The Journal of Experimental Biology 205, 2079–2087 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
JEB4141

Long-term fasting and realimentation in hypogean and epigean isopods: a proposed adaptive strategy for groundwater organisms

Frédéric Hervant[1,*] and David Renault[2]

[1]*Hydrobiologie et Ecologie Souterraines (UMR CNRS 5023), 6 rue Dubois, Université Claude Bernard-Lyon I, F-69622 Villeurbanne Cedex, France* and [2]*Station biologique (UMR 6553 CNRS), Université de Rennes I, F-35380 Paimpont, France*

*Author for correspondence (e-mail: hervant@univ-lyon1.fr)

Accepted 18 April 2002

Summary

The effects of long-term fasting and subsequent refeeding on digestive physiology and energy metabolism were investigated in a subterranean aquatic crustacean, *Stenasellus virei*, and in a morphologically similar surface-dwelling species, *Asellus aquaticus*. Metabolic response to food deprivation was monophasic in *A. aquaticus*, with an immediate, large decrease in all energy reserves. In contrast, *S. virei* displayed three successive periods of phosphageno-glucidic, lipidic and, finally, proteo-lipidic-dominant catabolism over the course of the nutritional stress. To represent the responses of subterranean crustaceans to food stress and renutrition, a sequential energy strategy was hypothesized, suggesting that four successive phases (called stress, transition, adaptation and recovery) can be distinguished. Based on these results, a general adaptive strategy for groundwater organisms was proposed. Their remarkable resistance to long-term fasting may be partly explained by (1) a depressed metabolism, during which they mainly subsist on lipid stores, (2) a prolonged state of glycogen- and protein-sparing, (3) low energetic requirements and (4) large body stores. In addition, these groundwater species displayed high recovery abilities during refeeding, showing an optimal utilization of available food and a rapid restoration of their body reserves. These adaptive responses might be considered for numerous subterranean organisms as an efficient energy-saving strategy in a harsh and unpredictable environment where fasting (and/or hypoxic) periods of variable duration alternate with sporadic feeding events (and/or normoxic periods). Therefore, food-limited and/or hypoxia-tolerant groundwater species appear to be good examples of animals representing a low-energy system.

Key words: starvation, refeeding, subterranean, surface, crustacean, intermediary metabolism, energy metabolism, digestive physiology, adaptive strategy, food-limited biotope.

Introduction

Alternating short periods of feeding and prolonged episodes of fasting are experienced by numerous subterranean (i.e. hypogean) aquatic organisms, as many groundwater ecosystems are characterized yearly by severely limited food supplies due to lack of autotrophic production and sporadic allochthonous input (Poulson, 1964; Hüppop, 1985; Hervant et al., 1997). Even in highly productive hypogean biotopes, food resources may be extremely patchy (Malard and Hervant, 1999). In addition, many hypogean aquatic species have to cope with periods of prolonged hypoxia (Hervant et al., 1996; Malard and Hervant, 1999). An organism's ability to withstand and recover from long periods of food shortage is a critical adaptation for survival in harsh, extreme, biotopes such as groundwaters. Undernourishment experienced during ontogeny and/or postembryonic development has important consequences for life history (Brzek and Konarzewski, 2001) and, although instantaneous ecological consequences of poor and spotty nutrition (and/or hypoxia) are sometimes difficult to distinguish, the reproductive potential of any animals experiencing such conditions may become reduced so the effects will manifest at the population level. It is therefore hypothesized that several hypogean species possess specific behavioral, physiological and/or metabolic adaptations that allow them to successfully exploit subterranean environments. These features suggest that subterranean species are excellent models to study the effects and responses to prolonged fasting.

Hervant et al. (1997) discovered that the hypogean aquatic isopod *Stenasellus virei* surviving prolonged fasting (exceeding 200 days) longer than the surface-dwelling species *Asellus aquaticus* and most other crustaceans previously studied. The hypogean amphipods *Niphargus virei* and *N. rhenorhodanensis* and the cave amphibian *Proteus anguinus* also showed high tolerance to starvation (Hervant et al., 1999b, 2001). During long-term fasting in these subterranean species, locomotory, ventilatory and metabolic rates were drastically reduced, whereas surface-related species exhibited only slight

decreases in these rates and responded with a transitory hyperactivity. Hervant et al. (1997) hypothesized that the ability of hypogean species to survive prolonged starvation probably involves their entering into a state of temporary torpor, during which they subsist only on endogenous energy reserves. Unfortunately, little information is available on the fasting-induced metabolic and physiological responses of these organisms. Therefore, identifying the changes in the digestive performance, biochemical composition and energy content of such organisms under conditions of food limitation and refeeding would improve our understanding of the competitive abilities of these hypogean species, and their ability to exist in food-limited biotopes.

This study was designed to examine whether the behavioral and whole-animal physiological responses (i.e. oxygen consumption) during prolonged food deprivation and subsequent refeeding that had previously been identified for the groundwater isopod *Stenasellus virei* (Hervant et al., 1997) are accompanied by specific changes in intermediary and energy metabolism (e.g. energy allocation patterns, qualitative and/or quantitative changes in body composition). We recorded some metabolic parameters (ammonia, arginine, arginine phosphate, glucose, glycerol, glycogen, proteins, triglycerides and non-esterified fatty acids) during a 180-day fasting period and a subsequent 15-day feeding phase in a subterranean aquatic isopod, *Stenasellus virei*. In addition, we investigated the feeding and digestive strategies (i.e. food-searching behavior and regulation of digestive performance) of this isopod during refeeding. To generalise the energy strategy for groundwater organisms, we undertook a parallel study during a 28-day fasting period and a subsequent 7-day feeding phase in the morphologically similar surface-dwelling isopod *Asellus aquaticus*.

Materials and methods

Animals and experimental conditions

Stenasellus virei Magniez (hypogean isopods, 12.2±0.8 mg wet mass) were collected from a groundwater system hydraulically connected with the river Tarn, using special pumps lowered into piezometers (11 m deep) in a phreatic system at Cantepau (AEP d'Albi, France). *Asellus aquaticus* L. (epigean isopods, 15.1±1.0 mg wet mass) were collected with a net from a backwater of the Rhône river at Balan (France). All animals were maintained in recirculating aquaria containing groundwater pumped from the aquifer of University Lyon 1. Tanks holding *S. virei* contained clay and stones removed from the collection sites. Tanks holding *A. aquaticus* contained stones and live plant material which they use for food, collected from the collection site for this species. *S. virei* were fed with minced meat every 2 weeks. All aquaria were kept in a controlled temperature facility (11±0.3 °C) in constant darkness.

Individuals of both species were acclimated to laboratory conditions for 2 months prior to separating into control and treatment groups. Adults of both groups (males only) were placed into 400 ml glass flasks (containing 250 ml water and pieces of fine plastic grid as an artificial substrate) for experimentation. Water in the flasks was renewed weekly. For both species, the isopods of the control group were fed as described above. Treatment groups were deprived of food for 180 days (hypogean species, *S. virei*) or 28 days (epigean species, *A. aquaticus*), according to their survival times while fasting (Hervant et al., 1997). Following the fasting periods, individuals were refed twice over a 15-day (*S. virei*) or 7-day (*A. aquaticus*) period. Throughout the study, mortality was considered negligible (<3 %) for both species.

Sample preparation and metabolite assays

To investigate changes in dry mass, water content and whole-body metabolites during food deprivation, ten individuals were sampled at intervals of 0, 15, 30, 60, 90, 120, 150 and 180 days of fasting for *S. virei*, and at 0, 7, 15, 21 and 28 days of fasting for *A. aquaticus*. Control (fed) organisms were removed as described above (for each point, N=10 individuals). To identify changes in dry mass, water content, digestive metabolism and whole-body metabolites during recovery from long-term fasting, individuals were fasted for either 180 days (*S. virei*) or 28 days (*A. aquaticus*), and then refed (see above). Ten refed individuals were sampled at intervals of 4 and 15 days for *S. virei*, and at 3 and 7 days for *A. aquaticus*.

Once removed, control, fasted and refed individuals were immediately anaesthetized by placing the animals for 5 min into a tricaine methane sulfonate solution (0.5 g l^{-1}) (Sandoz MS-222), rapidly dissected to remove the gut content, weighed (wet mass), frozen in liquid nitrogen, lyophilized (Virtis lyophilisator, Trivac D4B) and then re-weighed (dry mass). Lyophilized individuals were homogenized (as described in Hervant et al., 1995) and stored at –80 °C until body metabolites were assayed. Gut contents were also lyophilized and weighed (dry digesta mass). In addition, the time until the first defecation (i.e. passage time) was recorded following refeeding.

Ammonia excretion rates were determined for both species from a sample of incubation water in which control, fasted or refed animals (N=10) were held for 12 h, as described (Hervant et al., 1996, 1997). The following metabolites were assayed by standard enzymatic methods as described (Hervant et al., 1995, 1996): ammonia (NH_4^++NH_3), arginine, arginine phosphate, glucose, glycerol and glycogen. Total proteins, triglycerides and non-esterified fatty acids were extracted according to the methods of Elendt (1989) and Barclay et al. (1983) and then measured using specific test-kits (Bœhringer-Mannheim). All assays were performed using a Beckman DU-6 spectrophotometer set at 25 °C. The accuracy of each analysis was tested by assaying the samples with and without an added internal standard. The sensitivity of all assays was approximately 1 μmol g^{-1} dry mass for all metabolites. Enzymes, coenzymes and substrates used for enzymatic assays were purchased from Bœhringer (Mannheim, Germany) and Sigma Co. (St Louis, USA).

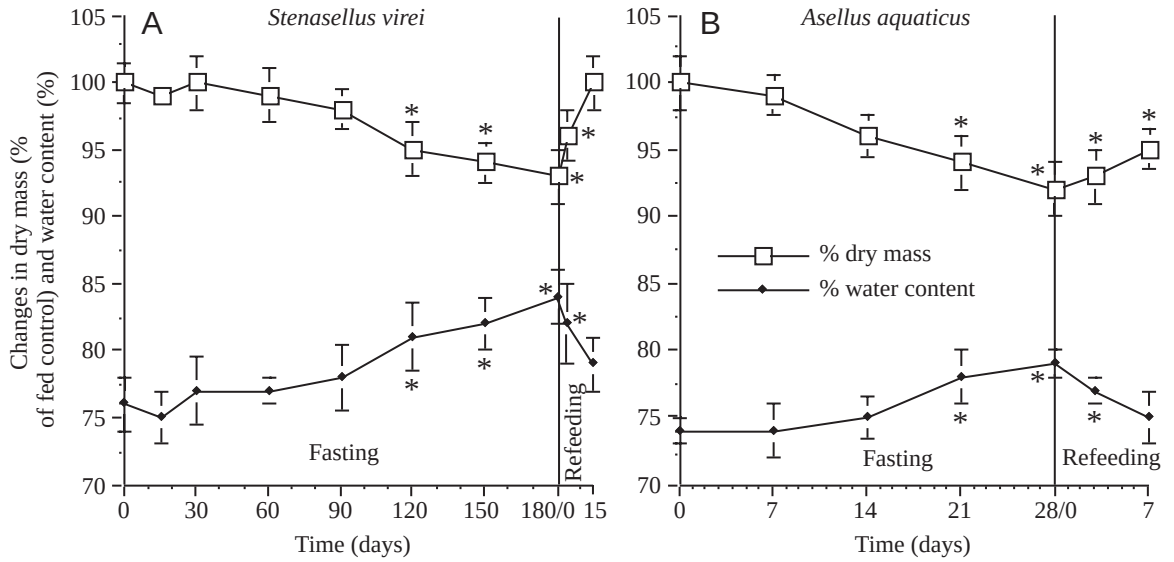

Fig. 1. Changes in dry mass (expressed as a percentage of the fed control; open squares) and percentage water content (filled triangles) during long-term fasting and subsequent refeeding in (A) the subterranean crustacean *Stenasellus virei* and (B) the surface-dwelling crustacean *Asellus aquaticus* at 11 °C, in darkness. Values are means ± S.E.M. for N=10 animals. *Value significantly different from fed control (at time 0) (P<0.05).

Statistical analyses

Values are presented as means ± S.E.M. Comparisons among means were conducted with a one-way analysis of variance (ANOVA), using a Bonferroni test for multiple comparisons as appropriate. For comparisons between means (at the P<0.05 level) and after verification of normality of values, a Tukey test was used. Statistical analyses were performed with the StatView 5® software package (Abacus).

Results

Control (fed) organisms showed no changes in behavioral, physiological or biochemical variables throughout the experimental period (not shown).

Body mass and water content

In control (fed) organisms, dry mass and water content did not vary significantly between sampling periods (not shown). Fasted animals showed a slight decrease in mean percentage dry mass (–7 % after 180 days in the hypogean *S. virei*, –8 % after 28 days in the epigean *A. aquaticus*), and a small increase (Fig. 1) in mean percentage water content (+8 % after 180 days in *S. virei*, +5 % after 28 days in *A. aquaticus*), although it was not significantly different until 120 days in the subterranean species and 21 days in *A. aquaticus*. With refeeding, both dry mass and water content resumed pre-fasting levels in *S. virei*, while dry mass showed a slight, but non-significant, recovery for *A. aquaticus* (Fig. 1).

Digestive responses during refeeding

Time of first defecation (i.e. passage time) did not differ between control and refed animals, but was significantly longer in *S. virei* (8.3±1.0 days) than in *A. aquaticus* (5.2±0.7 days) isopods (results not shown). Dry digesta mass (i.e. food intake) was 1.5-fold greater in refed than in control *S. virei*, but did not differ between control and refed *A. aquaticus* (results not shown).

Effect of fasting and subsequent refeeding on metabolite body levels in the subterranean S. virei

Arginine phosphate content decreased significantly by day 30 of fasting, reaching 73 % of its initial value (fed level) after 180 days of food deprivation, and quickly returned to the pre-fast value during refeeding (Fig. 2A). During the 180 days of fasting, only 8.5 μmol g^{-1} dry mass of arginine phosphate was metabolized. Arginine content showed a significant increase by day 14 of fasting (+19 %), then dramatically decreased from day 120 to 46 % of the control value after 180 days fasting (Fig. 2A).

Body glycogen content decreased by day 30 of food deprivation in *S. virei* (Fig. 2B), reaching 83 % of its initial content by day 60 (corresponding to a utilization of 53 μmol glycosylic unit g^{-1} dry mass). Glycogen levels then returned to the pre-fasting level after 120 days fasting. Glycogen content dramatically increased within the first week of refeeding (reaching 121 % of the fed value), before returning to the pre-fasting level (Fig. 2B). Moreover, we found no significant change in whole animal glucose content (Fig. 2C) in both fasting and refeeding periods.

Proteins were significantly metabolized after 120 days lack of food, until they reached 80 % of the fed level by day 180 (Fig. 2D), corresponding to a utilization of 0.11 g g^{-1} dry mass. During refeeding, protein content returned to pre-fasting levels by day 15 (Fig. 2D). Moreover, the ammonia excretion rate (NH_4^++NH_3, calculated from its cumulation in the flask water during an incubation period of 12 h) remained constant for 120 d of fasting, followed by a slight increase (Fig. 2D). During

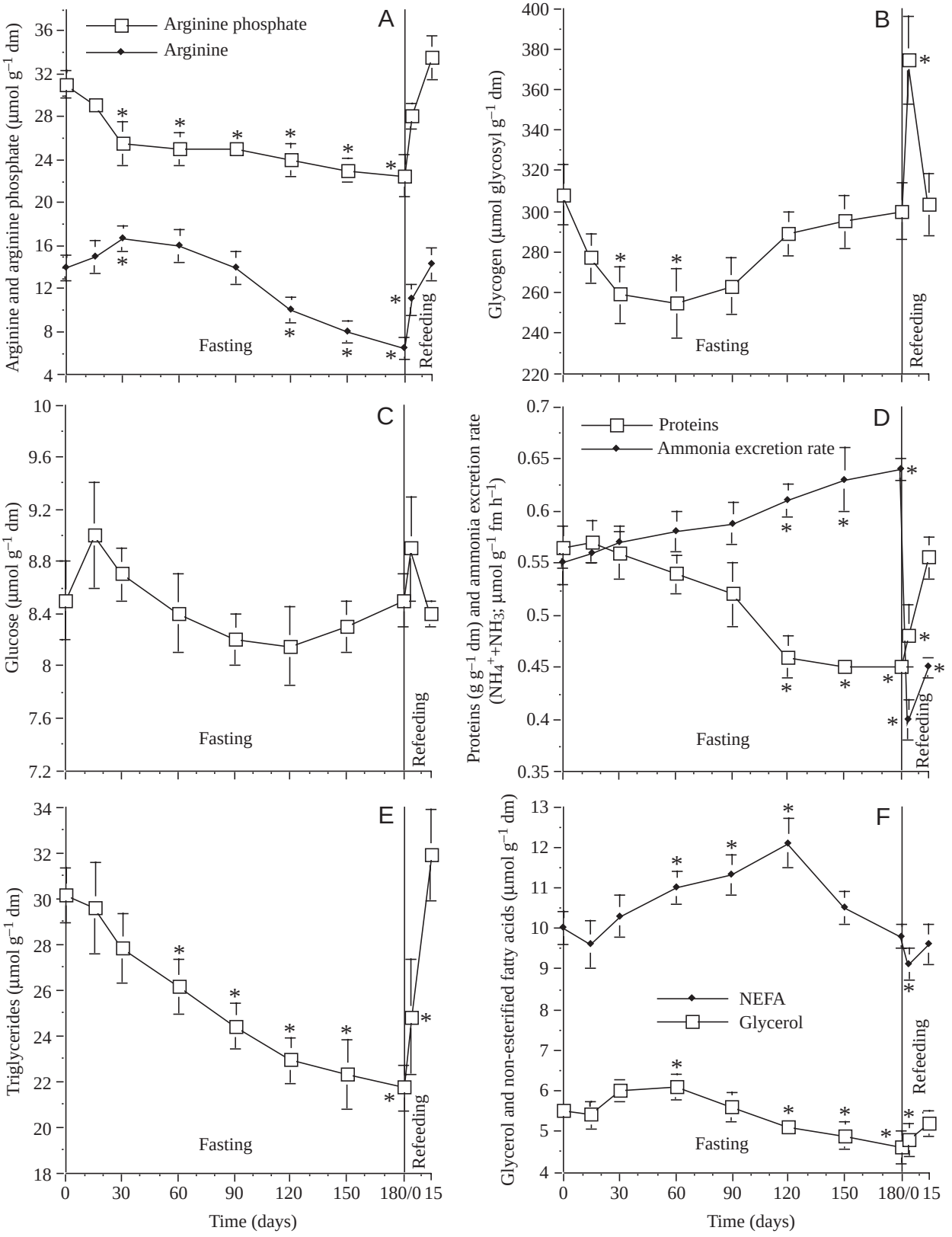

Fig. 2. Changes in the levels of metabolites during long-term fasting (180 days) and subsequent refeeding (15 days) in the subterranean crustacean *Stenasellus virei* at 11 °C, in darkness. Values are means ± S.E.M. for N=10 animals. *Value significantly different from fed control (at time 0) (P<0.05). dm, dry mass; fm, fresh mass. NEFA, non-esterified fatty acids.

refeeding, this rate immediately decreased to 73 % of the initial level (Fig. 2D).

Triglyceride (TG) stores had significantly decreased by day 60 of fasting, and reached 72 % of their initial value after 180 days of food deprivation (Fig. 2E), representing a total utilization of 8.4 μmol g^{-1} dry mass. With refeeding, TG content rebounded to the pre-fast level (Fig. 2E). In contrast, non-esterified fatty acids (NEFA) levels significantly increased between 60 and 120 days of food deprivation, before returning to the pre-fast level (Fig. 2F). Upon recovery from nutritional stress, NEFA content immediately decreased, before returning to control level (Fig. 2F). During fasting, glycerol content showed a significant increase on day 60 then immediately decreased, reaching 84 % of the fed level after 180 days (Fig. 2F). Refeeding resumed glycerol levels to the control value (Fig. 2F).

Effect of fasting and subsequent refeeding on metabolite body levels in the epigean A. aquaticus

A. aquaticus showed a significant decrease in arginine phosphate content by day 14 of fasting, losing 41 % of its initial content by day 28 (Fig. 3A), corresponding to a total utilization of 6.6 μmol g^{-1} dry mass. On refeeding, arginine phosphate content increased, reaching 80 % of its initial amount within 7 days (Fig. 3A). In contrast, arginine content showed the opposite response, increasing with fasting and decreasing with refeeding (Fig. 3A).

With fasting, whole-animal glycogen content immediately fell sharply to 33 % of its initial concentration within 28 days (Fig. 3B), corresponding to a utilization of 90 μmol glycosylic unit g^{-1} dry mass. Refeeding allowed a slow increase in glycogen to 60 % of the initial content within 7 days (Fig. 3B). Body glucose significantly decreased from 14 days fasting and reached 88 % of the initial content after 28 days (Fig. 3C). Glucose concentration returned to the fed value during refeeding (Fig. 3C).

Fasting lead to a significant decrease in body protein by day 14 and a total decline of 21 % by day 28 (Fig. 3D), representing a utilization of 0.11 g g^{-1} dry mass. During refeeding, *A. aquaticus* protein content slowly increased, reaching 87 % of the pre-fast level within 7 days (Fig. 3D). Ammonia excretion rate increased immediately with fasting, up to 115 % of the pre-fast level by day 28, and subsequently decreased with refeeding (Fig. 3D).

During fasting, TG dramatically and continuously decreased from day 14, reaching 45 % of the initial value by day 28 of the fast, a use of 10.7 μmol g^{-1} dry mass (Fig. 3E). Refeeding enabled the body TG content to slowly increase to 62 % of its initial content within 7 days (Fig. 3E). Body content of NEFA had significantly increased by day 14 of the fast, but was recovered with refeeding (Fig. 3F). Body glycerol concentration also increased from 14 days of fasting (Fig. 3F), but during refeeding rapidly decreased (Fig. 3F), then returned to the initial level after 7 days.

Discussion

Natural groundwater systems (mainly karstic and porous aquifers) are characteristically energy-poor habitats (Malard and Hervant, 1999). The low and infrequent food supply and/or alternating hypoxic and normoxic conditions encountered by many groundwater species (Hüppop, 1985; Hervant et al., 1997) are likely to be strong selective forces in the development of behavioral and metabolic adaptations for these organisms (Hervant et al., 2001).

Body mass and water content during long-term fasting

When animals experience periods of limited food, they have to rely on their own body reserves to fuel metabolic processes and the maintenance of homeostasis. The subterranean (i.e. hypogean) *S. virei* showed lower magnitudes of response to long-term fasting than the surface-dwelling *A. aquaticus*, with a 7.3-fold slower rate of relative mass loss. Some subterranean amphipods and amphibians and numerous epigean animals display fasting responses similar to that of *S. virei* (Hervant et al., 1999a, 2001, and references therein). These results suggest that hypogean organisms utilize their endogenous energy stores at a relatively low rate.

To maintain the necessary body volume (fixed by the exoskeleton in Arthropods) and internal turgidity during fasting, the lost tissue mass (used as metabolic fuel) must be replaced by water (Dall, 1974; Wilcox and Jeffries, 1976; Stuck et al., 1996). Both isopod species followed this pattern, displaying a significant (but low) increase in water content and a corresponding decrease in percentage dry mass during food deprivation.

Metabolic responses to long-term fasting

The capacity to withstand periods of inadequate/poor nutrition depends on the presence (i) of endogenous nutritive stores, and (ii) the necessary adaptive responses (i.e. adjustments in behavior, physiology, and/or energy and intermediary metabolism) to ensure that these stored metabolites are utilized efficiently. A general energy-conserving physiological response to starvation is a lowering of standard metabolic rate (SMR) (Fuglei et al., 2000). For both species, experimental data on fasting-induced changes in body composition indicated significant utilization of phosphagen (arginine phosphate), glycogen, triglycerides (TG) and proteins reserves.

Fed *S. virei* possesses large glycogen reserves, 2.3-fold greater than fed *A. aquaticus*, and significantly higher than those usually found in epigean crustaceans (reviewed in Hervant et al., 1996). In addition, the hypogean species possessed significantly greater arginine phosphate (×2.2) and TG (×1.5) reserves than *A. aquaticus*, allowing them to fuel their metabolism for a much longer time while fasting, thus prolonging their survival. For groundwater species, energy stores at the beginning of a fast have to be sufficient to allow survival for an unpredictable duration, but paradoxically, should not be too large, because body reserves are energetically costly to transport and might reduce mobility, thus increasing the risk of predation and/or reducing food-searching abilities. Consequently, energy stored during periods

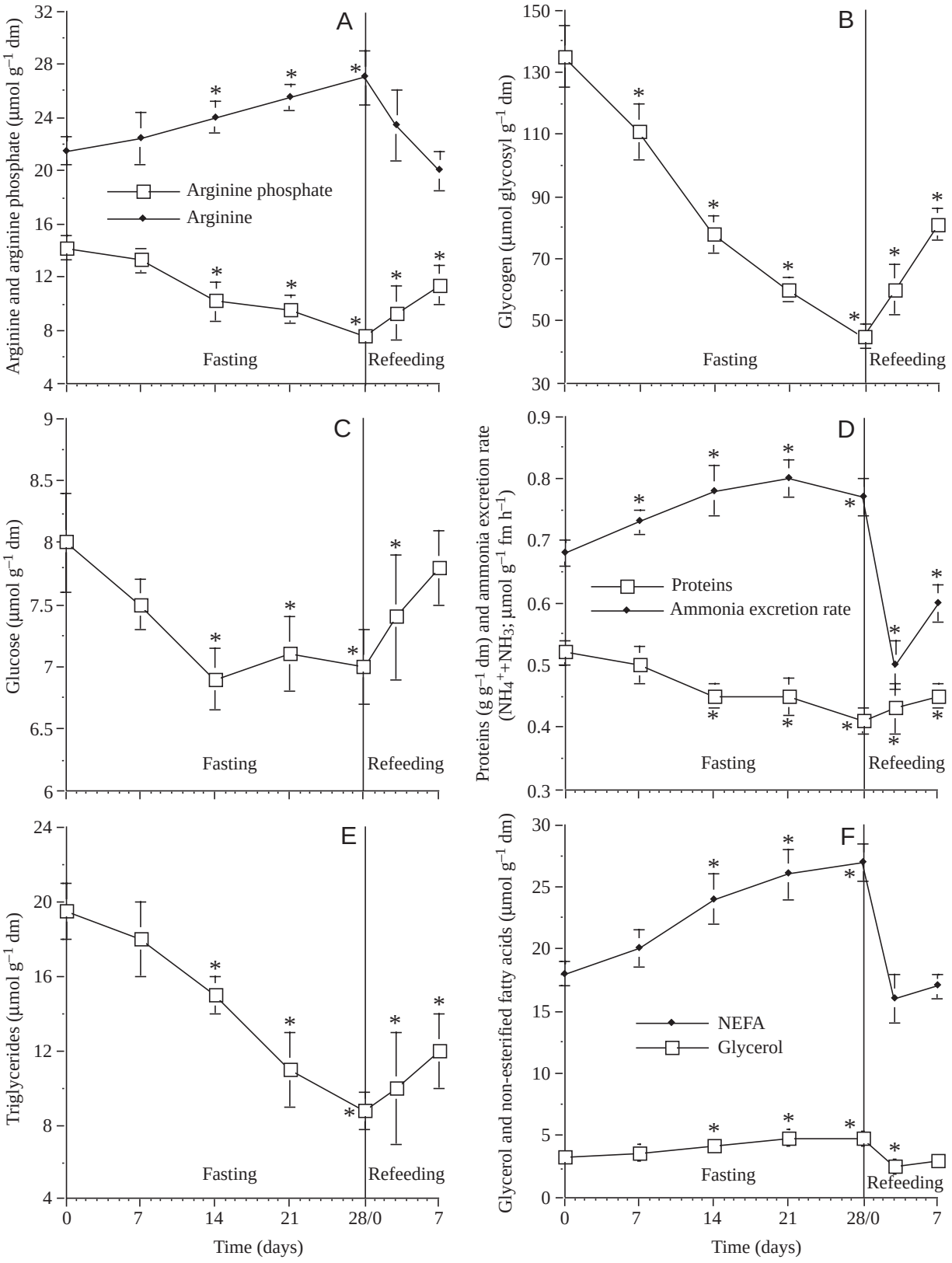

Fig. 3. Changes in the levels of metabolites during long-term fasting (28 days) and subsequent refeeding (7 days) in the surface-dwelling crustacean *Asellus aquaticus* at 11 °C, in darkness. Values are means ± S.E.M. for N=10 animals. *Value significantly different from fed control (at time 0) (P<0.05). dm, dry mass; fm, fresh mass. NEFA, non-esterified fatty acids.

of food abundance must always be adjusted to the highest tolerable nutritional stress for hypogean species feeding infrequently. The loss of energy during long-term fasts will therefore be reduced to a minimum. It has been found that the relative metabolic rate of *S. virei* while fasting (i.e. the metabolic rate during fasting divided by that before fasting) is considerably lower than that of *A. aquaticus* (Hervant et al., 1997), so approximately 50 % of the metabolic energy dissipated by the well-fed subterranean species was saved by the starving one, whereas under these conditions no energy was saved by the epigean crustacean. Hervant et al. (1997, 1999b, 2001) observed similar responses for epigean and hypogean amphipods and salamanders. In addition, fed groundwater species often possessed low resting metabolic and activity rates (Hüppop, 1985; Hervant et al., 1997). Although lowering metabolic rate may lead to a reduction in motility, increasing the risk of predation, hypogean animals usually suffer little from predatory pressure. Indeed, subterranean organisms can survive during long periods of food deprivation at a low energetic cost. Moreover, *S. virei* metabolized phosphagen, proteins and TG at low rates under conditions of food deprivation (5.1–8.2 times lower than the epigean *A. aquaticus*), metabolizing glycogen only at the beginning of the fast, but then, surprisingly, later resynthesizing it. This drastic reduction in energy use (which was initially low) sustains their metabolic reserves for as long as possible, therefore increasing survival time under fasting conditions.

Studies that have addressed the effects of fasting on crustaceans have demonstrated both qualitative and quantitative changes in body composition (reviewed in Stuck et al., 1996). The relative importance of metabolic reserves and their order of utilization varies among species (reviewed in Hervant et al., 1999b). Some species actually switch from one stored metabolite to another as prolonged starvation continues (Mayzeau, 1976; Elendt, 1989; Hervant et al., 1999b).

The epigean *A. aquaticus* demonstrated a monophasic response to food deprivation, characterized by an immediate, linear and large decrease in all of its energy reserves. In contrast, prolonged fasting by *S. virei* was characterized by three successive phases: (1) an immediate, but low, depletion of both glycogen and arginine phosphate stores (days 15–60), followed by (2) the utilization of triglycerides associated with glycogen resynthesis (days 60–120) and finally (3) a slow depletion (days 120–180) of both proteins (demonstrated by a slight increase in ammonia excretion rate) and lipids, always associated with a glycogen resynthesis.

In food-limited groundwater species, the rapidly usable carbohydrates and phosphagen stores served only as initial metabolic fuels, before being replaced by lipid reserves. The glycogen *de novo* synthesis observed in *S. virei* after day 60 may be a result of an increased conversion/utilization of amino acids (originating from proteolysis) and/or glycerol (from lipolysis) to glycogen, by glyconeogenesis. This hypothesis is supported by the decrease in both arginine (originating from the utilization of arginine phosphate) and glycerol observed with fasting in this animal. Moreover, the existence of a high glyconeogenic capability has been demonstrated recently in the subterranean crustacean *Niphargus virei* (Hervant et al., 1999a). In contrast, the epigean *A. aquaticus* did not show high glyconeogenic conversion rates of amino acids and/or glycerol during food deprivation.

If the utilized amounts of glycogen, proteins and lipids are completely oxidized to CO_2 and H_2O, then the energy provided by each metabolite can be derived (Elendt, 1989). In the hypogean species, lipids (representing approximately 60 % of the energy consumed during the 180 days fasting period) and proteins (40 % of energy consumed) were the most metabolized substrates in terms of total energy, while glycogen did not contribute to energy production. The epigean *A. aquaticus* had a different energy strategy: proteins (representing approximately 50 % of the energy losses during the 28 day fasting period) and total lipids (45 % of energy loss) were the most metabolized stores, whereas glycogen reserves, although dramatically depleted, seemed not to be preferentially used (5 % of energy loss). The calculated reduction of total energy content was only $34\,J\,g^{-1}$ dry mass day^{-1} for *S. virei*, *versus* $190\,J\,g^{-1}$ dry mass day^{-1} for *A. aquaticus*. Our data are in agreement with the metabolic rates given by Hervant et al. (1997, 2001) for fed and starved hypogean and epigean amphipods and salamanders.

These results demonstrate that the groundwater crustacean *S. virei* (i) has lower energetic requirements and is better adapted to long-term food shortage than the surface-dwelling *A. aquaticus*, and (ii) preferentially utilizes lipids in order to save carbohydrates and phosphagens (the two main fuels metabolized during oxygen deficiency in crustaceans; Zebe, 1991) and, like some mammals (Newsholme and Stuart, 1973; Fuglei et al., 2000), cave amphibians (Hervant et al., 2001) and birds (Le Maho, 1984), to save proteins (and therefore muscular mass) for as long as possible. Thus, this species can successfully withstand a hypoxic period subsequent to (or associated with) an initial nutritional stress, and can rapidly resume searching for food during short-term, sporadic, nutrition events.

Metabolic and digestive responses to refeeding

When food is available once more, it is ecologically very advantageous for organisms to quickly and completely restore the energy reserves that were depleted during nutritional stress, especially in harsh and unpredictable biotopes such as numerous groundwater systems. Refeeding resulted in a partial restoration of body stores within *A. aquaticus*, and in complete restoration within *S. virei*. For both species, the resynthesized body materials replaced the ‘excess’ water accumulated during fasting.

The food-limited *S. virei* resynthesized phosphagen, proteins and TG with high production rates, significantly higher (1.2- to 1.4-fold) than in the frequently feeding *A. aquaticus*. For *S. virei*, these resynthesis rates were 11.0- to 15.5-fold greater than utilization rates (calculated during the whole nutritional stress in starved animals), while *A. aquaticus* only showed a moderate increase (1.2- to 2.3-fold) in these ‘recovery indicators’. Cave amphipods and salamanders also showed

high resynthesis rates (Hervant et al., 1999b, 2001). As a consequence, the rate at which fat stores were deposited while groundwater organisms fed was largely higher than fat accumulation rates measured in numerous wild mammals and birds, including antarctic penguins, which experience prolonged periods of anorexia on land and hyperphagia at sea (reviewed in Groscolas and Robin, 2001).

In *S. virei*, as in other subterranean species (Hervant et al., 2001), body glycogen content displayed a large but transitory increase during refeeding, its concentration strongly exceeding the control level during the first week of refeeding. This response may represent an adaptation for the rapid storage of food energy to be mobilized later for the synthesis of body materials such as TG and proteins.

The ability to maintain and rapidly restore high levels of metabolic stores for use during food deficiency (and/or lack of oxygen; Malard and Hervant, 1999) allows groundwater organisms to fuel successfully an ensuing unpredictable fasting (and/or hypoxic) period and, therefore, to increase their competitive abilities.

Secor (2001) stated that the regulation of digestive performance is an adaptive response of feeding habits. Hervant et al. (1997) showed that immediately after the onset of refeeding, both species presented a large (and transitory) overshoot in oxygen consumption. This increase in metabolism was probably due to the added cost of digestive metabolism, together with any additional cost of upregulating the digestive tract (Secor, 2001). We suspect that both crustaceans regulate their digestive performance, especially the infrequently feeding *S. virei*, which exhibits a larger post-feeding metabolic response than the frequently feeding *A. aquaticus* (Hervant et al., 1997). Secor (2001) noted that infrequently feeding amphibians and reptiles possess lower SMR and experience a greater increase in metabolic rate during digestion than frequent feeders. The preliminary results presented by Hervant et al. (1997, 2001) reinforced this general hypothesis.

Compared to *A. aquaticus*, fasted *S. virei* consumed 50 % more food upon refeeding, leading to an acceleration in the resynthesis of depleted body stores. It was felt that if the intestine was going to significantly upregulate performance in the subterranean isopod, it would do so in response to this large digestive load. This feeding behavior appears to be a good adaptive response to an extreme biotope, often simultaneously unpredictable (concerning food and oxygen) and energy-poor, in which infrequent meals must be optimally utilized.

From the observed passage times, digestion rates appeared slower in the food-limited groundwater species than in the frequently feeding epigean species, probably maximizing assimilation of available nutrients. The ‘digestive efficiency’ (defined in this study as the gain in body mass per gram of O_2 consumed and per day, and calculated from the extra O_2 consumed beyond SMR during realimentation) (data in Hervant et al., 1997) was 1.2-fold higher in the infrequently feeding *S. virei* than in *A. aquaticus*. Hypogean and epigean amphipods and salamanders also showed a high digestive efficiency (Hervant et al., 1999b, 2001). There is obviously a selective advantage for an animal in such an harsh environment to use the available food energy optimally.

During refeeding, both species show a large hyperactivity (Hervant et al., 1997), corresponding to an active food-searching behavior. The preferential degradation of lipids as fuel for metabolism and the protein sparing observed during fasting may preserve essential functions such as locomotion (Fuglei et al., 2000). This protein sparing may be of prime necessity for subterranean organisms so that they can rapidly resume locomotory activity (e.g. food searching activity) when food becomes available again. This could be crucial, particularly in habitats where food competition occurs: animals whose locomotory capabilities are rapidly restored may have a significant advantage (by their higher ability to compete for limited food resources) for further population growth. Due to the higher muscular protein content and sensitivity to the presence of potential food generally shown by hypogean organisms (Uiblein et al., 1992; Hervant et al., 2001), nutrient detection was economical, more efficient and more rapid in *S. virei* (contact after a few seconds) than in *A. aquaticus* (a few minutes). This faster reaction may also be explained by a lower metabolic depression in active muscles than in other tissues, as shown for numerous fasted mammals and birds (Fuglei and Oritsland, 1999).

A proposed adaptive strategy for food-limited groundwater organisms

Mendez and Wieser (1993), reviewing numerous studies on fishes, pointed out that selection might have favored a sequential energy strategy in response to long-term fasting and subsequent refeeding, such that four successive phases (referred to as stress, transition, adaptation and recovery) can be distinguished on the basis of changes in oxygen consumption and spontaneous activity. Hervant et al. (2001) demonstrated the existence of a similar energy strategy in hypogean and epigean salamanders, based on behavioral, respiratory, haematological and metabolic responses. To provide a hypothetical model (i.e. a sequence of events) representing the responses of subterranean animals to long-term food stress, this nomenclature was also employed in the present study.

During the stress phase (days 0–15), both species increased locomotor activity (and therefore SMR; data in Hervant et al., 1997) at first, reflecting an increased food searching behavior.

During the transition phase (days 15–30 in *A. aquaticus*; days 15–60 in *S. virei*), both isopods responded to continued food deprivation by a reduction in SMR and spontaneous activity. Both reductions were more drastic in *S. virei* than in *A. aquaticus*. During this second phase, *S. virei* only catabolized carbohydrates and phosphagen stores, while *A. aquaticus* largely used all four stored metabolites. In addition, the subterranean isopod *S. virei* rapidly resynthesized its glycogen content (by the glyconeogenesis pathway: Hervant et al., 1999a).

During the adaptation phase in *S. virei* (after 60 days of fasting), energy metabolism shifted from a carbohydrate-dominated to a lipid-dominated form. At the end of this third period, metabolism progressively shifted from a lipid-dominated

to a lipid/protein-dominated form, suggesting that the hypogean crustacean studied could not prolong total cessation of protein metabolism after a 120-day food stress. For *S. virei*, the adaptation phase was characterized by stable metabolic and activity rates that remained at the reduced, minimal, levels reached at the end of the transition phase (data in Hervant et al., 1997). In contrast, no significant adaptation period was observed in the epigean *A. aquaticus*; this species seemed to directly enter a 'critical', lethal, phase (as defined by Le Maho, 1984).

During the recovery phase, both crustaceans responded to renutrition by an increase in both oxygen consumption and spontaneous activity (i.e. active food-searching behavior), and rapidly resynthesized all four energy reserves. Both adaptive responses were more efficient and more rapid in the groundwater species.

Based on the results of this study, we propose a general model of adaptive strategy for groundwater organisms, involving the ability to withstand long-term fasting and the efficient use of consumed food. Adaptation to prolonged fasting included (i) a 'sit-and-wait' behavior, i.e. a period of depressed metabolism during which the subterranean species subsisted on a high-energy reserve (mainly lipid stores), and (ii) the possession of low energetic requirements and large body stores. In addition, hypogean species displayed high recovery abilities during refeeding, showing optimal utilization of available food energy and therefore rapid restoration of the body reserves depleted during nutritional stress. All hypogean species studied (Hervant et al., 1997; 1999b; 2001; this study) appeared better adapted to long-term food deprivation and to unpredictable, short-term, energy inputs than surface-dwelling species. These adaptations allow subterranean organisms to tolerate a prolonged reduction in food availability by maximizing the length of time that metabolism can be fuelled by a given food ration and/or a given energy reserve. This supports the suggestion by Hoffmann and Parson (1991) that difficulties in obtaining food in stressful environments may select for conservative energy use.

These adaptive responses might be considered for numerous subterranean organisms as an efficient energy-saving strategy in a harsh and unpredictable environment where fasting (and/or hypoxic) periods of variable duration alternate with sporadic feeding events (and/or normoxic periods). Therefore, food-limited (and/or hypoxia tolerant) groundwater species appear to be good examples of animals representing a low-energy system.

This research was supported by funds from University Claude Bernard-Lyon I and the National Centre for French Scientific Research (CNRS). The authors thank C. Bou for his valuable assistance in collecting individuals of *Stenasellus virei*, and Prof. E. Pattee for his assistance in checking a first version of the manuscript. They are also pleased to acknowledge the constructive criticisms of the anonymous referees.

References

Barclay, M. C., Dall, W. and Smith, D. M. (1983). Changes in lipid and protein during starvation and the moulting cycle in the tiger prawn, *Penaeus esculentus* Haswell. *J. Exp. Mar. Biol. Ecol.* **68**, 229–244.

Brzek, P. and Konarzewski, M. (2001). Effect of food shortage on the physiology and competitive abilities of sand martin (*Riparia riparia*) nestlings. *J. Exp. Biol.* **204**, 3065–3074.

Dall, W. (1974). Indices of nutritional state in the western rock lobster *Panulirus longipes* (Milne Edwards). I. Blood and tissue constituents and water content. *J. Exp. Mar. Biol. Ecol.* **16**, 176–180.

Elendt, B. P. (1989). Effects of starvation on growth, reproduction, survival and biochemical composition of *Daphnia magna*. *Arch. Hydrobiol.* **116**, 415–433.

Fuglei, E. and Oritsland, N. A. (1999). Body composition, resting and running metabolic rates, and net cost of running in rats during starvation. *Acta Physiol. Scand.* **165**, 203–210.

Fuglei, E., Aanestad, M. and Berg, J. P. (2000). Hormones and metabolites of arctic foxes (*Alopex lagopus*) in response to season, starvation and re-feeding. *Comp. Biochem. Physiol.* **126A**, 287–294.

Groscolas, R. and Robin, J.-P. (2001). Long-term fasting and re-feeding in penguins. *Comp. Biochem. Physiol.* **128A**, 645–655.

Hervant, F., Garin, D., Mathieu, J. and Fréminet, A. (1999a). Lactate metabolism and glucose turn-over in the subterranean crustacean *Niphargus virei* during post-hypoxic recovery. *J. Exp. Biol.* **202**, 579–592.

Hervant, F., Mathieu, J. and Barre, H. (1999b). Comparative study on the metabolic responses of subterranean and surface-dwelling amphipod crustaceans to long-term starvation and subsequent refeeding. *J. Exp. Biol.* **202**, 3587–3595.

Hervant, F., Mathieu, J. and Durand, J. P. (2001). Behavioural, physiological and metabolic responses to long-term starvation and refeeding in a blind cave-dwelling salamander (*Proteus anguinus*) and a facultative cave-dwelling newt (*Euproctus asper*). *J. Exp. Biol.* **204**, 269–281.

Hervant, F., Mathieu, J., Barre, H., Simon, K. and Pinon, C. (1997). Comparative study on the behavioral, ventilatory and respiratory responses of hypogean and epigean crustaceans to long-term starvation and subsequent feeding. *Comp. Biochem. Physiol.* **118A**, 1277–1283.

Hervant, F., Mathieu, J., Garin, D. and Fréminet, A. (1995). Behavioral, ventilatory and metabolic responses to severe hypoxia and subsequent recovery of the hypogean *Niphargus rhenorhodanensis* and the epigean *Gammarus fossarum* (Crustacea: Amphipoda). *Physiol. Zool.* **68**, 223–244.

Hervant, F., Mathieu, J., Garin, D. and Fréminet, A. (1996). Behavioral, ventilatory and metabolic responses of the hypogean amphipod *Niphargus virei* and the epigean isopod *Asellus aquaticus* to severe hypoxia and subsequent recovery. *Physiol. Zool.* **69**, 1277–1300.

Hoffmann, A. A. and Parson, P. A. (1991). *Evolutionary Genetics and Environmental Stress*. 284 pp. Oxford: Oxford University Press.

Hüppop, K. (1985). The role of metabolism in the evolution of cave animals. *Nat. Speleol. Soc. Bull.* **47**, 136–146.

Le Maho, Y. (1984). Adaptations métaboliques au jeûne prolongé chez l'oiseau. *J. Physiol. (Paris)* **79**, 113–119.

Malard, F. and Hervant, F. (1999). Oxygen supply and the adaptations of animals in groundwater. *Freshwater Biol.* **41**, 1–30.

Mayzeau, P. (1976). Respiration and nitrogen excretion of zooplankton. IV. The influence of starvation on the metabolism and the biochemical composition of some species. *Mar. Biol.* **37**, 47–58.

Mendez, G. and Wieser, W. (1993). Metabolic responses to food deprivation and refeeding in juveniles of *Rutilus rutilus* (Teleostei: Cyprinidae). *Env. Biol. Fish.* **36**, 73–81.

Newsholme, E. and Stuart, C. (1973). *Regulation in Metabolism*. 349pp. London: John Wiley & Sons.

Poulson, T. L. (1964). Animals in aquatic environments: animals in caves. In *Handbook of Physiology*, section 4, *Adaptation to the Environment* (ed. D. B. Dill), pp. 749–771. Baltimore: Williams and Wilkins.

Secor, S. M. (2001). Regulation of digestive performance: a proposed adaptive response. *Comp. Biochem. Physiol.* **128A**, 565–577.

Stuck, K. C., Watts, S. A. and Wang, S. Y. (1996). Biochemical responses during starvation and subsequent recovery in postlarval white shrimp, *Penaeus vannamei*. *Mar. Biol.* **125**, 33–45.

Uiblein, F., Durand, J. P., Juberthie, C. and Parzefall, J. (1992). Predation in caves: the effects of prey immobility and darkness on the foraging behavior of two salamanders, *Euproctus asper* and *Proteus anguinus*. *Behav. Process.* **28**, 33–40.

Wilcox, J. R. and Jeffries, H. P. (1976). Hydration in the sand shrimp *Crangon septemspinosa*: relation to diet. *Biol. Bull. mar. biol. Lab. (Woods Hole)* **150**, 522–530.

Zebe, E. (1991). Arthropods. In *Metazoan Life without Oxygen* (ed. C. Bryant), pp. 218–237. New York: Chapman and Hall.