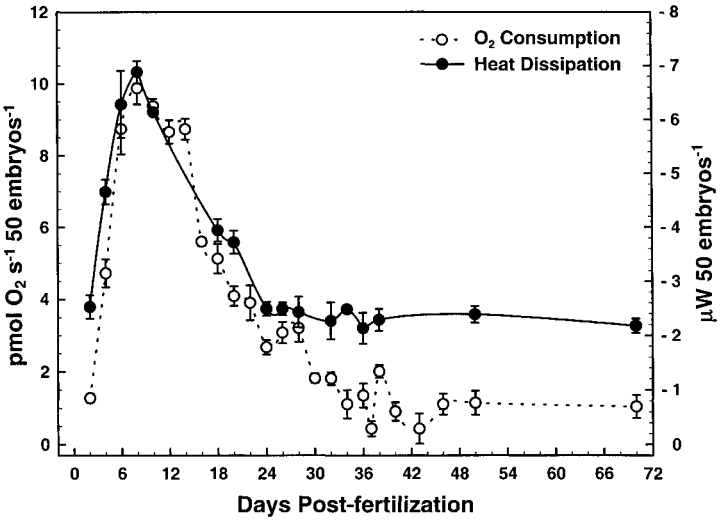

Fig. 4. Oxygen consumption and heat dissipation are depressed during diapause II in embryos of *A. limnaeus*. Decline in both indicators of metabolic rate begins 12 days prior to developmental arrest. Embroys enter diapause II at 24 dpf. Values are means±SEM (n=4–7 independent spawning events) (redrawn from [31]).

[20–25]. The basis for the decline in the rate of energy flow prior to the onset of diapause is unclear. The decline may not be associated with entry into diapause, but rather, may be due to the unique developmental pattern of annual killifish. Unlike typical teleost embryos, in annual killifish the cell movements associated with epiboly (cell migration of the blastoderm around the yolk) are separated from the formation of an embryonic axis [17,18]. The separation of these two processes can explain the decline in metabolism if the energetic cost of cell motility greatly out-weighs the cost of embryonic axis formation. An alternate hypothesis is that the metabolic decline is strictly associated with diapause and may be explained by a sequential exit of dividing cells from the cell cycle in an anterior to posterior fashion. Thus, while major developmental processes may be complete in the anterior region, the posterior may still be developing. Such a pattern could explain how somites are still being added in the posterior through 24 dpf, yet metabolism is already declining after 8 dpf (as a result of cell cycle arrest in the more anterior regions). Information on the energy metabolism and developmental patterns of annual killifish species that do not enter diapause II may help to clarify this point.

After embryos of *A. limnaeus* reach diapause II (24 dpf), rates of oxygen consumption continue to decline for an additional 8–10 days (Fig. 4). At 70 dpf, oxygen consumption is depressed by 90% compared to embryos at 8 dpf, thus clearly documenting metabolic downregulation in diapause. A slow decline in oxidative metabolism appears to be a common trait of diapause embryos. Levels et al. [26] report that embryos of the African annual killifish *Nothobranchius korthausae* reach a low in oxygen consumption after three weeks of diapause II. Clegg et al. [27] have shown in the brine shrimp, *Artemia franciscana*, that metabolism slowly declines upon entry into diapause over a period of five days. While rates of oxygen consumption decrease during diapause II in embryos of *A. limnaeus*, rates of heat dissipation remain constant which leads to CR ratios that are well above the oxycaloric equivalent (Table 2). During hypoxia, excess heat dissipation can typically be explained by accumulation of end products like lactate or ethanol [28–30]. However, in our studies *A. limnaeus* embryos were not hypoxic. While recruitment of anaerobic processes during normoxia may seem unexpected, there are a number of examples where anaerobic processes are known to contribute to the total energy flow of normoxic cells (for a brief review see [12]). Because of insignificant changes in glycogen content during development and diapause in *A. limnaeus* (Fig. 5a), the high CR ratios cannot be

Table 2
CR ratios during development and diapause in embryos of *A. limnaeus* (after [31])

Developmental stage	dpf[a]	dpdII[b]	CR ratio[c]
Late blastula	2	–	2209[d]±392
Axis formation	10	–	671[d]±15
Late somitogenesis	18	–	683[d]±71
Early diapause II	24	–	848[d]±85
Late diapause II	70	–	2860[d]±855
Early organogenesis	76	6	507±78
Late organogenesis	85	15	483[d]±13
Early diapause III	103	33	429±16
Late diapause III	145	75	833[d]±32

[a] dpf is days post-fertilization.
[b] dpdII is days post-diapause II.
[c] kJ mol^{-1} O_2, mean±SEM, n=4 independent spawning events.
[d] Mean is statistically different (p<0.05) from the oxycaloric equivalent for a mixed substrate metabolism (−450 kJ mol^{-1} O_2). Comparisons were made using a one sample t-test assuming the oxycaloric equivalent to be the parametric value.

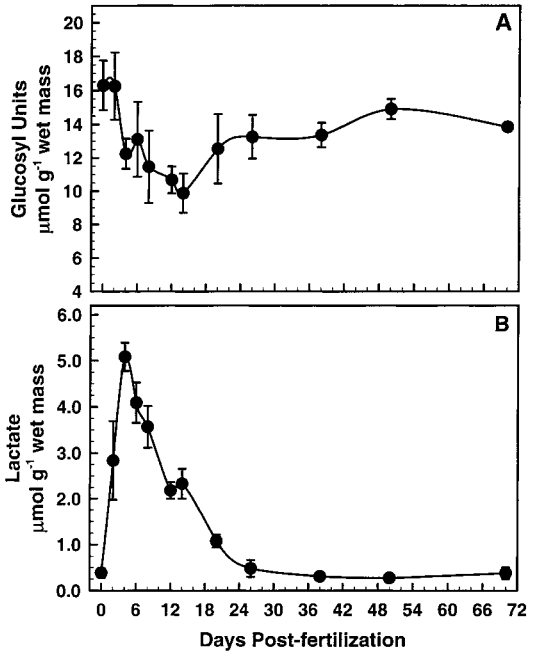

Fig. 5. (A) Glycogen content does not change significantly during early development and diapause II. (B) Lactate does not accumulate during diapause II in embryos of *A. limnaeus.* Values are means±S.E.M. (n=4 independent spawning events) (redrawn from [31]).

explained by glycogen-derived end products. Lactate remained low and actually declined after 12 dpf (Fig. 5b) and ethanol was not detected in acid extracts of embryos nor the media in which embryos were incubated (data not shown). A more thorough evaluation of possible metabolic end products and metabolic intermediates may clarify the reason for the high CR ratios observed in diapause II embryos.

Upon release from diapause II (fostered by long photoperiods, [31]), the metabolism of the embryos increases in an almost exponential fashion and reaches a high at 21 days post-diapause II (Fig. 6). At this time, oxygen consumption is over 200-fold greater than during diapause II. Oxygen consumption and heat dissipation slowly decline after the onset of diapause III (21 days post-diapause II; Fig. 6). At 75 days post-diapause II, the rate of oxygen consumption is depressed by 84% compared to the peak rates. Levels et al. [26] report similar rates of oxygen consumption for diapause III embryos of *N. korthausae,* although the time course is very different. Diapause III appears to be a different phenomenon compared to delayed hatching in fish embryos which is not associated with metabolic depression [32–34]. This lack of metabolic depression appears to limit survival of delayed hatching embryos of *L. tenuis* to about 2 weeks [32], while diapause III embryos of *A. limnaeus* can survive for well over 50 days. The ability to hatch quickly appears to be more important than increased survival time in species which exhibit delayed hatching, likely because the window for successful hatching is limited to each spring tide [32]. In contrast, the long duration of the dry season and relative instability and unpredictability of ephemeral pond habitats may have favored diapause over delayed hatching in annual killifish.

Post-diapause II metabolism is largely aerobic as indicated by CR ratios that are indistinguishable from the theoretical oxycaloric equivalent for aerobic metabolism of mixed substrates (Table 2). CR ratios increase slightly during late diapause III, which suggests a small contribution of anaerobic pathways to total energy flow in late diapause III embryos. Aerobic CR ratios agree with values reported in the literature for both early [24] and late embryos [25,28] of normoxic teleost fish embryos (reviewed in [13]).

Developing and diapausing embryos of *A. limnaeus* maintain a highly charged adenylate pool as evidenced

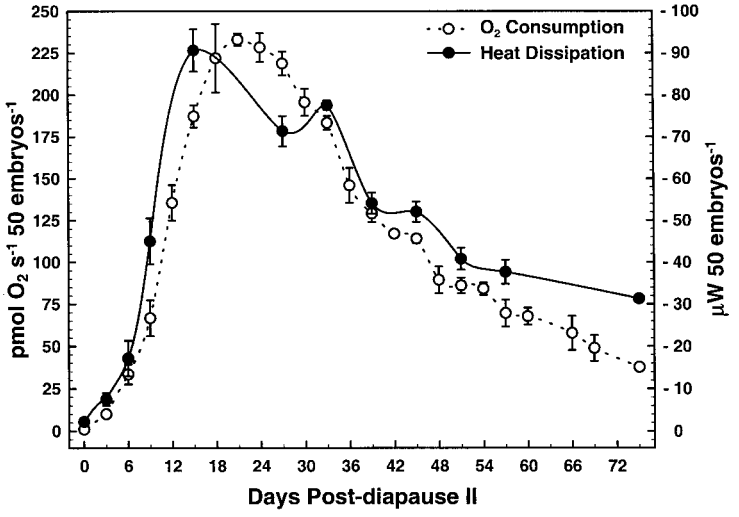

Fig. 6. Oxygen consumption and heat dissipation decline slowly after the onset of diapause III (21 days post-diapause II) in embryos of *A. limnaeus*. Values are means±S.E.M. (n=4 independent spawning events) (redrawn from [31]).

by high ATP/ADP ratios (Fig. 7a). ATP/ADP ratios for embryos of *A. limnaeus* are substantially higher than in other fish embryos [21,22]. The maintenance of a high energy charge in diapause embryos of *A. limnaeus* is consistent with other organisms that enter diapause and suggests a tightly-coupled downregulation of energy producing and energy consuming processes. Embryos of *A. franciscana*, an organism that does not maintain high AEC or ATP/ADP ratios during anaerobic quiescence (see Section 2.3), contain high levels of ATP during diapause as evidenced by ^{31}P-NMR [35,36]. As a likely consequence, these diapause embryos do not experience the pronounced decrease in intracellular pH that is associated with anaerobic quiescence in *A. franciscana* [35]. Thus, due to the high levels of adenylates in embryos of *A. limnaeus*, we predict no major changes in intracellular pH occur during diapause II [31].

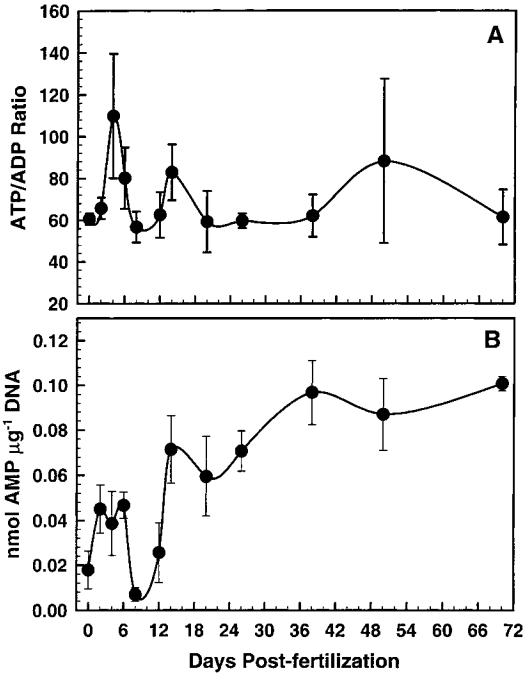

Fig. 7. (A) ATP/ADP ratios are high during diapause II in embryos of *A. limnaeus*. (B) AMP is elevated in diapause II embryos. Diapause II begins at 24 dpf. Values are means±S.E.M. (n=4 independent spawning events) (redrawn from [31]).

Levels of AMP are elevated in diapause II embryos of *A. limnaeus* despite high ATP/ADP ratios (Fig. 7b). AMP/ATP ratios correlate strongly with both indicators of metabolic rate (Fig. 8). This strong correlation suggests that AMP may be acting to regulate the depression of metabolism in diapause II embryos of *A. limnaeus*. Elevated levels of AMP activate the AMP-activated protein kinase (AMPK), which is known to phosphorylate and deactivate a number of key biosynthetic enzymes [37,38]. Additionally, AMPK may play a role in the control of cellular proliferation by preventing the activation of MAP kinases by Raf-1 [38]. The role of elevated AMP in the control of anabolic processes and cell proliferation

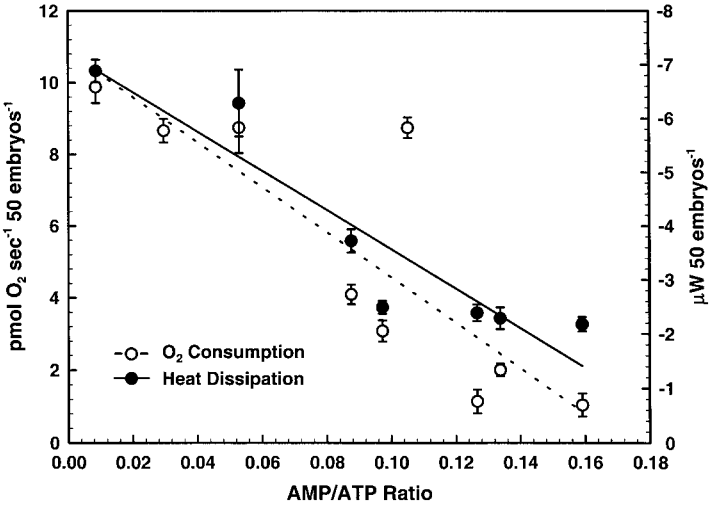

Fig. 8. There is a strong negative correlation between AMP/ATP ratios and both oxygen consumption ($r=-0.852$, $p=0.0035$) and heat dissipation ($r=-0.934$, $p=0.0021$) in early embryos of *A. limnaeus*. Values are means±SEM ($n=4$ independent spawning events). (redrawn from [31]).

in embryos of *A. limnaeus* is an area that deserves future attention.

Depression of metabolism is often accompanied by a depression in the rate of protein synthesis (cf. [7]). In embroys of *A. limnaeus*, a substantial (93%) decline in the relative rate of protein synthesis is observed during diapause II (Podrabsky and Hand, unpublished observations). This reduction in energy demand can account for 36% of the metabolic depression observed and implies that other energy consuming processes, such as ion transport, must be substantially down-regulated during diapause II. In contrast, depression of protein synthesis appears to be even more profound in diapausing embryos of the brine shrimp *A. franciscana* [27].

2.3. Quiescence in brine shrimp embryos

Encysted embryos of the brine shrimp *A. franciscana* are capable of acute metabolic arrest during anoxic exposure. Oxygen limitation is often prevalent within the thick windrows of cysts (and decaying algal mats) that accumulate along the shorelines of the hypersaline lakes inhabited by brine shrimp [39]. In

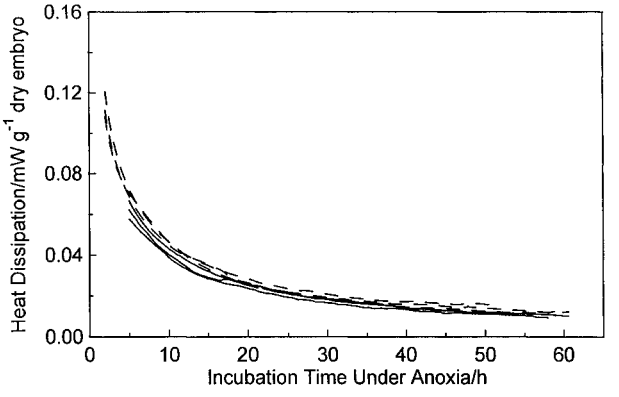

Fig. 9. Heat dissipation by *A. franciscana* embryos under anoxia in sealed ampoules. Data are from experiments using 5 cm^3 (dashed lines) and 25 cm^3 (solid lines) ampoules (redrawn from [18]). Measurable heat flux is still present after 50–60 h. (redrawn from [44]).

Table 3
Calorimetry data for aerobic, nitrogen perfused, and sealed-ampoule (anoxic) embryos of *A. franciscana* (after [44])

Experiment	Heat flow (mW g^{-1})	Percentage of aerobic value
Fully aerobic embryos[a] (flow through chamber) [41,42]	6.35±0.074 (n=5)[b]	–
Nitrogen perfused embryos (flow through chamber) [42]	0.0314[c]	0.49
Anoxic embryos[d] (sealed ampoule experiments; present study)	0.0128±0.0007 (n=6)[b]	0.20

[a] 5 h aerobic incubation.
[b] Values are means±one SE.
[c] Value at the termination of long-term experiment.
[d] Calculated at 50 h for sealed ampoules.

response to the lack of oxygen, these gastrula-stage embryos enter a state termed anaerobic quiescence and can withstand anoxia at room temperature for four years with remarkable survivorship [40].

Heat flow is depressed to extremely low levels [41–44] as embryos enter anaerobic quiescence (Fig. 9, Table 3). Calorimetric studies with sealed ampoules showed that heat dissipation dropped to 0.2% of aerobic values after 50 h of anoxia and the rate was still declining when experiments were terminated [44]. The absolute level of depression was lower with sealed ampoules than with the open-flow system (0.49%; Table 3) where embryos were perfused with nitrogen-saturated medium [42]. Thus, it is possible that trace quantities of oxygen (below the detection level of the polarographic oxygen sensors) were present in the flow-through studies. At these low heat flux values, even processes like protein degradation can make a quantitatively significant (16%) contribution to the heat flow [44]. These are the lowest heat dissipation values thus far reported for organisms under anoxia (cf. [11,45]). At some point during prolonged anoxia, it is not unreasonable to propose that the embryos may actually reach an ametabolic state (see arguments presented by Clegg [40]).

The largest intracellular pH (pH_i) transition ever measured for living cells occurs within minutes after exposure of *A. franciscana* embryos to anoxia. pH_i drops by at least one full unit to 6.7 within 20 min [46,47], and to as low as 6.3 after several hours [46]. The pH_i recovers to aerobic control values within minutes of reoxygenation, and this pattern is superimposable on the metabolic recovery after short term anoxia [41,48]. A qualitatively similar state of metabolic arrest can be promoted artificially by exposing the embryos to elevated levels of CO_2 in the presence of oxygen (aerobic acidosis) [49]. The pH_i of embryos under the artificial condition of aerobic acidosis is 6.8 [49], and the biochemical features describing the shutdown of carbohydrate metabolism are virtually identical to anoxia [50]. Exposure to aerobic acidosis, which markedly depressed heat flow, did not appreciably alter the CR ratio (-481 kJ mol^{-1} O_2) [41]. Thus, nonoxidative metabolism was not stimulated to compensate for the depression of aerobic metabolism under aerobic acidosis [41]. ATP levels drop substantially during anoxia [50,51], but under aerobic acidosis ATP does not change from control values for several hours [50,52]. Thus, the metabolic depression is not dependent upon the decrease in ATP levels. These and other observations (reviewed in [53]) provide support for pH_i as one cellular signal in the metabolic switching.

For there to be such a dramatic depression of energy flow during anoxia-induced quiescence, it is apparent that there must be a coordinated downregulation of energy demand and energy production. On the energy demand side, the energetically expensive processes depressed under anoxia in brine shrimp embryos include transcription and translation. Recent studies utilizing transcriptional run-on assays indicate that RNA synthesis is depressed by low oxygen and acidic pH in both nuclei (F. van Breukelen and S. Hand, unpublished observations) and mitochondria (B. Eads and S. Hand, unpublished observations). Similarly, translation is acutely depressed in the cytoplasm of these embryo cells, as judged by in vivo protein labelling [54,55] polysome profiles [56], and cell free translation assays [57].

Experiments with isolated mitochondria from *Artemia* embryos indicate that arrest of protein synthesis also occurs in this cellular compartment [58–60]. Fig. 10 shows the depression of protein synthesis that occurs in isolated mitochondria from *A. franciscana*

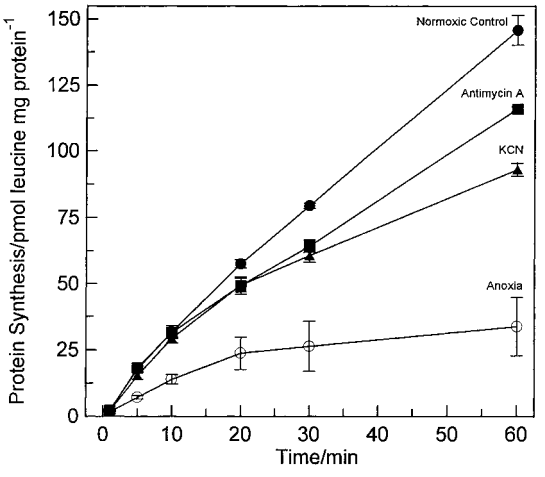

Fig. 10. Comparison of the effects of antimycin A, KCN, and anoxia on protein synthesis in isolated mitochondria from *A. franciscana* embryos. Filled circles are normoxic controls, squares are assays containing 100 μM antimycin A, triangles are assays containing 500 μM KCN, and open circles are anoxic assays. Data are means±SE for at least three independent assays from multiple mitochondrial preparations at each time point (redrawn from [60]).

embryos under anoxia. While mitochondrial translation was acutely inhibited by acidic pH, the process was also depressed by the removal of oxygen in the absence of any pH change [59,60]. Changes in adenine and guanidine nucleotides in the matrix did not appear sufficient to explain effect of anoxia. If the inhibition of protein synthesis by removal of oxygen was caused simply by blockage of the electron transport chain, then chemical anoxia should elicit a quantitatively identical response. Surprisingly, chemical anoxia (saturating levels of cyanide and antimycin A under aerobic conditions) did not inhibit protein synthesis nearly to the same degree as anoxia (Fig. 10). One explanation for this cyanide- and antimycin-insensitive, but hypoxia-sensitive, pattern of protein synthesis depression is the presence of a molecular oxygen sensor within the mitochondrion (cf. [61–63]). Multiple signals are apparently operative in promoting the suite of biochemical and physiological adjustments that accompany anaerobic quiescence in *A. franciscana* embryos.

Because biosynthesis is effectively shut off during anoxia-induced quiescence, the embryos cannot replenish macromolecules lost due to normal turnover. It is now clear that protein degradation is arrested under anoxia in these embryos [52,64–66], thereby stabilizing existing proteins until such time as biosynthesis resumes. Less is known about mRNA stability during quiescence in *A. franciscana* embryos (cf. [56,67]), and current studies are underway to evaluate the fate of this class of macromolecule as well.

3. Conclusions

Several key differences between diapause and quiescence become evident as a result of the preceding comparisons. Aside from being endogenously controlled, we know very little about the molecular mechanisms that promote entry into diapause, and much additional work will be required to unravel these events. In the case of quiescence, we focused in this review on the state induced by anoxia; it is apparent that both the transition in pH_i and the removal of molecular oxygen per se combine to coordinate this form of metabolic arrest in *A. franciscana* embryos. Transitions in pH_i are apparently not involved in entry into diapause [35].

Another difference between these two dormant states is the time period required to downregulate metabolism. For both vertebrates and invertebrates, the metabolic arrest accompanying diapause requires several days before maximal depression is achieved. In contrast, over 90% of the depression of energy flow during anoxia-induced quiescence occurs in the first hour after exposure to oxygen deprivation. It is plausible that diapause requires the specific expression of certain gene products, or conversely, the elimination of products via macromolecular turnover — both of which can require considerable periods of time. The application of differential display and other subtractive methods used to screen for state-specific RNA messages could prove useful in future studies of diapause. Perhaps the global controls of gene expression observed during entry into quiescence operate more quickly and do not require macromolecular synthesis or degradation. We do not have any evidence thus far from our studies with *A. franciscana* embryos that differential gene expression occurs during quiescence.

A final point that deserves mention is the marked difference in energy status between the two states. All

evidence so far indicates that a highly-charged adenylate pool is maintained during diapause, while during quiescence (at least for the anoxia-induced form) ATP levels drop precipitously over the first hour. This net ATP hydrolysis contributes to the intracellular acidosis that itself is a signal coordinating the downregulation of metabolism in *A. franciscana* embryos. Regardless of the ultimate reason for this dichotomy in energy status, the fact that these embryos can withstand such a large swing in intracellular ATP provides a contrasting perspective to the issue of proximal causes of eukaryotic cell death, for which ATP depletion has long been viewed as an indicator (e.g., [68]). Certainly ATP depletion, in and of itself, should not be considered the causative agent.

Acknowledgements

This work was supported by NSF Grant IBN-9723746 to SCH and Dean's Small Grant Awards and an EPO Graduate Research Award to JEP.

References

[1] J.H. Crowe, J.S. Clegg (Eds.), Anhydrobiosis, Dowden, Hutchinson and Ross, Stroudsburg, PA, 1973, 477pp.
[2] S.C. Hand, Adv. Comp. Environ. Physiol. 8 (1991) 1.
[3] D. Keilin, Proc. Roy. Soc. London B 150 (1959) 149.
[4] A. Mansingh, Can. Entomol. 103 (1971) 983.
[5] P.W. Hochachka, M. Guppy, Metabolic Arrest and the Control of Biological Time, Harvard University, Cambridge, MA, 1987, 227pp.
[6] M. Guppy, C.J. Fuery, J.E. Flanigan, Comp. Biochem. Physiol. 109B (1994) 175.
[7] S.C. Hand, I. Hardewig, Annu. Rev. Physiol. 58 (1996) 539.
[8] S.C. Hand, J. Exp. Biol. 201 (1998) 1233.
[9] S.H. Loomis, S.C. Hand, P.E. Fell, Biol. Bull. 191 (1996) 385.
[10] P.E. Fell, Int. J. Invertebr. Reprod. Dev. 11 (1987) 305.
[11] E. Gnaiger, J. Exp. Zool. 228 (1983) 471.
[12] E. Gnaiger, R.B. Kemp, Biochim. Biophys. Acta 1016 (1990) 328.
[13] S.C. Hand, In: R.B. Kemp (Ed.), Handbook of Thermal Analysis and Calorimetry, Vol. 4, Elsevier Science, Amsterdam, (1999) 469–510.
[14] S.H. Loomis, L.F. Ungemach, B.R. Branchini, S.C. Hand, P.E. Fell, Biol. Bull. 191 (1996) 393.
[15] J.E. Reiswig, T.L. Miller, Invert. Biol. 117 (1998) 1.
[16] T.L. Simpson, G.A. Rodan, Dev. Biol. 49 (1976) 544.
[17] J.P. Wourms, J. Exp. Zool. 182 (1972) 143.
[18] J.P. Wourms, J. Exp. Zool. 182 (1972) 169.
[19] J.P. Wourms, J. Exp. Zool. 182 (1972) 389.
[20] T.-O. Hishida, E. Nakano, Embryologia 2 (1954) 67.
[21] L.S. Milman, Y.G. Yurowitzki, In: A. Wolsky (Ed.), Monographs in Developmental Biology, Vol. 6, Karger, New York, 1973, 106pp.
[22] H. Boulekbache, Amer. Zool. 21 (1981) 377.
[23] W.A. Walsh, C. Swanson, C.-S. Lee, J.E. Banno, H. Eda, J. Fish Biol. 35 (1989) 347.
[24] K.T. Paynter, L. DiMichele, S.C. Hand, D. Powers, J. Exp. Zool. 257 (1991) 24.
[25] R.N. Finn, J. Widdows, H.J. Fyhn, Mar. Biol. 122 (1995) 157.
[26] P.J. Levels, R.E.M.B. Gubbels, J.M. Denuce, Comp. Biochem. Physiol. 84A (1986) 767.
[27] J.S. Clegg, L.E. Drinkwater, P. Sorgeloos, Physiol. Zool. 69 (1996) 49.
[28] E. Gnaiger, R. Lackner, M. Ortner, V. Putzer, R. Kaufmann, Eur. J. Physiol., Suppl. 131 (1981) R57.
[29] J. van Waversveld, A.D.F. Addink, G. van den Thillart, J. Comp. Physiol. B 159 (1989) 263.
[30] D. Johansson, G.E. Nilsson, E. Törnblom, J. Exp. Biol. 198 (1995) 853.
[31] J.E. Podrabsky, S.C. Hand, J. Exp. Biol. 202 (1999) 2567.
[32] R.S. Darken, K.L.M. Martin, M.C. Fisher, Physiol. Zool. 71 (1998) 400.
[33] L. DiMichele, M.H. Taylor, J. Exp. Zool. 214 (1980) 181.
[34] L. DiMichele, D.A. Powers, Physiol. Zool. 57 (1984) 46.
[35] L.E. Drinkwater, J.H. Crowe, J. Exp. Zool. 241 (1987) 297.
[36] L.E. Drinkwater, J.S. Clegg, In: R.A. Browne, P. Sorgeloos, C.N.A. Trotman (Eds.), Artemia Biology, CRC Press, Boca Raton, 1991, pp. 93–117.
[37] D.G. Hardie, D. Carling, Eur. J. Biochem. 246 (1997) 259.
[38] D.G. Hardie, D. Carling, M. Carlson, Annu. Rev. Biochem. 67 (1998) 821.
[39] J.S. Clegg, Trans. Amer. Micros. Soc. 93 (1974) 481.
[40] J.S. Clegg, J. Exp. Biol. 200 (1997) 467.
[41] S.C. Hand, E. Gnaiger, Science 239 (1988) 1425.
[42] S.C. Hand, J. Comp. Physiol. B 160 (1990) 357.
[43] F. Hontoria, J.H. Crowe, L.M. Crowe, F. Amat, J. Exp. Biol. 178.
[44] S.C. Hand, J. Exp. Zool. 273 (1995) 445.
[45] J. Widdows, In: A.M. James (Ed.), Thermal and Energetic Studies of Cellular Biological Systems, IOP Publishing Limited, Bristol, 1987, p.182.
[46] W.B. Busa, J.H. Crowe, G.B. Matson, Arch. Biochem. Biophys. 216 (1982) 711.
[47] K. Kwast, J.I. Shapiro, B.B. Rees, S.C. Hand, Biochim. Biophys. Acta 1232 (1995) 5.
[48] S.C. Hand, Pure Appl. Chem. 65 (1993) 1951.
[49] W.B. Busa, J.H. Crowe, Science 221 (1983) 366.
[50] J.F. Carpenter, S.C. Hand, J. Comp. Physiol. B 156 (1986) 451.
[51] D.M. Stocco, P.C. Beers, A.H. Warner, Dev. Biol. 27 (1972) 479.
[52] T. Anchordoguy, S.C. Hand, J. Exp. Biol. 198 (1995) 1299.
[53] S.C. Hand, Acta Physiol. Scand. 161 (1997) 543.

[54] J.S. Clegg, S.A. Jackson, In: A.H. Warner, J. Bagshaw, T. MacRae (Eds.), The Cellular and Molecular Biology of Artemia Development, Plenum Press, New York, 1989, p.1.
[55] G.E. Hofmann, S.C. Hand, Am. J. Physiol. 258 (1990) R1184.
[56] G.E. Hofmann, S.C. Hand, J. Exp. Biol. 164 (1992) 103.
[57] G.E. Hofmann, S.C. Hand, Proc. Natl. Acad. Sci. USA 91 (1994) 8492.
[58] K. Kwast, S.C. Hand, Am. J. Physiol. 265 (1993) R1238.
[59] K. Kwast, S.C. Hand, Biochem. J. 313 (1996) 207.
[60] K. Kwast, S.C. Hand, J. Biol. Chem. 271 (1996) 7313.
[61] M.A. Goldberg, S.P. Dunning, H.F. Bunn, Science 242 (1988) 1412.
[62] G.L. Semenza, P.H. Roth, H.M. Fang, G.L. Wang, J. Biol. Chem. 269 (1994) 23757.
[63] C.C. Tan, P.J. Ratcliffe, Am. J. Physiol. 261 (1991) F982.
[64] T. Anchordoguy, G.E. Hofmann, S.C. Hand, Am. J. Physiol. 264 (1993) R85.
[65] T. Anchordoguy, S.C. Hand, Am. J. Physiol. 267 (1994) R895.
[66] A.H. Warner, S.A. Jackson, J.S. Clegg, J. Exp. Biol. 200 (1997) 897.
[67] I. Hardewig, T. Anchordoguy, D. Crawford, S.C. Hand, Mol. Cell. Biochem. 158 (1996) 139.
[68] D.E. Atkinson, Cellular Energy Metabolism and its Regulation, Academic Press, New York, 1977, 293pp.