Effects of temperature and oxygen availability on water loss and carbon dioxide release in two sympatric saproxylic invertebrates

James D. Woodman^{a,b,*}, Paul D. Cooper^a, Victoria S. Haritos^b

^a School of Botany and Zoology, Australian National University, Canberra ACT 0200, Australia

^b CSIRO Entomology, GPO Box 1700, Canberra ACT 2601, Australia

Received 20 June 2006; received in revised form 28 January 2007; accepted 29 January 2007

Available online 2 February 2007

Abstract

Water loss and $V\text{CO}_2$ relative to temperature and oxygen tension was investigated in a log-dwelling onychophoran (*Euperipatoides rowelli*) and a sympatric, un-described millipede species using flow-through respirometry. Onychophorans possess a tracheal system featuring permanently open spiracles. Total body water loss was consistently very high in *E. rowelli* and there was a positive correlation with increasing temperature. CO_2 output was continuous, increasing with higher temperatures and decreasing under lower oxygen concentrations. The millipede which has occludible spiracles also showed continuous gas exchange; however water loss was up to an order of magnitude lower than *E. rowelli*. An ability to survive under hypoxia is apparent for both species and corresponds with reports of hypoxic conditions within rotting logs. The rotting log habitat common to both taxa is characterized by high relative humidity and typically cool temperatures that approach 0 °C at night in winter. Consequently, dispersal through the higher temperatures and lower humidity of the exposed and dry understorey between suitable habitat may be hazardous for *E. rowelli* due to high desiccation susceptibility.

© 2007 Elsevier Inc. All rights reserved.

Keywords: Desiccation; Dispersal; Hypoxia; Millipede; Onychophoran; Respirometry

1. Introduction

Desiccation is a major risk that faces all terrestrial organisms, but in particular, terrestrial invertebrates with a relatively high surface area to volume ratio (Endey, 1977; Hadley, 1994). Numerous adaptations have arisen to combat water loss including waterproof cuticles and the means for reducing water lost through excretion (Ahearn, 1970; Hadley, 1994). Compared to xeric species, more mesic species may experience relaxed selection for water conservation. Indeed, mesic species are normally buffered from desiccation within their habitat because of higher humidity and thus a reduced gradient for water loss.

Invertebrates living within rotting logs (saproxylic habitat) experience a high humidity, low temperature, buffered environment and have limited dispersal abilities over outside terrain

(Grove, 2002). These conditions may result in relaxed selection for water conservation. The saproxylic habitat may also be hypoxic: oxygen levels have been reported low as 0.5% due to a lack of flow-through air movement coupled with oxygen consumption by inhabitants (Paim and Beckel, 1964; Hicks and Harmon, 2002). Hypoxic atmospheres can stress an organism's ability to develop and survive (Greenlee and Harrison, 2004; Hoback and Stanley, 2001), but less obviously may affect water loss due to increased ventilation and elevated spiracular conductance (Lighton and Joos, 2002). The effects of hypoxia may be different for taxa with differing levels of complexity associated with the tracheal system and spiracular control (Schmitz and Harrison, 2004; Hoback and Stanley, 2001).

The soft-bodied Onychophora are unable to control respiratory water loss from their simple, open tracheal system (Campiglia and Lavallard, 1982). This contrasts with the Diplopoda which have a highly sclerotized, lipid impregnated cuticle that typically incorporates closable spiracles (Hopkin and Read, 1992). Onychophora feature numerous, non-closable spiracles which extend from the base of small pits in the cuticle

* Corresponding author. School of Botany and Zoology, Australian National University, Canberra ACT 0200, Australia. Tel.: +61 0262464031 (w).

E-mail address: james.woodman@csiro.au (J.D. Woodman).

scattered all over the body and are most abundant on the dorsal surface (Manton and Ramsey, 1937; Mendes and Sawaya, 1958). Manton and Ramsey (1937) reported that tracheae extending from each spiracle do not branch, but limited branching was later reported for another species (Lavallard and Campiglia-Reimann, 1966). The cuticular pits may be separated by as little as 80 μm , further distinguishing the Onychophora from the Diplopoda which typically have two spiracles on each sternite that are able to close and lead to numerous unbranched tracheae that ramify among the tissues (Manton and Ramsey, 1937; Hopkin and Read, 1992). The inability of the Onychophora to control their spiracular openings means water will be continually lost from the respiratory surface to the environment (Bicudo and Campiglia, 1985). For the Diplopoda, spiracles that are able to close restrict water movement between the tracheae and the external atmosphere reduce water loss and may permit some respiratory control (Hopkin and Read, 1992).

The phylum Onychophora are of special interest due to their 'living fossil' status and close affinities to the arthropods (Giribet et al., 2001). Studies of Onychophora have the potential to further our understanding of how adaptations in higher arthropod taxa evolved. In this paper we question whether oxygen tension and temperature affect evaporative water loss rates and $V\text{CO}_2$ in both an onychophoran (simple, open tracheal system) and an un-described co-occurring species of millipede (more complex tracheal system incorporating occlusible spiracles). We use this information to explore the extent to which both species are exclusively suited to the saproxylic habitat and interpret results with respect to dispersal and migration. Finally, we evaluate the hypothesis that micro-habitat specialization has resulted in relaxed selection for water conservation.

2. Methods

2.1. Collection site

The Tallaganda region of south-eastern New South Wales, Australia incorporates Tallaganda State Forest, Badja State Forest and Tallaganda National Park (longitude 149.5 °E, latitude 36 °S). The forest is temperate sclerophyll with abundant saproxylic habitat of fallen, decaying timber. It represents a continuous and narrow strip of forest 3–17 km wide (east–west) and approximately 100 km in length (north–south). It is surrounded by lower-lying tablelands except for a connection to the Great Dividing Range in the south.

2.2. Study species

Euperipatoides rowelli (previously included in *E. leuckarti*) Reid 1996 (Onychophora: Peripatopsidae) is a ovo-viviparous peripatopsid that exclusively inhabits saproxylic microhabitat in south-eastern New South Wales (Barclay et al., 2000a). It is found in unusually high densities throughout Tallaganda (over 1000/ha in many areas) (Barclay et al., 2000a) and has been the subject of previous ecological and morphological studies (e.g. Scott and Rowell, 1991; Barclay et al., 2000a,b; Sunnucks and

Wilson, 1999; Sunnucks et al., 2000). The similarly sized undescribed millipede species belongs to the speciose paradoxosomatid Polydesmida (Diplopoda: Polydesmida) (Bob Mesibov, pers. comm.) and co-occurs with *E. rowelli* within the saproxylic habitat of Tallaganda.

2.3. Sampling

Sampling for both *E. rowelli* and the millipede was carried out at 2 sites within Tallaganda, separated by approximately 60 km. Specimens were excavated opportunistically from decomposing logs by splitting the log, locating individuals and transferring them into a plastic specimen tube accompanied by a small quantity of the moist, woody substrate (Special Purposes Permit No. 09751; Scientific Investigation Licence No. A397, State Forests of NSW). Live specimens were stored in 24 hour darkness at 10 °C approximating natural habitat conditions. Millipedes were provided with ample decaying woody debris from their natural habitat as a food source whilst *E. rowelli* was periodically fed termites or small crickets.

2.4. The saproxylic environment: temperature and relative humidity

Temperature and relative humidity were recorded using 6 iButton data loggers (Model DS1923; Dallas semiconductor/Maxim integrated products, USA) calibrated using saturated salt solutions (Lide, 1999). Three were placed into rotting logs within pre-drilled holes to a depth of approximately 20 cm and sealed with cork after placement. The remaining three were suspended just above ground level at the base of a rotting log within a small wire mesh enclosure. Data were collected over a six month period spanning winter to summer from July 2005 to January 2006. Measurements of both temperature and relative humidity were logged once every 4 h. The data loggers were connected to a personal computer for both initial programming and the downloading of data using OneWireViewer software (Dallas semiconductor/Maxim integrated products, USA).

2.5. Gas exchange and water loss experiments

The simultaneous release of water and CO_2 under variable oxygen concentrations and temperatures were measured for both *E. rowelli* and the millipede. Data were recorded using flow-through respirometry that incorporated Li-Cor 7000 $\text{CO}_2/\text{H}_2\text{O}$ differential infrared gas analysis equipment that was subject to a calibration check using known concentrations of CO_2 before use (Li-Cor, Lincoln, USA). A custom-built chamber was used that consisted of clear, cylindrical perspex tubing and end caps with gas fittings internally secured with rubber rings (internal dimensions: radius 4.9 mm, length 25 mm, volume 1.89 cm^3). A single chamber loaded with a live specimen was introduced into the gas line within a dark incubator that controlled temperature ± 0.1 °C (Binder KB-series incubator, Germany). The input and output tubing from the analyzer was connected to the chamber initially to flush the chamber for approximately 5 min. Gas was drawn through the system by an

Fig. 1. Representative CO₂ (solid line) and H₂O (dotted line) output traces for (a) *E. rowelli* and (b) the millipede when exposed to normoxia at 15 °C. Both traces show the 40 minute period used for analysis during which activity was minimal.

Edwards E2M-1.5 high-vacuum pump (BOC-Edwards, BOC group Inc.). Each specimen was acclimated to the experimental temperature for approximately 15 min prior to beginning a trial. The experiments proceeded with a gas of known composition passing through the chamber at a rate of 100 mL min⁻¹. The gas mixtures used were made by diluting dry, CO₂-free air with dry high purity N₂. The ratio of each gas used to achieve the desired mixture was controlled by a Brooks 5878 mass-flow controller with three Brooks 5850TR mass-flow meters (one each for vacuum, O₂ and N₂) calibrated (using a calibrated digital flow meter before use) at 0–500 mL min⁻¹ (Brooks Instruments, Hatfield, USA). The use of mass-flow controllers permitted the gas mixture to be instantaneously varied as required without disruption to the gas line.

Raw data from the gas analyzer was logged on a personal computer using Li-Cor LI7000 data acquisition software (Version 1.0.1). The data from each trial were imported into Microsoft Excel where both water and CO₂ measurements (1 second recording frequency) over a 40 minute period (total trial duration of approximately 2 h) were selected for analysis. The initial 10 min of recording was not used due to flushing the chamber and animal acclimation. Specimens were visually

monitored periodically throughout each trial and data were selected to best represent a period of consistently low CO₂ output associated with the animal remaining largely stationary throughout. The total CO₂ and water vapour concentrations for each trial were subsequently converted from ppt and ppm to mean mg h⁻¹ and mean mL h⁻¹ (Fig. 1).

Specimens were weighed before each trial using an A&D company HR-200 balance (sensitivity 0.1 mg). Each individual of

Table 1				
Temperature and relative humidity measurements (mean±SD) from both within a rotting log and on the forest floor outside of a rotting log				
Season	Within a log (n=3)		Outside of a log (n=3)	
	Temperature (°C)	Humidity (%)	Temperature (°C)	Humidity (%)
Winter				
Day	4.4±1.2	100	8.2±2.4	84.3±8.5
Night	4.7±1.2	100	2.8±1.6	96.9±3.7
Spring				
Day	10±0.8	100	15.2±3.3	80±10.7
Night	10.3±0.9	100	9±0.7	97.7±1.4
Summer				
Day	17.8±0.4	95.1±8.2	20.6±1.3	83±5.7
Night	17.7±1	95±8.6	15.1±1.3	95.4±1.8

Table 2

The effect of temperature on total water loss rate and CO₂ release rate (mean±SD) for *E. rowelli* and the millipede under normoxic conditions

Temperature (°C)	<i>E. rowelli</i> (n=10 per treatment)			Millipede sp. (n=5 per treatment)		
	Mass (mg)	Mean H ₂ O output (mg h ⁻¹)	Mean CO ₂ output (μL h ⁻¹)	Mass (mg)	Mean H ₂ O output (mg h ⁻¹)	Mean CO ₂ output (μL h ⁻¹)
5	192±160	22.06±6.28	15.9±17.3	236±36	1.76±0.47	2.8±1.5
10	162±100	28.00±5.66	18.6±7.7	169±93	2.04±1.68	5.7±3.3
15	255±138	45.82±6.58	28.6±9.2	238±35	3.87±0.97	7.1±4
20	295±136	61.32±6.81	49.3±12	171±95	4.77±1.53	8.3±5.1

both *E. rowelli* and the millipede was used only once. After each trial, the specimen was returned to a specimen jar containing moist substrate collected from their natural habitat. Statistical analysis was completed using JMP statistical software (version 4.0) (SAS Institute Inc., USA). Mass, CO₂ output rates corrected to STP and water loss rates were all log transformed prior to analysis. Data was analyzed using generalized linear models with stepwise removal of non-linear factors. Species, temperature and oxygen concentration were all treated as nominal factors in the model. Data are presented as means and standard deviations unless indicated otherwise.

3. Results

3.1. Temperature and relative humidity within saproxylic habitat

Conditions on the forest floor ranged from -3 °C and 100% relative humidity in winter to 34 °C and 15% relative humidity in summer. Conditions within a rotting log ranged from 2 °C and 100% relative humidity in winter to 23 °C and 82% relative humidity in summer (Table 1).

3.2. Water loss and temperature

Approximate linear relationships were evident between log water loss rate and log mass in *E. rowelli* ($R^2=0.77$; slope=0.33) and the millipede ($R^2=0.40$; slope=0.46) at 10 °C. Water loss rates were positively correlated with mass ($F=35.55$, $df=1$, $p<0.0001$). Water loss at any one mass and temperature was up to an order of magnitude greater in *E. rowelli* than for the millipede ($F=1630.69$, $df=1$, $p<0.0001$).

Water loss rates were positively correlated with increasing temperature ($F=39.39$, $df=3$, $p<0.0001$) however there was no interaction effect. With reference to Table 2, *E. rowelli* experienced high water loss regardless of the treatment, but mass specific rates still increased from 5 to 10 °C ($Q_{10}=1.8$), 10

to 15 °C ($Q_{10}=1.98$) and 15 to 20 °C ($Q_{10}=1.63$). Mass specific millipede water loss rate also increased from 5 to 10 °C ($Q_{10}=1.83$), 10 to 15 °C ($Q_{10}=2.62$) and 15 to 20 °C ($Q_{10}=2.06$).

3.3. Water loss and oxygen tension

Oxygen tension had a significant effect on water loss for both *E. rowelli* and the millipede ($F=5.71$, $df=4$, $p=0.0003$), but each taxa was shown to respond differently to decreasing oxygen tensions ($F=6.5$, $df=4$, $p=0.0001$). Water loss increased in the millipede and decreased in *E. rowelli* with decreasing oxygen availability. All five of the millipedes showed a negative slope for water loss from low to high oxygen tensions.

3.4. Carbon dioxide output and temperature

VCO_2 was significantly different between species ($F=154.32$, $df=1$, $p<0.0001$) with approximately linear relationships evident as a function of mass in both *E. rowelli* ($R^2=0.51$; slope=0.62) and the millipede ($R^2=0.73$; slope=0.71). VCO_2 was positively affected by mass ($F=80.41$, $df=1$, $p<0.0001$), but the interaction term for CO₂ output rate and mass was not significant.

VCO_2 was also positively correlated with increasing temperature ($F=32.56$, $df=3$, $p<0.0001$). With reference to Table 2, *E. rowelli* shows what is interpreted to be a three-tiered temperature response: low metabolic rate at 5 °C, similar and intermediate response to both 10 and 15 °C, and an elevated metabolic rate at 20 °C. Despite a lower rate of CO₂ output, the millipede data show a similar three-tiered response to that observed for *E. rowelli*.

3.5. Carbon dioxide output and oxygen tension

VCO_2 in *E. rowelli* and the millipede responded differently to decreasing oxygen availability ($F=16.74$, $df=4$, $p<0.0001$).

Table 3

The effect of oxygen tension on total water loss rate and CO₂ release rate (mean±SD) for *E. rowelli* and the millipede at 10 °C

Oxygen tension (%)	<i>E. rowelli</i> (n=10 per treatment)			Millipede sp. (n=5 per treatment)		
	Mass (mg)	Mean H ₂ O output (mg h ⁻¹)	Mean CO ₂ output (μL h ⁻¹)	Mass (mg)	Mean H ₂ O output (mg h ⁻¹)	Mean CO ₂ output (μL h ⁻¹)
2	200±136	31.99±6.34	9.0±3.2	231±25	5.02±2.71	8.1±4.2
5	286±140	34.5±5.95	13.6±5.3	226±27	4.62±2.34	8.8±3.5
10	202±129	31.85±6.44	15.4±5.6	222±29	3.91±2.29	4.7±1.5
15	266±129	63.42±15.53	19.8±4.8	226±30	3.64±1.63	5.6±2.4
21	162±100	38.00±5.66	18.6±7.7	169±93	2.04±1.68	5.7±3.3

Fig. 2. A representative trace for *E. rowelli* showing CO₂ (solid line) and water flux (dotted line) for a single individual at 20 °C. Note the significant depression of both CO₂ and water release upon the individual rolling-up into a tightly curled position.

E. rowelli decreased $\dot{V}\text{CO}_2$ as oxygen tension decreased. The data show a decrease in metabolic rate for *E. rowelli* by as much as 48% in response to hypoxia (Table 3). The millipede showed a different relationship: CO₂ release rate increased at 5 and 2%.

3.6. Characteristic 'rolling-up' behaviour

The 'rolling-up' behaviour that *E. rowelli* displays when stressed or shocked was observed during a small number of the trials ($n=4$ out of 80). During periods spent 'rolled-up' the animal constricts the ventral surface and curls up tightly. A comparison of means t -test revealed a significant decrease in both $\dot{V}\text{CO}_2$ ($t=136.44$, $p<0.0001$) and water flux ($t=74.16$, $p<0.0001$) during the period spent rolled-up relative to before. Upon rolling-up, release of CO₂ was observed to immediately drop by as much as 50%, whilst water loss was observed to immediately drop by as much as 60% (Fig. 2).

4. Discussion

An inability to control water loss may limit the time that *E. rowelli* can be exposed to conditions outside of a rotting log. The specific habitat requirements of *E. rowelli* are thus indicative of microhabitat specialization. *E. rowelli* was consistently subject to very high water loss rates in dry air which increased with increasing temperature. Morrison (1946) demonstrated a two-fold advantage in water loss for two onychophoran species (*Epiperipatus* sp. and *Oroperipatus* sp.) over an earthworm and a two-fold disadvantage when compared to a mesic centipede (Morrison, 1946). Our results for *E. rowelli* show even higher rates of water loss than either of the previously investigated onychophorans. By contrast, the millipede had water loss rates up to an order of magnitude lower than *E. rowelli*. It is interesting that two species living within the same, environmentally buffered habitat have such contrasting water loss rates. The mean water loss (expressed as percentage initial mass) of *E. rowelli* was $20.3\% \text{ h}^{-1}$ in dry air at 10 °C, whilst the millipede lost merely $1.4\% \text{ h}^{-1}$ under the same conditions.

In line with the dramatic difference in total water loss, the relationship slope between water loss and oxygen tension for the millipede was statistically different from that in *E. rowelli*, showing the millipede loses water at a greater rate as oxygen availability is reduced. This trend was not observed for *E. rowelli* which showed reduced water loss at lower oxygen tensions despite a consistently high water loss rate irrespective of the treatment. This result is most probably due to individuals becoming slightly more quiescent with each decrease in oxygen tension.

Gas exchange in *E. rowelli* and the millipede was continuous, but the millipede did show evidence of occludible spiracles (data not shown). For *E. rowelli*, a decrease in $\dot{V}\text{CO}_2$ was observed during exposure to increasing degrees of hypoxia at a constant temperature of 10 °C. During periods of severe hypoxia all individuals remained motionless and were never observed to compensate for low oxygen availability via bodily movements. This result is similar to that of another onychophoran, *Peripatus acacioi*, which also experienced a reduction (albeit less dramatic) in mass specific metabolic rate at 20 °C over the same oxygen tension range (Mendes and Sawaya, 1958). In contrast, the millipede showed increased $\dot{V}\text{CO}_2$ at 10 °C during severe hypoxia probably at least partly attributable to the buffering of lactate by mobilized cutaneous carbonate. Previously, the Brazilian millipede, *Pseudonannolene tricolor*, which may also encounter hypoxic conditions, showed reduced oxygen consumption at 25 °C in response to oxygen tensions below 10% (Penteado and Hebling-Beraldo, 1991). Occasional bouts of movement (excluded from periods used for analysis) were observed at both 5 and 2% oxygen suggesting a behavioural response whereby the animal may try to move to locate an area of higher oxygen availability. It is also possible that the animal may move to increase gas exchange by heightened ventilation of the tracheal system. Importantly, both species were observed to promptly return to a normal state after each treatment free of any obvious immediate or delayed ill-effects.

At higher temperatures, elevated metabolic rates resulted in an increase in $\dot{V}\text{CO}_2$ for both *E. rowelli* and the millipede. A

decrease in $V\text{CO}_2$ as temperature decreases was to be expected, particularly as onychophorans are able to withstand very cold conditions and become torpid during winter (Mendes and Sawaya, 1958). Coinciding with our measurements within rotting logs (Table 1), it appears both taxa function optimally at 10–15 °C and ~100% relative humidity. Below approximately 10 °C individuals show a low metabolic rate, whilst above 15 °C the rate of water loss was very high. The 10–15 °C temperature range contrasts with forest floor conditions outside of logs at Tallaganda for much of the year, particularly in summer months with 34 °C with 15% relative humidity recorded. During such hot and dry periods, the most extreme conditions recorded within a log were 23 °C with 82% relative humidity, providing direct evidence for environmental buffering within the saproxylic habitat.

To summarise, both *E. rowelli* and the millipede show increased $V\text{CO}_2$ in response to increased temperature. *E. rowelli* decreases CO_2 production in response to both low temperature and hypoxia (and may dorsoventrally roll-up as discussed later), whilst the millipede increases CO_2 output when exposed to hypoxic conditions.

4.1. Water loss and its implications for distribution patterns and dispersal

Previous research on Australian Onychophora has reported that most foraging and movement is observed at night due to cooler and more humid conditions (Tait et al., 1990). Despite such favourable conditions, *E. rowelli* dispersal is likely to remain hazardous, particularly during summer months; although upon initial colonization of a log, complex behaviours and pheromonally-based dispersal systems may result in more direct travel for subsequent individuals (Reinhard and Rowell, 2005; Barclay et al., 2000b).

High rates of water loss may limit the dispersal ability of *E. rowelli*, and therefore directly influence gene flow and patterns of distribution (Scott and Rowell, 1991; Barclay et al., 2000a). We have shown that when exposed to dry air at 20 °C, *E. rowelli* will desiccate after only 2–3 h whilst the millipede remains relatively unaffected (typically <10% reduction in mass). Whilst a small proportion of water lost from both *E. rowelli* and the millipede can be attributed to respiratory transpiration, the majority is assumed to have been lost across the cuticular surface (Chown, 2002). Indeed, the millipede features a hardened cuticle that is approximately four times the thickness of that in the soft bodied *E. rowelli* (Woodman et al., unpublished data) which may contribute to the difference in water loss rates between the species. From our trials, it was noted that the maximum tolerable water loss for *E. rowelli* is relatively high and may exceed 50% of initial mass; however the exceptionally high rate of water loss prevents any extended buffering effect against desiccation in dry air. Importantly, the buffering effect is likely to be greater with higher relative humidity in the natural environment. That *E. rowelli* is so common over much of its range is testament to microclimatic conditions and the very high density of suitable habitat within Tallaganda. Previous research on a co-occurring saproxylic

funnelweb spider at Tallaganda has shown the typical distance between fallen rotting logs rarely exceeds 23 m in most areas (Woodman et al., 2006).

Despite such plentiful habitat, this 'Onychophoran hotspot' still fosters remarkable patterns of local endemism and population structuring on a scale of drainage catchments and sub-catchments (each at most only tens of kilometres apart) for several specialist saproxylic taxa (Sunnucks et al., 2006; Garrick et al., 2004; Woodman et al., 2006; Sunnucks and Wilson, 1999). It is probable that despite being established along the length of the forest in moderate to high densities, *E. rowelli* dispersal and migration may still be physiologically limited to the extent of potentially segregating populations relative to understorey terrain conditions. From our data we can speculate that population structuring for the millipedes however, would be diffuse due to the greater potential for successful dispersion and thus increased gene flow throughout the forest.

4.2. Characteristic rolling-up behaviour as a strategy to combat stressful gaseous conditions?

For periods during several of the trials at either 15 or 20 °C and normoxia, *E. rowelli* was observed to roll-up dorsoventrally. Curiously, this was never observed for the millipede during our trials which like other polydesmid millipedes can also roll-up in a similar manner (Appel, 1988). For *E. rowelli*, animals roll-up when a log is first opened and the animals are exposed to the external environment (pers. obs.). Rolling-up also occurs when *E. rowelli* is stressed or shocked and becomes disoriented or threatened beyond the defensive scope of its projectile adhesive secretion (pers. obs.). Compared to data from immobile individuals, rolling-up corresponded to a further dramatic and immediate decrease in water loss and CO_2 output (Fig. 2). The rolling behaviour is curious for several reasons. Firstly, if it represents a means by which an individual can better tolerate stressful environmental conditions, then why was it so rare in its appearance? Secondly, from a morphological standpoint, a majority of the open and exposed spiracles on an onychophoran have been reported to exist over the dorsal body surface (Mendes and Sawaya, 1958) which remains largely exposed during the period spent rolled-up. Thirdly, if the rolling-up behaviour represents nothing more than a defense reaction mechanism, then what would trigger it during a period when no apparent acute disturbance was involved? This characteristic behaviour may be simply a recoil defense mechanism, but the findings presented here argue for a possible function in reducing water loss and CO_2 release to lower levels than those whilst elongated and largely immobile. Whether such a function is adaptive remains unclear and requires further investigation.

4.3. Concluding remarks

The saproxylic habitat hosts organisms that are well adapted to moist, dark, climatically buffered rotting timber. Such organisms exhibit remarkable population structure over highly restricted spatial scales (e.g. Garrick et al., 2004) and are generally unable to withstand extended exposure to foreign

environments. We have shown *E. rowelli* to be susceptible to desiccation as very high levels of water loss cannot be actively regulated (with the exception of the rolling-up behaviour). Successful dispersal for *E. rowelli* is thus most likely when traveling at night after rain in spring, summer or autumn (Table 1). In contrast, water loss from a co-occurring un-described species of millipede evaporated from the animal at a rate up to an order of magnitude lower. During field sampling it was noted that the millipedes were typically found in close proximity to the outermost log layer, perhaps indicating they experience drier conditions than *E. rowelli* and thus feature greater resistance to desiccation. Both *E. rowelli* and the millipede were tolerant of hypoxia, but at a constant temperature the millipede lost water at a greater rate as oxygen tension was reduced.

Assuming the absence of any as yet unknown desiccation avoiding strategies, the sensitivity of *E. rowelli* (and to a lesser extent the millipede) to the external environment outside of a log has the potential to magnify the effects of human-induced ecosystem modification and also climate change (Salinger, 2005). As such the priority conservation of discontinuous and transient habitat such as that provided by rotting logs is supported to preserve and foster exclusive species with limited physiological flexibility.

Acknowledgements

The authors thank Dave Rowell and Paul Sunnucks for their input into both this manuscript and greater on-going Tallaganda research program. For technical assistance we also thank Greg Dojchinov. This research was financially supported by a PhD scholarship awarded to James Woodman by the Bulk Handling Companies of Australia.

All experiments as part of the research presented herein were conducted in compliance with the current laws of Australia.

References

- Ahearn, G.A., 1970. The control of water loss in desert tenebrionid beetles. *J. Exp. Biol.* 53, 573–595.
- Appel, A.G., 1988. Water relations and desiccation tolerance of migrating garden millipedes (Diplopoda: Paradoxosomatidae). *Environ. Entomol.* 17, 463–466.
- Barclay, S., Ash, J.E., Rowell, D.M., 2000a. Environmental factors influencing the presence and abundance of a log-dwelling invertebrate. *J. Zool. (London)* 250, 425–436.
- Barclay, S., Ash, J.E., Rowell, D.M., 2000b. Pheromonally mediated colonisation patterns in the velvet worm *Euperipatoides rowelli* (Onychophora). *J. Zool. (London)* 250, 437–446.
- Bicudo, J.E.P.W., Campiglia, S.S., 1985. A morphometric study of the tracheal system of *Peripatus acacioi* Marcus and Marcus (Onychophora). *Respir. Physiol.* 60, 75–82.
- Chown, S.L., 2002. Respiratory water loss in insects. *Comp. Biochem. Physiol. A* 133, 791–804.
- Campiglia, S.S., Lavallard, R., 1982. Water loss from *Peripatus acacioi* Marcus and Marcus (Onychophora) under conditions of experimental immobilization. *Vie Milieu* 32, 171–174.
- Endey, E.B., 1977. Water balance in land arthropods (Zoophysiology and Ecology, vol. 9. Springer–Verlag, Berlin.
- Garrick, R.C., Sands, C.J., Rowell, D.M., Tait, N.N., Greenslade, P., Sunnucks, P., 2004. Phylogeography recapitulates topography: very fine-scale endemism of a saproxylic ‘giant’ springtail at Tallaganda in the Great Dividing Range of south-eastern Australia. *Mol. Ecol.* 13, 3329–3344.
- Giribet, G., Edgecombe, G.D., Wheeler, W.C., 2001. Arthropod phylogeny based on eight molecular loci and morphology. *Nature* 413, 157–161.
- Greenlee, K.J., Harrison, J.F., 2004. Development of respiratory function in the American locust *Schistocerca americana*. *J. Exp. Biol.* 207, 497–508.
- Grove, S., 2002. Saproxylic insect ecology and the sustainable management of forests. *Ann. Rev. Ecol. Syst.* 33, 1–23.
- Hadley, N.F., 1994. Water Relations of Terrestrial Arthropods. Academic Press Inc., San Diego, CA.
- Hicks, W.T., Harmon, M.E., 2002. Diffusion and seasonal dynamics of O₂ in woody debris from the Pacific Northwest, USA. *Plant Soil* 243, 67–79.
- Hoback, W.W., Stanley, D.W., 2001. Insects in hypoxia. *J. Insect Physiol.* 47, 533–542.
- Hopkin, S.P., Read, H.J., 1992. The Biology of Millipedes. Oxford University Press, Oxford.
- Lavallard, R., Campiglia-Reimann, S., 1966. Structure et ultrastructure de l'appareil trachéen chez *Peripatus acacioi* Marcus et Marcus. *C.R. Acad. Sc. Paris* 263, 1728–1731.
- Lide, D.R., 1999. CRC Handbook of Chemistry and Physics. CRC Press, London.
- Lighton, J.R.B., Joos, B., 2002. Discontinuous gas exchange in the Pseudoscorpion *Garypus californicus* is regulated by hypoxia, not hypercapnia. *Physiol. Biochem. Zool.* 75, 345–349.
- Manton, S.M., Ramsey, J.A., 1937. Studies on the onychophora III. The control of water loss in Peripatopsis. *J. Exp. Biol.* 14, 470–472.
- Mendes, E.G., Sawaya, P., 1958. The oxygen consumption of “onychophora” and its relation to size, temperature and oxygen tension. *Rev. Bras. Biol.* 18, 129–142.
- Morrison, P.R., 1946. Physiological observations on water loss and oxygen consumption in *Peripatus*. *Biol. Bull.* 91, 181–188.
- Paim, U., Beckel, W.E., 1964. The carbon dioxide related behaviour of the adults of *Orthosoma brunneum* (Forster) (Coleoptera: Cerambycidae). *Can. J. Zool.* 42, 295–304.
- Penteado, C.H.S., Hebling-Beraldo, M.J.A., 1991. Respiratory responses in a Brazilian millipede, *Pseudonannolene tricolor*, to declining oxygen pressures. *Physiol. Zool.* 64, 232–241.
- Reinhard, J., Rowell, D.M., 2005. Social behaviour in an Australian velvet worm, *Euperipatoides rowelli* (Onychophora: Peripatopsidae). *J. Zool. (London)* 267, 1–7.
- Salinger, M.J., 2005. Climate variability and change: past, present and future — an overview. *Clim. Change* 70, 9–29.
- Schmitz, A., Harrison, J.F., 2004. Hypoxic tolerance in air-breathing invertebrates. *Respir. Physiol. Neurobiol.* 141, 229–242.
- Scott, I.A.W., Rowell, D.M., 1991. The population biology of *Euperipatoides leuckartii* (Onychophora: Peripatopsidae). *Aust. J. Zool.* 39, 499–508.
- Sunnucks, P., Wilson, A.C.C., 1999. Microsatellite markers for the Onychophoran *Euperipatoides rowelli*. *Mol. Ecol.* 8, 899–900.
- Sunnucks, P., Curach, N.C., Young, A., French, J., Cameron, R., Briscoe, D.A., Tait, N.N., 2000. Reproductive biology of the Onychophoran *Euperipatoides rowelli*. *J. Zool. Lond.* 250, 447–460.
- Sunnucks, P., Blacket, M.J., Taylor, J.M., Sands, C.J., Ciavaglia, S.A., Garrick, R.C., Tait, N.N., Rowell, D.M., Pavlova, A., 2006. A tale of two flatties: different responses of two terrestrial flatworms to past environmental climate fluctuations at Tallaganda in montane southeastern Australia. *Mol. Ecol.* 15, 4513–4531.
- Tait, N.N., Stutchbury, R.J., Briscoe, D.A., 1990. Review of the discovery and identification of Onychophora in Australia. *Proc. Linn. Soc. N. S. W.* 112, 153–171.
- Woodman, J.D., Ash, J.E., Rowell, D.M., 2006. Population structure in a funnelweb spider (Hexathelidae: Hadronyche) along a forested rainfall gradient. *J. Zool.* 268, 325–333.