

Climatic conditions, diapause and migration in a troglophile caddisfly

V. SALAVERT*, C. ZAMORA-MUÑOZ*, M. RUIZ-RODRÍGUEZ*, A. FERNÁNDEZ-CORTÉS[‡] AND J. J. SOLER[†]

*Departamento de Biología Animal, Universidad de Granada, Granada, Spain

[†]Estación Experimental de Zonas Áridas, Consejo Superior de Investigaciones Científicas, Almería, Spain

[‡]Departamento de Ciencias de la Tierra y del Medio Ambiente, Universidad de Alicante, Alicante, Spain

SUMMARY

1. Factors explaining the end of diapause include environmental conditions such as daily photoperiod, temperature and humidity. However, because all these factors are almost constant inside deep caves, they would hardly affect diapause termination in many animal taxa that use such habitats in which to aestivate or hibernate (such as bats and some insects, snakes or frogs).

2. An innate biological signal, based on body reserves (mass), could determine the end of diapause in cave-dwelling animals. Another possibility is that the diapausing animals may use as a stimulus convective air circulation, produced by a fall in temperature outside the cave to a value below that inside (i.e. temperature inversions). Here, we explored these two non-exclusive hypotheses explaining the end of diapause and the start of migration in the caddis *Mesophylax aspersus*. This species aestivates as an adult in caves, as a physiological adaptation to seasonal drought and stream drying.

3. The variation in body mass of individuals entering and leaving the cave was similar, suggesting no role for the 'reserve level' hypothesis in breaking diapause. However, the onset of a temperature inversion in autumn coincided with the breaking of diapause followed after a few days by the migration of the caddisflies.

4. We conclude that the seasonal air currents, produced by thermal inversions, could be the migration stimulus for caddisflies and many other animals that spend a period of diapause in caves.

Keywords: cave, convective air circulation, *Mesophylax aspersus*, summer diapause, temporary streams

Introduction

Timing the onset of migration, often associated with seasonal reproduction in particular areas, is crucial for many birds, mammals (Sandell *et al.*, 1991; Hoover, 2003) and insects (Dingle, 1996; Hardie, Gibson & Wyatt, 2001). Most of the putative stimuli for migration are related to environmental conditions (e.g. Hill & Gatehouse, 1993), but the possibility of

innate biological signals, such as those based on energy (i.e. fat) reserves, must be considered (Saunders, 2002).

Caves are used as a temporal refuge by some insects (e.g. Bouvet, 1971; Tercafs, 2000) and endothermic (bears, bats) and ectothermic (snakes and frogs) vertebrates (e.g. Sexton & Hunt, 1980; Resetarits, 1986; Humphries, Speakman & Thomas, 2005). Caves are quite constant environments in terms of day length, temperature and humidity (e.g. Culver, 1982) and it seems unlikely that the troglophile fauna uses variations in conditions in the timing of events such as migration. However, a phenomenon known as 'cave breathing', convective currents due mainly to changes

Correspondence: V. Salavert & C. Zamora-Muñoz, Departamento de Biología Animal, Facultad de Ciencias, Campus Fuentenueva s/n, Universidad de Granada, E-18071, Granada, Spain. E-mail: salavert@ugr.es; czamora@ugr.es

in atmospheric pressure or to differences between the temperature inside and outside the cave (Christoforou, Salmon & Cass, 1996; Fernández-Cortés, 2005; Fernández-Cortés, Calaforra & Sánchez-Martos, 2006), could inform animals about the environment outside the caves. In this sense, the onset and breaking of dormancy in troglophile frogs and snakes have been related to the reversals in the thermal gradient between soil surfaces inside and outside the cave (Sexton & Hunt, 1980; Resetarits, 1986). Dormancy is a state of reduced metabolism, and two types can be distinguished ecologically: hibernation in winter or aestivation in summer (Saunders, 2002). For other animals inhabiting deep caves, such as bats, other factors as external ambient temperature or barometric pressure have been suggested as important for migration decisions (Berková & Zupal, 2006). In any case, because caves are used by many animals for hibernation and aestivation, and abandoning the cave at an optimal time profoundly affects reproductive success, the study of factors potentially explaining migration decision in troglophile animals would help to understand the biology of these species.

Some caddisfly species, belonging to the '*Stenophylax* group' (Schmid, 1957), are called 'subtroglophile' because their adults are cave-dwelling (Bournaud, 1971; Botosaneanu, 1974). The larvae of these species exploit temporary waters and the adults undergo aestivation, or summer diapause, delaying reproduction until autumn or winter when larval habitats are refilled or rain is imminent (Bouvet, 1971; Botosaneanu, 1974; Denis, 1977; Wiggins, Mackay & Smith, 1980; Williams, 2006). This diapause incurs a minimum energetic expenditure during this period (Bouvet, 1971; Botosaneanu, 1974; Denis, 1974). Mating takes place after aestivation and before migration (e.g. Botosaneanu, 1974; V. Salavert, C. Zamora-Muñoz & M. Ruiz-Rodríguez, unpubl. data), and both sexes leave the caves (Bouvet, 1975; V. Salavert, C. Zamora-Muñoz & M. Ruiz-Rodríguez, unpubl. data).

Although several studies deal with the ecology of this troglophile group (e.g. Bournaud & Bouvet, 1969; Bournaud, 1971; Bouvet, 1975; Malicky, 1987) our knowledge of them is quite limited. Very little is known about how adults locate caves, or which factors induce them to leave this subterranean habitat. The survival of troglophile caddisflies largely depends upon the match between the time at when they break their diapause and leave the cave, and the

onset of rain. The study of factors that determine the migration from the cave to oviposit are therefore particularly interesting in these insects.

Here, we tested two non-exclusive hypotheses to explain the end of the aestivation and the initiation of migration from the cave. The first hypothesis (H1) suggests that energy reserves (indicated by body mass) act as an internal signal that determines the breaking of the summer diapause. Troglophile caddisflies do not feed as adults (Bournaud, 1971; Malicky, 1987), and survive mainly on the fat reserves accumulated by the larva (Bournaud, 1971). Thus, assuming a constant rate of body mass lost during the diapause, the time taken to consume reserves could indicate the optimal time for abandoning the cave. Flight is a very energetically expensive activity that produces great physiological demands on the individual (Dudley, 2001; Goldsworthy & Joyce, 2001). Thus, errors on the time of leaving the cave would be under strong selection. The second hypothesis (H2) suggests that an abiotic factor, such as a thermal inversion (Christoforou *et al.*, 1996; Fernández-Cortés *et al.*, 2006), induces the breaking of diapause and leaving the cave. In particular, troglophile insects could use air currents to infer environmental conditions outside the caves and hence, to 'decide' to migrate.

The first hypothesis (H1), infers an internal biological signal that does not depend on environmental conditions but on body condition. It firstly predicts that, because most individuals would leave the cave upon reaching a similar body condition, the variation of body mass of the individuals captured at departure time (autumn) would be smaller than that estimated for those captured when they arrived at the cave (spring). Moreover, because body condition of diapausing individuals at a target date would largely depend on body condition when they arrive to the cave, as well as on their arrival date, H1 secondly predicts that the migration should not be concentrated in a short period, because it depends on individual body mass and date of entering the cave.

From the second hypothesis (H2) we predict, first, that thermal inversion should coincide with the date when caddisflies end their diapause. Fulfilment of this prediction would suggest that insects might detect and use the convective circulation of air to determine the start of migration. Secondly, if the migration is induced by this environmental change, most individuals would start migration on the same date. In other

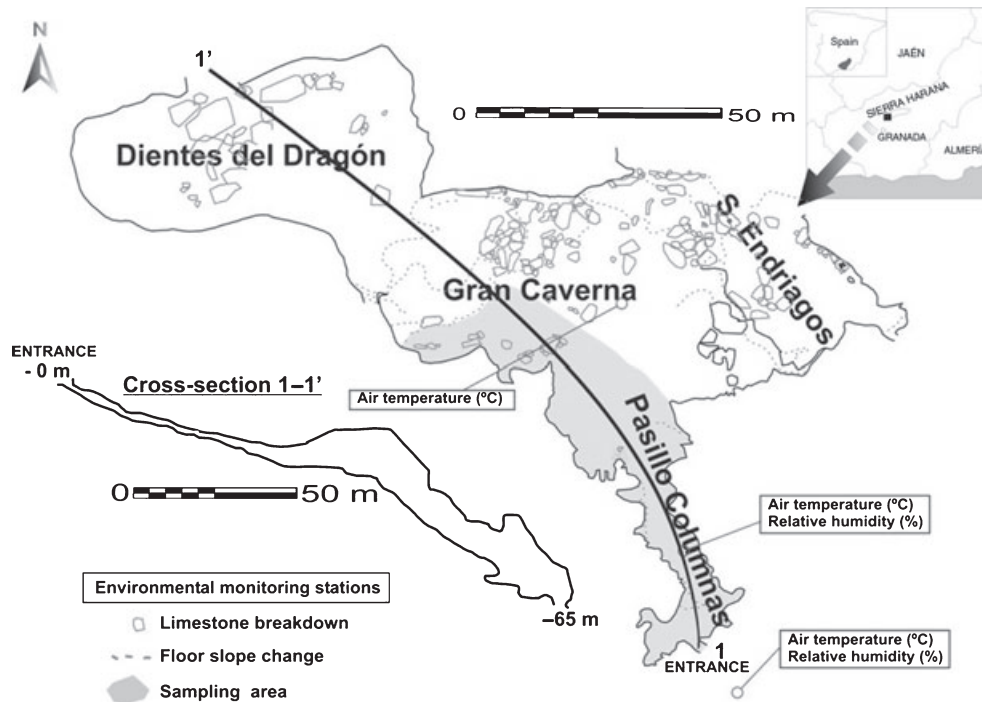


Fig. 1 Partial topography of the Cueva del Agua (based upon: González-Ríos & Marín, 1994) showing: (1) sampling area for trogliphile caddisflies (grey area), (2) spatial location of the environmental monitoring stations (cross-section 1-1').

words, a concentrated migration predicts a leptokurtic frequency distribution of captured individuals, with a kurtosis value significantly higher than zero. In contrast, in a non-concentrated migration (as in the second prediction of H1), the frequency distribution of individuals at the departure period will follow a normal distribution or with a kurtosis term significantly smaller than zero.

Methods

Study site

The Cueva del Agua de Iznalloz is located in south-east Spain (Granada) in the occidental area of the Sierra Harana mountains. This cave is a subterranean laboratory for environmental hydro-geological research, intensely monitored since 1995 (González-Ríos & Marín, 1994; Barranco *et al.*, 2004; Fernández-Cortés *et al.*, 2006). The only known natural entrance to the cave lies at 1746 m a.s.l. (the maximum altitude within the cave) and the cave is 165 m deep. The caddisfly sampling area, with a length of approximately 135 m, extended from the entrance through the 'Pasillo de las Columnas' gallery as far as the

chamber of 'Gran Caverna' (Fig. 1). The sampling area for the environmental variables studied, extended from the entrance through the 'Pasillo de las Columnas' gallery and the chamber of 'Gran Caverna', as far as the 'Dientes del Dragón' zone, covering a depth of 65 m (cross-section 1-1', Fig. 1).

Species and sampling methods

The study species was *Mesophylax aspersus* (Rambur 1842). This species is the most abundant in the *Stenophylax* group (Limnephilidae) found in the Cueva del Agua (V. Salavert, C. Zamora-Muñoz and M. Ruiz-Rodríguez, pers. obs.). The sampling procedure consisted of a visual inspection of the sampling area (Fig. 1), searching actively for adults caddisflies on the walls and ceiling of the cave. Individuals were caught by hand nets or directly with forceps. Taking account of the arrival and departure times of the caddis, the sampling period covered the spring (March–May) and autumn (October–December) of 2000, 2002, 2003 and 2004. During these periods, the cave was visited every 2–3 days. We also visited the cave once per week in each summer, though caddisflies were never located (Tinaut, 1995; V. Salavert,

C. Zamora-Muñoz and M. Ruiz-Rodríguez, pers. obs.), probably because they hide in the fissures. After capture, individuals were transported to the laboratory in isothermal boxes (6–10 °C), where they were weighed on a digital balance (accuracy 0.0001 g; SV-120A, Gram Precision, Barcelona, Spain), and the lengths of both right and left forewings were measured to the nearest 0.01 mm using a digital calliper (Calb112; SESA Tools, Guipúzcoa, Spain) under a binocular microscope. Caddisflies were held in a small transparent glass tube while they were weighed and measured, to keep them still. Wing length was calculated as the mean value of right and left forewings. Most of the individuals were returned to the cave, except a few individuals per year that were kept in the laboratory for other scientific purposes.

Study of environmental variables

Replenishment of air within caves results from convective air circulation, such that cool air enters the cave from outside and warm air is expelled (see Christoforou *et al.*, 1996), and occurs in the Cueva del Agua. This circulation is provoked by temperature inversions caused by a fall in outside temperature to a value below that in the cave. Thermal inversions are most common during the winter (November–April), at the beginning and at the end of each day (Fernández-Cortés *et al.*, 2006).

To study climatic conditions both inside and outside the cave, data were collected using solid-state data loggers at three locations: outside the cave entrance and in the Pasillo de las Columnas (Thermos Data mod. TH; Digital Analog Systems, Roma, Italy), and within the Gran Caverna (Thermos Data mod. TE; Digital Analog Systems) (Fig. 1). Hourly measurements of temperature and relative humidity (RH) were made over 4 years (2000, 2002, 2003 and 2004). In 2004, the data-logger in the Pasillo de las Columnas was out of service from 15 October to 12 November. Temperature was measured using a resistive sensor with a range between –25 and 100 °C, with an accuracy of ±0.2 °C (0–70 °C). The resolution of air temperature was 0.1 °C for recordings outside the cave and in the Pasillo de las Columnas and 0.01 °C in the Gran Caverna chamber. RH was measured using a capacitive sensor that operates between 0% and 100% RH, with an accuracy of ±2% between 0% and 75%

RH. The resolution of the RH sensor was 0.1%. The temperature gradient was quantified as the difference in temperature between each of the monitoring stations inside and outside the cave. Two types of inversion of the thermal gradient (i.e. interchange of the air in the cave) can be distinguished: (i) partial inversion (convective circulation is restricted to the air between the exterior of the cave and the Pasillo de las Columnas) and is calculated as the difference in temperature between outside the cave and the Pasillo de las Columnas and (ii) total inversion (the denser and cooler outside air can reach as far as the Gran Caverna, displacing warmer, less denser air toward the exterior) and is calculated as the difference in temperature between outside the cave and the Gran Caverna.

Light at the three locations was measured by a luxometer with a range of 0.1 lux–199.9 Klux (Delta HD8366, Productos de Conservación S.A., Madrid, España).

Statistical analyses

The body condition of individuals arriving at and departing the cave was compared by means of ANCOVA, with wing length as the controlling variable (covariable), body mass as the dependent variable, and period and sex as the fixed factors (García-Berthou, 2001). Any interaction between fixed factors would inform us of whether the change in body condition between arrival and departure depended on the sex.

The Levene test of homogeneity of variances (Quinn & Keough, 2002) was used to compare the variance of individual body mass upon arrival and departure. In addition, the kurtosis term associated with the frequency distribution of observed caddisflies departure dates was used to explore whether migration from the cave was more or less synchronous than that expected from a normal distribution (i.e. the kurtosis term differed from zero).

To determine both the date when the number of captures was maximal and the time when migration had finished, we estimated a logistic curve of the accumulated frequencies of captures on different dates. Briefly, the number of captured caddisflies was fitted to a logistic equation of the form:

$$\text{Number of individuals} = A/[1 + e^{-K(t-t_{50})}]$$

where Number of individuals is the accumulated number of individuals captured during autumn, the departure period from the cave (t); A represents the approximate asymptote of the curve (i.e. time when migration ends); K is the capture rate constant (i.e. slope); e is the base of natural logarithms and t_{50} is the date at the inflection point of the capture curve, which indicates the date when the number of captures reached the maximum. Statistical analyses were performed using STATISTICA software[®] (StatSoft, Inc., 2006).

Results

Body condition upon arrival and departure

As expected, the body mass of females and males at departure from the cave (no. females = 82; males = 312), was significantly lighter than that on arrival (no. females = 54; males = 49) (ANCOVA controlling for wing length, effect of period, $F_{1,420} = 47.59$, $P < 0.0001$; Fig. 2). Although males were on average lighter than females (ANCOVA controlling for wing length, effect of sex, $F_{1,420} = 72.55$, $P < 0.0001$), both sexes lost weight at the same rate (ANCOVA controlling for wing length, interaction between sex and period, $F_{1,420} = 0.44$, $P = 0.51$; Fig. 2).

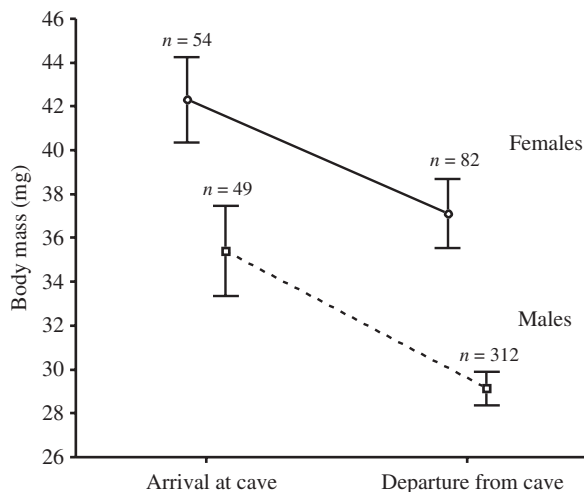


Fig. 2 Comparison of body mass of female and male caddisflies on arrival at (spring) and departure from (autumn) Cueva del Agua. ANCOVA analysis with wing length as the controlling variable (covariable), body mass as the dependent variable and the period and sex as the fixed factors. Represented values are the covariates at their means and their ± 0.95 confidence intervals (denoted by the vertical bars).

Moreover, the variances of body mass estimated for both males and females on departure from the cave were not significantly less than variances estimated on arrival at the cave (Levene Tests of homogeneity of variances, females $F_{1,134} = 3.83$, $P = 0.052$; males $F_{1,359} = 1.63$, $P = 0.203$; both male and female together, $F_{1,495} = 1.83$, $P = 0.18$). Further, the body mass of both migrating males and females was quite variable, since variances were twice the estimated mean body mass values (females, mean = 40.62, variance = 112.20, $n = 82$; males, mean = 28.04, variance = 40.90, $n = 312$). Finally, the relationship between body mass and date of capture during the departure period was not significant ($r = 0.058$, $P = 0.252$, $n = 395$). These results suggest that in neither females nor males does body condition determine the onset of migration back to the river.

Individual distribution of migration dates

Most caddisflies left the cave within a 20-day period during the autumn, most captures occurring during November (6–26 November). The median capture date was between the 13 and 20 November, depending on the year (Fig. 3a). For each of the years studied, males and females left the cave on similar dates ($t < 1.85$, d.f. > 80 , $P > 0.067$).

The frequency distribution of departure dates differed significantly from a normal distribution (Fig. 3a; Shapiro-Wilk, $W = 0.986$, $P < 0.0001$). This was partially because the distribution was sharper than could be fitted to a normal distribution (Fig. 3a; although with a non-significant kurtosis term, k (SE) = 0.193 (0.220), $t_{493} = 0.877$, $P = 0.380$), indicating that the departure of caddisflies from the cave was concentrated over a short period. Similar results were obtained when we analysed separate frequency distributions for both sexes (males, Shapiro-Wilk, $W = 0.987$, $P < 0.005$, k (SE) = 0.168 (0.255), $t_{362} = 0.659$, $P = 0.511$; females, Shapiro-Wilk, $W = 0.956$, $P = 0.002$, k (SE) = 0.719 (0.488), $t_{95} = 0.231$, $P = 0.818$). In agreement with this interpretation, the frequency distribution of captures during the arrival period to the cave did not follow a normal distribution either (Fig. 3b; Shapiro-Wilk, $W = 0.957$, $P < 0.001$). The shape of the curve was flatter than a normal distribution [platycurtic, k (SE) = -0.785 (0.435), although this was not quite significant, $t_{121} = 1.790$, $P = 0.074$; Fig. 3b). Overall, our results suggest that departure

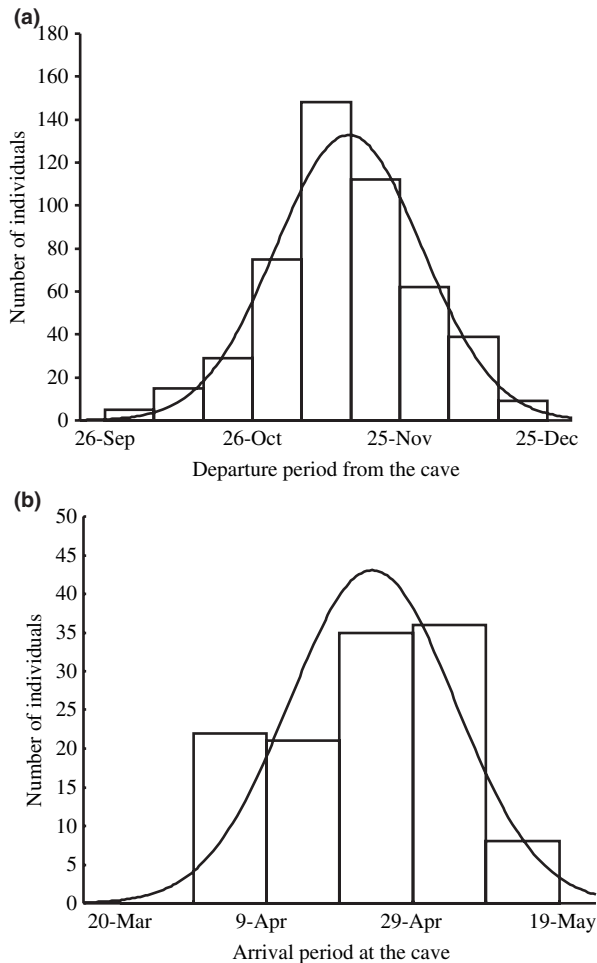


Fig. 3 Frequency distribution of individuals captured during the study upon (a) departure from and (b) arrival at the Cueva del Agua.

from the cave was synchronous but did not depend on individual body mass.

Influence of abiotic factors in the migration

The mean air temperature in the Cueva del Agua decreased gradually with distance from the Cave entrance and depth (mean daily temperature recorded in years studied, at exterior = 11 °C, Pasillo Columnas = 10.9 °C and Gran Caverna = 8.99 °C; and daily thermal amplitude recorded in years studied, at exterior = 7 °C, Pasillo Columnas = 0.4 °C and Gran Caverna = 0.02 °C). Temporal variation of air temperature also had a spatial component, with the variation being less pronounced with increasing distance from

the cave entrance (Fig. 4). At the station closest to the cave entrance (Pasillo de las Columnas), temporal variations in RH were closely linked to hygrothermic changes outside the cave, with values of RH approaching saturation (mean daily humidity over the study outside the cave was 72%, at Pasillo Columnas 99.9% and at Gran Caverna 100%; mean amplitude of daily humidity amplitude recorded outside the cave was 31.7%, at Pasillo Columnas 0.7% and at Gran Caverna 0%; Fig. 4). Beyond 7 m from the entrance (at the start of Pasillo de las Columnas) daylight was not detected and beyond 18 m the air was saturated for the greater part of the year.

An inversion in the thermal gradient is indicated by values >0 in Fig. 5a. Partial thermal inversions were more frequent and intense than total inversions. However, both types of inversions were less frequent during the late spring and summer (May–September) and peak intensities were recorded exclusively during late autumn, winter and early spring (October to beginning of May; see Fig. 5). The distribution of thermal inversions over 1 day (Fig. 5b) indicated that they were more frequent during the early morning (06:00 hours, 5.2%) due to a sharp drop in the outside temperature associated with frosts. A second peak of higher intensity occurred at the beginning of the night (21:00 hours, 4.7%), as outside air temperature fell sharply, which occurred most often in autumn and winter.

Because variations in partial and total thermal inversions gave similar results in every year studied, only results on partial inversion dates are presented for the rest of the analyses, to avoid repetition. Mean hourly values of interior–exterior thermal differences for the cave increased significantly from the late summer to the beginning of winter, independently of study year ($r = 0.729$ for year 2002, $r = 0.804$ for year 2003 and $r = 0.890$ for year 2004, $P < 0.0001$ in all the cases; Fig. 6), as the temperature outside the cave fell during autumn. During this period, thermal inversions (partial inversion, specifically) started on 23 September in 2002 (Fig. 6a), 1 October in 2003 (Fig. 6b) and 9 October in 2004 (Fig. 6c). This climatological change in the cave (i.e. a thermal inversion) produces air currents, which the caddisflies could detect and, consequently, might induce the breaking of diapause and the onset of migration from the cave.

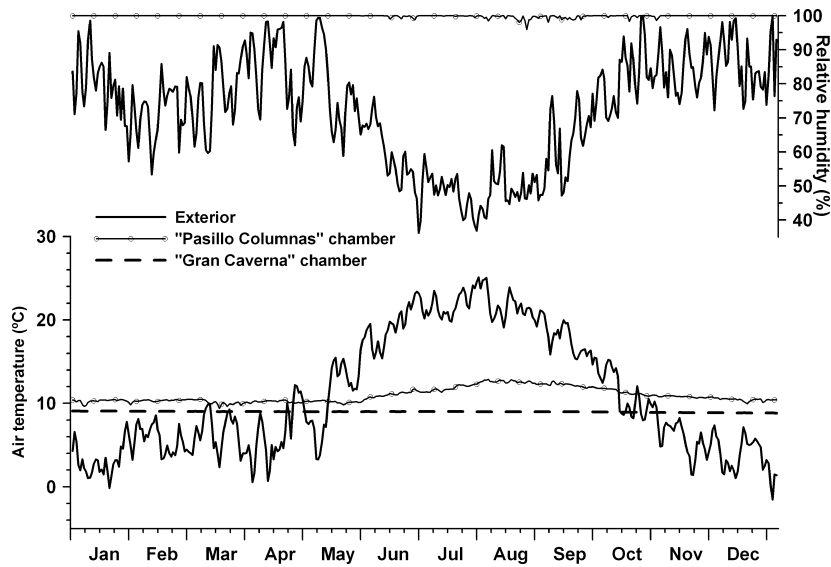


Fig. 4 Mean daily temperature and relative humidity of the air outside and inside the Cueva del Agua, estimated with data from the years 2000, 2002–04.

The accumulated number of caddisflies captured over every year sampled was a significant fit to a logistic equation (Fig. 6). The date of the first capture of caddisflies after the summer was 1 October in 2002, 7 October in 2003 and 6 October in 2004. Approximately half of the total individuals were captured between the 13 and 20 of November, depending on the year ($t_{50} = 15$ of November for 2002, $t_{50} = 13$ of November for 2003 and $t_{50} = 19$ of November for 2004; Fig. 6).

The first capture of a caddis occurred about 1 week after thermal inversion first occurred (8 and 6 days in 2002 and 2003 respectively). Moreover, the capture rate was very similar in the 3 years of the study ($K = 0.09$ for 2002, $K = 0.12$ for 2003 and $K = 0.09$ for 2004). These findings together are consistent with the second hypothesis, and infer that abiotic factors induce migration.

Discussion

Summer diapause or aestivation is a form of dormancy induced before the height of summer. The function of summer diapause in insects such as weevils, chrysomelid beetles, carabid beetles, crane-flies and caddisflies is to delay the reproductive phase until conditions are more favourable, in the autumn or winter (Masaki, 1980). The start of the diapause in adult caddisflies (which inhibits ovarian development during summer) is conditioned by photoperiod during the larval stage (Bouvet, 1974; Denis, 1977).

In troglophile caddisflies, once diapause has begun factors such as low temperature (<14 °C), low light intensity and an ambient saturated humidity (close to 100%) of some caves are important for survival (Bournaud, 1971; Bouvet, 1974, 1975; Malicky & Winkler, 1974). The Cueva del Agua, but not other caves in the area, satisfy these conditions for troglophile caddisflies such as *M. aspersus*, *Stenophylax crossotus* McL. 1884, *Stenophylax espanioli* Schmid, 1957 and *Micropterna fissa* McL. 1875 (Tinaut, 1995; V. Salavert, C. Zamora-Muñoz & M. Ruiz-Rodríguez, unpubl. data).

The general statement that breaking of diapause depends on environmental stimuli is true for some species but not for others (Tauber & Tauber, 1976, 1981). In contrast to the end of an overwintering diapause, which is mainly determined by photoperiod, moisture and food, factors explaining the breaking of summer diapause are not well known except for a few species (Tauber & Tauber, 1976; Masaki, 1980). There is evidence, however, suggesting that the end of the ovarian diapause in some epigeous and troglophile caddisflies, is associated with a short-day photoperiod (Novák & Sehnal, 1963; Gower, 1967; Svensson, 1972; Malicky & Winkler, 1974). Nevertheless, photoperiod is unlikely to explain the end of diapause in our cave-dwelling caddisflies. As in most other studies performed in caves (e.g. Bournaud, 1971; Bouvet, 1971, 1974), the effect of the photoperiod is rapidly lost in the Cueva del Agua because, for aestivation, most caddisflies flew deep into the cave,

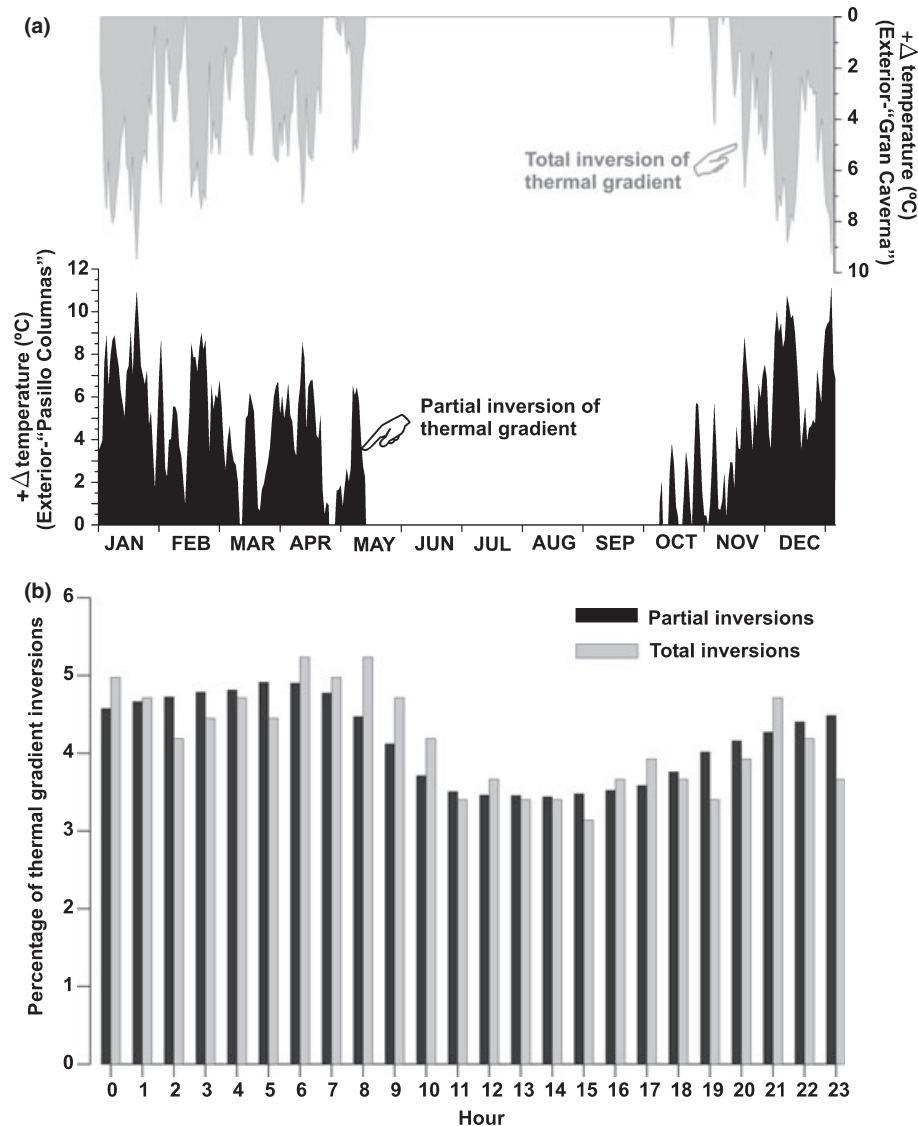


Fig. 5 (a) Mean hourly values (years 2000, 2002–04) of thermal differences between internal [Pasillo de las Columnas (partial thermal inversion) and Gran Caverna (total thermal inversion)] and external air temperature (differences >0 indicate partial or total thermal inversion). (b) Hourly distribution of both types of thermal gradient inversions (throughout an average day of the period 2000 and 2002–04).

far from the influence of daylight. Neither can declining temperature bring the end of ovarian diapause in troglophile caddisflies, because temperature inside the caves is almost constant and laboratory experiments with these species (from genera *Microp-terna* and *Stenophylax*) showed that two populations kept at different temperatures both had a synchronous diapause (Bouvet, 1971, 1974).

We hypothesized that a decline in body reserves could be the stimulus that ends the diapause in troglophile caddisflies. This hypothesis is plausible

because much energy is necessary to fly a long distance to oviposition sites (Dudley, 2001). Since *M. aspersus* mates after diapause and the females have to migrate to water for oviposition (Botosaneanu, 1974; V. Salavert, C. Zamora-Muñoz & M. Ruiz-Rodríguez, unpubl. data), an 'alarm signal' indicating a shortage of energy would be advantageous. The body mass of females and males of *M. aspersus* declined between arrival at and departure from the cave. This presumably reflects the energy costs of diapause, combined with a lack of food intake. It has

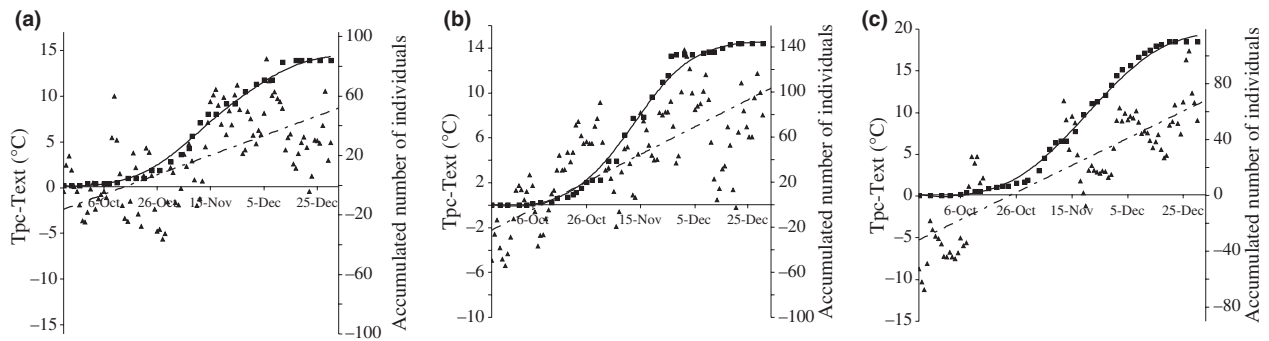


Fig. 6 Mean hourly values of thermal differences between internal (from 'Pasillo de las Columnas') and external air temperatures (Tpc-Text, triangles), from late summer to the beginning of winter, and the accumulated number of individuals (squares) captured over departure period from years 2002 (a), 2003 (b) and 2004 (c). The dotted line is a fit to the temperature, and the solid curve is a fit to the accumulated number of individuals.

been suggested that caddisflies might ingest water with organic material (e.g. amino acids, bacteria and protozoa) that percolates through cave walls (Bouvet, 1975). However, it is clear from our data, as well as from previous articles, that any such feeding cannot account for all the energy used, and that they survive mainly on the reserves of adipose tissue accumulated during larval life (Bournaud, 1971). In fact, under laboratory conditions and supplied only with water, the adults of these troglophile species survived during the summer, and the females were able to mature their ovaries and lay viable eggs (Bouvet, 1975).

The hypothesis of the existence of a biological signal, based on body reserves, did not fit our results because we found a great variation in the body mass of individuals leaving the cave for reproduction, which was not associated with the date of departure (see Results). In addition, variation in the body mass of individuals arriving did not differ from that estimated for those leaving the cave, as predicted from the 'food reserves' hypothesis. In any case, because it is possible that individuals adjust their body reserves before aestivation, we cannot completely refute the hypothesis that physical condition served as an internal signal for the termination of the diapause.

The other hypothesis tested suggests that abiotic factors were responsible for the end of the aestivation period of troglophile caddisflies. This hypothesis was founded on the well-known existence of air currents inside caves, which are mainly caused by differences in temperature inside and outside caves (see Christoforou *et al.*, 1996; Fernández-Cortés *et al.*, 2006). The study of abiotic factors in the Cueva del Agua

revealed that, although temperature inside the cave was almost constant throughout the year, the thermal gradient (differences between internal and external temperature) fluctuated depending on the seasons. Inversions of this thermal gradient provoke convective air circulation in this cave (Fernández-Cortés *et al.*, 2006; this article) that, according to our hypothesis, could be detected by caddisflies and induce the end of aestivation. The use of this cue inside the caves to begin migration in caddisflies has the advantage that thermal inversions are most frequent at the end of autumn and at the beginning of winter (October–January) (Fig. 5). More importantly, in all the years studied, the onset of high intensity thermal inversions coincided with the time of capturing the first migrating caddisflies after the aestivation period, and most of the captures were made within a short time. At that time, the resumption of flow in temporary rivers is most probably and, therefore, it is the optimum time for reproduction of these species with summer diapause. The movement of air masses in the cave, as outside temperature falls in autumn, indicates that seasonal rains are imminent. In Mediterranean-type streams, the discharge regime generally follows that of the rainfall pattern, and high flows commence abruptly in autumn or early winter (Gasith & Resh, 1999). A reversal in the thermal gradient of the soil surface has been used to explain the entry and exit from hibernation of snakes and frogs (Sexton & Marion, 1981; Resetarits, 1986). As in convective air circulation in caves, use of the thermal gradient in the soil ensures that snakes do not emerge from hibernation

until temperature conditions outside caves are beneficial and predictable (Sexton & Hunt, 1980).

To our knowledge, the seasonal airstreams produced by inversions of cave thermal gradients have never been measured or used to explain the end of the diapause and departure time in troglophile animals inhabiting deep caves (see studies of Berková & Zukal, 2006 on bats, and Tercafs, 2000 on moths, e.g.). It has been shown that some insects and spiders are able to detect the direction and frequency of air current under both laboratory and natural conditions (Barth *et al.*, 1995; Jacobs, 1995; Paydar, Doan & Jacobs, 1999). Caddisflies have long antennae, and raised areas of the integument of the head and thorax bear dense aggregations of setae (setose warts), which probably function as sensory organs (Wiggins, 2004). Air currents could be detected by the mechanoreceptor sensilla of caddisflies and could inform them, for instance, about the route out of the cave in the absence of light.

Not all caves have the same environmental properties in relation to the frequency and characteristics of thermal inversion (see Christoforou *et al.*, 1996). Thus, because of the advantages related to the use of air currents, the fact that populations of troglophile caddisflies are not found aestivating in some subterranean cavities (Bournaud, 1971; Bouvet, 1975, 1977; see before) even suggests that the abiotic conditions in such caves do not produce convective air circulation or that the thermal inversion phenomenon does not occur at the right time (i.e. at the end of autumn).

Nothing is known about how caddisflies find and choose the caves in which they aestivate, but it is supposed that it depends on interior climatic conditions. Because individuals choosing the wrong place would not reproduce, traits that facilitate finding optimal places for aestivations (i.e. caves with convective air circulation mainly occurring at the optimal time for migration) would rapidly become fixed in the population. In the cave studied here, peak intensities of thermal inversion are recorded from autumn to early spring and, within this period, at the beginning and at the end of each day (Fig. 5b, and Fernández-Cortés *et al.*, 2006). Consequently, air emanating from the cave could also be used by adults looking for aestivation places, or for detecting appropriate caves for diapause. In accordance with this possibility, we have made direct observations at the entrance of the Cueva del Agua at night, when caddisflies arrive, and

have observed that they never fly into the cave directly. Rather, they land near the cave entrance, pause outside and then crawl inside. Once in the cave, the individuals usually walk a few metres, stay near the entrance for 1 day and subsequently mostly fly deep inside the cave (V. Salavert, C. Zamora-Muñoz & M. Ruiz-Rodríguez, pers. obs.).

In summary, we present evidence for the importance of an environmental factor in caves that could determine essential life history characteristics that include breaking the diapause and the onset of migration towards oviposition areas. Such air currents in caves could also be relevant to other troglophile taxa that can detect them.

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