

Comparison of routine metabolic rates of the stygobite, *Gammarus acherondytes* (Amphipoda: Gammaridae) and the stygophile, *Gammarus troglophilus*

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SUMMARY

1. Reduced metabolic rate among cave organisms compared with surface species has long been suggested as an adaptation to food shortage in cave environments. However, comparisons of metabolic rates between species have not often included closely related surface and cave species. By measuring metabolic rate across three seasons and over a range of body sizes, we examined the hypothesis that the routine metabolic rate of *Gammarus acherondytes*, a federally listed stygobitic amphipod, is lower than that of the closely related stygophilic *Gammarus troglophilus*. To determine if human activities increased the supply of organic matter to caves, we also examined the relationship between residential development and bacterial contamination in water wells.
2. For *G. acherondytes*, the slope of the overall relationship between oxygen consumption and body dry mass did not differ from zero and did not vary seasonally, whereas for *G. troglophilus* it was positive and higher in summer than in winter and spring. These results provide insights into a potential novel metabolic adaptation among stygobites. Higher metabolic rate in young *G. acherondytes* would allow efficient use of typically transient energy sources and a low metabolic rate at larger body sizes would increase survival through periods of food scarcity.
3. The number of wells with faecal coliform contamination was weakly but positively correlated with the number of residential building permits, indicating that surface land-use changes probably increase the availability of energy in groundwater systems inhabited by *G. acherondytes*. This may give stygophilic animals, with higher metabolic rates, a competitive advantage in the caves, thus reducing the abundance of stygobites such as *G. acherondytes*.

Keywords: amphipods, caves, Crustacea, groundwater contamination, metabolic rate

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Introduction

Beyond the entrance and twilight zones, caves lack light and therefore photoautotrophs and the autochthonous carbon available to epigeal terrestrial and aquatic ecosystems (Poulson & White, 1969; Hüppop, 2000; Poulson & Lavoie, 2000). Scarcity of food, coupled with other characteristics of cave environments such as constant darkness, stable temperature and high humidity (Poulson & White, 1969; Howarth, 1983; Langecker, 2000) have influenced the evolution

of a variety of adaptations – morphological, physiological and behavioural – of cave-limited animals (stygobites) that are often quite different from those of their nearest surface-dwelling relatives (Culver, 1982; Christiansen, 1992; Culver, Kane & Fong, 1995). Stygobites generally have a lower metabolic rate (Culver, 1982; see Hüppop, 1985 for review; Hervant *et al.*, 1997; Hervant, Mathieu & Messana, 1998), which results in the conservation of energy reserves (Hervant & Renault, 2002) and the capacity to survive longer periods of food scarcity relative to epigean or facultative cave animals (stygophiles) (Biswas, 1991; Hervant *et al.*, 1997; Hervant & Renault, 2002; Issartel *et al.*, 2005). These physiological adaptations also make stygobites vulnerable to replacement by more metabolically active taxa – stygophiles and epigean species – if the availability of energy (quantity and frequency) in caves increases. This can result in profoundly altered cave communities (e.g. Malard *et al.*, 1994, 1996; Simon & Buikema, 1997) and may lead to the extirpation, or extinction of highly troglomorphic taxa, because many stygobites are endemic to small geographic areas (e.g. Culver & Sket, 2000).

Energy enters caves in various ways, including the faeces of animals which feed on the surface and use caves as roosts or shelters (Poulson, 1972; Culver, 1982; Gnaschini & Trajano, 2000), the death of surface animals which wander or fall into caves (Hüppop, 2000), roots of plants (Howarth, 1983), diffuse percolation of energy-laden water from the surface (Simon, Benfield & Macko, 2003), and discrete flow of water and associated organic matter into caves (e.g. via sinkholes and sinking streams) (Poulson & White, 1969). The latter combined with changes in surface land-use such as arable agriculture, urban development and faulty septic systems are linked to the increased abundance of organic matter and bacteria in caves (Malard *et al.*, 1996; Panno *et al.*, 1996; Simon & Buikema, 1997). Such energy rapidly becomes available to grazers including amphipods and isopods, via bacteria and other microorganisms in biofilms (Simon *et al.*, 2003). Septic leachate increased food availability and resulted in the loss of stygobitic species in a cave in the south-eastern U.S.A. (Simon & Buikema, 1997), while replacement of stygobites by stygoxene taxa in aquifers affected by septic effluent has been recorded in France (Malard *et al.*, 1994, 1996). These studies suggest that land use change, especially increased

urbanisation of aquifer recharge areas (analogous of stream catchments) negatively affect stygobitic faunas. Run off from agricultural fields combined with septic leachate also may be increasing the energy available in caves of the Sinkhole Plain of Illinois, U.S.A., and threatening the survival of stygobites in those caves. This is of special interest because the stygobitic amphipod, *Gammarus acherondytes* (Hubricht and Mackin), which is a federally listed species in the U.S., is endemic to a 230 km² area of the Sinkhole Plain in Illinois, U.S.A. [U.S. Fish and Wildlife Service (USFWS), 2002].

Here we test the hypothesis that the seasonal metabolic rate of *G. acherondytes*, a stygobite, is lower than that of the co-occurring stygophilic amphipod, *Gammarus troglophilus* (Hubricht and Mackin). In addition, we also evaluate trends in faecal coliform bacteria in wells, as a measure of organic pollution and energy availability to the shallow karst aquifers to assess the fate of populations of these congeneric amphipods in caves of Illinois' Sinkhole Plain.

Methods

Study site

The Sinkhole Plain in south-western Illinois, U.S.A. is characterised by numerous caves, high sinkhole density (>90 sinkholes km⁻²) and shallow karst aquifers (Panno *et al.*, 2003). Discharge in cave streams increases rapidly after rain events, indicating little interaction with the overlying soils. Land use is predominantly agriculture (arable and livestock pasture), with dramatic residential growth since 1987 (Panno *et al.*, 1996). Consequently, agricultural runoff and septic leachate contribute organic matter and bacteria (faecal coliforms) to the cave streams (Panno *et al.*, 1996; Taylor, Webb & Panno, 2000; Panno *et al.*, 2001, 2003; Taylor *et al.*, 2003).

Amphipods were collected from Illinois Caverns (90°8'W, 38°14'N), a 9.6 km long cave in Monroe County, Illinois with a drainage basin of approximately 5.4 km² (Aley, Moss & Aley, 2000). Discharge of the perennial cave stream, measured near the main entrance, averaged $4.8 \times 10^{-3} \text{ m}^3 \text{ s}^{-1}$ (range 4.8×10^{-4} to $725.3 \times 10^{-3} \text{ m}^3 \text{ s}^{-1}$ for the period February 1999 to May 2003; Taylor *et al.*, 2000, 2003) and mean annual water temperature was 13.2 °C (range 11.8–15.6 °C for the period February 1999 to May 2003; Taylor *et al.*,

2000). Other physico-chemical parameters are summarised in Taylor *et al.* (2000).

Collection of amphipods for experiments

Amphipods were collected seasonally (summer 24 July and 18 August 2002, winter 23 February 2003, and spring 4 May and 15 May 2003) to coincide with low, medium and high flows, respectively, and to cover the full seasonal range of water temperature in Illinois Caverns. Both species were captured in the cave from the substratum using small aquarium nets. The sampling site was upstream of the cave entrance, which gives access to the stream partway along its subterranean course. In the field, a stereo microscope was used to separate the species, based on morphological features (e.g. Holsinger, 1972; Lewis, 2000). Each species was placed into 4 L aerated containers, packed in ice to maintain cave temperature and transported to the laboratory. In the laboratory, amphipods were transferred to 8 L glass aquaria containing water and several large rocks from the cave stream. The amphipods were maintained in darkness at 12 ± 1.0 °C for a minimum of 1 day before respiration experiments were started. In August 2002, amphipods were allowed to acclimate for 4 days before experiments were started because the cave stream temperature was 16 °C.

Dissolved oxygen (DO) concentration, water temperature and specific conductivity were measured in the stream once during each collection period with a model 85 YSI (Yellow Springs Instrument, OH, U.S.A.) multi probe. Discharge was measured with a Global Flow Probe (FP101, Global Water, Gold River, CA, U.S.A.) or converted from a stage-discharge relationship for Illinois Caverns (Taylor *et al.*, 2000).

Oxygen consumption experiments

Respiration, measured as the mass of oxygen consumed per unit time, was measured in glass static respirometers similar to those of Culver & Poulson (1971) with a size of 6.5 cm inside diameter, providing a circular path 2 cm wide \times 1.6 cm high which held 55 mL of water. Lids were made of 1.5 cm thick polyvinyl chloride (PVC) with double O-rings. Four small (approximately 5 \times 5 \times 5 mm) aquarium stones were placed in each respirometer as substratum (cf. Culver & Poulson, 1971). Preliminary trials showed

that the addition of the stones greatly reduced the proportion of time amphipods spent swimming and closely approximated their activity in the cave stream. We did not starve animals prior to experiments because we wanted to measure and compare routine metabolic rates, which includes oxygen consumed during normal activities related to maintenance of the individual (Brett & Groves, 1979). Each experimental trial lasted a minimum of 4 h and individual amphipods were used only once. Amphipods were chosen randomly and experiments were carried out around the clock. This avoided any bias due to the potential presence of a circadian rhythm or size of amphipod being run at only a certain time of day.

The partial pressure of oxygen was recorded once every 10 s with a Clark-style microelectrode (MI-730; Microelectrodes Inc., Bedford, NH, U.S.A.) connected to a computer. To calibrate probes prior to each run, they were sequentially immersed for 10–20 min in 0.22- μ m-filtered cave water that was 100% air-saturated, 0% (nitrogen bubbled, for a minimum of 30 min), and 100% air-saturated, until the voltage had stabilised at the same reading before placing them in 0%. Probes were then inserted through an O-ring sealed hole in the lid of each respirometer. Water temperature and barometric pressure during calibration and each trial were recorded with a temperature logger (H8 Hobo; Onset Computer Co., Pocasset, MA, U.S.A.) and a digital barometer (Fisher Scientific, Palantine, IL, U.S.A.), respectively. To calculate the initial oxygen content in each respirometer we used equations in American Public Health Association (APHA) (1995; Table 4500-O:I). Temperatures for each seasonal experiment were selected to reflect the long-term seasonal average temperature and were 12.2 ± 1.0 °C for July and August 2002, and 11.3 ± 1.0 °C for February 2003 and May 2003. All chambers were tested for leaks, probe oxygen consumption, potential bacterial respiration and probe stability. Because the stones were cold sterilised with 70% ethanol before each trial, we assumed they did not contribute to oxygen consumption in the chambers. Each chamber and probe combination was tested separately with 0.22 μ m-filtered cave water containing 100% and 0% oxygen concentration for a minimum of 4 h.

Activity of amphipods was observed at half-hourly intervals during initial experiments. Amphipods of both species moved slowly around the chambers, but

predominantly sat still among the aquarium stones. Thus we are confident that the measured rates of oxygen decline are representative of routine metabolism. A total of 57 and 62 trials were completed with *G. acherondytes* and *G. troglophilus*, respectively. Specifically, 23 and 26 trials were completed in July/August 2002, 20 and 20 in February 2003, and 14 and 16 in May 2003 with *G. acherondytes* and *G. troglophilus*, respectively.

Analysis

All amphipods were photographed with the aid of a digital camera (Fuji FinePix 6800Zoom, Fuji Photo Film Co., Japan) mounted on a Leica MZ-9.5 (Leica Microsystems, Wetzlar GmbH, Germany) dissecting microscope after each trial and held in tanks before returning them to the cave at the end of the season. Because amphipods were returned, it is possible but highly unlikely (14–23 amphipods collected each time versus >6000 *G. acherondytes* in the cave, Lewis, 2003) that individuals were recaptured over the course of the study. We treated seasons as independent samples.

Dry mass of amphipods was estimated from head capsule lengths (measured from digital images) using a species-specific head capsule length-dry mass relationship (Taylor *et al.*, 2003). Oxygen consumption ($\mu\text{g O}_2 \text{ animal}^{-1} \text{ h}^{-1}$) was calculated from the slope of the linear regression of oxygen concentration in the respirometer versus time between hours 1 and 4 for each individual trial. The 3-h interval was chosen to allow the amphipods to consume detectable amounts of oxygen, while avoiding the potentially confounding problems of metabolic waste buildup and declining oxygen concentration, which are criticisms of static respiration chambers (Kedwards *et al.*, 1996). The first hour was not analysed to avoid bias in results related to increased respiration from handling the amphipods and their acclimation to the respirometers (e.g. Wilhelm *et al.*, 1997; Hervant *et al.*, 1998).

To compare rates of oxygen consumption between seasons and species, we used analysis of covariance (ANCOVA) with the rate of oxygen consumption as the response variable, body dry mass as the covariate and season or species as the grouping variable. Relationships were \log_{10} - \log_{10} transformed prior to analysis to conform to expected relationships between surface area and body mass (Peters, 1983). This also satisfied

assumptions of homogeneity of variance and normality (Sokal & Rohlf, 1981).

Respiration rates of other amphipod species

To place our measured rates of oxygen consumption in context, we surveyed the literature for respiration rates of other amphipod species from hypogean and epigean habitats. All published rates were converted to $\mu\text{g O}_2 \text{ animal}^{-1} \text{ h}^{-1}$, a standard unit for respiration studies with invertebrates (e.g. Franke, 1977; Sandeman & Lasenby, 1980; Kedwards *et al.*, 1996) and standardised to a 5 mg (dry mass) animal for comparison (e.g. Hoback & Barnhardt, 1996). Standardisation to whole animal rates avoids the erroneous comparison of specific metabolic rates (i.e. $\text{O}_2/\text{unit body mass}/\text{time}$) among studies that use animals differing in mass, owing to the negative relationship between mass specific oxygen consumption and body mass (e.g. Peters, 1983; Schmidt-Nielsen, 1984; Packard & Boardman, 1999). For respiration rates presented per unit mass, the average mass of the species reported was used in combination with the Power Law:

$$B = aM^\alpha$$

where B is the oxygen consumption; M is body mass; a is a constant and α is the scaling constant. We set α at 0.75 (Hendriks, 1999; Dodds, Rothman & Weitz, 2001) to estimate the metabolic rate of a 5 mg individual. Dry mass was estimated at 15% of wet mass and ash free dry mass was converted to dry mass by assuming an ash content of 15% (e.g. Nalepa, 1991). Volume of oxygen ($\mu\text{L O}_2$) was converted to mass ($\mu\text{g O}_2$) by multiplying by 1.43 (Gnaiger, 1983; Peters, 1983). The mean dry mass for *Gammarus fossarum* (Koch), *Niphargus virei* (Chevreux) and *N. rhenorhodensis* (Schellenberg) from the Hervant *et al.* (1997, 1998) studies were obtained by multiplying the cited fresh weight by 0.227, 0.244 and 0.252, respectively. These conversions were supplied by the author (Hervant, personal communication) for the 1998 study. No temperature corrections were made, and temperatures at which each study was conducted were recorded.

Faecal coliform bacterial contamination of groundwater in Monroe County

Faecal coliform bacterial contamination of shallow water wells in Monroe County, Illinois, as an indicator

of organic pollution, was assessed by examining records obtained from the Monroe-Randolph Bi-County Health Department. Data included the number of wells sampled, the number of wells with coliform bacteria, and the percentage of wells with faecal coliform contamination for each year between 1986 and 2003. The number of building permits issued in Monroe County between 1986 and 2003 was obtained from the county courthouse in Waterloo, Illinois. We examined the relationship of the percentage of wells with faecal coliforms as a function of the number of building permits. Because the number of wells tested dropped markedly after 1995, we also examined the relationship between the percentage of wells with faecal coliforms as a function of the number of wells tested per year.

Results

Values for dissolved oxygen, water temperature and specific conductance during the study were within the range previously recorded for Illinois Caverns (Table 1). Discharge ranged from 5.2 to $725.3 \times 10^{-3} \text{ m}^3 \text{ s}^{-1}$, the latter being the highest recorded for Illinois Caverns (Table 1).

The total number of amphipods collected per visit varied from <5 to >100 and was negatively related to discharge and turbidity in the stream. Precopula pairs of amphipods were not removed from the cave stream to minimise our impact on recruitment. Amphipods used in the respiration experiments varied in size (dry mass and length) from 0.88 to 34.5 mg and 5.6 to 17.5 mm for *G. acherondytes*, and 0.88 to 68.8 mg and 6.4 to 25.3 mm for *G. troglophilus*, respectively. Adult *G. acherondytes* were smaller than those of *G. troglophilus*, and few very large *G. acherondytes* were collected or seen in the cave.

Measurement of respiration

Probe response was rapid and linear over the calibration range, conforming to specifications by the manufacturer. Probe stability was excellent and oxygen did not leak into chambers, as evidenced by the constant flat line output at the residual probe voltage for 0% saturation. Boundary layer and stirring artefacts were not observed during the experiments because of the small ($25 \text{ }\mu\text{m}$) cathode diameter and the continuous movement of water in the chamber attributed to pleopod beating.

A total of 57 and 62 respiration trials were completed with *G. acherondytes* and *G. troglophilus*, respectively. For both species, individual respiration rate was highly variable ($\text{CV} = 47\%$ for *G. acherondytes* and $r^2 = 0.54$ and 0.39 for *G. troglophilus*, respectively; Fig. 1) and several amphipods had extremely low rates (Fig. 1). For *G. acherondytes*, oxygen consumption did not increase with body size and seasonal regressions were not different from zero ($P > 0.05$) except in May 2003 ($P < 0.001$). Although this resulted in a significant interaction term (date $\times \log_{10}$ dry mass, $F_{2,55} = 5.0$, $P = 0.010$) in the ANCOVA analysis for *G. acherondytes*, we examined the group effect because the positive slope was because of only two data points outside the general cluster of the data. No seasonal differences ($P = 0.28$) in the respiration rate was detected and it was on average $8.8 (\pm 0.93 \text{ SE}) \text{ }\mu\text{g O}_2 \text{ animal}^{-1} \text{ h}^{-1}$ (Fig. 1a). In contrast, oxygen consumption increased with amphipod dry mass for *G. troglophilus* in all seasons ($P < 0.05$; Fig. 1b). Slopes of seasonal regressions were similar (ANCOVA, $F_{2,58} = 1.6$, $P = 0.20$), but elevations of regressions differed significantly (ANCOVA, $F_{2,60} = 42.6$, $P < 0.001$). *Post hoc* comparisons showed that the respiration rate for February and May 2003 (adjusted

Table 1 Basic water chemistry data on dates of amphipod collections at Illinois Caverns (Monroe County, IL, U.S.A.). Means for 1999–2000 (Taylor *et al.*, 2000) given for comparison.

| Season | Date | Water temperature °C | Specific conductance ($\mu\text{S cm}^{-1}$) | Dissolved oxygen (mg L^{-1}) | Dissolved oxygen (% saturation) | Discharge [$\text{m}^3 \text{ s}^{-1}$ ($\times 10^{-3}$)] |
|--------|------------------|-------------------------|---|--|------------------------------------|--|
| Summer | 24 July 2002 | 13.3 | 617 | 10.16 | 97.1 | 7.8 |
| | 18 August 2002 | 16.0 | 609 | 10.00 | 101.0 | 5.22 |
| Winter | 23 February 2003 | 11.5 | 458.3 | 10.26 | 94.6 | 26.61 |
| Spring | 15 May 2003 | 13.8 | 585 | 10.13 | 98.1 | 725.27 |
| | (1999/2000) | 13.2 | 543.5 | 9.07 | – | 4.84 |

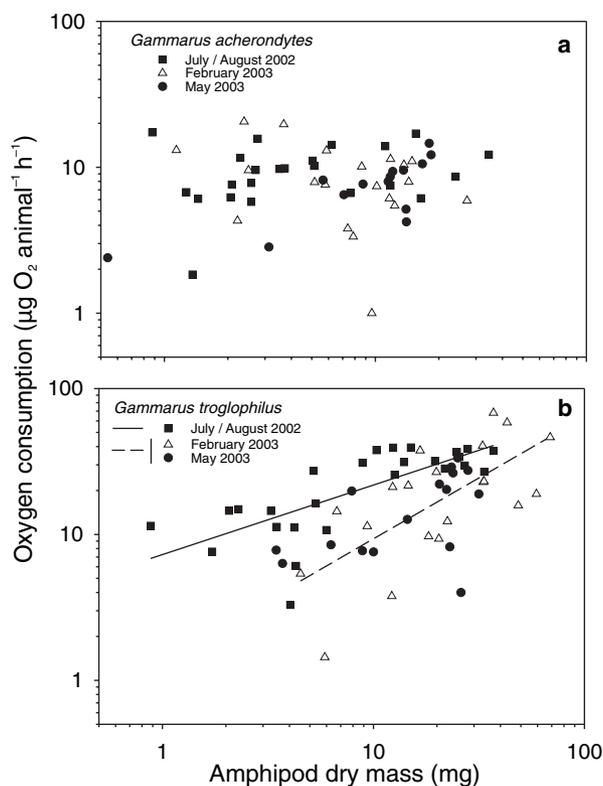


Fig. 1 The seasonal relationships between oxygen consumption and amphipod dry mass for *Gammarus acherondytes* (a) and *Gammarus troglophilus* (b) from Illinois Caverns, IL, U.S.A. Because the relationship for *G. acherondytes* was not significant ($P_{\text{slope}} = 0.41$), oxygen consumption $8.77 (\pm 0.93 \text{ SE}; n = 57)$ did not vary with body weight. The regression equations in the form \log_{10} oxygen consumption ($\mu\text{g O}_2 \text{ animal}^{-1} \text{ h}^{-1}$) = $\log_{10} a$ [$\pm 95\%$ CI (confidence interval)] + $b(\pm 95\%$ CI) \log_{10} amphipod dry mass (mg) for *G. troglophilus* are: July/August 2002 ($\mu\text{g O}_2 \text{ animal}^{-1} \text{ h}^{-1}$) = $0.862(\pm 0.19) + 0.477(\pm 0.18) \log_{10}$ amphipod dry mass (mg) ($n = 26$; $r^2 = 0.54$; $P < 0.001$); and February and May 2003 ($\mu\text{g O}_2 \text{ animal}^{-1} \text{ h}^{-1}$) = $0.339(\pm 0.38) + 0.682(\pm 0.30) \log_{10}$ amphipod dry mass (mg) ($n = 36$; $r^2 = 0.39$; $P < 0.001$).

mean = $12.8 \mu\text{g O}_2 \text{ animal}^{-1} \text{ h}^{-1}$) was about half of that in July/August 2002 (adjusted mean = $25.4 \mu\text{g O}_2 \text{ animal}^{-1} \text{ h}^{-1}$; Fig. 1b). Lack of a significant relationship between body mass and oxygen consumption for *G. acherondytes* precluded a statistical comparison between the two species.

Respiration rates of other amphipod species

The respiration rate of other amphipod species from different aquatic habitats varied from 1.3 to $79.3 \mu\text{g O}_2 \text{ animal}^{-1} \text{ h}^{-1}$ (Table 2), a range which includes the rates we recorded for *G. acherondytes*

and *G. troglophilus*. Among stygobites, the rate for *G. acherondytes* was approximately two times lower than that for *G. minus* from caves in Virginia and approximately three to 4.5 times higher than those recorded for *Niphargus verei* and *N. rhenorhodanensis* from France (Table 2). Among stygophiles, the rate for *G. troglophilus* was similar to that recorded for *G. minus* from groundwater springs in Virginia (Table 2). Overall, rates of oxygen consumption among stygobites were approximately two times lower than those of stygophiles or epigeal species.

Faecal coliform contamination of wells

Faecal coliforms were detected in water wells in Monroe County, Illinois, in all years (1986–2003). The proportion of water samples from wells containing faecal coliforms and the number of building permits issued in Monroe County was positively but weakly correlated ($P = 0.155$, $r^2 = 0.24$, for 1986 to 1995 when between 255 and 480 wells were sampled per year; and $P = 0.218$, $r^2 = 0.10$ for the period 1986 to 2003; Fig. 2a). Overall, there was a general positive trend in the number of building permits issued per year in Monroe County since 1986, whereas the number of water wells sampled declined (Fig. 2b). A strong positive relationship ($P < 0.001$, $r^2 = 0.63$) was found for the percentage of wells with faecal coliforms as a function of the number of wells sampled (Fig. 2c). The presence of faecal coliforms strongly suggests that faecal matter enters the cave ecosystems.

Discussion

The two to 4.5 times higher oxygen consumption among mature individuals of the stygophilic *G. troglophilus* compared with the stygobitic *G. acherondytes* supports the general trend that subterranean organisms have a lower metabolic rate than related surface species (Poulson, 1964; Mösslacher & Creuzé des Châtelliers, 1996; Hervant *et al.*, 1997, 1998). In addition, the rates of oxygen consumption measured for *G. acherondytes* and *G. troglophilus* fall within the range reported for other amphipod species, including those from hypogean environments (Table 2). A lower metabolic rate should be advantageous for organisms in food-poor habitats such as caves (Hüppop, 1985), or in interstitial spaces where they can experience large

Table 2 Respiration rates of amphipods from a variety of hypogean (below ground) and epigean (above ground) habitats

| | Species | Rate | °C | Habitat | Source | | |
|------------------------------|--|---|------------------------|---------------------|------------------------------|------------------------------|--|
| Hypogean | Stygobite | <i>Gammarus acherondytes</i> | 8.8 | 11–12 | CS | This study | |
| | | <i>Gammarus minus</i> I (Say) | 15.1 | 13 | CS | Culver & Poulson, 1971 | |
| | | Benedict's Cave | | | | | |
| | | <i>Gammarus minus</i> II | 9.2 | 13 | CS | Culver & Poulson, 1971 | |
| | | Coffman's Cave | | | | | |
| | | <i>Stygobromus spinatus</i> * (Holsinger) | 11.3 | 13 | CS | Culver & Poulson, 1971 | |
| | | McClung's Cave | | | | | |
| | | <i>Stygobromus emarginatus</i> * (Hubricht) | 3.5 | 13 | CS | Culver & Poulson, 1971 | |
| | | Court St. Levisay's Cave | | | | | |
| | | <i>Niphargus virei</i> (Chevreux) | 1.5 [†] | 11 | SP | Hervant <i>et al.</i> , 1997 | |
| | | 4.9 [‡] | | SP | | | |
| | | 3.2 | 11 | SP | Hervant <i>et al.</i> , 1998 | | |
| | <i>Niphargus rhenorhodanensis</i> (Schellenberg) | 1.4 [†] | 11 | IN | Hervant <i>et al.</i> , 1997 | | |
| | | 3.0 [‡] | | | | | |
| | | 3.8 | 11 | IN | Hervant <i>et al.</i> , 1998 | | |
| | | Stygobite [mean (range)] | 6.0 (1.4–15.1) | 12 (11–13) | | | |
| | Stygophile | <i>Gammarus troglophilus</i> | 15.7 | 12 | CS | This study (July/August) | |
| | | | 5.2 | 11 | CS | This study (February) | |
| | | | 8.3 | 11 | CS | This study (May) | |
| | | <i>Gammarus minus</i> III | 7.4 | 13 | SP | Culver & Poulson, 1971 | |
| Fort Spring | | | | | | | |
| <i>Gammarus minus</i> III | | 18.3 | 13 | SP | Culver & Poulson, 1971 | | |
| U.S. no. 219 Spring | | | | | | | |
| <i>S. tenuis potamacus</i> * | | 8.3 | 13 | SP | Culver & Poulson, 1971 | | |
| Fairfax Co. Va. | | | | | | | |
| | | Stygophile [mean (range)] | 11.2 (6.5–18.3) | 12.4 (11–13) | | | |
| Epigean | <i>Pontoporeia hoyi</i> (Kröyer) | 1.5 | 10 | PL | Johnson & Brinkhurst, 1971 | | |
| | <i>Pontoporeia hoyi</i> | 1.9 | 12 | PL | Johnson & Brinkhurst, 1971 | | |
| | <i>Pontoporeia hoyi</i> | 1.5 | 4 | PL | Nalepa, 1991 | | |
| | <i>Diporeia hoyi</i> (Bousfield) | 10.9 | 7 | PL | Wilhelm <i>et al.</i> , 1997 | | |
| | <i>Pontoporeia affinis</i> (Lindström) | 2.4 | 4.6 | PL | Cederwall, 1979 | | |
| | <i>Pontoporeia femorata</i> (Kröyer) | 1.3 | 4.6 | PL | Cederwall, 1979 | | |
| | <i>Paramoera walkeri</i> (Stebbing) | 3.0 | 2 | OA | Opaliński, 1974 | | |
| | <i>Hyalella azteca</i> (Saussure) | 4.1 | 15 | LL | Mathias, 1971 | | |
| | <i>Gammarus fossarum</i> (Koch) | 5.2 [†] | 11 | SS | Hervant <i>et al.</i> , 1997 | | |
| | | 9.1 [‡] | | SS | | | |
| | | 8.0 | 11 | SS | Hervant <i>et al.</i> , 1998 | | |
| | | 4.0 | 12 | SS | Franke, 1977 | | |
| | <i>Gammarus pulex</i> (Linnaeus) | 4.4 | 10 | SS | Soumalainen, 1958 | | |
| | | 5.4 | 15 | SS | Soumalainen, 1958 | | |
| | | 79.3 | 20 | SS | Rumpus & Kennedy, 1974 | | |
| | | 39.4 | 10 | SS | Rumpus & Kennedy, 1974 | | |
| | | 9.4 | 10 | SS | Nilsson, 1974 | | |
| | | 12.9 | 15 | SS | Nilsson, 1974 | | |
| | | Epigean [mean (range)] | 11.3 (1.3–79.3) | 10 (2–15) | | | |

The habitat of species is indicated as follows: Hypogean: CS, cave stream; IN, interstitial (including deep hyporheic zone); SP, karst groundwater spring; Epigean: SS, surface stream; LL, littoral lake; PL, profundal lake; OA, oceanic Antarctica.

All rates have been converted to $\mu\text{g O}_2 \text{ animal}^{-1} \text{ h}^{-1}$ and are given for a 5 mg individual (see Methods for conversions to standardise rates). Authorities of species are given with each species name.

*Formerly *Stygonectes*, interpolated from Fig. 2 and Table 1 of Culver & Poulson, 1971.

[†]Animals in starved condition.

[‡]Animals in fed condition.

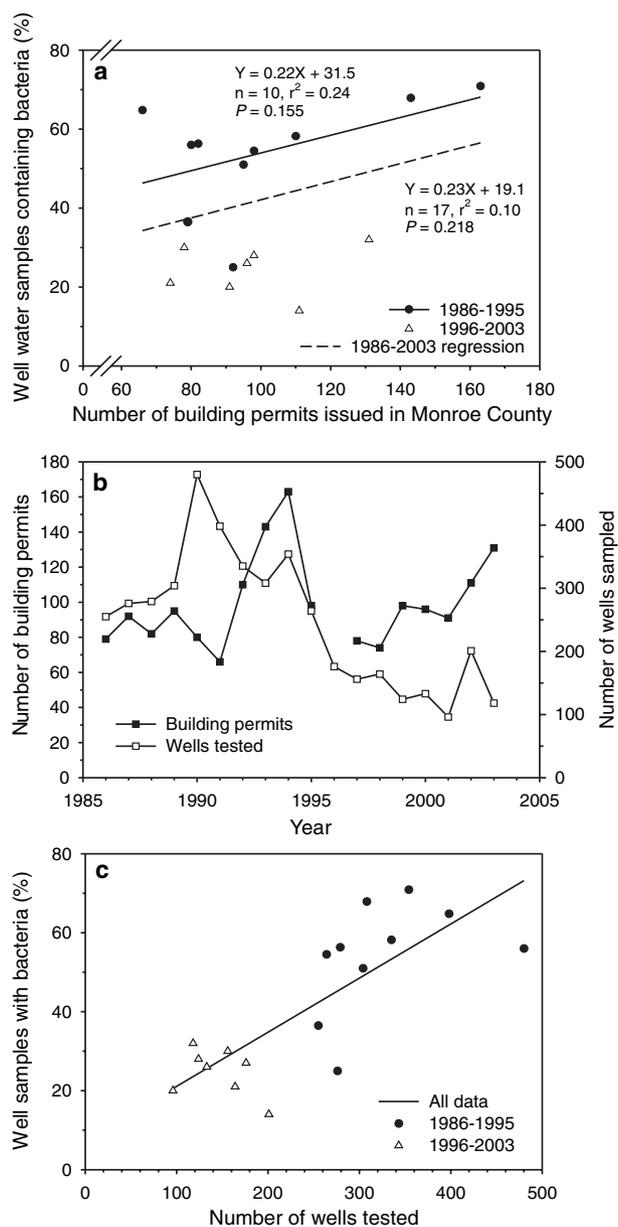


Fig. 2 Relationships between (a) water samples of wells containing bacteria and the number of building permits issued in Monroe County, (b) the number of building permits and wells sampled between 1985 and 2003 and (c) the number of wells with bacteria and number of wells tested between 1985 and 2003. Least squares linear regressions in the form $Y = m(\pm 95\% \text{ CI})X + b(\pm 95\% \text{ CI})$ are: for all data in (a) well samples with bacteria (%) = $0.23 (\pm 0.48)$ number of building permits + $19.1 (\pm 39.3)$ ($n = 17; r^2 = 0.1; P = 0.22$); for data from 1986 to 1995 in (a) well samples with bacteria (%) = $0.22 (\pm 0.37)$ number of building permits + $31.5 (\pm 33.5)$ ($n = 10; r^2 = 0.24; P = 0.16$); for all data in (c) well samples with bacteria (%) = $4.6 (\pm 1.9)$ number of wells tested + $58 (\pm 143)$ ($n = 18; r^2 = 0.62; P < 0.001$).

fluctuations in oxygen availability (Henry & Danielopol, 1999; Malard & Hervant, 1999).

The lack of a significant positive slope in the regression between the oxygen consumption rate as a function of body mass for *G. acherondytes* is difficult to explain and may be a novel result. Generally, metabolism (B) is related to organism mass (M) via the Power Law (see eqn 1 above; Peters, 1983; Schmidt-Nielsen, 1984; West, Brown & Enquist, 1997, 1999; Dodds *et al.*, 2001), in which the scaling exponent (α) is in the range of $2/3$ to $3/4$ (see West *et al.*, 1997, 1999 and Dodds *et al.*, 2001 for debate about $\alpha = 2/3$ and $\alpha = 3/4$). To our knowledge, such a result – the lack of a significant positive slope approximating the widely accepted value of $2/3$ to $3/4$ used for most animals – has not been previously reported for cave invertebrates. However, Adams & Johnson (2001) reported a negative relationship between respiration rate and body mass of Ozark cavefish (*Amblyopsis rosae* Eigenmann) in Logan Cave, Arkansas. Although the regressions for *G. troglophilus* were positive, only one slope parameter was close to $3/4$, while the other two were much lower than $2/3$. One possible interpretation of this result is that it represents an optimisation of metabolic rate for cave environments. In general, animals tend to produce offspring under favourable environmental conditions, including optimum temperature and abundant food. Although temperature varies little in cave streams, the temporal availability of food can be pronounced (e.g. Hüppop, 1985 and references therein). Young amphipods with high rates of metabolism could make good use of transient food patches into which they are born. A high metabolic rate would allow rapid growth and would facilitate survival under adverse environmental conditions because young could acquire energy reserves rapidly (e.g. Kolding & Fenchel, 1981; Wilhelm & Schindler, 2000). For *G. acherondytes*, a low metabolic rate at a body size larger than 1.5, 9.3 and 5.7 mg in July/August 2002, February 2003 and May 2003, respectively, relative to *G. troglophilus*, would reduce energy expenditure between temporally variable periods of food availability. It could be argued that our results are not novel but the relationship just indicates lower energy efficiency among small-bodied relative to large-bodied individuals (e.g. Peters, 1983; Schmidt-Nielsen, 1984). However, if we recalculate our rates for *G. acherondytes* per unit mass, the current slope of zero translates to a slope of -1 . Typically, the slope of

specific metabolism versus body mass ranges from -0.25 to -0.33 (Peters, 1983; Schmidt-Nielsen, 1984). Thus a slope of -1 would indicate a large inefficiency and would be contrary to reduced metabolism and high efficiency advocated for hypogean relative to epigeal organisms (e.g. Hervant *et al.*, 1997, 1998; Malard & Hervant, 1999; Hervant & Renault, 2002). Hervant & Renault (2002) have shown experimentally that hypogean animals recover depleted energy stores faster than epigeal animals following starvation. We suggest that small-bodied or young *G. acherondytes* may have what appears to be an inefficient specific metabolic rate because they rapidly and efficiently increase body mass or energy reserves (cf. Hervant & Renault, 2002). We are unaware of any simultaneously measured oxygen consumption and growth rates for stygobites or stygophiles spanning a range of body sizes from newborn young to adults to address this issue and thus our data suggest an area for possible future research.

Several other explanations for the constant metabolic rate of *G. acherondytes* are possible. First, the lack of a positive relationship could be related to differential activity among individuals tested. Although we did not monitor activity continuously (e.g. Davies, Wrona & Kalarani, 1992; Hervant & Mathieu, 1997; Wilhelm *et al.*, 1997), observations of the entire size range of amphipods during initial calibration trials and occasional observations during each experiment showed that amphipods of both species spent the majority of time sitting among the aquarium stones and infrequently crawled or swam in the respirometers. This was the basis for our decision to proceed with the experiments *in lieu* of continuously recording activity and we are confident that the rates are representative of routine metabolism for both species. However, it does not ensure that activity did not contribute to individual variation and thus obscure a potential relationship. High variability is inherent with the measurement of individual oxygen consumption rates and is common in respiratory studies (Culver & Poulson, 1971; Hervant *et al.*, 1997, 1998; Wilhelm *et al.*, 1997). To overcome this problem, groups of similar sized animals are often tested (e.g. Nilsson, 1974). However, using groups of animals has the associated problem of not knowing if measured rates were influenced by the presence of conspecifics, which is known to modify the behaviour of invertebrates

(Burns, 1995, 2000). Second, the range in body size and dry mass of *G. acherondytes* used in the experiments may have been too small to detect a positive relationship. The animals we tested included most of the size range of *G. acherondytes* except extremely small individuals (i.e. newly born young). For the same size range as *G. acherondytes*, we did find a positive relationship for *G. troglophilus*, indicating that the size range of animals used was unlikely to be the source of the lack of a positive relationship. Lastly, it is unlikely that variation among the oxygen probes contributed to the observed relationship because we ensured that probes and chambers were randomly assigned for each trial and species. In addition, probe stability and accuracy, as indicated by the calibrations before each experiment, was excellent over the course of the study. We conclude that the unusual mass-independent oxygen consumption relationship we recorded for *G. acherondytes* is real.

Because of the lack of equivalent data for other amphipods from cave streams or hypogean interstitial habitats, unlike the case for epigeal species (e.g. Johnson & Brinkhurst, 1971; Mathias, 1971, Fig. 9; Nilsson, 1974, Fig. 7; Franke, 1977, Fig. 14), it is impossible to place the relationship between respiration rate and body mass we observed for *G. acherondytes* in context. The fact that two of the slope estimates for *G. troglophilus* were also below $2/3$ may indicate a fundamental difference between epigeal and hypogean animals in the allometric relationship between metabolism and body mass thought to exist among all animals (West *et al.*, 1997, 1999). This lack of comparable data, suggests a potential avenue of future research for cave biologists.

The seasonality observed in the respiration rate of *G. troglophilus* may indicate its surface origin, because it is similar to temperature-dependent metabolic rates reported for epigeal species (Mathias, 1971; Franke, 1977; Sandeman & Lasenby, 1980). Along the Mississippi River in south-western Illinois, *G. troglophilus* commonly occurs in streams in caves (Peck & Lewis, 1978), but is also found in epigeal habitats including spring runs and their associated streams (Jenio, 1980). These streams experience annual and diurnal temperature changes that exceed the $3-4$ °C annual range typical in cave streams of the Sinkhole Plain in Illinois (Taylor *et al.*, 2000). In addition to temperature-mediated

seasonal changes, the respiration rate of *G. troglophilus* may be affected by the availability of food washed into the cave. In February and early May (winter and mid-spring), the frequency of storms and the production of organic matter outside the caves is low. Little new material is available for transport into the caves. However, in late spring and summer, storms are more frequent (Taylor *et al.*, 2000) and production from surface plants, including shed leaves, are available for transport into the caves. Thus high metabolic rates during summer may allow this amphipod species to utilise the increased food more efficiently.

The low metabolic rate of stygobites, which make them ideally suited to food-poor hypogean environments, may also be the source of their demise. Our results, as well as those of others (e.g. Malard *et al.*, 1994, 1996; Simon & Buikema, 1997) suggest a direct link between the decline in abundance of stygobites and the increase of organic matter and stygophiles. Typically, traces of septic leachate or, as in our case, the presence of faecal coliforms (e.g. Panno *et al.*, 1996; Taylor *et al.*, 2000, 2003) in caves or aquifers generally indicate inputs of organic matter. Often these increases are due to alterations in surface land-use and are associated with intensified agricultural use or urbanisation (e.g. Fig. 2b). Simon *et al.* (2003) experimentally showed that organic matter rapidly becomes available to amphipods in caves via bacterial biofilms. If food ceases to be a limiting factor in hypogean environments, it may be expected that stygophilic species would become more abundant and even potentially replace stygobites. In Illinois' Sinkhole Plain, *G. acherondytes*, with its constant respiration rate, would be at a disadvantage at large body sizes compared with *G. troglophilus*, because many life history characteristics such as growth, age at reproduction and number of offspring per reproductive event are tied to metabolic rate. Our research thus suggests a possible mechanism by which replacement of stygobites by stygophiles may occur in hypogean environments. Unfortunately, the high failure rate of rural septic systems in the Sinkhole Plain (approximately 50%, Panno *et al.*, 1996), coupled with the current rapid urbanisation, is problematic. Conservation of *G. acherondytes* and other stygobites will require a concerted effort to curb unnatural inputs of energy into groundwater ecosystems.

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