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COMPARATIVE STUDY ON THE METABOLIC RESPONSES OF SUBTERRANEAN AND SURFACE-DWELLING AMPHIPODS TO LONG-TERM STARVATION AND SUBSEQUENT REFEEDING

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Summary

The effects of long-term starvation and subsequent refeeding on intermediary and energy metabolism were investigated in two subterranean aquatic crustaceans, *Niphargus rhenorhodanensis* and *Niphargus virei*, and in a morphologically similar surface-dwelling species, *Gammarus fossarum*. The metabolic response to prolonged food deprivation was monophasic in *G. fossarum*, showing an immediate, linear and large decline in all of the energy reserves. In contrast, both subterranean organisms displayed successive periods of glucidic, proteo-glucidic then lipidic-dominant catabolism during food deprivation. In both subterranean species, lipids (51 % of the energy consumed during a 180-day starvation period) and proteins (44 %) were the most metabolized substrates in terms of total energy, whereas glycogen (5 %) contributed little energy. *G. fossarum* displayed a different energetic strategy: proteins comprised 56 % of the energy losses during a 28-day starvation period, total lipids some 39 % and glycogen reserves only 5 %. We propose an energy strategy for food-limited subterranean crustaceans involving the possession of (1) higher amounts of stored arginine phosphate, triglycerides and glycogen and (2) lower utilization rates of stored metabolites than *G. fossarum* and numerous other surface-dwelling crustaceans, making the fueling of food deprivation possible for a longer time. In addition, these species had a faster and more efficient assimilation of available nutrients during recovery from food deprivation, enabling preparation for a new nutritional stress. These specific adaptive responses might be considered, for *N. virei* and *N. rhenorhodanensis*, as an efficient energy-saving strategy for an environment where extended starvation periods alternate with sporadic feeding events, therefore improving their competitive advantages.

Key words: intermediary metabolism, energy metabolism, starvation, catabolism, refeeding, amphipod, *Niphargus rhenorhodanensis*, *Niphargus virei*, *Gammarus fossarum*.

Introduction

Numerous groundwater ecosystems are characterized by severely limited food supplies during most of the year because of the lack of autotrophic production and sporadic allochthonous input. Due to the temporal and spatial patchiness of food availability in most groundwater biotopes, periods of prolonged starvation are common events in the life of subterranean (i.e. hypogean) organisms (Poulson, 1964; Hüppop, 1985, 1986; Hervant et al., 1997). The ability to withstand and recover from long periods of nutritional stress is a critical adaptation for survival of any organism in food-limited systems. Therefore, several hypogean species are thought to have evolved behavioral, physiological and/or metabolic adaptations that allow them to exploit harsh subterranean environments successfully. However, needs and life styles differ considerably between hypogean species (Danielopol and Rouch, 1991) and so their adaptations to food limitation might also differ. Although instantaneous ecological consequences of poor nutrition are sometimes hard to distinguish, the reproductive potential of animals may become reduced and the effects will be manifested at the population level through a smaller amount of offspring or reduced survival capacity of the young.

The subterranean aquatic crustaceans *Niphargus rhenorhodanensis* and *N. virei* can survive without feeding for periods well in excess of 1 year (Gibert and Mathieu, 1980; Mathieu and Giber, 1980). In a previous paper (Hervant et al., 1997), it was pointed out that both hypogean amphipod species and the hypogean isopod *Stenasellus virei* are better adapted to lack of food than surface-related species and all crustaceans previously studied, with survival times exceeding 200 days. During long-term starvation, the locomotory, ventilatory and metabolic rates were drastically lowered in subterranean

species, whereas surface-dwelling species showed lesser decreases in these rates and responded with a marked and transitory hyperactivity. The higher reduction in activity and metabolic rates showed by hypogean species would ensure their survival during long periods of food deprivation. Hervant et al. (1997) stated that resistance to prolonged starvation would probably involve a state of temporary torpor, during which subterranean organisms subsist on high energy reserves. Thus, knowledge of changes in the biochemical composition and energy content of these species under food limitation and refeeding could improve our understanding of their competitive abilities.

Here we report on investigations designed to elucidate whether the behavioral and the whole animal physiological responses (i.e. oxygen consumption), investigated in a previous paper (Hervant et al., 1997), are accompanied during prolonged food deprivation and subsequent refeeding by changes in the intermediary and energy metabolism of hypogean crustaceans (e.g. energy allocation patterns, and qualitative and/or quantitative changes of body composition). This was partly performed by recording some metabolic parameters during a 180-day starvation period and a subsequent 15-day feeding phase in two subterranean aquatic crustaceans (*N. rhenorhodanensis* and *N. virei*). For a comparison, a parallel study was performed during a 28-day starvation period and a subsequent 7-day feeding phase in a morphologically close (but not taxonomically closely related, as most stygobites are either phylogenetic or distributional relicts) surface-dwelling crustacean, *Gammarus fossarum*.

Materials and methods

Animals and experimental conditions

Niphargus rhenorhodanensis (Schellenberg) (hypogean amphipods, 13.0±0.8 mg fresh mass, *N*=30, adults) were collected using traps sunk into the sediment of an interstitial ecosystem (Chalamont, Dombes Forest, France). *Niphargus virei* (Chevreux) (hypogean amphipods, 90.6±4.1 mg fresh mass, *N*=30, adults) were collected with a net placed at the emergence spring of a karst system at Gueux, near Dijon, France. *Gammarus fossarum* (Koch) (epigean amphipods, 32.7±3.5 mg fresh mass, *N*=30, adults) were collected with a net from a swiftly flowing river (Le Renaison, Pouilly-les-Nonains, France). All animals were maintained in recirculating aquaria containing groundwater pumped from the aquifer of the University Lyon 1, together with clay and stones removed from the collection sites. *N. virei* and *N. rhenorhodanensis* were fed with minced meat every 2 weeks. *G. fossarum* was fed with minced beef meat every week. Aquaria were kept in the dark at 11±0.4 °C.

Animals of all three species were acclimated to laboratory conditions for 2 months, then separated into control and treatment groups. Adults of both groups (males only) were transferred individually into glass flasks (containing 250 ml water, and pieces of fine plastic grid as an artificial substrate) for experimentation. The incubation water was renewed weekly. The control group was fed as described above. The treatment group was deprived of food for 180 days (in both hypogean species) or 28 days (in the epigean *G. fossarum*), according to their survival times under conditions of starvation (Mathieu and Gibert, 1980; Hervant et al., 1997). After this starvation period, individuals of the treatment group were refed twice (two supplies of food, the second after a period of 4 days for *G. fossarum* and 9 days for both *Niphargus* species) during 15 days for hypogean crustaceans or 7 days for *G. fossarum.*

Sample preparation and metabolite assays

Ammonia (NH_4^++NH_3) excretion rates were determined on a sample of incubation water in which control, starved or refed animals were held for 24 h, as described by Hervant et al. (1996). To investigate changes in mass, water content and key metabolites during food deprivation, individuals were maintained under conditions of starvation and removed (except if animals moulted during the experiment, due to the significant utilization of stored metabolites for cuticle formation; Elendt, 1989) at intervals of 15, 30, 60, 90, 120, 150 and 180 days for *N. virei* and *N. rhenorhodanensis*, and 7, 15, 21 and 28 days for *G. fossarum*. To investigate changes in mass, water content and key metabolites during recovery from long-term lack of food, individuals were starved for either 180 days (*N. virei* and *N. rhenorhodanensis*) or 28 days (*G. fossarum*), before subsequent refeeding (see above). Individuals were then removed (except if animals had moulted) at intervals of 4 and 15 days for both *Niphargus* species, and 3 and 7 days for *G. fossarum*. Once removed, control, starved and refed individuals were immediately weighed (wet mass), frozen in liquid nitrogen before being lyophilized (VIRTIS lyophilisator, Trivac D4B) then reweighed (dry mass). The lyophilized animals were homogenized (as described by Hervant et al., 1995) and stored in capped vials at −75 °C until ready for assays of key metabolites.

The following metabolites were determined as described in Hervant et al. (1995, 1996) by standard enzymatic methods (Bergmeyer, 1985): ammonia (NH_4^++NH_3), arginine and arginine phosphate, ATP, glucose, glycerol and glycogen. Total proteins, total lipids, triglycerides and non-esterified fatty acids (NEFA) were extracted according to the methods of Elendt (1989) and Barclay et al. (1983), and then determined using specific test-combinations (Bœhringer-Mannheim). All assays were performed in a recording spectrophotometer (Beckman DU-6) at 25 °C, except for the ATP assays, which were performed in an LKB 210 luminometer. Enzymes, coenzymes and substrates used for enzymatic assays were purchased from Bœhringer (Mannheim, Germany) and Sigma Co. (St Louis, USA).

Analytical procedures, units and statistics

The accuracy of each analysis had been previously tested by assaying the samples with and without an added internal standard. The sensitivity of the assays used was approximately 1 μmol g^{-1} dry mass for all metabolites, except for glycogen (5 μmol g^{-1} dry mass) and ATP (0.1 μmol g^{-1} dry mass). During

all the experiments, mortality was considered negligible (mortality <4 %) for all three species. The use of the terms 'utilization rate' and 'production rate' has only a comparative value in this study, since metabolite utilization/production was not constant over time. Values are presented as means ± S.E.M. For analyses between means (at the $P<0.05$ level) and after verification of normality of the values, a Student's *t*-test was used, whereas tests among means were conducted with a one-way ANOVA, using a Bonferroni test for multiple comparisons as appropriate.

Results

Body mass and water content

Both dry mass and water content showed no changes in fed (control) animals during all experiments (not shown). Starved animals showed a slight decrease (Fig. 1A,B) in mean percentage dry mass (–6 % after 180 days in the hypogean *N. virei* and *N. rhenorhodanensis*, –6 % after 28 days in the epigean *G. fossarum*), and a small increase (Fig. 1C,D) in mean percentage water content (+6.5 % after 180 days in *N. virei* and *N. rhenorhodanensis*, +4 % after 28 days in *G. fossarum*) in all three species, although it was not significantly different until 180 days in both subterranean species and 21 days in *G. fossarum*. During refeeding, both dry mass and water content returned to pre-starvation levels in both *Niphargus* species, whereas these parameters showed no significant recovery in *G. fossarum* (Fig. 1).

*Effects of starvation and subsequent refeeding on metabolite concentrations in the subterranea*n Niphargus virei *and* Niphargus rhenorhodanensis

No significant changes in the concentration of whole animal ATP (Fig. 2A) and glucose (Fig. 2H) were observed during the 180-day starvation period in either *Niphargus* species ($P>0.05$). Arginine phosphate concentration decreased to 88 % (in *N. virei*) and 76 % (in *N. rhenorhodanensis*) of its initial value (fed level) after 180 days of food deprivation (Fig. 2B). After 180 days starvation, only 3.5 µmol arginine phosphate g^{-1} dry mass were utilized in *N. virei* and 7.6 µmol arginine phosphate g^{-1} dry mass in *N. rhenorhodanensis*. In both hypogean species, the arginine phosphate concentration rapidly returned to the fed value (pre-starved control) during refeeding (Fig. 2B). Arginine concentration changed in a pattern similar to that of arginine phosphate (Fig. 2B).

Glycogen content decreased from 60 days in both species, reaching 63 % (*N. virei*) and 55 % (*N. rhenorhodanensis*) of its initial concentration after 180 days of food deprivation (Fig. 2C), which corresponded to a utilization of 89 µmol glycosylic unit g^{-1} dry mass (*N. virei*) and

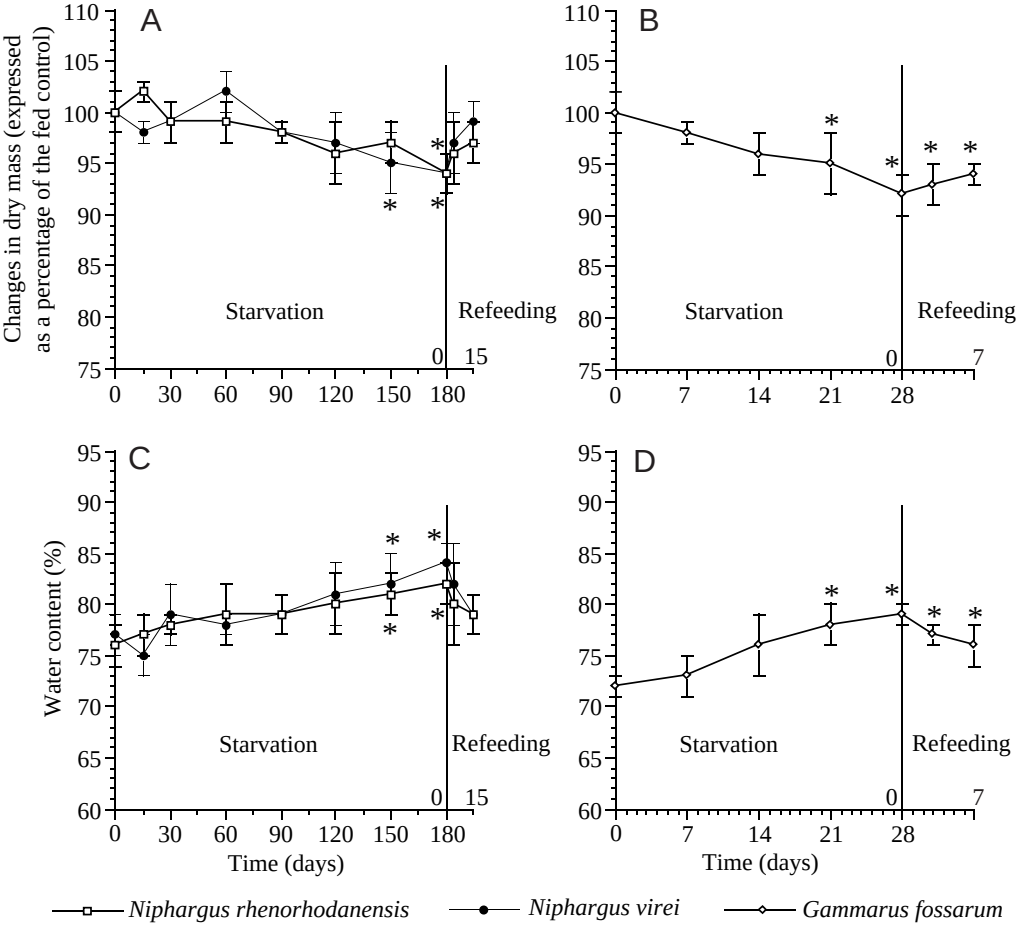

Fig. 1. Changes in dry mass (expressed as a percentage of the fed control) and percentage water content during long-term starvation and subsequent refeeding for the subterranean crustaceans *Niphargus rhenorhodanensis* and *Niphargus virei* (A,C), and for the surface-dwelling crustacean *Gammarus fossarum* (B,D) at 11 °C, in darkness. Values are means ± S.E.M. for $N=10$ animals. An asterisk indicates a value that was significantly different from the fed control ($P<0.05$).

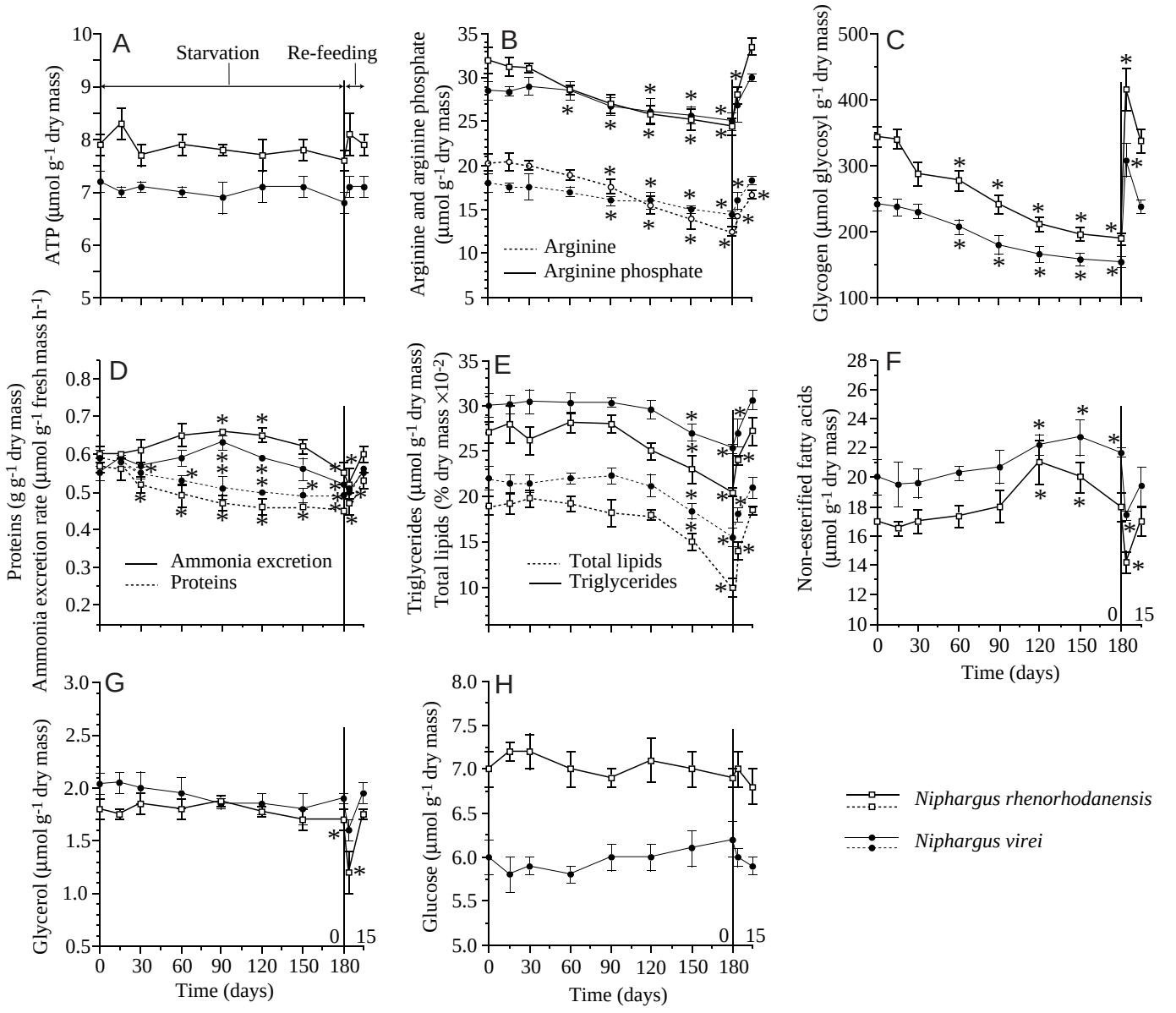

Fig. 2. Changes in the levels of metabolites during long-term starvation (180 days) and subsequent refeeding (15 days) for the subterranean crustaceans *Niphargus rhenorhodanensis* and *Niphargus virei* at 11 °C, in darkness. Values are means ± S.E.M. for N=10 animals. An asterisk indicates a value that was significantly different from the fed control (P<0.05). Ammonia in (D) = NH_3+NH_4^+.

155 µmol glycosylic unit g^{-1} dry mass (*N. rhenorhodanensis*). Glycogen content showed a significant overshoot during the first week of recovery from nutritional stress (reaching 127 % of the fed value in *N. virei* and 121 % in *N. rhenorhodanensis*) before returned to the pre-starvation level (Fig. 2C).

During lack of food, proteins were metabolized after 30 days in both species, reaching 83 % (*N. virei*) and 79 % (*N. rhenorhodanensis*) of the fed level by day 180 (Fig. 2D), which corresponded to a utilization of 0.10 g g^{-1} dry mass (*N. virei*) and 0.12 g g^{-1} dry mass (*N. rhenorhodanensis*). During refeeding, protein content returned to pre-starvation levels in both hypogean species by day 15 (Fig. 2D). Moreover, the ammonia excretion rate (NH_4^++NH_3, calculated from the cumulative ammonia excretion during an incubation period of 24 h) changed according to a triphasic pattern in both *Niphargus* species. No significant changes were observed up to 60 days of starvation, but then there was an increase up to 120 days, then a decrease (Fig. 2D). During refeeding, this rate returned to the pre-starvation level in both species (Fig. 2D).

During starvation, total lipids were metabolized only after 150 days in both species, reaching 70 % (*N. virei*) and 53 % (*N. rhenorhodanensis*) of the initial value after 180 days (Fig. 2E), corresponding to a utilization of 0.065 g g^{-1} dry mass (*N. virei*) and 0.09 g g^{-1} dry mass (*N. rhenorhodanensis*). During refeeding, lipid stores returned to control levels in both *Niphargus* species (Fig. 2E). Triglyceride (TG) contents changed in a similar pattern to that of total lipids. TG stores decreased from 150 days and reached 84 % (*N. virei*) and 76 %

(*N. rhenorhodanensis*) of the initial value after 180 days of food deprivation (Fig. 2E), equating to a utilization of 4.7 μmol g^{-1} dry mass (*N. virei*) and 6.6 μmol g^{-1} dry mass (*N. rhenorhodanensis*). During recovery, TG content returned to the fed level in both species (Fig. 2E). NEFA concentrations significantly increased between 120 and 150 days of food deprivation, then returned to fed level in *N. rhenorhodanensis* and *N. virei* (Fig. 2F). During recovery from nutritional stress, the NEFA content immediately decreased then returned to the control level in both species (Fig. 2F). Glycerol concentration showed no changes during food deprivation in either subterranean species (Fig. 2G). During recovery, glycerol content decreased, then rapidly returned to the control level (Fig. 2G).

Effects of starvation and subsequent refeeding on metabolite concentrations in the epigean Gammarus fossarum

During starvation, the whole body ATP content decreased from 21 days and reached 72 % of its initial concentration after 28 days in *G. fossarum* (Fig. 3A). The ATP concentration returned to the fed value (pre-starved control) during refeeding (Fig. 3A). Arginine phosphate concentration immediately fell sharply under starvation, reaching 6 % of its initial value (fed level) on day 28 (Fig. 3B), which corresponded to a utilization of 7.5 μmol g^{-1} dry mass. Arginine phosphate content slowly increased during refeeding, reaching only 25 % of its initial value after 7 days of recovery (Fig. 3B). Arginine concentration changed in a pattern similar to that of arginine phosphate (Fig. 3B).

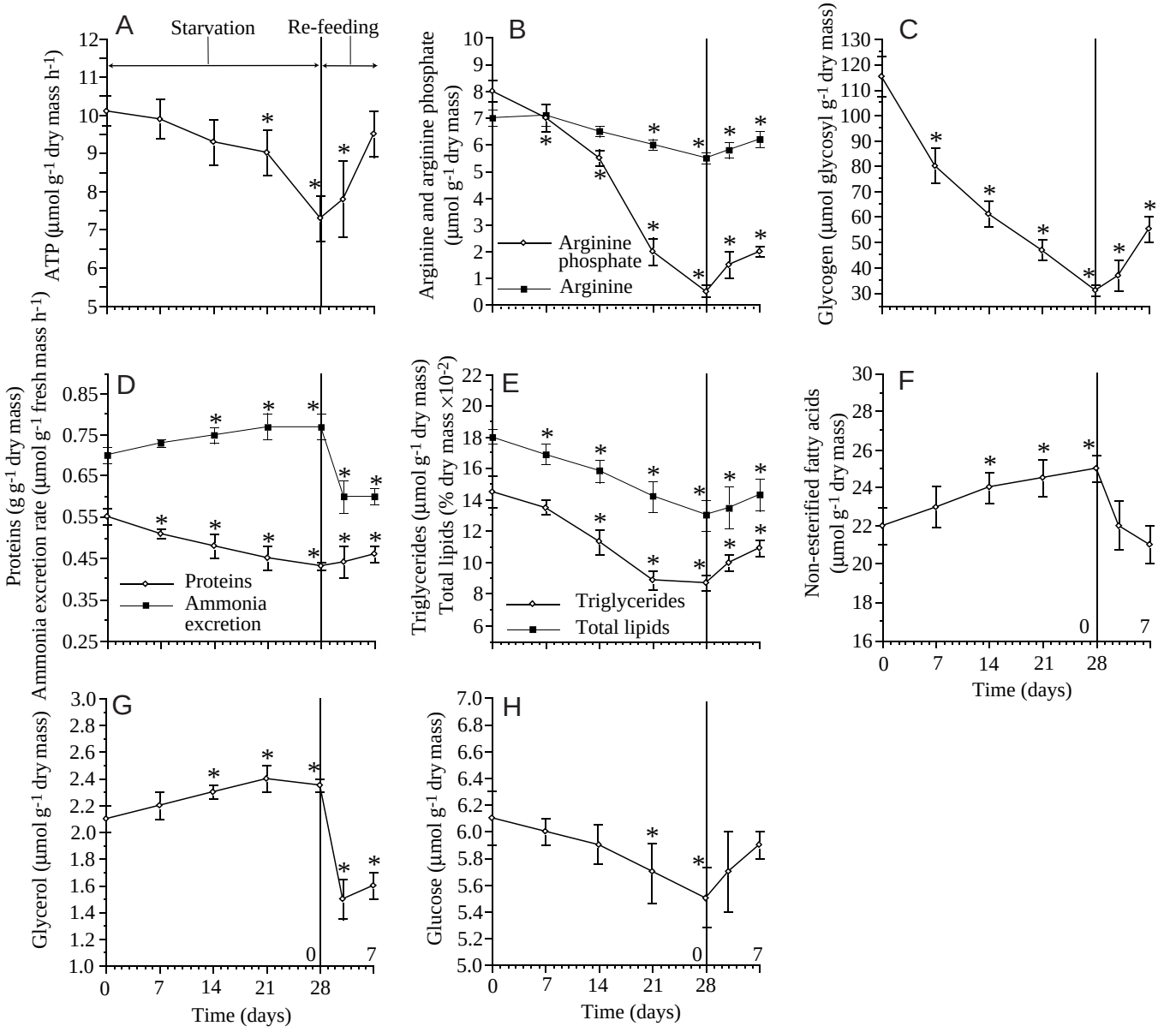

Fig. 3. Changes in the levels of metabolites during long-term starvation (28 days) and subsequent refeeding (7 days) for the surface-dwelling crustacean *Gammarus fossarum* at 11 °C, in darkness. Values are means ± S.E.M. for N=10 animals. An asterisk indicates a value that was significantly different from fed control (P<0.05). Ammonia in (D) = NH_3+NH_4^+

Glycogen content immediately decreased during starvation, to 27 % of its initial concentration after 28 days (Fig. 3C), which corresponded to a utilization of 84 μmol glycosylic unit g^{-1} dry mass. During recovery from nutritional stress, glycogen content slowly increased, reaching only 48 % of its initial value after 7 days (Fig. 3C).

During lack of food, protein levels immediately decreased, reaching 78 % of the fed level after a 28-day period (Fig. 3D), equating to a utilization of 0.12 g g^{-1} dry mass. During refeeding, the protein content slowly increased, reaching 84 % of the fed level after 7 days (Fig. 3D). Ammonia excretion rate increased from 14 days, and was 110 % of the fed level on day 28 (Fig. 3D). During refeeding, this rate immediately decreased to 86 % of the initial level after 7 days recovery (Fig. 3D).

During starvation, total lipids immediately decreased, reaching 72 % of the initial value after 28 days (Fig. 3E), equating to a utilization of 0.05 g g^{-1} dry mass. During recovery, lipid stores slowly increased, reaching only 79 % of the fed value after 7 days (Fig. 3E). The TG content changed in a pattern similar to that of total lipids, reaching 59 % of the initial value after 28 days food deprivation (Fig. 3E). This corresponds to a utilization of 5.8 μmol g^{-1} dry mass. During recovery, TG content increased, reaching 75 % of the fed value after 7 days (Fig. 3E). The NEFA concentration significantly increased after 14 days of food deprivation (Fig. 3F). During recovery, NEFA content showed no significant changes (Fig. 3F). The glycerol concentration also increased after 14 days of starvation (Fig. 3G), but during recovery rapidly decreased (Fig. 3G) to 76 % of the fed value after 7 days.

During starvation, the glucose content decreased after 21 days and reached 90 % of its initial concentration after 28 days (Fig. 3H). The glucose concentration rapidly returned to fed value during refeeding (Fig. 3H).

Discussion

Body mass and water content during long-term starvation

During starvation, energy is derived solely from metabolic stores (e.g. lipids, proteins and/or glycogen), and therefore body tissues are lost due to catabolic activities. To maintain the necessary body volume (fixed by the exoskeleton in arthropods) and internal turgidity during starvation, the lost tissue mass must be replaced by water (Dall, 1974; Wilcox and Jeffries, 1976; Stuck et al., 1996). All three studied crustaceans followed this pattern, displaying a significant (but low) increase in water content and a corresponding decrease in percentage dry mass during food deprivation. Wilcox and Jeffries (1976) and Stuck et al. (1996) concluded that percentage water content can be used as a short-term, sensitive indicator of nutritional stress in crustaceans. The slow and weak changes in whole body hydration in response to starvation and subsequent refeeding observed in our study do not support this hypothesis.

Both subterranean amphipods showed the lowest responses to long-term starvation, particularly indicated by a rate of decrease in percentage dry mass that was ninefold slower than in the surface-dwelling *G. fossarum*. Similar responses to starvation (but showing higher decrease in percentage dry mass than in both *Niphargus* species) have been previously reported for lobsters (Dall, 1974; Sasaki et al., 1986) and shrimps (Cuzon et al., 1980; Regnault, 1981; Barclay et al., 1983; Stuck et al., 1996). These results indicate that hypogean amphipods have a very low utilization rate of metabolic stores.

Metabolic responses to long-term food deprivation

Energy storage during food abundance and metabolic energy-saving adjustments triggered by food limitation are seen in most organisms, including subterranean organisms, and influence both the geographic and temporal distribution of a species. Periods of starvation are encountered by most epigean and hypogean animals, but they can adjust their metabolism to the lack of food by utilizing metabolites stored during times when food is abundant.

Many data about the influence of starvation in crustaceans concern qualitative and quantitative changes of body composition (reviewed by Stuck et al., 1996). The relative importance of these metabolic reserves and their order of utilization varies with species. In some species, carbohydrate stores (mainly glycogen) are used first, then lipids and finally proteins (Chaisemartin, 1971; Cuzon and Ceccaldi, 1972; Regnault, 1981; Stuck et al., 1996). In others, glycogen contributes little energy during food deprivation, the metabolic stores used being predominantly lipids (Schafer, 1968; Sasaki et al., 1986; Percy, 1993) or proteins (Marsden et al., 1973; Mayzeau, 1976; Barclay et al., 1983), or both simultaneously (Mayzeau, 1976; Hiller-Adams and Childress, 1983). Moreover, some species switch from one stored metabolite to another as prolonged starvation progresses (Mayzeau, 1976; Elendt, 1989). Among crustaceans, neutral lipids (mainly triglycerides, TG) are preferentially catabolized during starvation, while polar lipids (phospholipids and cholesterol) are conserved due to their role as structural components of cell membranes (Heath and Barnes, 1970; Bourdier and Amblard, 1989; Stuck et al., 1996). In the tiger prawn *Penaeus esculentus*, the abdominal 'muscle mass' contributes the most proteins and lipids during a 14-day starvation period (Barclay et al., 1983). Taxonomic differences, ecology and/or geography have all been invoked to explain the adoption of a particular energetic strategy by a given species (Percy, 1993).

For both subterranean species, *Niphargus virei* and *N. rhenorhodanensis*, and for the surface-dwelling *Gammarus fossarum*, experimental data on starvation-induced changes in body composition indicated a significant utilization of phosphagen (arginine phosphate), glycogen, TG, total lipid and protein reserves. The body materials utilized were replaced by water. These data accord with the results given by Gibert and Mathieu (1980) and Mathieu and Gibert (1980) for hypogean Niphargids. Under food deprivation, *N. virei* and *N. rhenorhodanensis* metabolized phosphagen, glycogen, proteins, TG and lipids with very low utilization rates, from 5- to 135-fold lower than in the epigean *G. fossarum*. Contrary to

the results for *N. virei* and *N. rhenorhodanensis*, a rapid and drastic reduction in total lipids (particularly a total depletion of TG stores in a few days) as a response to starvation has been reported for the lake-dwelling copepod *Acanthodiaptomus denticornis* (Bourdier and Amblard, 1989) and for larval, adult and sub-adult lobsters (reviewed by Stuck et al., 1996).

Fed *N. virei* and *N. rhenorhodanensis* had large glycogen reserves, two- to threefold greater than in fed *G. fossarum*, and significantly higher than those usually found in epigean crustaceans (reviewed by Hervant et al., 1996). Both hypogean species also showed significantly greater arginine phosphate and TG reserves than *G. fossarum*, making the fueling of energy metabolism possible for a longer time under starvation. In addition, *N. virei* displayed higher lipids and protein stores than *G. fossarum*. The possession of higher metabolic stores would ensure prolonged survival during periods of food deprivation.

Metabolic response to prolonged food deprivation was monophasic in the epigean *G. fossarum*, showing an immediate, linear and large decrease in all of their energy reserves. In contrast, starvation developed in three successive phases in both hypogean amphipods, with an 'immediate' but low (from 15-120 days) depletion of glycogen stores, followed by a transitory (and low) utilization of proteins (from 30 to 120 days, with reciprocal changes, according to a triphasic pattern, in ammonia excretion rate), then finally lipids (from day 150). Both subterranean organisms displayed successive periods of glucidic, proteo-glucidic, and finally lipidic-dominant catabolism in the course of the 150-day experiment.

All three organisms showed a release of NEFA (probably to hemolymph) at the same time as lipid mobilization from lipid depots occurred. Thus, NEFA seemed to play a role as a transport form for lipids between fat reserves and utilization places in amphipods, similar to their role in vertebrates (Larsson and Lewander, 1973).

If the utilized amounts of glycogen, proteins and lipids were completely oxidized to CO_2 and H_2O, then the energy provided by each metabolite would be derivable, according to Elendt (1989). In both hypogean species, lipids (51 % of the energy consumed during the 180-day starvation period) and proteins (44 %) were the most metabolized substrates in terms of total energy, whereas glycogen contributed little energy (5 %). Some studies on surface-dwelling crustaceans have also reported that glycogen contribution appeared negligible during prolonged starvation (for reviews, see Regnault, 1981; Dittrich, 1991; Stuck et al., 1996). For hypogean crustaceans, this may be a result of an increased conversion/utilization of amino acids (originating from proteolysis) or glycerol (from lipolysis) to glycogen, by glyconeogenesis. This hypothesis is supported by the large decrease in arginine content (originating from the utilization of arginine phosphate), and by the absence of glycerol release observed in both hypogean amphipods. Moreover, the existence of a high glyconeogenic capability (from lactate) has been demonstrated recently in *N. virei* (Hervant et al., 1999).

The epigean *G. fossarum* displayed a different energy strategy: proteins (56 % of the energy losses during the 28-day starvation period) and total lipids (39 %) were the most metabolized stores, whereas glycogen reserves seemed not to be preferentially used (5 %). During food deprivation, this species did not show high glyconeogenic conversion rates of amino acids and/or glycerol.

The total energy content was reduced by 29 joules g^{-1} dry mass day^{-1} in *N. virei*, 38 joules g^{-1} dry mass day^{-1} in *N. rhenorhodanensis* and 179 joules g^{-1} dry mass day^{-1} in *G. fossarum*. In comparison, Percy (1993) reports a decline in energy content from 230 to 65 joules g^{-1} dry mass day^{-1} (at only 3 °C) during a 4-week starvation period in the arctic amphipod *Themisto libellula*. Our data accord with the metabolic rates given by Hervant et al. (1997) for fed and starved *N. virei*, *N. rhenorhodanensis* and *G. fossarum*.

For both *Niphargus* species, prolonged lack of food resulted in a rapid decrease in oxygen consumption for 2-3 months before leveling off, and in a drastic decrease in the rates of locomotory activity and ventilation. Relative respiration rates of hypogean organisms during starvation (i.e. the respiration rate during starvation divided by that before starvation) were considerably lower than that of the epigean species (Hervant et al., 1997). In this way, about 50 % of the metabolic energy dissipated by well-fed subterranean species was saved by starving individuals. Indeed, hypogean organisms can survive during long periods of food deprivation at a lower energetic cost. This probable drop in energy demand (associated with a very low standard metabolic rate; Hervant et al., 1997) sustained metabolic reserves for as long as possible, therefore increasing survival time under starvation. A similar saving of energy has been shown by Dall and Smith (1986) in the tiger prawn *P. esculentus*. In addition, *N. virei* and *N. rhenorhodanensis* maintained a high ATP concentration in tissues during the whole nutritional stress, whereas there was a significant decrease in ATP content in *G. fossarum*. No effect of fasting on glucose level was observed in both *N. virei* and *N. rhenorhodanensis*, demonstrating that these species can maintain their glucose homeostasis during a prolonged nutritional stress, whereas *G. fossarum* showed substantial hypoglycemia. Gluconeogenesis (from glycerol and/or amino acids) probably significantly contributed to glucose production in *Niphargus*, maintaining, in particular, the glucose supply to the nervous system.

These results demonstrate that both subterranean amphipods (1) have lower energetic requirements and are better adapted to long-term food deprivation than the surface-dwelling *G. fossarum*, and (2) that they preferentially utilize lipids in order to save carbohydrates and phosphagen (the two main fermentable fuels metabolized during oxygen deficiency in crustaceans; reviewed by Zebe, 1991; Hervant et al., 1996; Malard and Hervant, 1999) and, like some mammals (Newsholme and Start, 1973) or birds (Le Maho, 1984), to save muscular proteins. Therefore, these species could withstand a prolonged hypoxic period subsequent (or associated) to an initial nutritional stress, and could rapidly renew searching for food (i.e an active habitat exploration, Danielopol et al., 1994).

These results corroborate those of Hervant et al. (1997), concerning starvation-induced changes in oxygen consumption for five epigean and hypogean crustacean species.

Metabolic responses to refeeding

It is ecologically very important for organisms to recover quickly and completely from nutritional stress when food is available once more. This recovery corresponds to a restoration of high energy compounds. Renutrition resulted in a partial restoration of the proteins, lipids, triglycerides, phosphagen and glycogen reserves within 7 days in *G. fossarum*, and in a total restoration of the same stored metabolites within 15 days in *N. virei* and *N. rhenorhodanensis*. In addition, the resynthesized body materials replaced the 'excess' water stored during starvation.

N. rhenorhodanensis and *N. virei* resynthesized phosphagen, glycogen, proteins, TG and lipids with very high production rates, from 1.2- to 3-fold higher than in *G. fossarum*. There is a selective advantage for an animal in such an harsh environment to use the available food energy optimally. This ability to maintain and rapidly to restore high amounts of metabolic stores used during food (and/or oxygen: Malard and Hervant, 1999) deficiency allowed subterranean amphipods to fuel a new starvation (and/or hypoxic) period successfully and, therefore, increased their competitive abilities.

All three species also show a large hyperactivity (Hervant et al., 1997) corresponding to an active food searching behavior. Due to their higher muscular protein content and to their higher sensitivity to the presence of potential food (Hervant et al., 1997), such a response to refeeding will be stronger and more rapid (a few seconds) in starved hypogean amphipods than in *G. fossarum* (a few minutes). This suggests that not only is there a sparing of protein during fasting, but also a selective utilization of muscular proteins, saving those that are involved in essential functions such as locomotion.

In contrast to *G. fossarum*, both subterranean species displayed a large but transitory overshoot during recovery, its concentration largely exceeding the control level during the first week of refeeding. This metabolic response may represent an adaptive tactic for the fastest and most efficient way of storing food energy, later to be mobilized for the synthesis of body materials such as triglycerides and proteins.

An adaptive strategy for hypogean crustaceans

Hervant et al. (1997) proposed an energy strategy for a hypogean organism, involving the ability to withstand long-term starvation and the efficient use of consumed food. Resistance to starvation would probably involve a state of temporary torpor ('sit-and-wait' strategy), during which the subterranean crustaceans subsisted on a high-energy reserve, mainly lipid stores. During this inactive state, the locomotory, ventilatory and respiratory rates were drastically lowered.

This study confirms the existence of this strategy. During prolonged starvation, both species of *Niphargus* displayed (1) higher amounts of stored arginine phosphate, neutral lipids and glycogen, and (2) lower stored metabolite utilization rates than *G. fossarum* and numerous other epigean crustaceans (Bourdier and Amblard, 1989; Stuck et al., 1996; Hervant et al., 1996), making the fuelling of food deprivation possible for a longer time. In addition, these species assimilated available nutrients faster and more efficiently than most surface-dwelling species during recovery from food deprivation, making the fueling of a new nutritional stress (or a new hypoxic period) possible. These specific adaptive responses might be considered for *N. virei* and *N. rhenorhodanensis* (and probably numerous other subterranean organisms) as an efficient energy-saving strategy to an environment where more or less extended starvation periods alternate with sporadic feeding events.

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