OXYGEN CONSUMPTION AND VENTILATION IN DECLINING OXYGEN TENSION AND POSTHYPOXIC RECOVERY IN EPIGEAN AND HYPOGEAN CRUSTACEANS

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ABSTRACT

Respiratory and ventilatory responses to declining O2 tension and posthypoxic recovery were investigated in 3 hypogean and in 2 epigean aquatic crustaceans. The aims of this study were to determine how these species reacted to declining O2 tension, to investigate their changes in ventilation and metabolic rate during progressive hypoxia, and to extend our knowledge on the fate of anaerobic end products during subsequent recovery. Hypogean organisms had normoxic O2 consumption rates 1.7–3.5 times lower than the epigean species. All 5 crustaceans were able to maintain their O2 consumption rates (1) at relatively constant levels and (2) independent of Po2 between normoxia and the critical Po2. Hypogean species also possessed lower critical Po2 than epigean ones, which may indicate that these organisms are better adapted to low O2 content and are better equipped to stay aerobic under hypoxia. For all species, posthypoxic recovery resulted in a high O2 debt. The payment of this debt was smaller in hypogean than in epigean species. The main explanations of the lower O2 debt shown by hypogean organisms are the lower energetic expenditures noticed during hypoxia, partly due to a decrease in locomotory and ventilatory activities.

Aquatic subterranean biotopes, including porous, fissured, and karstic aquifers, are characterized by several main properties. These environments are relatively stable with respect to abiotic factors, such as darkness, high moisture, temperature, and water chemistry, as well as to biotic factors, such as predator and food limitations, because of the lack of autotrophic production and sporadic allochthonous input. In addition to depigmentation and reduction (or loss) of eye structures, hypogean species have evolved a number of characteristics which allow them to successfully exploit the subterranean environment. These include the elongation of the body and/or appendages, the development of extraoptic sensory structures, and especially a reduced metabolic rate compared with closely related epigean species (for review, see Hüppop, 1985).

Numerous aquatic subterranean organisms have to cope with periodic hypoxia or near anoxia, with occasionally a very rapid switch from high to low oxygen partial pressure (Po2) (for review, see Hervant and Mathieu, 1995). In temporarily anaerobic interstitial or karstic habitats, the survival of aquatic animals may require specific biochemical, behavioral, and/or physiological adaptations, including respiratory and ventilatory adaptations (Hochachka and Somero, 1973). These responses are often connected with a metabolic rate depression (Brooks et al., 1991). However, we know very little about the adaptations that contribute to extend the survival of hypogean organisms in their alternately hypoxic and normoxic habitats.

Classically, aerobic organisms have been described as metabolic conformers if their O2 consumption varies directly with Po2 or as regulators if O2 consumption is independent of this parameter. Aquatic invertebrates show very different responses to declining O2 tension, with O2 consumption and ventilatory patterns ranging from conformity to regulation. Many crustaceans are able to maintain their rate of O2 consumption independent of Po2 (oxyregulation) over a wide range of O2 tensions from normoxia to a critical Po2 (Pc). Therefore, Pc is a good indicator of adaptation to low Po2 (Bridges and Brand, 1980; Vernberg, 1983; McMahon and Wilkens, 1983; Taylor and Spicer, 1989). Some of the variabilities observed in O2 consumption may be attributed to different degrees of complexity of the respiratory apparatus and control systems or to the severity of environmental hypoxic stress normally encountered. Other differences probably reflect the anaerobic metabolic pathways used, the general activity, and a possible decrease in metabolic rate during lack of O2 (for reviews, see Herreid, 1980; McMahon and Wilkens, 1983).

In the last 20 years, survival, behavior,
physiology, and metabolism of aquatic epigean crustaceans (especially marine decapods) in anaerobiosis has been extensively studied (for review, see Zebe, 1991). Many epigean crustaceans show little tolerance to severe hypoxia or anoxia. They are generally very mobile (Zebe, 1991) and, therefore, can easily move to sites of higher $P_{O_2}$ (behavioral compensation). Consequently, it is probably not necessary for them to become adapted to long-term environmental hypoxia. On the contrary, some aquatic hypogean populations living permanently in ground waters generally cannot escape anaerobic conditions and cannot select oxygenated waters. Therefore, it is necessary for them to become adapted to long-term environmental hypoxia.

Previous investigations have shown that three species of hypogean aquatic crustaceans, Niphargus rhenorhodanensis (Schellenberg), Stenasellus virei (Dollfus), and Niphargus virei (Chevreux), which encounter hypoxic conditions for several months every year during the hydrological cycle, display very high survival times under severe hypoxia (Hervant and Mathieu, 1995; Hervant et al., 1995, 1996, 1997b; Hervant, 1996). The most significant adaptive characteristics of these subterranean species are the presence of large amounts of stored glycogen and phosphagen and the ability to reduce metabolic rates during severe hypoxia by reducing levels of activity, ventilation, and glycolysis (Hervant and Mathieu, 1995; Hervant et al., 1995, 1996, 1997b; Hervant, 1996).

The present investigation extends the study of locomotory, ventilatory, and metabolic responses of these subterranean crustaceans to severe hypoxia and subsequent recovery. The aims of this study were: (1) to determine how subterranean species (from different biotopes) reacted to declining $O_2$ tension, (2) to determine why they displayed a survival time in severe hypoxia longer than that of most epigean crustaceans (for reviews, see Zebe, 1991; Hervant and Mathieu, 1995), and (3) to verify whether a low normoxic metabolic rate is common among subterranean amphipods.

This was partly performed by recording $O_2$ consumption and ventilatory activity (number of pleopod beats/min) in declining $O_2$ tension in three hypogean aquatic crustaceans, Niphargus rhenorhodanensis, Niphargus virei (amphipods), and Stenasellus virei (isopod). For a comparative analysis, a parallel study was performed with two epigean aquatic crustaceans, Gammarus fossarum (Koch) (amphipod) and Asellus aquaticus (L.) (isopod). In addition, respiration and ventilation measurements in all populations during posthypoxic recovery allowed us to assess the difference in the repayment of the $O_2$ debt (defined as the extra $O_2$ consumed after a period of lack) observed in severe hypoxia between hypogean and epigean species.

**MATERIALS AND METHODS**

**Animals.**—Niphargus rhenorhodanensis (hypogean amphipods, fresh weight = 12.0 ± 1.8 mg) were collected from an interstitial aquatic environment (Chalamont, Dombes Forest, France), with traps sunk into the sediment. Niphargus virei (hypogean amphipods, fresh weight = 88.9 ± 4.0 mg) were collected using a net placed at the emergence spring of a karst system at Gueux, near Dijon, France. Gammarus fossarum (epigean amphipods, fresh weight = 33.5 ± 4 mg) were collected from a swiftly flowing river (Le Renaison, Pouilly-les-Nonains, France) with a net. Stenasellus virei (hypogean isopods, fresh weight = 2.5 ± 0.1 mg) were collected in ground water hydraulically connected with the river Tarn, using special pumps lowered into piezometers in an interstitial system at Cantepau (AEP d’Albi, France). Asellus aquaticus (epigean isopods, fresh weight = 16.2 ± 0.9 mg) were collected with a net in a backwater of the Rhône River at Balan, France. All animals were maintained in separate recirculating aquaria containing ground water (pumped from the aquifer of the University Lyon 1). The tanks containing N. virei, N. rhenorhodanensis, S. virei, and G. fossarum contained clay and stones removed from the collection site. Tanks containing A. aquaticus contained stones and live plant material, which the isopod used for food, collected from the collection site for this species. Niphargus virei, N. rhenorhodanensis, and S. virei were fed with minced meat every 2 weeks. Gammarus fossarum was fed with minced meat every week. All aquaria were kept in the dark in a controlled temperature facility (11°C), and individuals of all species (males only, of the same size and intermolt period) were removed for experimentation as required. Each individual was starved for 2 days before an experiment, in order to ensure that the large overshoot in oxygen consumption due to digestive metabolism (Hervant et al., 1997a) did not modify the results.

**Respirometry in Various $O_2$ Partial Pressures.**—Many hypogean organisms are very sensitive to the unnaturally high flow rates used in continuous flow respirometers (Ginet, 1960). Consequently, $O_2$ consumption and ventilatory activity of individuals were measured under various $P_{O_2}$, using a modified Warburg constant volume system, as described in Hervant et al. (1997a). In order to measure the $O_2$ consumption at different $O_2$ tensions, air/N₂ gas mixtures with the required $P_{O_2}$ were prepared as described in Hervant et al. (1997a). Gas mixtures were passed through the respirometer (then $P_{O_2}$ checked in the respirometer) according to the method of Kleinzeller (1965). For each $P_{O_2}$ (150, 120, 100, 80, 60, 50, 40, 30, 20, and 10 Torr), the simultaneous measurement of $O_2$ consumption and ventilation were made with 8 respirometers. The Warburg reaction vials were placed in a water...
RESULTS

pH Changes and CO₂ Concentrations in the Incubation Water

The pH of the incubation water was 7.1 ± 0.3, and no significant pH changes (P > 0.05) were measured in hypoxic water or in reoxygenated water for any organism. The CO₂ concentration was 609 ± 48 mol/l in control incubation water, and no significant changes (P > 0.05) were measured in hypoxic water or in reoxygenated water, for any organism.

O₂ Consumption and Ventilation in Normoxia

In normoxia, hypogean organisms showed lower metabolic rates than epigean ones (P < 0.05, Fig. 1). Niphargus virei showed a very low normoxic O₂ consumption rate, with a value 1.4 times lower than that of S. virei, twice lower than that of N. rhenorhodanensis, 3.1 times lower than that of G. fossarum, and 4.2 times lower than that of A. aquaticus (Fig. 1). Stenasellus virei, N. rhenorhodanensis, G. fossarum, and A. aquaticus presented the same ventilatory activity (P > 0.05, Fig. 2).

Respiration and Ventilation in Declining O₂ Partial Pressure

For each species, O₂ consumption and the rate of pleopod beating varied with declining O₂ tension (between 150 and 10 Torr) (P < 0.05). However, respiratory and ventilatory responses differed among the five crustaceans. All five species were oxyregulators and maintained their rate of O₂ consumption constant and independent of P0₂ between normoxia (P0₂ = 150 Torr) and the critical O₂ pressure (Pc) (P > 0.05, Fig. 1). Pc values (calculated as described by Yeager and Ultsch, 1989) are presented in Table 1.

In all species, no significant relationships were found between Pc and body dry weight (0.09 < r < 0.17, P > 0.05). The regression lines for the changes of O₂ consumption (between normoxia and Pc) versus P0₂ were: y = 16.8 + 0.44 x for S. virei (r = 0.63, P < 0.05); y = 12.9 + 0.13 x for N. virei (r = 0.93, P < 0.05); y = 23.3 + 0.62 x for N. rhenorhodanensis (r = 0.91, P < 0.05); y = 26.6 + 2.7 x for G. fossarum (r = 0.77, P < 0.05), and y = 30.9 + 4.1 x for A. aquaticus (r = 0.84, P < 0.05).

Among the three hypogean species and between both epigean species, there was no difference in the slope of the regression line for bath, and the entire respirometer system was kept in a temperature-controlled chamber at 11°C ± 0.1°C, in very low energy red light (about 50 lux). In order to measure their O₂ consumption, individuals were directly transferred to the respiration vials. O₂ consumption and the number of pleopod beats were measured as described by Hervant et al. (1997b).

At the end of an experiment, animals were immediately removed and frozen in liquid N₂, lyophilized, using a VIRTIS Trivac D4B lyophilizer (Virtis Company, New York, New York), and then weighed. For the incubation water, the pH was determined, using a TACUSSEL Issis 20000 pH-meter, accuracy ± 0.01 pH (Tacussel Instruments, Lyon, France) and CO₂ concentrations were measured using the Gran titration method (Talling, 1973), in order to verify that deoxygenation did not induce an alkalosis due to a decrease of Pco₂.
changes in $O_2$ consumption (between normoxia and Pc) versus $P_0$, ($P > 0.05$). In contrast, the slopes of regression lines were significantly different between epigean and hypogean species ($P < 0.05$).

For each species a “starting” value was chosen as a $P_0$ where the rate of pleopod beats was significantly different from normoxic value ($P < 0.05$, Fig. 2). In all species (except $S$. virei, not measured), there was a linear increase in the rate of pleopod beats between the starting $P_0$ value and Pc. These restarting values differed among the four crustacean species: 120 Torr for $G$. fossarum, 100 Torr for $A$. aquaticus, and 80 Torr for both species of $N$. rhenorhodanensis. There were no differences among the four species in the slope of the regression line for the number of pleopod beats versus $P_0$ between the starting $P_0$ and the Pc ($P > 0.05$).

Near the Pc, each species hyperventilated, with a ventilation rate $3.5 \pm 0.2$ times greater than the normoxic rate in both epigean species and $2.7 \pm 0.1$ times higher in both species of $N$. virei (significantly different between epigean and hypogean species, $P < 0.05$) (Fig. 2).

Below the Pc, all the species showed dramatic decreases in $O_2$ consumption and ventilatory activity (except $S$. virei, not measured). There were no differences among the four species in the slope of the regression line for the number of pleopod beats versus $P_0$ between the Pc and 10 Torr ($P > 0.05$) (Fig. 2).

Respiration and Ventilation during Hypoxic Prestress and Posthypoxic Recovery

All species except $N$. virei showed a high but transitory hyperventilation during the hypoxic prestress (before the recovery phase.
Fig. 2. Ventilatory activity in declining P₀₂ for the epigean crustaceans Gammarus fossarum and Asellus aquaticus, and for the hypogean crustaceans Niphargus rhenorhodanensis and Niphargus virei, at 11°C in darkness. Values are means ± SE for N = 8 animals, E = epigean species, H = hypogean species.

Fig. 3. At a P₀₂ lower than 0.2 Torr (near anoxia), O₂ consumption could be considered negligible (Fig. 4).

All species showed a high hyperventilation (Fig. 3) and an overshoot in O₂ uptake immediately after the onset of posthypoxic recovery (Fig. 4), with values much higher than those found in normoxia. After 4–5 h recovery, ventilation and respiration showed a gradual decline, taking about 9 h for N. virei, N. rhenorhodanensis, S. virei, and A. aquaticus and 15 h for G. fossarum, before both rates resumed normoxic levels (and initial O₂ and ventilation patterns were restored).

Each species (except S. virei, not measured) showed the highest hyperventilation just after the onset of recovery (at 1 h), with a ventilation rate 2.6 ± 0.1 times higher than normoxic rate for both species of Niphargus (not significantly different between both hypogean species, P > 0.05), 3.1 ± 0.1 times higher for A. aquaticus, and 3.4 ± 0.1 times higher for G. fossarum (significantly different between epigean and hypogean species, P < 0.05).

Similarly, the five species displayed their highest overshoot in O₂ consumption just after the onset of recovery (at 1 h), with values approximately twice normoxic rates (Fig. 4).

The excess volume of O₂ consumed during recovery (i.e., in excess of that which would have been consumed in normoxia without hypoxic stress, calculated as the area between the observed curve (during the 24 h of recovery) and the normoxic curve (Fig. 4), is presented in Table 1 for the five species. Excess volumes were significantly different between each species, P < 0.05, except between G. fossarum and A. aquaticus. This additional poststress O₂ consumption repre-
sented the payment of the O₂ debt (Herreid, 1980) incurred during the 5 h of anaerobiosis; no measurable O₂ uptake occurs at Po₂ < 0.2 Torr (near anoxia).

In comparison, the O₂ deficit theoretically incurred during the period of severe hypoxia (calculated as the area under the normoxic curve, during the 5 h of anaerobiosis) is presented in Table 1 for the five species. All these theoretical values are not significantly different from observed O₂ debt (P > 0.05), except for *A. aquaticus* and *G. fossarum*.

**DISCUSSION**

**O₂ Consumption in Normoxia**

O₂ consumption rate is generally regarded as a good index of aerobic metabolic activity. Hypogean organisms are generally believed to have a lower metabolic rate than closely related epigean species (for review, see Huppop, 1985; Ercolini et al., 1987; Hervant et al., 1997a).

The present study showed that the hypogean *N. virei*, *S. virei*, and *N. rhenorhodensis* were no exceptions to this almost general rule, with O₂ consumption rates 2–4 times lower than the epigean *A. aquaticus* and *G. fossarum* (Fig. 1). The presence of a low metabolic rate (and a low activity level; Hervant and Mathieu, 1995; Hervant et al., 1995, 1996, 1997a) in taxonomically different groups of hypogean animals led to the supposition that this was one of the most important adaptations to subterranean life (Vandel, 1964). Poor and discontinuous food supplies and alternately hypoxic and normoxic waters are the most important factors controlling adaptations of metabolic rates and activity in aquatic hypogean organisms (for reviews, see Huppop, 1985; Hervant and Mathieu, 1995; Hervant et al., 1995, 1996, 1997a), since these adaptations are generally considered to be the result of selection for energy efficiency.

Contrasting results have been obtained when comparing the subterranean characid *Asyanax jordani* Hubbs and Innes to the epigean *Asyanax mexicanus* (Filippi) (see Schlager and Breder, 1947) and some cave and surface amphipods, such as *Stygobromus emarginatus* (Hubricht), *Stygobromus spinatus* (Holsinger), *Stygobromus tenuis potomacus* (Holsinger) and *Gammarus minus* Say (see Culver and Poulson, 1971); no differences in respiratory rates of the subterranean and surface species were found. A suggestion
explaining these results is the absence, in both instances, of food limitation (Culver and Poulson, 1971) and perhaps of long-term hypoxia. Therefore, it appears that reduced metabolic rate is not universal in hypogean organisms, but may be more related to the energetic state of each subterranean biotope, which indeed can be highly energetic, for example, bat guano caves.

Respiration and Ventilation in Declining Po2

The respiratory response of an animal to reduced Po2 may be one of two different kinds. In one case, the animal will maintain its O2 consumption relatively unchanged by means of compensatory mechanisms (behavioral, ventilatory, and/or circulatory compensations, changes in respiratory pigments, and/or surface area for O2 conductance). In the other case, the O2 consumption will decrease with the falling Po2. Animals of the first type are called oxyregulators and animals of the second type oxyconformers. These two types are merely two ends of the large spectrum of respiratory responses of different species. Animals never fully belong to one or the other type and below the Pc, a regulator becomes a conformer. In many regulators, O2 consumption shows a slight dependence on the Po2, even above the Pc value. An efficient regulator, well adapted to low Po2 conditions, would have a low Pc point, but generally (as in this study) an abrupt transition point does not exist, which complicates the interpretation (for review, see Herreid, 1980).

Moreover, for numerous species, the Pc value is well known to be affected by numerous endogenous factors, such as level of activity, physiological state, size in some species (especially the smallest), or stage in the molt cycle, as well as by environmental factors, such as temperature, time of day, laboratory stress, or salinity (Herreid, 1980). Consequently, it is very difficult to make successful and meaningful interspecific comparisons of respiratory responses to declining O2 partial pressure, especially with subterranean amphipods that are very sensitive to environmental variations (Ginet, 1960), without taking into account these physiological characteristics and without taking special precautions such as performing the study with unstressed animals, with animals of the same sex and size and in the same intermolt period, and measuring respiration and ventilation at the same temperature and the same period of day.

All species investigated were able to maintain their O2 consumption rates at constant levels and independent of Po2 between normoxia and Pc (Table 1). Hypogean species possessed lower Pc values than the epigean ones (Table 1) and, therefore, probably maintained an aerobic metabolism for a longer time in declining Po2 instead of partly switching to a low energy anaerobic metabolism. In subterranean crustaceans, maintenance of the aerobic respiration rate under hypoxic conditions was certainly helped by their lower metabolic rates. This hypothesis is probably also true for hypoxia-tolerant burrowing decapods possessing Pc values between 10 and 45 Torr (for review, see Taylor and Atkinson, 1991).

In the five crustaceans investigated, O2 consumption fell drastically below the Pc value (Fig. 1), but Hervant et al. (1995, 1996, 1997b) showed an important anaerobic compensation for severe hypoxic conditions (Po2 < 0.2 Torr) in these species, indicating a heterometabolic conformity pattern (changes in oxygen consumption reflect changes in anaerobic input, as defined by Herreid, 1980).

The study of ventilation under declining O2 concentrations (Fig. 2) led to similar conclusions as in previous investigations. In crustaceans, O2 uptake rates are mainly regulated, between normoxia and Pc, by increasing ventilation during decreasing O2 concentration (Walshe-Maetz, 1956; Herreid, 1980; McMahon and Wilkens, 1983).

All the results presented above and previous ones (Hervant and Mathieu, 1995; Hervant et al., 1995, 1996, 1997b) show that these hypogean species are better adapted to low O2 content, and are better equipped to remain aerobic under hypoxia, than both epigean species, even the hypoxia-tolerant A. aquaticus (see Hervant et al., 1996). These differences probably explain the higher survival times in hypoxia found for Niphargus (see Hervant and Mathieu, 1995).

Respiration and Ventilation during Posthypoxic Recovery

Aquatic arthropods differ in tolerance to hypoxia or anoxia. Many species, including numerous crustaceans, contract O2 debts that are repaid upon return to normoxia, but other invertebrates do not (Taylor et al., 1977; Butler et al., 1978; Herreid, 1980). O2 debt is a
Fig. 3. Ventilatory activity in normoxia (3 h), severe hypoxia (5 h), and during subsequent recovery (24 h) for the epigean crustaceans *Gammarus fossarum* and *Asellus aquaticus*, and for the hypogean crustaceans *Niphargus rhenorhodanensis* and *Niphargus virei*, at 11°C in darkness. Values are means ± SE for *N* = 8 animals, *E* = epigean species, *H* = hypogean species.

widespread phenomenon, reported for numerous invertebrates (for reviews, see Herreid, 1980; Ellington, 1983) and occurs for several reasons: replenishing O$_2$, ATP, and phosphagen stores depleted during severe hypoxia; the oxidation of anaerobic end products (mainly lactate or alanine) once normoxic conditions return; or the conversion of these end products back into storage products, such as glycogen (Herreid, 1980). The repayment of the debt during recovery from anaerobic stress involves a significant increase of O$_2$ consumption (and, therefore, in the rate of pleopod beats).

The present study showed that duration and/or intensity of hyperventilation (Fig. 3) in hypogean organisms were lower than in epigean ones. For both epigean species, O$_2$ consumption curves (Fig. 4) showed a supernormal O$_2$ debt (Herreid, 1980). The excess O$_2$ consumed during posthypoxic recovery greatly exceeded the O$_2$ deficit predicted during severe hypoxia (Table 1). This kind of pattern has been observed in several crustaceans (Taylor et al., 1977; Butler et al., 1978), and it indicates increased energetic expenditures, especially those linked to locomotion and ventilation, in these animals (Hervant and Mathieu, 1995; Hervant et al., 1995, 1996, 1997b).

On the contrary, the hypogean crustaceans studied here showed a pattern close to a normal O$_2$ debt (Herreid, 1980). The excess O$_2$ consumed during posthypoxia was almost equal to the O$_2$ deficit predicted during severe hypoxia (Table 1), since these organisms mainly convert their anaerobic end products back into stored glycogen during reoxygenation (Hervant et al., 1995, 1996, 1997b). Such a pattern was also found by Taylor et al.
Fig. 4. O2 consumption in normoxia (3 h), severe hypoxia (5 h), and during subsequent recovery (24 h) for the epigean crustaceans *Gammarus fossarum* and *Asellus aquaticus*, and for the hypogean crustaceans *Niphargus rhenorhodanensis*, *Niphargus virei*, and *Stenaxellus virei*, at 11°C in darkness. Values are means ± SE for N = 8 animals, dw = dry weight, E = epigean species, H = hypogean species.

(1977) in the shore crab *Carcinus maenas* (L.).

Recent observations in the hypogean *S. virei*, *N. virei*, *N. rhenorhodanensis*, and in the epigean *A. aquaticus* and *G. fossarum* showed that part of the end products was excreted and part metabolized (Hervant et al., 1995, 1996, 1997b). We have calculated the amount of O2 needed for the complete oxidation of the unexcreted end products, according to the following equations:

1 lactate + 3 O2 → 3 CO2 + 3 H2O
1 alanine + 3 O2 → 3 CO2 + 2 H2O + 1 NH3
1 succinate + 5.5 O2 → 4 CO2 + 3 H2O

Calculation of O2 demand for end-product oxidation (ratio of anaerobic end products utilized during a 24-h recovery period, from Hervant et al., 1995, 1996, 1997b) is listed in Table 2. Moreover, part of the O2 consumed during posthypoxic recovery would account for the resaturation of body fluids. It is clear that these O2 requirements exceeded the excess volume of O2 consumed during recovery (O2 debt) by hypogean species (Table 2, P < 0.05). Thus, a high proportion of the accumulated end products (and especially lactate) served as fuel for the resynthesis of glycogen via the glyconeogenesis pathway, as previously shown by Hervant et al. (1995, 1996, 1997b). Recent experiments using injection of labeled glucose and lactate revealed that the glucconeogenesis rate in *N. virei* during posthypoxic recovery was higher than all rates previously measured for epigean crustaceans (Hervant, 1996). On the contrary, O2 requirements were lower than the observed
Table 2. Fate of anaerobic end products during a 24-h posthypoxic recovery period, theoretical $O_2$ demand for end-products oxidation, and excess $O_2$ consumed during recovery for the epigean crustaceans Gammarus fossarum and Asellus aquaticus, and for the hypogean crustaceans Niphargus renorhodanensis and Niphargus virei, at 11°C in darkness. Values are means ± SE for $N =$ 8 animals. Calculation methods in the Discussion section. *Values from Hervant et al. (1995, 1996, 1997).

<table>
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<th>Species</th>
<th>Origin</th>
<th>Amount of anaerobic end-products metabolized during a 24-h posthypoxic recovery period</th>
<th>Theoretical $O_2$ demand for end-products oxidation [1]</th>
<th>Excess $O_2$ consumed during recovery [2]</th>
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<tr>
<td>Niphargus virei</td>
<td>hypogean</td>
<td>lactate: 100 μmol/g dw alanine: 13 μmol/g dw succinate: 1.5 μmol/g dw</td>
<td>347 μmol $O_2$/g dw</td>
<td>76 ± 4 μmol $O_2$/g dw</td>
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<td>Niphargus renorhodanensis</td>
<td>hypogean</td>
<td>lactate: 178 μmol/g dw alanine: 23 μmol/g dw succinate: 2 μmol/g dw</td>
<td>614 μmol $O_2$/g dw</td>
<td>103 ± 6 μmol $O_2$/g dw</td>
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<td>Stenasellus virei</td>
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<td>lactate: 106 μmol/g dw alanine: 18 μmol/g dw succinate: 0 μmol/g dw</td>
<td>372 μmol $O_2$/g dw</td>
<td>156 ± 13 μmol $O_2$/g dw</td>
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<td>Gammarus fossarum</td>
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<td>lactate: 8 μmol/g dw alanine: 6 μmol/g dw succinate: 0 μmol/g dw</td>
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<td>405 ± 21 μmol $O_2$/g dw</td>
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<td>Asellus aquaticus</td>
<td>epigean</td>
<td>lactate: 35 μmol/g dw alanine: 18 μmol/g dw succinate: 0 μmol/g dw</td>
<td>160 μmol $O_2$/g dw</td>
<td>421 ± 23 μmol $O_2$/g dw</td>
<td>263%</td>
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</table>

extra $O_2$ consumption for both epigean species (see Results section, $P < 0.05$), indicating that a high proportion of the unexcreted end products may have been used for oxidation, as also previously shown by Hervant (1996) and Hervant et al. (1995, 1996, 1997b). Hypogean species applied a strategy of lactate removal different from that observed in epigean crustaceans, favoring lactate-supported gluconeogenesis and glyconeogenesis and rapid glycogen replenishment instead of rapid lactate removal via oxidative pathways during recovery from anaerobiosis (Hervant, 1996; Hervant et al., 1995, 1996, 1997b). These results do not support the idea of a close relationship between the size of the $O_2$ debt and the amount of accumulated end products. Another explanation for the lower $O_2$ debt observed in subterranean species is the lower energetic expenditures noticed under severe hypoxia (due to a decrease in locomotory and ventilatory activities; Hervant and Mathieu, 1995).

In conclusion, the lower $O_2$ debt observed in these hypogean species might be explained in two ways. First, a decrease in general metabolism during hypoxia might be correlated with the decreased use of metabolic reserves (glycogen, arginine phosphate) observed during the anaerobic stress (Hervant et al., 1995, 1996, 1997b), and second, these organisms used preferably the glyconeogenesis pathway from end products during posthypoxic recovery (Hervant, 1996; Hervant et al., 1995, 1996, 1997b), which requires less $O_2$ than end-product oxidation (Herreid, 1980). Moreover, the occurrence of a low metabolic rate in normoxia, a further reduction in their metabolic rate by lowering energetic expenditures, and a high glycogen resynthesis ability are ecologically very advantageous for these subterranean aquatic organisms, which have to cope frequently with alternation of hypoxic and normoxic phases, with sometimes a very rapid switch from high to low $P_0$ (Hervant, 1996). Finally, the ability among hypogean crustaceans to maintain and to rapidly restore a high amount of fermentable fuels (without feeding) is considered a very efficient adaptive strategy that allows them to extensively exploit a moving mosaic of hypoxic and normoxic patches, such as in some deep phreatic aquifers.

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