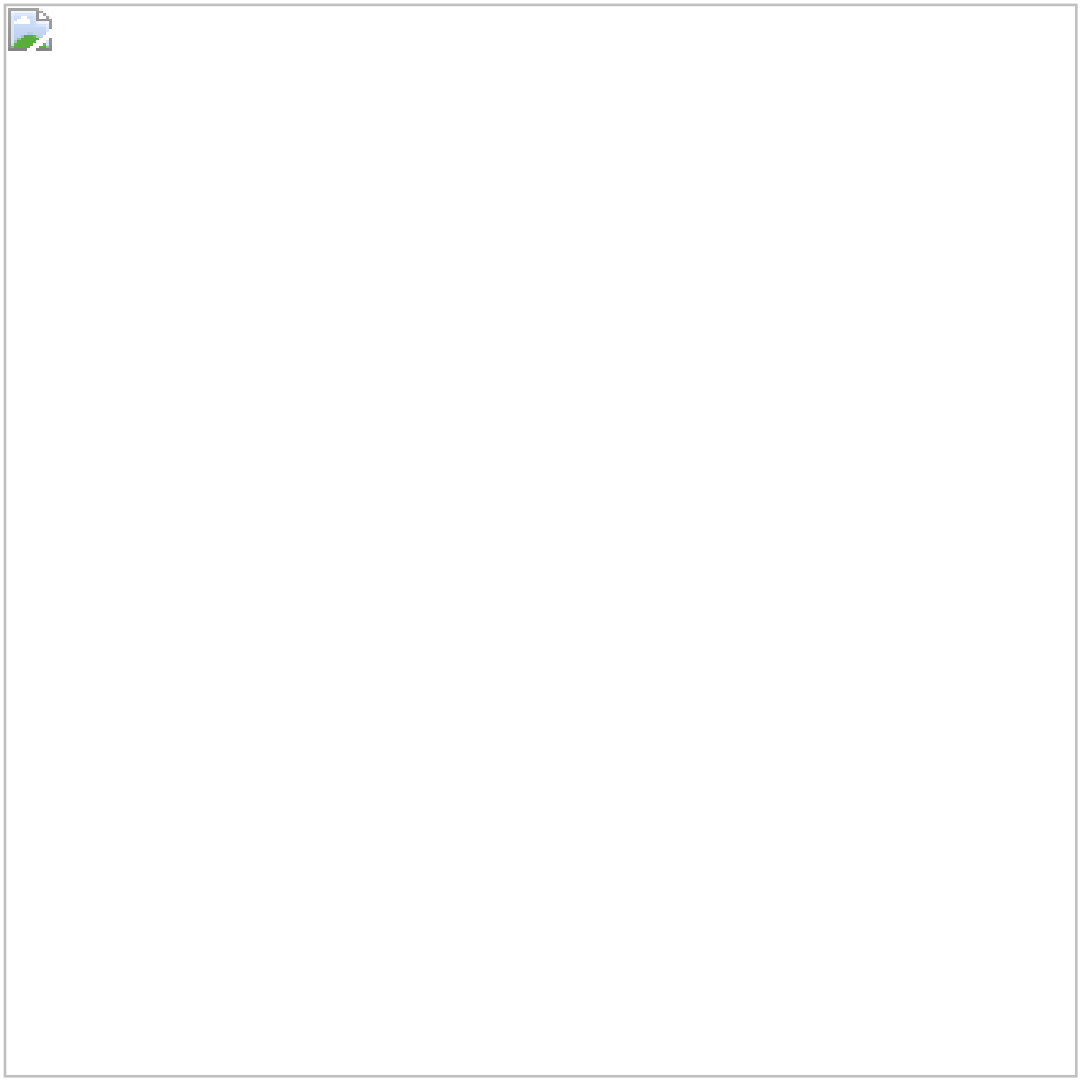

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Discontinuous gas exchange in a tracheate arthropod, the pseudoscorpion *Garypus californicus*: Occurrence, characteristics and temperature dependence.

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Abstract

The discontinuous gas exchange cycle of the pseudoscorpion *Garypus californicus*, mean mass 5.9 mg, is rudimentary and is characterized by bursts of CO_2 at frequencies ranging from 3.6 mHz at 15 °C to 13.3 mHz at 35 °C. The mean volume of CO_2 emitted per burst is 3.6 μl g^{-1} at 25 °C, about a tenth of the amount emitted by tracheate arthropods with a well developed discontinuous gas exchange cycle. Interburst CO_2 emission is high and increases with temperature, reaching near 45% of total CO_2 production rate at 35 °C. No fluttering spiracle phase is evident. The metabolic rate of *G. californicus* at 25 °C (8.4 μW) is typical of other arthropods. We infer from the high rate of interburst CO_2 emission in *G. californicus* that trans-spiracular O_2 partial pressure gradients are small and that spiracular conductance is correspondingly high, which may lead to high rates of respiratory water loss relative to arthropods with more stringent spiracular control and higher CO_2 buffering capacity. The typical moist, hypogeal environments and small body sizes of pseudoscorpions correlate well with their respiratory physiology.

Abbreviation:

C phase	closed spiracle
DGC	discontinuous gas exchange cycle
O phase	open spiracle
VCO_2	rate of CO_2 emission

Introduction

The phylogeny of the arthropods is interesting in many respects, but the independent evolution of tracheation in several distinct clades is particularly noteworthy. Hexapods are the best known tracheate arthropods, and have developed a fairly complex spiracular control strategy for managing CO_2 and O_2 gas exchange in their tracheal system (see reviews by Kestler, 1985, Sláma, 1994, Lighton, 1994, 1996, 1998). This system is often referred to as the discontinuous gas exchange cycle, or DGC (*sensu* Lighton, 1996).

Recent evidence suggests that the DGC evolved independently in several non-hexapod clades of tracheate arthropods, including the ticks (Lighton *et al.*, 1993a; Lighton and Fielden, 1995), solphugids (Lighton and Fielden, 1996) and diplopods (Klok et al., 2002). Karel Sláma has described respiratory cyclicity in a pseudoscorpion (Sláma, 1995); however, analysis of the DGC requires recordings of rates of CO_2 emission (VCO_2), which his microrespirometric system does not provide. The analysis of VCO_2 has been shown by direct spiracular observation to be an excellent indicator of spiracular activity (Lighton *et al.*, 1993b).

We here quantify the DGC of a pseudoscorpion, *Garypus californicus*, a native of seashore habitats on the western coast of the United States, as a function of temperature and thus metabolic flux rate. Pseudoscorpions possess two pairs of spiracles that are located laterally on the posterior margins of abdominal sternites 3 and 4. The spiracles open into four short tracheal trunks from which smaller branches ramify to supply the body. Pseudoscorpions are of particular interest because a clade of closely related tracheate arthropods, the solphugids (Shultz, 1990, Wheeler and Hayashi, 1998), have evolved a DGC that closely resembles that of insects (Lighton and Fielden, 1996).

Materials and Methods

Animals

Specimens of *Garypus californicus* were collected in the Northern hemisphere during Fall (September) from beneath dried kelp and rocks at the Hopkins Marine Station, Pacific Grove, California. They were maintained at 25 ± 3 °C at an ambient L/D cycle in a 20 x 20 x 5 cm polyethylene container with a floor of moist sand and pleated filter paper. They were fed twice weekly on wingless fruit flies.

Respirometry

The DGC of *Garypus californicus* was quantified with a flow-through respirometry system (Sable Systems TR-2; www.sablesystems.com) as described elsewhere (Lighton, 1991a; Lighton and Berrigan, 1995). The mass flow rate of CO_2-free air through the system was 20 ml min^{-1} regulated by a mass flow control valve (Sierra Instruments; http://www.sierrainstruments.com). For each run, we removed an individual from the pseudoscorpion colony, weighed it to 0.1 mg, and placed it in a glass and aluminum respirometry cuvette with Viton O-ring seals (Sable Systems RM-1). The animal's temperature was regulated (± 0.2 °C) by a Peltier effect temperature cabinet (Sable Systems PELT-4 equivalent), and its activity level was monitored within the *ca.* 5 ml cuvette using a Sable Systems AD-1 activity detector. Only data from motionless animals were used. Data were acquired with DATACAN V software (Sable Systems), using a sampling interval of 2 seconds, finite impulse response digital filtration within each sample, and a typical recording duration of 5250 samples. Two to four successive recordings were typically made on a given animal at a given temperature, after which it was returned to the colony.

The effect of temperature on DGC parameters was tested with analysis of variance (ANOVA) supplemented by Studentized multiple range tests (Newman-Keuls) where indicated. Regressions were calculated by least squares with axis transformation where noted, and compared with analysis of covariance (ANCOVA). Statistical software was written by JRBL and validated against known data sets (Zar, 1996) and/or other programs (SYSTAT IV). Means are accompanied by standard deviations (SD) or standard errors (SE) and sample sizes (N).

Results

We analyzed 771 DGCs by an approximately equal number (10-11) of haphazardly sampled animals at 15, 25 and 35 °C, with a mean mass of 5.90 ± 1.28 SD mg. Larger numbers of DGCs were analyzed at higher temperatures because they occurred at a faster rate. We analyzed 137 DGCs from 11 animals at 15 °C, 181 DGCs from 10 animals at 25 °C, and 453 DGCs from 11 animals at 35 °C. The data were analyzed by individual within each temperature treatment. Because the pseudoscorpion colony had approximately 19 inhabitants, and because of the random (or more precisely, haphazard) selection technique, *i.e.* selecting a new individual at random and then replacing the previously measured individual, some pseudoscorpions will have been measured more than once in different, but never consecutive, runs. This is an inevitable consequence of the restricted pool size of our experimental organisms. We have no reason to believe that our sampling technique introduced any bias, by virtue of its inherently random nature.

Typical CO_2 emission data by a 6.02 mg *G. californicus* at 15 °C are shown in Fig. 1. The DGC in our pseudoscorpions was generally characterized by incomplete spiracular closure during the constricted spiracle or "C" phase, which therefore is something of a misnomer in this group. We retain the term because spiracular closure was, on occasion in some individuals, close to or indistinguishable from zero at low temperatures. In addition, in no arthropod are the spiracles totally constricted. The reader may wish to think of the C phase as used here as an "interburst phase." No evidence was found for an F (fluttering spiracle) phase. For purposes of analysis we divided the DGC of *G. californicus* into the C and O (Open-spiracle) phases; again, we remind the reader that these terms should be understood as shorthand references to the interburst and burst phases of the DGC, respectively, rather than as references to conventional C and O phases.

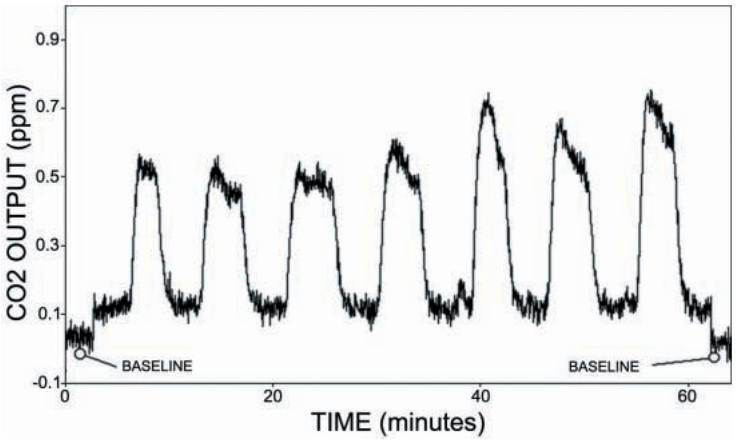

Figure 1. Typical CO_2 emission data by a 6.02 mg pseudoscorpion, *Garypus californicus*, at 15 °C. Flow rate was 20 ml min^{-1}.

Overall metabolic flux rates

At 25 °C, the mean VCO_2 of our animals was 1.126 ± 0.567 SD µl hr^{-1}, or 0.201 ± 0.101 SD ml g^{-1} hr^{-1}, N = 181 DGCs by 10 animals, mean mass 5.65 ± 1.31 mg. Assuming aerobic catabolism of palmitate (respiratory quotient 0.72), this corresponds to a metabolic rate of 8.44 ± 4.25 SD µW, which does not differ significantly from the value expected for a consensus arthropod of that mean body mass and temperature (Lighton *et al.*, 2001); 11.5 µW (t = 0.68; P = 0.4).

As might be expected because of the small range of body masses, there was no correlation between body mass and metabolic rate (ANCOVA of log body mass *vs.* log metabolic rate across the three temperature treatments, $F[1, 28]$ = 1.43, P[no correlation] > 0.2).

By regressing the logarithm of metabolic rate against temperature, the slope of that relationship (0.0336) was found to correspond to the temperature sensitivity of metabolism. Expressed as a Q_{10}, it is $10^{0.336}$ or 2.16. Using this model, temperature explains 72% of metabolic rate variance ($F[1, 30]$ = 76.9; P < 0.0001).

DGC Frequency

The DGC frequency increased exponentially with temperature, with a slope of 0.0261 for log-transformed frequency *vs.* temperature, corresponding to a Q_{10} of $10^{0.261}$ or 1.82; temperature explained 57% of DGC frequency ($F[1, 30]$ = 39.5; P < 0.0001). At 25 °C mean DGC frequency was 5.19 ± 2.66 SD mHz, corresponding to a mean cycle length of 193 seconds or slightly over three minutes.

C Phase

C phase duration increased with DGC duration (Fig. 2), with a dimensionless shared slope of 0.64 across temperatures (r^2 = 0.71; $F[1, 30]$ = 75.4; P < 0.001). The intercept is -36 ± 61 seconds,

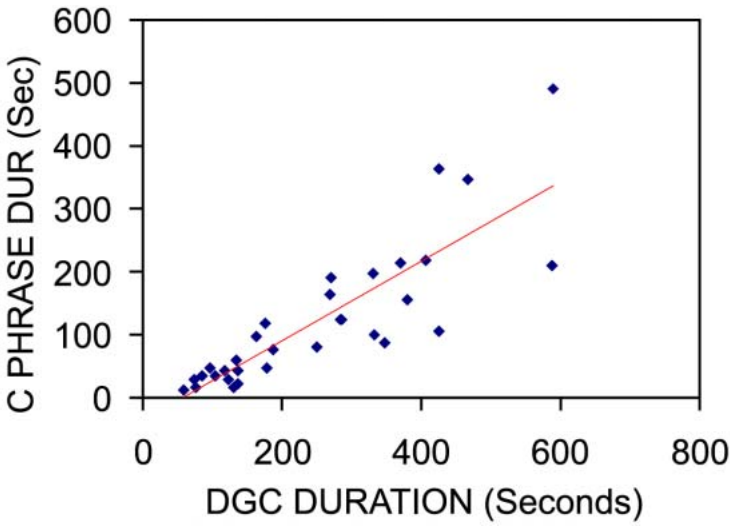

Figure 2. The relation between overall DGC duration and C phase duration in *Garypus californicus*. Each point represents the mean value for one individual. The slopes of the line is 0.67. See text.

which does not differ significantly from zero. C phase length decreased significantly ($P < 0.05$) as temperature increased, reflecting the increase in DGC frequency, and thus the reduction in DGC duration, as temperature rose (see DGC frequency above).

C phase VCO_2 increased steeply and significantly with temperature, with a Q_{10} of 2.75 ($r^2 = 0.70$, $F[1, 30] = 68.5$, $P < 0.0001$). Because of the rapid increase of C phase VCO_2 with temperature, its contribution to total CO_2 release increased with temperature ($Q_{10} = 2.75/2.16 = 1.27$) until at 35 °C it approached 45% of total CO_2 emission.

O Phase

O phase duration increased with DGC duration, with a dimensionless shared slope of 0.36 across temperatures ($r^2 = 0.45$; $F[1, 30] = 24.5$; $P < 0.001$). The intercept is 36 ± 61 seconds, which does not differ significantly from zero. O phase duration was strongly affected by temperature, decreasing from 201 ± 24 SE seconds at 15 °C to 106 ± 17 SE seconds at 25 °C and 71 ± 8 SE seconds at 35 °C (Fig 3). O phase volume rose modestly between 15 and 25 °C, from 3.06 ± 0.41 SE µl mg^{-1} to 3.63 ± 0.52 SE µl mg^{-1}. It then declined steeply at 35 °C to 2.14 ± 0.24 SE µl mg^{-1}. Thus the effect of temperature on O phase volume, though marginally significant by ANOVA ($F[2, 29] = 3.48$; $P < 0.05$), was not directional, reaching a peak value at the intermediate temperature of 25 °C. O phase VCO_2, in contrast, increased exponentially with temperature ($F[1, 30] = 45.3$; $P < 0.0001$) because of the rapid reduction of O phase duration with temperature. The Q_{10} of O phase VCO_2 was 1.97.

Discussion

It is clear from these results that although *G. californicus* does employ a DGC, it is a marginal one, lacking stringent spiracular closure. In fact the C phase contributed up to 45% of overall CO_2 release at high temperatures, whereas the DGC of *G. californicus* took the form of small peaks of CO_2 release "riding" on a large interburst baseline. Although solphugids, a clade of closely related tracheate arthropods (Shultz, 1990, Wheeler and Hayashi, 1998), have independently evolved a stringent, three-phase DGC that closely resembles that of insects (Lighton and Fielden, 1996), pseudoscorpions have apparently developed only a modest degree of spiracular control.

Some of this difference may be explained by total-animal CO_2 buffering capacity. Pseudoscorpions emit only about a tenth of the volume of CO_2 per unit mass in the O phase that hexapods and other tracheate arthropods do (see, e.g., Lighton *et al.*, 1993; Quinlan and Lighton, 1999). For example, the fairly closely related clade of the solphugids release 20 µl mg^{-1} (Lighton and Fielden 1996), approximately six to eightfold more. The ability of pseudoscorpions to regulate CO_2 release is therefore minimal compared with animals that employ the "classic" DGC. This is reflected in their high DGC frequency, about double to eight-fold that of similar sized insects (Lighton, 1991b, 1996). Investigation of CO_2 buffering capacity in pseudoscorpions would be worthwhile; it is worth noting, in this context, that the primary trigger for the burst phase in pseudoscorpions has recently been shown to be hypoxia, rather than the more conventional hypercapnia (Lighton and Joos, 2002).

The temperature dependence of the pseudoscorpions' DGC revealed no surprises. While overall metabolic Q_{10} was 2.16, the Q_{10} of DGC frequency was 1.82. Thus DGC frequency increased with temperature more slowly than rate of CO_2 emission, indicating that pseudoscorpions modulate both frequency and volume of gas exchange to accommodate changes in metabolic flux rates. The increase of CO_2 volume per DGC with increasing metabolic flux rates was mostly confined to the interburst phase. Although the O phase VCO_2 increased with temperature with a Q_{10} 1.97, O phase volume decreased because of a steep decrease in O phase duration. The majority of insects studied so far modulate DGC frequency, rather than volume, to accommodate changes in metabolic flux rates (see Lighton 1998 and 1996 for discussions).

It is certainly in the magnitude of interburst CO_2 emission that the DGC of *G. californicus* differs most radically from other tracheate arthropods that employ a DGC. The fact that interburst VCO_2 increases rapidly with temperature demonstrates that *G. californicus* is unable to sustain sufficient oxygen flux rates without losing a large amount of CO_2 and thus water vapor at the same time. This in turn suggests that the trans-spiracular gradient of oxygen partial pressure is minimal in this species. The likely

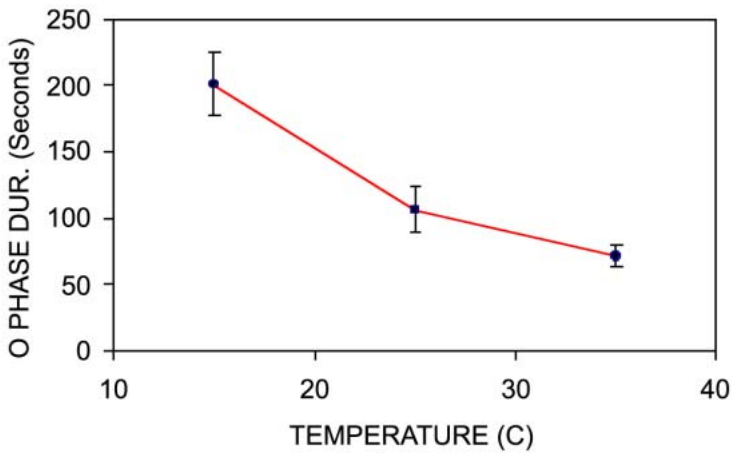

Figure 3. The relation between O phase duration and temperature in *Garypus californicus*. See text. Bars are standard errors.

presence of a minimal trans-spiracular oxygen partial pressure gradient, and an accompanying high rate of interburst CO_2 release, suggests from respiratory water loss considerations that the O phase trigger in *G. californicus* may be predominantly hypoxic rather than hypercapnic. Examining the effects of ambient oxygen partial pressure on the DGC of this species can test this hypothesis. This work has now been performed and a hypoxic O phase trigger in *G. californicus* has been confirmed (Lighton and Joos, 2002).

If *G. californicus* is unable to maintain a protracted and steep oxygen partial pressure gradient across its spiracles, then its rate of respiratory water loss in its interburst phase will be higher than in comparable animals that employ a "classic" DGC with a fully constricted C phase and a long F phase marked by low VCO_2 and endotracheal oxygen partial pressures as low as 4 kPa (see Lighton, 1998 and references therein). This is amenable to experimental testing. It is perhaps significant that pseudoscorpions are limited to marginal habitats in areas where water vapor pressure is likely to be high, such as in soil litter and under stones. Of course, the link (if any) between the DGC and respiratory water loss is somewhat controversial (see Lighton, 1998 for a discussion). Notwithstanding the above, the niche range of pseudoscorpions appears to be wider than that of another tracheate arthropod, the harvestperson (opiliones), in which spiracular control is minimal, and no cyclicity, let alone discontinuity, of external CO_2 emission is evident (Lighton 2002).

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References

Kestler A. 1985. Respiration and respiratory water loss. In: Hoffmann KH. Editor. *Environmental Physiology and Biochemistry of Insects*, pp 137-83. Springer Verlag, Berlin.

Klok, C.J., Mercer, R.D. & Chown, S.L. 2002. Discontinuous gas exchange in centipedes and its convergent evolution in tracheated arthropods. Journal of Experimental Biology 205, 1031-1036.

Lighton JRB. 1991a. Measurements on insects. In: Payne CA, editor. *Concise Encyclopedia on Biological and Biomedical Measurement Systems*, pp 201-208. Pergamon Press, Oxford.

Lighton JRB. 1991b. Ventilation in Namib Desert tenebrionid beetles: Mass scaling, and evidence of a novel quantized flutter phase. *Journal of Expeimental Biology* 159: 249-268.

Lighton JRB. 1994. Discontinuous ventilation in terrestrial insects. *Physiological Zoology* 67: 142-162.

Lighton JRB. 1996. Discontinuous gas exchange in insects. *Annual Review of Entomology* 41: 309-324.

Lighton JRB. 1998. Notes from underground: Towards ultimate hypotheses of cyclic, discontinuous gas exchange in tracheate arthropods. *American Zoologist* 38: 483-491.

Lighton JRB. 2002. Lack of discontinuous gas exchange in a tracheate arthropod, *Leiobunum townsendi* (Arachnida, Opiliones). *Physiological Entomology* (in press)

Lighton JRB, Berrigan D. 1995. Questioning paradigms: Caste-specific ventilation in harvester ants, *Messor pergandei* and *M. julianus* (Hymenoptera: Formicidae). *Journal of Expeimental Biology* 198: 521-530

Lighton JRB, Brownell PH, Joos B, Turner R. 2001. Low metabolic rate in scorpions: Implications for population biomass and cannibalism. *Journal of Expeimental Biology*. 204: 607-613

Lighton JRB, Fielden LJ. 1995. Mass scaling of standard metabolism in ticks: A valid case of low metabolic rates in sit-and-wait strategists. *Physiological Zoology* 68: 43-62.

Lighton JRB, Fielden LJ. 1996. Gas exchange in wind spiders: Evolution of convergent respiratory strategies in solphugids and insects. *Journal of Insect Physiology* 42: 347-357.

Lighton JRB, Fielden L, Rechav Y. 1993a. Characterization of discontinuous ventilation in a non-insect, the tick *Amblyomma marmoreum* (Acari: Ixodidae) *Journal of Expeimental Biology* 180, 229-245.

Lighton JRB, Fukushi T, Wehner R. 1993b. Ventilation in *Cataglyphis bicolor*: regulation of CO2 release from the thoracic and abdominal spiracles. *Journal of Insect Physiology* 39, 687-699.

Lighton JRB, Garrigan D. 1995. Ant breathing: Testing regulation and mechanism hypotheses with hypoxia. *Journal of Expeimental Biology* 198, 1613-1620

Lighton JRB, Joos B. 2002. Discontinuous gas exchange in the pseudoscorpion Garypus californicus is regulated by hypoxia, not hypercapnia. *Physiological Biochemistry and Zoology* (in press).

Lighton JRB, Wehner R. 1993. Ventilation and respiratory metabolism in the thermophilic desert ant, *Cataglyphis bicolor* (Hymenoptera, Formicidae). *Journal of Comparative Physiology* 163, 11-17.

Quinlan MC, Lighton JRBL. 1999. Respiratory physiology and water relations of three species of *Pogonomyrmex* harvester ants (Hymenoptera: Formicidae). *Physiological Entomology* 24: 293-302.

Shultz JW. 1990. Evolutionary morphology and phylogeny of Arachnida. *Cladistics* 6:1-38.

Sláma K. 1994. Regulation of respiratory acidemia by the autonomic nervous system (coelopulse) in insects and ticks. *Physiological Zoology* 67, 163-174.

Sláma K. 1995. Respiratory cycles of *Chelifer cancroides* (Pseudoscorpiones) and Galeodes sp. (Solifúgae). *European Journal of Entomology* 92: 543-552.

Wheeler WC, Hayashi CY. 1998. The phylogeny of the extant chelicerate orders. *Cladistics* 14:173-192.

Zar JH. 1996. *Biostatistical Analysis*, 3rd edition. Prentice Hall. Upper Saddle River, New Jersey