Investigating onychophoran gas exchange and water balance as a means to inform current controversies in arthropod physiology

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Summary
Several controversies currently dominate the fields of arthropod metabolic rate, gas exchange and water balance, including the extent to which modulation of gas exchange reduces water loss, the origins of discontinuous gas exchange, the relationship between metabolic rate and life-history strategies, and the causes of Palaeozoic gigantism. In all of these areas, repeated calls have been made for the investigation of groups that might most inform the debates, especially of taxa in key phylogenetic positions. Here we respond to this call by investigating metabolic rate, respiratory water loss and critical oxygen partial pressure (P_c) in the onychophoran ***Peripatopsis capensis***, a member of a group basal to the arthropods, and by synthesizing the available data on the Onychophora.

The rate of carbon dioxide release ($\dot{V}_{CO}2$) at 20°C in ***P. capensis*** is 0.043 ml CO_2 h^{-1}, in keeping with other onychophoran species; suggesting that low metabolic rates in some arthropod groups are derived. Continuous gas exchange suggests that more complex gas exchange patterns are also derived. Total water loss in ***P. capensis*** is 57 mg H_2O h^{-1} at 20°C, similar to modern estimates for another onychophoran species. High relative respiratory water loss rates (~34%; estimated using a regression technique) suggest that the basal condition in arthropods may be a high respiratory water loss rate. Relatively high P_c values (5–10% O_2) suggest that substantial safety margins in insects are also a derived condition. Curling behaviour in ***P. capensis*** appears to be a strategy to lower energetic costs when resting, and the concomitant depression of water loss is a proximate consequence of this behaviour.

Keywords: metabolism, hypoxia, respiratory water loss, cuticular water loss, discontinuous gas , exchange, invertebrate, velvet worm, respirometry

Introduction
The factors influencing variation in arthropod metabolic rates and gas exchange patterns have been the subject of investigation for at least the past 60 years. Despite much research over this period (e.g. Lighton and Garrigan, 1995; Westneat et al., 2003; Hetz and Bradley, 2005; White et al., 2007), several major controversies remain. Most prominent among these are the extent to which selection for reduction of water loss might alter both metabolic rate and gas exchange pattern (e.g. Lighton, 1998; Chown, 2002;Gibbs and Johnson, 2004; Lighton and Turner, 2008), the evolutionary origins of discontinuous gas exchange (DGE) (Marais et al., 2005), the relationship between life-history strategies and their mean metabolic rates (Reinhold, 1999; Lighton et al., 2001; Klok et al., 2002; Terblanche et al., 2004), and the likelihood that gas exchange abilities might limit overall size, so accounting for gigantism during the hyperoxic Palaeozoic (Greenlee et al., 2007; Kaiser et al., 2007).

Typical of these debates are calls for a broader phylogenetic coverage, especially of groups that are thought to possibly contribute to resolving the question at hand. For example, Kaiser et al. (Kaiser et al., 2007) called for investigations of tracheal proportions in taxa closely related to those that experienced Palaeozoic gigantism, whereas Greenlee et al. (Greenlee et al., 2007) argued that reduced safety margins for gas exchange under hypoxia might be detected in the largest of insects. Lighton et al. (Lighton et al., 2001) concluded that the number of published metabolic measurements is sparse, and that, in consequence, the direction of the relationship between low metabolic rates and cannibalism cannot be established. Similarly, Marais et al. (Marais et al., 2005) argued that limited investigations of gas exchange patterns across the

Arthropoda constrain comparative investigations of the reasons for the origin of DGE. In a different vein, Chown (Chown, 2002) argued that a null expectation for cuticular ***versus*** respiratory water loss had not been articulated. Therefore, comparisons amongst particular taxa without some reference to a baseline expectation, such as for species that have continuously open spiracles during rest, might prove to be unhelpful for resolving the significance of the contribution of respiratory water loss to overall water balance.

From the perspective of a comparative approach to addressing these controversies (e.g. Blomberg et al., 2003; Garland et al., 2005), perhaps the most obvious gap is the absence of information for taxa basal to the arthropods. That the Tardigrada should not have been investigated in this respect is unsurprising as a consequence of their small size and experimental intractability. However, the relative absence of data on the Onychophora is surprising, especially given their phylogenetic position basal to the arthropods (Giribet et al., 2001; Grimaldi and Engel, 2005; Dunn et al., 2008) and renewed interest in the group (e.g. Monge-Nájera, 1995; Sunnucks et al., 2000;Reinhard and Rowell, 2005). The few studies of onychophoran metabolism and water balance undertaken to date (Manton and Ramsay, 1937; Morrison, 1946;Bursell and Ewer, 1950; Mendes and Sawaya, 1958; Woodman et al., 2007) suggest that their gas exchange is mediated ***via*** a simple open tracheal system with large numbers of non-closable spiracles, and limited or no branching (Lavallard and Campiglia-Reimann, 1966; Bicudo and Campiglia, 1985).

In consequence, information on gas exchange and water balance in the Onychophora would substantially inform debates about the likely basal pattern of gas exchange in the arthropods, baseline expectations for cuticular ***versus***respiratory water loss, whether low metabolic rates in ticks, scorpions, centipedes and whip-spiders are a basal condition, and perhaps also the extent to which low critical oxygen partial pressure in insects (reviewed by Hoback and Stanley, 2001; Schmitz and Harrison, 2004; Harrison et al., 2006) can be considered derived.

Thus, the principal aim of this paper is to investigate gas exchange and water balance of the onychophoran ***Peripatopsis capensis*** Grube 1866, to address these questions. Specifically, we characterize the pattern of gas exchange, determine the relative contributions of cuticular and respiratory transpiration to total water loss, document standard metabolic rate for comparison with other taxa, and measure metabolic and water loss rates under declining oxygen partial pressures (P_{O2}) to identify the critical P_{O2} for resting metabolism (P_c). Recently, Woodman et al. (Woodman et al., 2007) suggested that the curling behaviour displayed by the Australian species ***Euperipatoides rowelli*** restricts water loss. Therefore, we also determine whether such behaviour in ***P. capensis*** affects water loss at different temperatures.

MATERIALS AND METHODS

Animals

Extant Onychophora include ~100 known species from temperate regions of the southern hemisphere and the tropics (Grimaldi and Engel, 2005). They are typically found in dark, moist microhabitats in forests, have a highly malleable body covered by numerous sensory papillae, and possess glands that secrete an adhesive slime used for defence and prey capture (Hamer et al., 1997;Benkendorff et al., 1999; Barclay et al., 2000; Walker et al., 2006). The study species, ***Peripatopsis capensis*** (Peripatopsidae), is confined to the southern parts of the Western Cape Province of South Africa and is usually found in humid indigenous forests and bushy ravines of mountain slopes (Hamer et al., 1997).

P. capensis individuals were collected in September 2006 (***N***=8) and February 2007 (***N***=7) from a milkwood (***Sideroxylon inerme***) forest (140 m altitude, exact location withheld for conservation reasons). Individuals were found under bark of fallen logs where temperature and relative humidity (AZ Instruments, Taichung, Taiwan), measured at the time of collection, were 13.4±5.5°C and 74.3±7.6% (mean ± s.e.m.; ***N***=10), respectively. Individuals were placed, on the day of collection, in a climate-controlled chamber (Labcon, Johannesburg, South Africa) at 14.4±0.4°C with a 12 h:12 h L:D photoperiod, and they remained at this temperature for 10 days before experiments commenced. One or two individuals were

placed in single non-air-tight plastic containers (17 cm×12 cm×6 cm) with a layer of sand covered by leaf litter and bark. Moisture was maintained at ~80% relative humidity (RH) by regularly spraying the litter with distilled water and by placing vials containing saturated salt (NaCl) solution within the containers (Winston and Bates, 1960). Individuals were fed isopods, springtails and fruit flies (one to four, depending on prey size) once per week, and none of the individuals showed a consistent decline in body mass over the course of the study. Food items were removed at least 24 h prior to experiments to ensure that animals were post-absorptive during respirometry.

Respirometry

Flow-through respirometry was undertaken over several days between 08.00 h and 18.00 h in humidified air to measure CO_2 production in conditions similar to the natural environment. Data were obtained at five different temperatures randomized as 15, 10, 20, 25, 5°C, and the order of individuals was consistent on each day to avoid diurnal effects that might confound temperature effects within individuals. Individuals were placed at 15°C for 2 days between temperature trials. One week after the completion of these trials, the same protocol was repeated, but dry air was used to enable simultaneous measurement of CO_2 production and water loss. For each trial, individuals were weighed to 0.1 mg (Mettler Toledo AX-504 electronic balance; Columbus, OH, USA) and placed in a darkened 9 ml glass cuvette kept at the temperature of the trial using a climate-controlled chamber (±0.4°C; Labcon). Individuals were allowed to settle for 15 min prior to recordings.

Dry, CO_2-free bottled air (21% O_2, balance N_2) scrubbed using soda lime, silica gel and Drierite® (Xenia, OH, USA), passed through a mass flow control valve (Sierra Instruments, Monterey, CA, USA) set at a STP-corrected flow rate of 100 and 200 ml $min^{-1}$for dry and humidified trials, respectively. The air flow was increased in the humidified trials to enhance stabilization and response times of the $\dot{V}_{CO2}$ (rate of carbon dioxide release) reading. Dry air was directed through the cuvette or in the case of the humidified trials, to a bubbler (flask containing distilled water placed inside a water bath adjusted to the temperature required to obtain 70% RH) before reaching the cuvette. The air was then guided to a calibrated CO_2/H_2O analyzer (Li 7000 infrared gas analyzer; LiCor, Lincoln, NE, USA). The temperature inside the cuvette (using a 36 SWG T-type thermocouple), activity pattern (infrared AD-1 activity detector; Sable Systems International, Las Vegas, NV, USA), $\dot{V}_{CO2}$ and $\dot{V}_{H2O}$ (rate of water loss) were recorded and stored via the LiCor and its software. Baseline readings for the empty cuvette were taken before and after each individual trial, which lasted ~1–2 h for humid and ~30–60 min for dry trials. Sex was not determined because finding the male papilla (Hamer et al., 1997) requires prolonged handling which induces additional water loss.

To characterize interactions among behaviour and gas exchange traces, the behaviour of six individuals acclimated at 15°C was filmed with a webcam (Logitech, Fremont, CA, USA) while measuring $\dot{V}_{CO2}$ and $\dot{V}_{H2O}$ at 15°C in 21% O_2 dry air and using a dim light to enhance the clarity of the image. Because of their photonegative behaviour (Newlands and Ruhberg, 1978), individuals moved frequently in the chamber and variations in patterns of gas exchange with changing behaviour were identified.
To measure the effects of P_{O2} on gas exchange, the same respirometry methods were used to record $\dot{V}_{CO2}$, $\dot{V}_{H2O}$ and activity of six individuals at 15°C. Each individual was exposed to five pre-determined P_{O2} (21, 15, 10, 5 and 2.5%) for 30 min in descending order to prevent an increase in metabolic rate that is associated with prior exposure to hypoxia. Individuals were held at 15°C (other conditions as above) for 3 days between measurements at eachP_{O2}. The 15% and 2.5% P_{O2} were obtained by mixing dry CO_2-free 21% or 5% O_2 with the appropriate ratio of pure N_2. The other concentrations originated from purchased dry CO_2-free bottled air. All P_{O2} were verified with a calibrated O_2 analyzer (Ametek S-3A/II, AEI Technologies, Pittsburgh, TN, USA).

Data analysis and statistics

Data were initially analyzed using ExpeData software version 1.0.24 (Sable Systems International, Las Vegas, NV, USA). Differential CO_2 (in parts per million) and H_2O (in parts per thousand) data were corrected for baseline drift and transformed to $\dot{V}_{CO2}$ (in ml h^{-1}) and $\dot{V}_{H2O}$ (in mgh^{-1}) using standard transformations (Lighton, 1991). For all trials, we assumed that standard metabolic rate (SMR) equalled the mean $\dot{V}_{CO2}$ for periods of zero activity (i.e. resting) with lowest stable data (usually lasting~ 2–10 min). For water loss rates

(WLR), the mean $\dot{V}_{H2O}$ was calculated from when the water trace stabilized until the end of the recording. Metabolic rate, WLR and body mass data distributions were $\log_{10}$ transformed prior to analyses to obtain, or in a few cases improve, the normality of the data. The SMR– and WLR–temperature and SMR– and WLR–body mass relationships were investigated using ordinary least-squares regressions. Repeated measures ANOVA and ANCOVA were performed in SAS version 8.0 (SAS Institute, Cary, NC, USA) to investigate the extent to which variation in $\dot{V}_{CO2}$ (and $\dot{V}_{H2O}$ for dry trials) could be explained by variation in temperature, body mass and their interaction. An unstructured covariance matrix was used in proc-mixed with a reduced maximum-likelihood estimation method (Littell et al., 1996).

Individual and temperature were entered as categorical variables and body mass as a continuous variable in the model. Relative humidity was included in an additional model to identify the effect of humidity treatment (70% ***versus*** 0% RH) on SMR. We used a similar approach to test for an effect of gas exchange pattern (now adding this as a categorical variable to the model described above; see the Results section) on SMR and resting $\dot{V}_{H2O}$ (obtained from the same resting period as for SMR). To assess the effect of P_{O2} on $\dot{V}_{CO2}$ and $\dot{V}_{H2O}$, periods of inactivity with continuous gas exchange were selected to avoid potentially confounding effects of variation in gas exchange pattern across oxygen trials. A repeated measures model was again used with individual and P_{O2} entered as categorical variables and body mass as a continuous variable. Critical $P_{O2}$for resting metabolism (P_c) was defined as the P_{O2} below which metabolism could no longer be sustained (Tang, 1933; Prosser and Brown, 1961).

Following the method of Gibbs and Johnson (Gibbs and Johnson, 2004), an ordinary least-squares regression of $\dot{V}_{H2O}$ (dependent variable) and $\dot{V}_{CO2}$(independent variable) was undertaken for each individual–temperature trial (mean r^2 of 0.55; range: 0.20–0.98). Cuticular water loss (CWL) was estimated as the $\dot{V}_{H2O}$ where $\dot{V}_{CO2}$ equalled zero (***y***-intercept) assuming that in the absence of CO_2 exchange, water loss must be entirely or predominantly cuticular (Gibbs and Johnson, 2004). Although the regression method provides CWL estimates that are generally as repeatable (e.g. Chown et al., 2006; Gray and Chown, 2008) as those found using other methods [e.g. the hyperoxic switch (Lighton et al., 2004)], this technique is not without its problems. In species with continuous gas exchange, the estimation of CWL is obtained by extrapolating beyond the measured $\dot{V}_{CO2}$ data to the ***y***-intercept, which might bias respiratory water loss (RWL) estimates (see Gray and Chown, 2008).

However, since preliminary trials using the hyperoxic switch technique on ***P. capensis*** revealed no spiracular response following exposure to extreme hyperoxia, and several studies report a lack of spiracular control in other onychophoran species (Manton and Ramsay, 1937; Lavallard and Campiglia-Reimann, 1966), which is a prerequisite for using the alternative techniques, the regression method was the only option available for estimating CWL. To this end, we used whole recording time periods minus the first few minutes during which traces stabilized (total period ~40 min). The difference between total water loss and CWL provided an estimate of RWL (Gibbs and Johnson, 2004). One negative value of CWL, considered biologically meaningless, was excluded from the analysis (see Gray and Chown, 2008). Except for the repeated measures models, analyses were performed using STATISTICA v.7 (Statsoft, Tulsa, OK, USA) and significance was accepted at $P<0.05$.

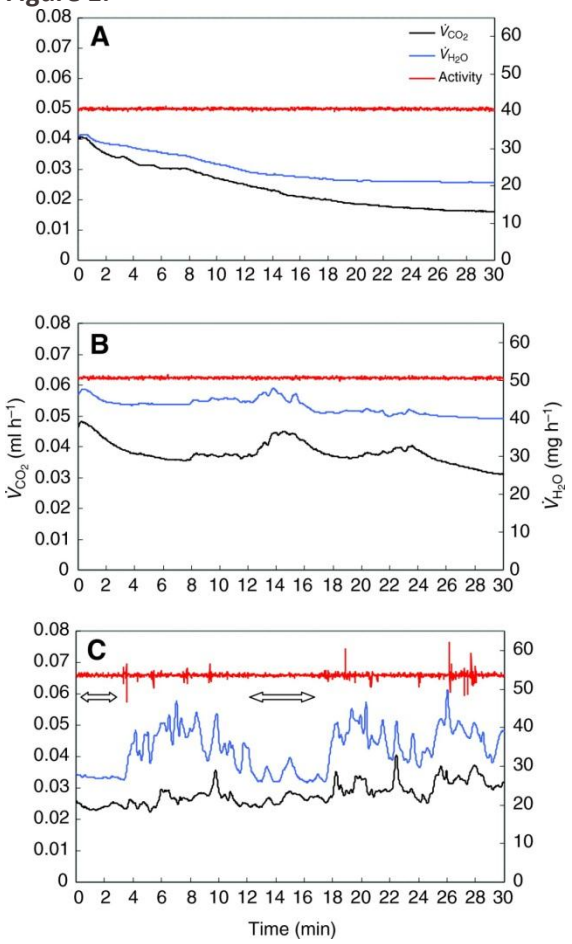
Results

Gas exchange patterns

The gas exchange recordings of ***Peripatopsis capensis*** can be grouped in three categories: (1) downregulated $\dot{V}_{CO2}$ (referred to `downregulated' hereafter; Fig. 1A), where the $\dot{V}_{CO2}$ clearly declines in an ongoing manner; (2) continuous $CO_2$exchange interspersed with downregulated episodes (`interspersed'; Fig. 1B), where $\dot{V}_{CO2}$ clearly declines and then increases in alternating episodes; and (3) continuous CO_2 exchange (`continuous'; Fig. 1C) during which gas exchange is continuous with no indication of downregulation. Observed and imaged behaviour confirmed that the downregulated pattern occurred when the animal assumed a curled position and remained immobile (Fig. 2; this behaviour can be seen in Movie 1 in supplementary material). Continuous gas exchange occurred both during inactivity and activity (Fig. 1C), although most individuals remained inactive inside the darkened cuvettes.

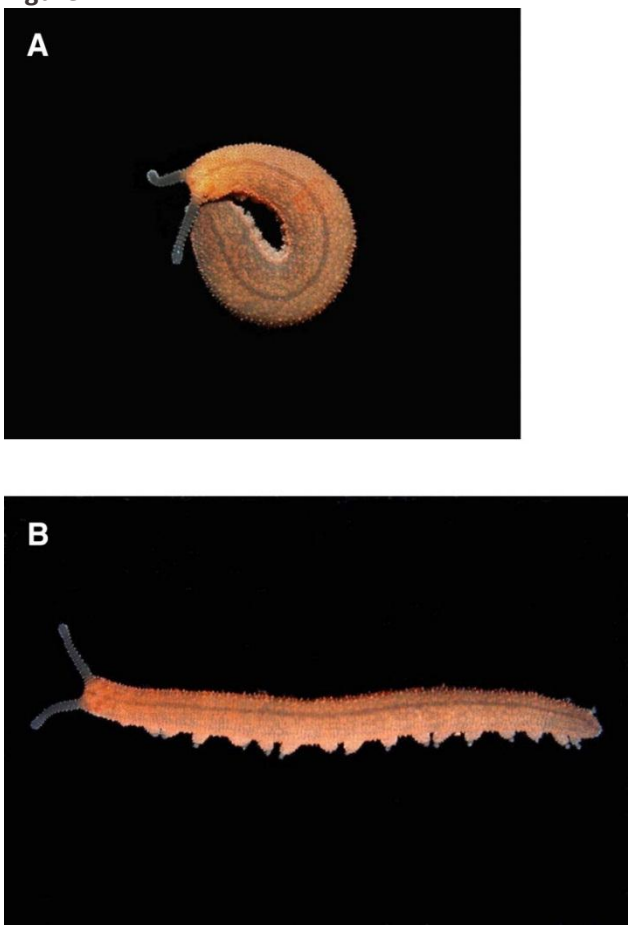
We quantified the proportion of individuals with downregulated, interspersed and continuous patterns across temperature trials and humidity treatments but did not compare the latter since flow rates and sampling periods differed between these treatments. The majority of individuals tested in humid conditions (from 5°C to 25°C) had interspersed patterns (60%) whereas 27% and 13% had continuous and downregulated patterns, respectively (Fig. 3A). Temperature had no significant effect on the proportions of individuals with each pattern [5×3 contingency test, ***G***=15.2, ***P***>0.05 (Sokal and Rohlf, 1995), p. 738]. However, in dry conditions, interspersed and continuous patterns were most common (45% each), with downregulated patterns occurring more frequently at 5°C and continuous patterns occurring more frequently at 25°C (Fig. 3B; 5×3 contingency test, ***G***=22.6, ***P***<0.05).

Figure 1.



Three typical 30 min CO2 and H2O release patterns of Peripatopsis capensis at 21% O2: (A) downregulated (recorded at 5°C), (B) interspersed (at 15°C) and (C) continuous gas exchange (at 15°C). Note that during downregulated events (body curling-up behaviour), CO2 decreases consistently whereas H2O remains constant. Activity is shown (not to scale) by the red line and represents the variance of activity (variation in A and B is from random instrument noise; spikes in C indicate activity). In C, the horizontal arrows indicate periods of stable continuous respiration during resting used to calculate mean standard metabolic rate.

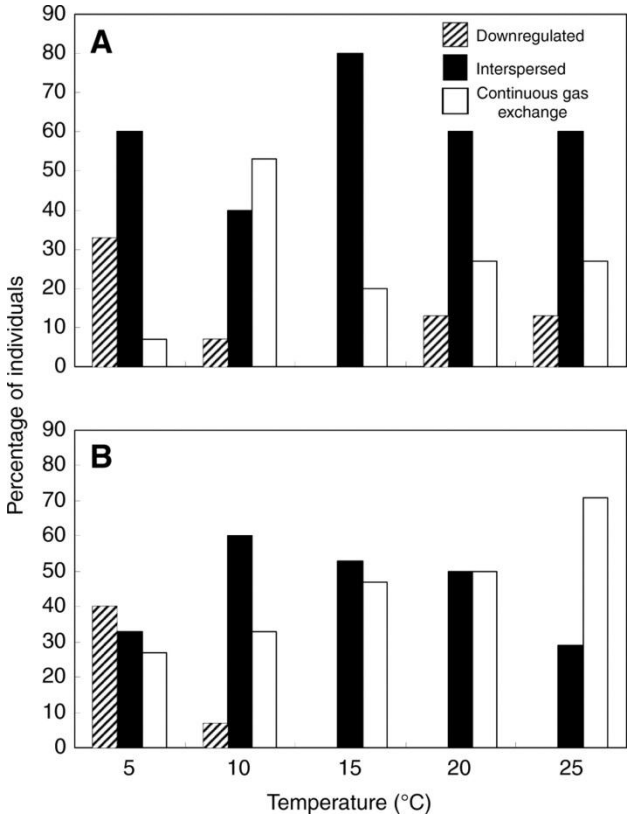
Figure 2.



Juvenile Peripatopsis capensis in (A) curling and (B) elongated body positions.

During downregulation, $\dot{V}_{CO2}$ declined continuously, whereas $\dot{V}_{H2O}$ declined initially and then remained constant (Fig. 1A,B). Mean $\dot{V}_{CO2}$ and $\dot{V}_{H2O}$ of the last 3 min prior to and at the end of downregulated episodes were compared using eight interspersed traces recorded at 15°C, and these indicated that$\dot{V}_{CO2}$ and $\dot{V}_{H2O}$ were significantly lower during downregulated periods than prior to them (paired *t*-tests; mean $\dot{V}_{CO2}$: t=11.06, d.f.=7, P<0.0001; mean$\dot{V}_{H2O}$: t=4.37, d.f.=7, P<0.01). The percentage decline ranged from 5 to 17% in$\dot{V}_{CO2}$ and from 3 to 9% for $\dot{V}_{H2O}$.

Figure 3.



Proportion of individuals with each gas exchange pattern at different temperatures (5, 10, 15, 20 and 25°C) and humidity treatments: (A) 70% RH, (B) 0% RH.

The standard, mass-adjusted $\dot{V}_{CO2}$ differed significantly among patterns in dry trials and among all test temperatures (pattern type×temperature×body mass, $F_{11,50}$=24.96, P<0.0001). The model indicated that standard $\dot{V}_{CO2}$during interspersed was lower than during continuous recordings, but since the downregulated pattern was only present at 5°C (N=6) and 10°C (N=1), there was insufficient variation between test temperatures to calculate a P value. Therefore, a sub-section of the data (from 15–25°C) was analyzed to exclude this pattern type and to determine whether or not the former interaction effect was simply due to the presence of downregulated patterns at 5°C and 10°C. The same significant interaction (pattern×temp×mass, $F_{6,31}$=27.05,P<0.0001) was found in the reduced dataset. Furthermore, to clarify the effect of pattern type, data from the 5°C test temperature were analyzed since this is the only temperature at which several individuals show each of the three $\dot{V}_{CO2}$patterns.

This analysis showed that mass-adjusted $\dot{V}_{CO2}$ was lower during downregulated than during interspersed, which in turn had lower $\dot{V}_{CO2}$ than continuous gas exchange (ANCOVA, $F_{2,11}$=5.9, P<0.018). In sum, these complementary analyses highlight clear differences in mass-adjusted $\dot{V}_{CO2}$among pattern types irrespective of the dataset used. Therefore, in general, curling behaviour led to a reduction of mass-adjusted $\dot{V}_{CO2}$.
The standard mass-adjusted $\dot{V}_{H2O}$ also differed significantly among pattern types in dry trials across all test temperatures (pattern×temperature×mass,$F_{11,50}$=4.58, P<0.0001). Even though the effect of pattern was significant across the full dataset the model was unable to resolve the estimate for the downregulated pattern.

Regardless, the interspersed pattern was characterized by a lower resting $\dot{V}_{H2O}$ than the continuous one. This result was confirmed by re-analysing the 15–25°C dataset, which showed the same significant interaction (pattern×temperature×mass; $F_{6,31}$=4.14, P=0.0036). Data from the 5°C test temperature, with all three patterns represented, indicated that standard, mass-adjusted $\dot{V}_{H2O}$ did not differ significantly among

the three patterns ($F_{2,11}$=1.034, P=0.39). Therefore, from the available data, curling behaviour did not lower $\dot{V}_{H2O}$ at 5°C, but did so at higher temperatures (from 10 to 25°C).

Metabolism and water loss

Temperature and body mass had significant positive effects on standard metabolic rate (SMR) in both dry and humidified trials (Tables 1, 2, 3). Relative humidity did not have an effect on SMR overall, but a significant interaction between temperature and humidity treatment (Table 3; Fig. 4) demonstrated that responses to temperature differed among the two humidity groups. Specifically, the increase in log $\dot{V}_{CO2}$ was larger from 15 to 20°C in the 70% RH group than in the 0% RH group. This difference was not caused by variation in the type of patterns across groups since between 15 and 20°C the change in pattern in the 70% RH group (an increase in the downregulation pattern) (Fig. 3) was incompatible with such a conclusion.

Table 1.
Standard metabolic rate, water loss rate and body mass of *Peripatopsis capensis* measured at five temperatures in dry and humid air conditions

	0% relative humidity				70% relative humidity		
T (°C)	$\dot{V}_{CO2}$	$\dot{V}_{H2O}$	Body mass (mg)	N	$\dot{V}_{CO2}$	Body mass (mg)	N
5	0.0181±0.0025	16.90±1.43	491.3±73.6	15	0.0178±0.0023	515.4±79.8	15
10	0.0227±0.0022	30.78±2.25	500.5±77.3	15	0.0209±0.0025	500.4±75.8	15
15	0.0345±0.0036	43.15±2.55	513.6±79.8	15	0.0245±0.0024	495.4±77.1	15
20	0.0428±0.0042	57.01±2.70	506.4±82.7	14	0.0601±0.0074	500.5±76.7	15
25	0.0589±0.0061	76.30±4.39	488.1±81.6	14	0.0751±0.0002	519.7±80.0	15

Values are means ± s.e.m. $\dot{V}_{CO2}$, standard metabolic rate (estimated as ml $CO_2 h^{-1}$); $\dot{V}_{H2O}$, water loss rate (mg H_2O h^{-1})

Table 2.
Ordinary least-squares regression relationships between standard metabolic rate and temperature at 0% and 70% relative humidity and water loss rate at 0% RH of *Peripatopsis capensis*

Variable	Intercept ± s.e.m.	Slope ± s.e.m.	r^2	d.f.	F	P
0% RH						
SMR (ml h^{-1})	−1.94±0.05	0.027±0.003	0.50	1, 71	73.38	<0.0001
WLR (mg h^{-1})	1.13±0.03	0.031±0.002	0.76	1, 71	227.6	<0.0001
70% RH						
SMR (ml h^{-1})	−2.07±0.07	0.035±0.004	0.49	1, 73	71.65	<0.0001

SMR, standard metabolic rate ($\log_{10} \dot{V}_{CO2}$); WLR, water loss rate ($\log_{10} \dot{V}_{H2O}$)

Table 3.
Results of repeated measures models testing for the effects of temperature, $\log_{10}$ body mass, the temperature × body mass interaction on standard metabolic rate of *Peripatopsis capensis* under 70% relative humidity and 0% RH conditions, the same effects on water loss rate, and the effects of humidity on standard metabolic rate

Effect	d.f.	*F*	*P*
Standard metabolic rate (at 70% RH)			
T	4, 65	30.79	<0.0001
M	1, 65	150.08	<0.0001
T×*M*	4, 65	1.39	0.25
Standard metabolic rate (at 0% RH)			
T	4, 63	29.79	<0.0001
M	1, 63	218.62	<0.0001
M×*T*	4, 63	0.57	0.69
Water loss rates ($\log_{10}\ \dot{V}_{H2O}$)			
T	4, 63	164.20	<0.0001
M	1, 63	404.78	<0.0001
M×*T*	4, 63	5.96	<0.001
Effect of RH on standard metabolic rate ($\log_{10}\ \dot{V}_{CO2}$)			
T	4, 65	43.03	<0.0001
M	1, 64	259.29	<0.0001
RH	1, 67	2.28	0.13
M×*T*	4, 65	1.03	0.40
RH×*T*	4, 68	7.47	<0.0001
RH×*M*	1, 67	7.99	0.006

T, temperature (°C); *M,* body mass (mg); RH, relative humidity; d.f., degrees of freedom; *F, F* ratio

Temperature (*T*) and body mass (*M*) had significant positive effects on total water loss rate (WLR; Tables 2 and 3) and the significant interaction of temperature and mass (Table 3) indicated that at different temperatures, the effect of mass on WLR varied. Indeed, a significant negative linear relationship (slope WLR–*T* relationship=0.0281–0.0068×$\log_{10}M$; r^2=0.394, $F_{1,13}$=8.465,*P*=0.012) was found between the individual slopes of the WLR–*T* relationships and body mass, indicating that small individuals lost water relatively faster at higher temperatures than larger individuals.

However, the relationship between individual slopes of SMR–*T* relationships and respective body masses was not significant (slope SMR–*T*=0.0257–0.0042×$\log_{10}M$; r^2=0.074, $F_{1,13}$=1.041,*P*=0.33) indicating that small individuals were not more metabolically sensitive to temperature, nor was the opposite the case. Cuticular water loss rates also increased significantly with temperature and body mass, although the interaction between temperature and mass was not significant (Table 4; *T*: $F_{4,62}$-10.93, *P*<0.0001; *M*: $F_{1,62}$=26.84, *P*=0.0001;*T*×*M*: $F_{4,62}$=1.53, *P*=0.19). Similarly, temperature and body mass had a significant positive effect on respiratory water loss, and the interaction between temperature and mass was not significant (Table 4; *T*: $F_{4,62}$=7.09,*P*<0.0001; *M*: $F_{1,62}$=16.38, *P*=0.0001; *T*×*M*: $F_{4,62}$=1.07, *P*=0.38).

Table 4.

Cuticular and respiratory water loss rates at five temperatures and their percentages relative to total water loss rates

Temperature (°C)	CWL	%CWL	RWL	%RWL	*N*
5	12.03±1.50	68.8±4.6	4.87±0.70	31.2±4.6	15
10	19.93±1.50	63.4±3.5	11.31±1.10	36.6±3.5	14
15	27.40±2.42	63.4±4.2	15.75±1.80	36.6±4.2	15
20	35.65±3.47	63.3±6.0	21.36±3.40	36.7±6.0	14
25	55.81±4.35	74.2±4.6	20.49±4.11	25.8±4.6	14

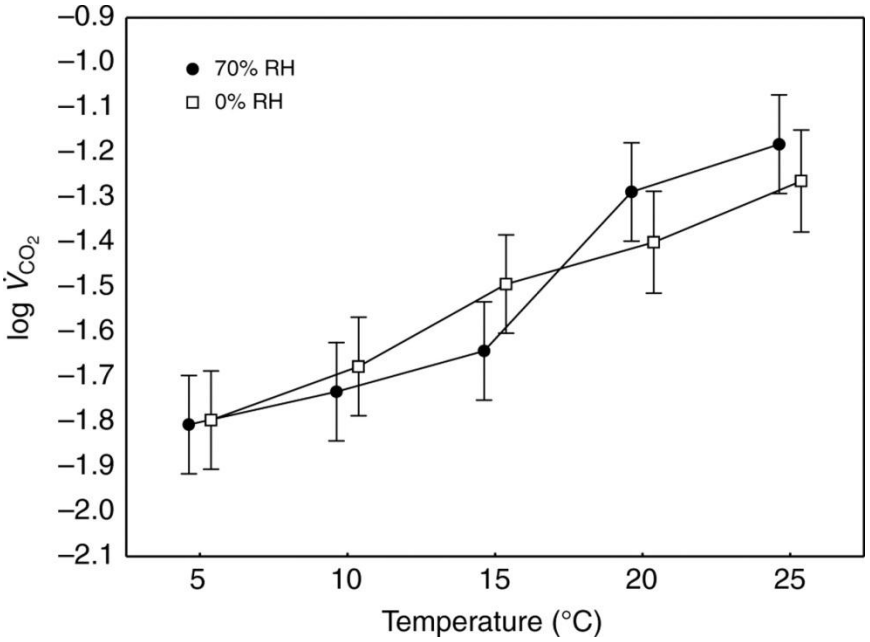
CWL, cuticular water loss (mg H_2O h^{-1}); RWL, respiratory water loss (mg H_2O h^{-1})
Values are means ± s.e.m.

Oxygen partial pressure effects

During hypoxic trials, gas exchange was mostly continuous. Only two out of 24 recordings had downregulated episodes during hypoxia. Mean resting $\dot{V}_{CO2}$remained relatively constant from 21% to 10% O_2, and declined at 5% and 2.5% O_2, by 19% and 41% relative to normoxia, respectively (Fig. 5A). This decrease was not significant when using a repeated measures model that included allP_{O2} treatments ($F_{4,20}$=1.19, P=0.34). However, paired t-tests between treatments showed that comparisons of log $\dot{V}_{CO2}$ between 21–15%, 15–10% and 21–10% were not significantly different (t=0.32, P=0.76; t=0.24, P=0.82;t=0.16, P=0.87, respectively with d.f.=5 in all tests) whereas $\dot{V}_{CO2}$ at 5% and 2.5% were significantly lower than all other P_{O2} treatments (5–10%: t=3.12,P=0.03; 5–15%: t=4.37, P=0.007; 5–21%: t=2.11, P=0.04; 2.5–10%: t=3.26,P=0.02; 2.5–15%: t=3.53, P=0.02; 2.5–21%: t=3.69, P=0.01; d.f.=5 in all tests).

These results suggest that the critical oxygen partial pressure (P_c) for this species lies between 5% and 10% O_2. The use of a piecewise linear regression (non-linear regression procedure in STATISTICA) resulted in a breakpoint at log $\dot{V}_{CO2}$=–1.7332, also indicating a P_c between 5% and 10% O_2. Resting $\dot{V}_{H2O}$did not change across P_{O2} trials (Fig. 5B; $F_{4,20}$=1.23, P=0.33; all paired t-tests were non-significant, 0.10<P<0.91 for all).

Figure 4.



Effects of temperature and humidity on standard metabolic rate (ml CO2 h–1) of Peripatopsis capensis. Values are means ± 95% confidence intervals.

Discussion

Gas exchange pattern and rate

Across a variety of temperature and moisture conditions, continuous gas exchange is the most common gas exchange pattern exhibited by ***Peripatopsis capensis***. The occurrence of continuous gas exchange and the presence of non-occludible spiracles in other onychophoran species (e.g. Woodman et al., 2007), and their phylogenetic position basal to the Arthropoda (Giribet et al., 2001; Dunn et al., 2008), suggest that continuous gas exchange is likely the ancestral state for the arthropods. Other forms of gas exchange pattern such as cyclic and discontinuous gas exchange can therefore be considered more derived, and indeed probably evolved several times within the Arthropoda (Lighton, 1998; Lighton and Joos, 2002; Klok et al., 2002). A recent study has suggested that either continuous or cyclic gas exchange is the ancestral condition in the insects (Marais et al., 2005). Although the current data cannot be used to resolve this question, they support the idea that continuous gas exchange may be the more likely basal state.

Given its body mass, the mean SMR of ***P. capensis*** at 25°C (447.4±45 μW;***N***=15, mass of 488.1±78.8 mg and assuming an RQ of 0.72) was not significantly different from the value expected for a similar-sized arthropod [501.3 μW, RQ=0.72; equation 1 of Lighton and Fielden (Lighton and Fielden, 1995); 526.5 μW, RQ=0.72 (Lighton et al., 2001)]. By contrast, the SMR of ***P. capensis*** was significantly higher than the values estimated from the scaling relationships of `anomalous' arthropods, which have typically low SMR (Lighton and Fielden, 1995; Klok et al., 2002; Terblanche et al., 2004). For example, the SMR values for a 488 mg animal derived from the tick (Lighton and Fielden, 1995), scorpion (Lighton et al., 2001), centipede (Klok et al., 2002) and whip-spider (Terblanche et al., 2004) scaling relationships were 71.4, 128.3, 181.34 and 135.6 μW, respectively.

Assuming that onychophorans have a SMR similar to or higher than that recorded for ***P. capensis*** (0.64μW mg^{-1} at 20°C), as is the case for ***Epiperipatus brasiliensis*** (0.89 μW mg^{-1}) (Morrison, 1946), ***Peripatus acacioi*** (0.50 μW mg^{-1}) (Mendes and Sawaya, 1958) and ***Euperipatoides rowelli*** (1.27 μW mg^{-1}) (Woodman et al., 2007), the basal phylogenetic position of Onychophora suggests that the low metabolism of `anomalous' arthropods is a derived condition. Further, explicit comparative studies incorporating

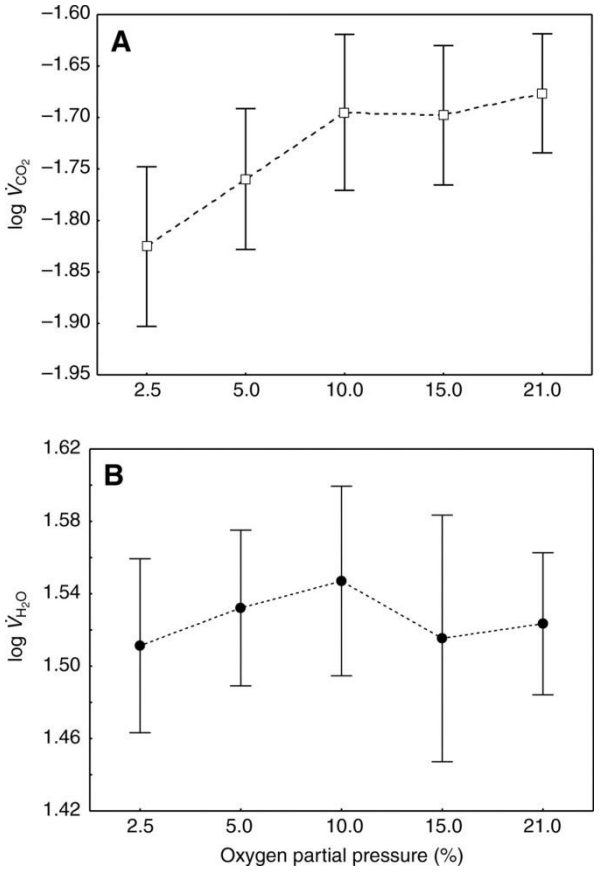
information on life-history strategies of the species used in such comparisons should be undertaken to assess the extent to which low metabolic rates are indeed associated with or constrained by variation in life histories (Lighton and Fielden, 1995; Reinhold, 1999; Lighton et al., 2001). Such a study would also contribute to further understanding of the significance of such variation relative to that of body mass and temperature in determining metabolic rate variation among species (e.g. White et al., 2006).

Water loss

Despite the frequent reports that onychophorans are highly susceptible to desiccation, few studies have quantified water loss rates (WLR) within the group and undertaken comparisons among species or with other taxa (Morrison, 1946; Woodman et al., 2007). One reason may be the difficulty of estimating area-independent WLR or cuticular permeability (Loveridge, 1980) of onychophorans given that they readily change body shape, confounding estimates of body surface area. Here, we sought to overcome this problem using an established framework (Zachariassen, 1996; Addo-Bediako et al., 2001) that provides interspecific least-squares estimates of the relationships between SMR and WLR for xeric and mesic insect species. ***Peripatopis capensis***lies well above the regression lines (3×95% CI) for both mesic and xeric insects, indicating that for its SMR, it has a much larger WLR (76.3 mg h^{-1} at 25°C) than insects inhabiting a wide range of environments.

Although comparisons with other Onychophora show that ***P. capensis*** has a lower WLR than ***Epiperipatus brasiliensis*** (Peripatidae; 222.2 mg h^{-1} at 24°C, 788 mg, placed in a dry-air container), ***Oroperipatus corradi*** (Peripatidae; 167.5 mg h^{-1}at 24°C, 423 mg, dry-air container) (Morrison, 1946), and a ***Peripatopsis*** spp. (263.3 mg h^{-1} at 30°C, 303 mg, with 27.5% RH and 7 m s^{-1} air flow) (Manton and Ramsay, 1937), different methods and the likelihood of unaccounted activity in the previous studies may explain the differences. Using flow-through respirometry, Woodman et al. (Woodman et al., 2007) reported a WLR of 61.33 mg h^{-1} (20°C, mass=295 mg, flow rate of 100 ml min^{-1}) for***Euperipatoides rowelli***, similar to the rate we documented. Thus, in keeping with claims in the literature (Morrison, 1946; Woodman et al., 2007), the Onychophora can be considered a group with high WLR, or `extremely mesic'.

Figure 5.



The effect of oxygen partial pressure on (A) metabolic rate (ml CO2 h–1) and (B) water loss rate (mg H2O h–1) during resting periods of Peripatopsis capensis at 15°C. Values are means ± s.e.m.

Although cuticular water loss (CWL) was found to be the largest avenue of water loss in ***P. capensis***, respiratory water loss (RWL; ~34%) also contributed substantially to total water loss. Given that onychophorans have an open tracheal system with numerous non-occludible spiracles (e.g. Bicudo and Campiglia, 1985), it is unsurprising that RWL contributes substantially to water loss. However, perhaps more importantly, the relative contributions of RWL estimated for this species provide a baseline expectation against which the significance of RWL in taxa that have occludible spiracles, and that in some cases show discontinuous gas exchange, can be assessed. Although comparisons among relative WLR expressed as percentages are problematic because they may fail to assess adequately the extent to which CWL and RWL have been modulated (Chown, 2002), they are widely used as a first gauge of the significance of RWL.

In this regard it is clear that the null expectation for RWL is indeed for a high RWL, given that more than 95% of the values reported in the literature [mostly for insects (see Chown, 2002; Johnson and Gibbs, 2004; Lighton et al., 2004; Schilman et al., 2005; Gray and Chown, 2008)] lie well below a RWL of 23%. However, it should be noted that complex changes to RWL might evolve from such a basal state to the current condition. For example, if CWL is reduced to a minimum, the relative contribution of RWL is expected to be high (Zachariassen et al., 1987; Zachariassen, 1991). In consequence, it may well be that the RWL–environmental water availability relationship is non-linear or U-shaped among arthropods and their allies.

Critical oxygen partial pressure

According to the repeated measures ANCOVA, $\boldsymbol{P}_{O2}$ had no overall effect on SMR of ***P. capensis***. However, common tests used to identify critical oxygen partial pressures ($\boldsymbol{P}_c$) such as breakpoint regression and pairwise ***t***-test techniques (e.g. Ultsch et al., 1978; Greenlee and Harrison, 2004), indicated that the $\boldsymbol{P}_c$ of ***P. capensis*** lies between 5% and 10% O_2. These discrepancies may have resulted from the fact that the

repeated measures model is a conservative approach given its high sensitivity to the within and between group variances (and small degrees of freedom), whereas pairwise ***t***-tests and regression techniques are less conservative. Moreover, the P_c of ***P. capensis*** is comparable to those found in other Onychophora: ***Peripatus acacioi*** (Mendes and Sawaya, 1958) ($10<P_c<15\%$ O_2 at 20°C) and ***Euperipatoides rowelli***[(Woodman et al., 2007) re-analysis of their data in Table 3, $5<P_c<10\%$ at 10°C)]. Therefore, these results suggest that onychophorans regulate metabolism down to intermediate levels of hypoxia and are not oxyconformers (see Schmitz and Harrison, 2004).

In addition, they have lower safety margins than most insects [range 2–5 kPa (Keister and Buck, 1964; Greenlee and Harrison, 2004; Schmitz and Harrison, 2004)] while being consistent with most non-insect invertebrates [5–12 kPa (Penteado and Hebling-Beraldo, 1991;Greenlee and Harrison, 2004; Schmitz and Harrison, 2004)]. Therefore, the high safety margins of insects may represent a derived condition, and one which may have interacted with the constraints set by the demands of metabolism during flight, or walking activity, to determine body size (see Dudley, 1998;Kaiser et al., 2007). Explicit comparative investigations of P_c and tracheal dimensions across species in this group, and across other, seldom-investigated arthropod taxa may provide additional, and much-needed insight into the mechanisms underlying Palaeozoic gigantism in arthropods (see alsoBerner et al., 2003).

Gas exchange, water loss and behaviour

The current study also demonstrated that behaviour has fundamental consequences for gas exchange in ***P. capensis***: $\dot{V}_{CO2}$ and $\dot{V}_{H2O}$ declined when individuals curled up. In ***E. rowelli*** this behaviour was rare (4 out of 80 respirometry trials) (Woodman et al., 2007), whereas in ***P. capensis***, periods of downregulation were relatively common. Importantly, the declines in $\dot{V}_{CO2}$ and$\dot{V}_{H2O}$ in ***P. capensis*** differed in form, such that $\dot{V}_{CO2}$ declined continuously while $\dot{V}_{H2O}$ remained relatively constant following an initial decline. If water loss restriction was the primary role of curling, this behaviour might be expected most commonly under high temperature and dry conditions, whereas the data indicate that curling occurred less frequently as temperature increased. Thus, it appears that curling may take place not necessarily to reduce water loss, but as a precursor to metabolic downregulation. Thus, curling may be a strategy to lower energetic costs when resting, whereas the depression of water loss is likely a proximate consequence of this behaviour.

Acknowledgements

This study was supported by the DST–NRF Centre of Excellence for Invasion Biology and by the Claude Leon Foundation Fellowship to S.C.T. We thank Savel Daniels, Sarah Davies, Sue Jackson, Elrike Marais and John Terblanche for logistical and field assistance, Andre Mader for his photographic skills, and two referees for their helpful comments. We are grateful to Brummer Olivier for permission to collect on the reserve he manages. Research was conducted under permit (#AAA007-000013-0035) from CapeNature (South Africa).

References

Addo-Bediako, A., Chown, S. L. and Gaston, K. (2001). Revisiting water loss in insects: a large scale view. *J. Insect Physiol.* **47**,1377 -1388.

Barclay, S., Ash, J. E. and Rowell, D. M. (2000). Environmental factors influencing the presence and abundance of a log-dwelling invertebrate,*Euperipatoides rowelli* (Onychophora: Peripatopsidae). *J. Zool.* **250**,425 -436.

Benkendorff, K., Beardmore, K., Gooley, A. A., Packer, N. H. and Tait, N. N. (1999). Characterisation of the slime gland secretion from the peripatus, *Euperipatoides kanangrensis* (Onychophora: Peripatopsidae).*Comp. Biochem. Physiol.* **124B**,457 -465.

Berner, R. A., Beerling, D. J., Dudley, R., Robinson, J. M. and Wildman, R. A. (2003). Phanerozoic atmospheric oxygen. *Annu. Rev. Earth Planet. Sci.* **31**,105 -134.

Bicudo, J. E. P. W. and Campiglia, S. S. (1985). A morphometric study of the tracheal system of *Peripatus acacioi* Marcus and Marcus (Onychophora). *Respir. Physiol.* **60**,75 -82.

Blomberg, S. P., Garland, T., Jr and Ives, A. R. (2003). Testing for phylogenetic signal in comparative data: behavioural traits are more labile.*Evolution* **57**,717 -745.

Bursell, E. and Ewer, D. W. (1950). On the reactions to humidity of*Peripatopsis moseleyi* (Wood-Mason). *J. Exp. Biol.* **26**,335 -352.

Chown, S. L. (2002). Respiratory water loss in insects. *Comp. Biochem. Physiol.* **133A**,791 -804.

Chown, S. L., Marais, E., Picker, M. D. and Terblanche, J. S. (2006). Gas exchange characteristics, metabolic rate and water loss of the Heelwalker, *Karoophasma biedouwensis* (Mantophasmatodea: Austrophasmatidae). *J. Insect Physiol.* **52**,442 -449.

Dudley, R. (1998). Atmospheric oxygen, giant paleozoic insects and the evolution of aerial locomotor performance. *J. Exp. Biol.* **201**,1043 -1050.

Dunn, C. W., Hejnol, A., Matus, D. Q., Pang, K., Browne, W. E., Smith, S. A., Seaver, E., Rouse, G. W., Obst, M., Edgecombe, G. D. et al. (2008). Broad phylogenomic sampling improves resolution of the animal tree of life. *Nature* **452**,745 -749.

Garland, T., Jr, Bennett, A. F. and Rezende, E. L. (2005). Phylogenetic approaches in comparative physiology. *J. Exp. Biol.* **208**,3015-3035.

Gibbs, A. G. and Johnson, R. A. (2004). The role of discontinuous gas exchange in insects: the chthonic hypothesis does not hold water. *J. Exp. Biol.* **207**,3477 -3482.

Giribet, G., Edgecombe, G. D. and Wheeler, W. C. (2001). Arthropod phylogeny based on eight molecular loci and morphology. *Nature* **413**,157-161.

Gray, E. M. and Chown, S. L. (2008). Bias, precision and accuracy in the estimation of cuticular and respiratory water loss: a case study from a highly variable cockroach, *Perisphaeria* sp. *J. Insect Physiol.* **54**,169 -179.

Greenlee, K. J. and Harrison, J. F. (2004). Development of respiratory function in the American locust *Schistocerca americana*. I. Across-instar effects. *J. Exp. Biol.* **207**,497 -508.

Greenlee, K. J., Nebeker, C. and Harrison, J. F. (2007). Body size-independent safety margins for gas exchange across grasshopper species. *J. Exp. Biol.* **210**,1288 -1296.

Grimaldi, D. and Engel, M. S. (2005). *Evolution of the Insects*. Cambridge: Cambridge University Press.

Hamer, M. L., Samways, M. J. and Ruhberg, H. (1997). A review of the Onychophora of South Africa, with discussion of their conservation. *Ann. Natal Mus.* **38**,283 -312.

Harrison, J., Frazier, M. R., Henry, J. R., Kaiser, A., Klok, C. J. and Rascón, B. (2006). Responses of terrestrial insects to hypoxia or hyperoxia.*Resp. Physiol. Neurobiol.* **154**,4 -17.

Hetz, S. K. and Bradley, T. J. (2005). Insects breathe discontinuously to avoid oxygen toxicity. *Nature* **433**,516 -519.

Hoback, W. W. and Stanley, D. W. (2001). Insects in hypoxia. *J. Insect Physiol.* **47**,533 -542.

Johnson, R. A. and Gibbs, A. G. (2004). Effect of mating stage on water balance, cuticular hydrocarbons and metabolism in the desert harvester ant,*Pogonomyrmex barbatus. J. Insect Physiol.* **50**,943 -953.

Kaiser, A., Klok, C. J., Socha, J. J., Lee, W.-K., Quinlan, M. C. and Harrison, J. F. (2007). Increase in tracheal investment with beetle size supports hypothesis of oxygen limitation on insect gigantism. *Proc. Natl. Acad. Sci. USA* **104**,13198 -13203.

Keister, M. and Buck, J. (1964). Respiration: some exogenous and endogenous effects of rate of respiration. In *Physiology of the Insecta*. Vol.**3**(ed. M. Rockstein), pp.618 -658. New York: Academic Press.

Klok, C. J., Mercer, R. D. and Chown, S. L. (2002). Discontinuous gas-exchange in centipedes and its convergent evolution in tracheated arthropods. *J. Exp. Biol.* **205**,1019 -1029.

Lavallard, R. and Campiglia-Reimann, S. (1966). Structure et ultrastructure de l'appareil trachaéen chez *Peripatus acacioi* Marcus et Marcus (Onychophore). *C. R. Acad. Sci.* **263**,1728 -1731.

Lighton, J. R. B. (1991). Measurements on insects. In *Concise Encyclopedia on Biological and Biomedical Measurement Systems* (ed. C.A. Payne), pp.201 -208. Oxford: Pergamon Press.

Lighton, J. R. B. (1998). Notes from the underground: towards ultimate hypotheses of cyclic, discontinuous gas-exchange in tracheate arthropods.*Am. Zool.* **38**,483 -491.

Lighton, J. R. B. and Fielden, L. J. (1995). Mass scaling of standard metabolism in ticks: a valid case of low metabolic rates in sit-and-wait strategists. *Physiol. Zool.* **68**, 43-62.

Lighton, J. R. B. and Garrigan, D. (1995). Ant breathing: testing regulation and mechanism hypotheses with hypoxia. *J. Exp. Biol.* **198**,1613-1620.

Lighton, J. R. B. and Joos, B. (2002). Discontinuous gas exchange in the pseudoscorpion *Garypus californicus* is regulated by hypoxia, not hypercapnia. *Physiol. Biochem. Zool.* **75**,345 -349.

Lighton, J. R. B. and Turner, R. J. (2008). The hygric hypothesis does not hold water: abolition of discontinuous gas exchange does not affect water loss in the ant *Camponotus vicinus. J. Exp. Biol.* **211**,563 -567.

Lighton, J. R. B., Brownell, P. H., Joos, B. and Turner, R. J. (2001). Low metabolic rate in scorpions: implications for population biomass and cannibalism. *J. Exp. Biol.* **204**,607 -613.

Lighton, J. R. B., Schilman, P. E. and Holway, D. A. (2004). The hyperoxic switch: assessing respiratory water loss rates in tracheate arthropods with continuous gas exchange. *J. Exp. Biol.* **207**,4463 -4471.

Littell, R. C., Millinken, G. A., Stroup, W. W. and Wolfinger, R. D.(1996). *SAS systems for mixed models*. Cary, NC: SAS Institute.

Loveridge, J. P. (1980). Cuticular water relations techniques. In *Cuticule Techniques in Arthropods* (ed. T. A. Miller), pp.301 -366. New York: Springer.

Manton, S. M. and Ramsay, J. A. (1937). Studies on the Onychophora III. The control of water loss in *Peripatopsis.J. Exp. Biol.* **14**,470 -472.
Marais, E., Klok, C. J., Terblanche, J. S. and Chown, S. L. (2005). Insect gas exchange patterns: a phylogenetic perpective. *J. Exp. Biol.***208**,4495 -4507.

Mendes, E. G. and Sawaya, P. (1958). The oxygen consumption of ``Onychophora" and its relation to size, temperature and oxygen tension.*Rev. Bras. Biol.* **18**,129 -142.

Monge-Nájera, J. (1995). Phylogeny, biogeography and reproductive trends in the Onychophora. *Zool. J. Linn. Soc.* **114**,21 -60.

Morrison, P. R. (1946). Physiological observations on water loss and oxygen consumption in *Peripatus.Biol. Bull.* **91**,181 -188.

Newlands, G. and Ruhberg, H. (1978). Onychophora. In *Biogeography and Ecology of Southern Africa* (ed. M. J. A. Werger), pp.677 -684. The Hague: W. Junk.

Penteado, C. H. S. and Hebling-Beraldo, M. J. A. (1991). Respiratory responses in a Brazilian millipede, *Pseudonannolene tricolor*, to declining oxygen pressures. *Physiol. Zool.* **64**,232 -241.

Prosser, C. L. and Brown, F. A. (1961).*Comparative Animal Physiology. 2nd edn.* London: Saunders.

Reinhard, J. and Rowell, D. M. (2005). Social behaviour in an Australian velvet worm, *Euperipatoides rowelli* (Onychophora: Peripatopsidae). *J. Zool.***267**, 1-7.

Reinhold, K. (1999). Energetically costly behaviour and the evolution of resting metabolic rate in insects. *Funct. Ecol.* **13**,217 -224.

Schilman, P. E., Lighton, J. R. B. and Holway, D. A. (2005). Respiratory and cuticular water loss in insects with continuous gas exchange: comparison across five ant species. *J. Insect Pysiol.* **51**,1295 -1305.

Schmitz, A. and Harrison, J. (2004). Hypoxic tolerance in air-breathing invertebrates. *Resp. Physiol. Neurobiol.* **141**,229 -242.

Sokal, R. R. and Rohlf, F. J. (1995).*Biometry. 3rd edn.* New York: W. H. Freeman.

Sunnucks, P., Curach, N. C., Young, A., French, J., Cameron, R., Briscoe, D. A. and Tait, N. N. (2000). Reproductive biology of the onychophoran *Euperipatoides rowelli. J. Zool.* **250**,447 -460.

Tang, P.-S. (1933). On the rate of oxygen consumption by tissues and lower organisms as a function of oxygen tension. *Q. Rev. Biol.* **8**,260 -274.

Terblanche, J. S., Klok, C. J., Marais, E. and Chown, S. L. (2004). Metabolic rate in the whip-spider, *Damon annulatipes* (Arachnida: Amblypygi). *J. Insect Physiol.* **50**,637 -645.

Ultsh, G. R., Boschung, H. and Ross, M. J. (1978). Metabolism, critical oxygen tension, and habitat selection in darters (Etheostoma).*Ecology* **59**, 99-107.

Walker, N. H., Roberts, E. M., Roberts, T., Spitteri, G., Streubig, M. J., Hartland, J. L. and Tait, N. N. (2006). Observations on the structure and function of the seminal receptacles and associated accessory pouches in ovoviviparous onychophorans from Australia (Peripatopsidae; Onychophora). *J. Zool.* **270**,531 -542.

Westneat, M. W., Betz, O., Blob, R. W., Fezzaa, K., Cooper, W. J. and Lee, W.-K. (2003). Tracheal respiration in insects visualized with synchrotron X-ray imaging. *Science* **299**,558 -560.

White, C. R., Philips, N. F. and Seymour, R. S. (2006). The scaling and temperature dependence of vertebrate metabolism. *Biol. Lett.* **2**,125-127.

White, C. R., Blackburn, T. M., Terblanche, J. S., Marais, E., Gibernau, M. and Chown, S. L. (2007). Evolutionary responses of discontinuous gas exchange in insects. *Proc. Natl. Acad. Sci. USA* **104**,8357-8361.

Winston, P. W. and Bates, D. H. (1960). Saturated salt solutions for the control of humidity in biological research. *Ecology* **41**,232 -237.

Woodman, J. D., Cooper, P. D. and Haritos, V. S. (2007). Effects of temperature and oxygen availability on water loss and carbon dioxide release in two sympatric saproxylic invertebrates. *Comp. Biochem. Physiol.***147A**,514 -520.

Zachariassen, K. E. (1991). Routes of transpiratory water loss in a dry-habitat tenebrionid beetle. *J. Exp. Biol.* **157**,425 -437.

Zachariassen, K. E. (1996). The water conserving physiological compromise of desert insects. *Eur. J. Entomol.* **93**,359 -367.

Zachariassen, K. E., Anderson, J., Maloiy, G. M. O. and Kamau, J. M. Z. (1987). Transpiratory water loss and metabolism of beetles from arid areas in East Africa. *Comp. Biochem. Physiol.* **86A**,403 -408.