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Standard metabolic rates of *Lepisma saccharina* and *Thermobia domestica*: Effects of temperature and mass

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ABSTRACT

Silverfish, *Lepisma saccharina* L., and firebrats, *Thermobia domestica* (Packard), are two common thysanuran pests in the urban environment. Both species can survive for extended periods without feeding, suggesting that they have some metabolic modifications compared with other insects which cannot tolerate extended starvation. To investigate potential metabolic modifications and to compare silverfish and firebrats, we measured the standard metabolic rate of both species at five temperatures (10, 20, 25, 30, 40 °C) across a range of body masses using closed system respirometry. Temperature had a stronger effect on firebrat mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) than on silverfish mass specific $\dot{V}_{O_2}$ for adults (>0.00700 g: firebrat Q_{10} = 2.32, silverfish Q_{10} = 2.07) and immatures (<0.00700 g: firebrat Q_{10} = 2.86, silverfish Q_{10} = 2.57). In addition, temperature had a stronger effect on the mass specific $\dot{V}_{O_2}$ of immatures than adults for both firebrats and silverfish. Respiratory quotients showed complex relationships with temperature from 10 to 40 °C, indicating a change in metabolic substrate with temperature. These results are interpreted with respect to the life histories and environment of both species. Finally, metabolic rates are compared with those of ticks and other arthropods.

1. Introduction

Metabolic rates have been reported for a vast number of arthropod taxa including ants (Lighton, 1988; Vogt and Appel, 1999), beetles (Burges, 1960), cockroaches (Dingha et al., 2009), crickets (Hack, 1997), fleas (Fielden et al., 2001), moths (Schneiderman and Williams, 1953), spiders (Anderson, 1970), termites (Shelton and Appel, 2001), and ticks (Fielden et al., 1999; Lighton and Fielden, 1995). In many cases, metabolic rates can be used to gain insight into an organisms' life history or adaptation to a particular environment. Lighton and Fielden (1995) used metabolic rates and mass scaling to better understand the ability of ticks to survive extended periods of starvation in comparison to other arthropods. Vogt and Appel (1999) used metabolic rates to gain a better insight into differences in diets between fire ant castes (*Solenopsis invicta* Buren). In addition, scaling of metabolic rates with mass is currently an area of debate. The 3/4-power law has been the accepted model of mass scaling for years, but it has recently undergone scrutiny with other models predicting mass scaling coefficient ranging from 0.67 to 1 (Chown et al., 2007; Glazier, 2005; Kozlowski et al., 2003; West et al., 1997, 2002). Despite the large number of taxa which have been measured and the uncertainty about the 3/4-power law, two primitive insect species whose respiratory physiologies have received almost no attention are firebrats, *Thermobia domestica* (Packard), and silverfish, *Lepisma saccharina* L.

Firebrats and silverfish are two common thysanuran pest species of the urban environment. Both species consume proteins and starchy materials and have the potential to cause extensive damage if left untreated (Adams, 1933; Lindsay, 1940; Meek, 2011; Sweetman, 1938, 1939). In addition, both species are capable of surviving for greater than two years under the right conditions (Adams, 1933; Lindsay, 1940; Meek, 2011; Sweetman, 1938, 1939). Firebrats and silverfish are frequently prone to bouts of forced starvation, which they are able to endure. Lindsay (1940) reported that one silverfish survived for almost a year without food. Both species undergo ametabolous development with an indeterminate number of instars; both species continue to molt even after reaching sexual maturity (Adams, 1933; Lindsay, 1940; Meek, 2011; Sweetman, 1938, 1939).

Despite these similarities, there are several distinct and important differences between the species. Firebrats usually inhabit warm to hot areas (32–41 °C), with high relative humidity (76–85%) (Sweetman, 1938). Such environments can include boiler rooms, steam tunnels or bakeries. Silverfish tend to inhabit cooler environments (22–27 °C) with high relative humidity (75–97%) (Sweetman, 1939). Due to their preference for cooler environments, silverfish tend to be found in environments closer in proximity to humans, such as basements, bathrooms, and attics. Within their optimal temperature range, firebrats can complete their lifecycle (egg to adult) in about 2–4 months (Sweetman, 1938),

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whereas silverfish generally require over a year to complete their lifecycle (Lindsay, 1940).

Currently, thysanuran respiratory physiology and specifically thysanuran standard metabolic rates (SMR), respiratory quotients (RQ) and Q_{10}s have received almost no attention. Standard metabolic rate is defined as the total amount of O_2 consumed or CO_2 excreted, over a given time, at a given temperature for post-absorptive ectothermic animals at rest (Moyes and Schulte, 2008). Respiratory quotients (RQ) is defined as the total amount of CO_2 excreted divided by the total amount of O_2 consumed and can be used as an indicator of what substrate is being metabolized. Specifically, theoretical RQ values range from 1 (pure carbohydrate metabolism) to 0.835 (pure protein metabolism) to 0.7 (pure fat metabolism) (Livesey and Elia, 1988). However, these values are best understood in a relative context because actual values often differ from theoretical values. Q_{10} is defined as the change in metabolic rate for a 10 °C change in temperature (Chown and Nicolson, 2004). Thysanuran metabolic rates have only been reported in the literature once by Edwards and Nutting (1950), who measured the metabolic rate of firebrats across a range of temperatures. However, no information was provided on the size of the animals tested or if they measured and corrected for activity, which significantly affects measurement of SMRs (Bartholomew et al., 1985; Lighton and Duncan, 1995; Lighton and Feener, 1989).

Due to the limited information on thysanuran respiratory physiology, and differences in the temperature preferences of firebrats and silverfish, it would be useful to compare the effects of temperature and mass on the metabolic rates of both species. In addition, information on these primitive insects could prove useful in gaining a better understanding of mass scaling and the ongoing debate regarding the 3/4-power law. In the present study, we measured both O_2 consumption and CO_2 production across a range of temperatures and masses for both species, accounting for movement. In addition, we calculated RQs and Q_{10}s for both species across the same range of temperatures. Finally, we compared the results for Thysanura as a group to values reported for ticks and other arthropods (Lighton and Fielden, 1995).

2. Materials and methods

2.1. Experimental animals

Separate firebrat and silverfish colonies were maintained at Auburn University, Auburn, Alabama, USA. The firebrat colonies were started in 1985 and have been maintained continuously thereafter. Firebrats were maintained in 48.6 L plastic coolers (54 × 30 × 30 cm; The Coleman Company, Golden, CO, USA) at 31 ± 1 °C in rolls of corrugated cardboard harborage. Temperature was maintained within each cooler with a 100 W incandescent light bulb surrounded by a 3 L clay pot (Wal-Mart Stores, Inc., Bentonville, Arkansas, USA). Coolers were provisioned with five 70 ml glass water jars covered with lids and fashioned with water wick (Absorbal Inc., Wheat Ridge, CO, USA). Firebrats were provided dry oatmeal (The Quaker Oats Company, Chicago, IL, USA) and pieces of dry Purina® dog chow (Nestle Purina Pet Care, St. Louis, MO, USA).

Silverfish colonies were initiated in 2010 and maintained in 19.0 L plastic containers (40 × 28 × 17 cm; VWR International, Radnor, PA, USA) at 24 ± 1 °C. Containers were filled with shredded white copy paper (various types) and cardboard for harborage. Containers were provisioned with two 70 ml glass water jars covered with lids and fashioned with water wick. Silverfish were provided dry oatmeal and pieces of dry Purina® dog chow.

Prior to testing, groups of silverfish and firebrats were removed from their colonies and placed into 50 ml glass beakers (one for each species), provisioned with wetted water wick, and covered with Parafilm® (American National Can, Chicago, IL, USA). Individuals were counted before and after isolation to ensure that they did not consume one-another. This procedure ensured that all animals were post-absorptive before testing. These containers were maintained in identical conditions to the original colonies and animals were isolated for a minimum of 24 h before testing.

2.2. Respirometry equipment

After both species were isolated for 24 h, individuals were weighed to the nearest 0.00001g on a Mettler-Toledo AX205 digital balance (Mettler-Toledo GmbH, Greifensee, Switzerland), and placed into 3 ml respirometry chambers fashioned from 3 ml plastic syringes (Becton, Dickinson and Company, Rutherford, NJ, USA). Six 1.4 mm diameter holes were drilled in each syringe barrel past the last gradation where the plunger enters the barrel. After animals were placed into individual respirometry chambers, the syringe plunger was inserted to close the syringe but leave the drilled holes open and animals were given at least 1 h to acclimate. After animals were acclimated, respiratory chambers containing animals were placed on a manifold for a minimum of 6 min, with dry, CO_2 free air flowing into the manifold at a rate of 230 ml min^{-1}. This procedure purged the respirometry chambers of all ambient CO_2 and H_2O. After purging, a 26 gauge intradermal bevel needle (Becton, Dickinson and Company, Rutherford, NJ, USA) was attached to the respirometry chamber and the volume was adjusted (depending on the life stage and temperature) by pushing the plunger past the drilled holes. Each respirometry chamber was sealed by attaching a rubber stopper (size 000) to the needle. Each syringe was placed into an incubator at one of five temperatures (10, 20, 25, 30, 40 °C) in a completely randomized design. A minimum of 10 replicates were used for each temperature, with one replicate being equal to one respirometry chamber. Most sizes were incubated between 2 and 7 h, depending on mass. However, 10 °C proved difficult to get results for some of the smaller sizes (<0.00700g) and therefore they were tested between 18 and 50 h at 10 °C. The incubator was illuminated with 15 W white fluorescent light and animals were monitored for activity using a SONY® DCR-SX85 video camera (Sony Corporation, Minato, Tokyo, Japan). Video recordings (MPEGs) were reviewed using Windows Media Player Classic version 6.4.9.1 (© 2002–2009). Playback was done manually, but an average play rate of 1 min per sec was used and allowed for adequate measurements of total distance traveled. After incubation was complete, an air sample (0.5–1.0 ml) from each respirometry chamber was injected into the respirometry system (described below) which measured the concentration of O_2 and CO_2. Following testing, each animal was re-weighed to the nearest 0.00001 g. It is important to note that for each group that was tested we also included at least one control respirometry chamber (syringe). The control respirometry chamber contained no animals, but underwent the same procedures as the syringes containing animals. After injecting air samples from the control syringes, we were able to adjust the experimental syringes for residual air which leaked in during the experiment. Oxygen showed no change in the control syringes (no O_2 leakage) and CO_2 leakage into the control syringes typically accounted for less than 2% of the total measured CO_2 in the experimental syringes.

The respirometry system used to measure O_2 and CO_2 was plumbed as follows: prior to entering the injection port (for injection of air samples from respirometry chambers), ambient air was forced through a FT-IR Whatman purge-gas generator (Whatman Inc., Haverhill, MA, USA) which scrubbed the air of both CO_2 and H_2O. Next, the CO_2- and H_2O-free air was forced into a 100 L mixing tank and then into an open manifold, where it equalized to atmospheric pressure. This dry, CO_2-free air was pulled through

the respirometry system past an injection port, where air samples were injected from each respirometry chamber. This air was drawn through a Li-Cor 6251 CO_2 analyzer (LI-COR Inc., Lincoln, NE, USA), a Drierite®–Ascarite®–Drierite® (W.A. Hammond Drierite Company LTD., Xenia, OH, USA; Thmoas Scientific, Swedesboro, NJ, USA) tube to remove all CO_2 before O_2 analysis, a Sable Systems Oxzilla II O_2 analyzer (Sable Systems, Henderson, NV, USA), and a Sable Systems mass flow system MFS2 (Sable Systems, Henderson, NV, USA), which pulled air through the system at 100 ml min^{-1} at STP. This system was properly calibrated (zeroed and spanned) regularly. To span we used 100 ppm Certified CO_2 (Airgas South, Theodore, AL, USA) and to zero we used 0 ppm CO_2 generated by passing air through the FT-IR Whatman purge-gas generator and a Drierite®–Ascarite®–Drierite® tube. Data were recorded and analyzed using Datacan V acquisition and analysis software (Sable Systems, Henderson, NV, USA). The exact incubation period (from the removal of the respirometry chamber from the manifold to when the air sample was injected) was recorded for each animal. Additional information on this type of closed system respirometry can be found in Lighton (1991) and Vogt and Appel (1999).

2.3. Statistical analysis

A *t*-test (PROC TTEST, SAS-Institute, 1985) was used to compare active versus inactive individuals and determine what level of activity was acceptable. Analysis of covariance (PROC GLM, SAS-Institute, 1985) was used to assess the effects of age (main effect; adults and immatures) and temperature (covariate) on respiratory rates of both silverfish and firebrats. Analysis of covariance was also used to assess the effects of species group (main effect; Thysanura, ticks, other arthropods) and mass (covariate) on metabolism. Multiple regression (PROC GLM, SAS-Institute, 1985) was used to model how variables including temperature and mass affect metabolic rate ($\dot{V}_{O_2}$) and RQ. Analysis of variance (PROC GLM, SAS-Institute, 1985) was used to compare mean RQ and $\dot{V}_{O_2}$ between temperatures and groups. An LSD test was used to determine individual differences among the means. Significance for all tests was determined at $p < 0.05$ (SAS-Institute, 1985). $\dot{V}_{O_2}$ is reported as both ml h^{-1} and ml g^{-1} h^{-1} (mass specific), as specified in the text. Q_{10}s are also reported and discussed. All means are reported with standard errors (±SE).

3. Results

3.1. Activity

Active individuals were identified and removed from the study if they moved >80mm h^{-1} within respirometry chambers. Mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) was compared using a *t*-test between animals which were defined as showing no movement (0–10 mm h^{-1}) and those which were defined as showing limited movement (11–80 mm h^{-1}). The no movement group ranged from 0 to 10 mm h^{-1} because very few individuals did not move (0 mm h^{-1}) for the duration of the experiment. However, this group represents individuals which did not even move the length of the syringe per hour and can therefore be considered as not moving. The *t*-test revealed no significant differences between groups (no movement, limited movement) for firebrats ($p = 0.2522$) and silverfish ($p = 0.1739$) and therefore, all animals moving <80 mm h^{-1} were included in the study. Overall, we discarded 121 firebrat samples (30% of all tested) and 24 silverfish samples (7% of all tested) due to activity (movement) which was determined to significantly enhance $\dot{V}_{O_2}$ measurements.

3.2. Division of adults and immatures

The effects of age and temperature on mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) were assessed for both firebrats and silverfish. Two age groups were established based on our data and biological information of both species: adults (>0.00700 g) and immatures (<0.00700 g) (Table 1). Analysis of covariance (age as main effect, temperature as covariate) revealed age, temperature, and the interaction variable (age * temperature) to have a significant effect on mass specific metabolic rate for both firebrats ($F_{3,282} = 1535.0$, $p < 0.0001$) and silverfish ($F_{3,334} = 965.4$, $p < 0.0001$). Further analysis revealed the interaction between age and temperature to be significant for both firebrats ($F_{1,282} = 53.96$, $p < 0.0001$) and silverfish ($F_{1,334} = 46.12$, $p < 0.0001$), indicating that adults and immatures of both species have mass specific metabolic rates which scale differently with temperature. This is illustrated in Fig. 1, as adult $\dot{V}_{O_2}$ is less affected by temperature than immature $\dot{V}_{O_2}$. Consequently, both adults and immatures were modeled independently.

The largest adult males and females (>0.02500 g) were also compared for both species. Analysis of covariance (sex as main effect, temperature as covariate) revealed no significant interaction between sex and temperature for both firebrats ($F_{1,65} = 3.63$, $p = 0.0611$) and silverfish ($F_{1,90} = 3.09$, $p = 0.0823$), indicating that male and female $\dot{V}_{O_2}$ scaled similarly with temperature. Therefore, all adults were combined and modeled together.

3.3. Adult Metabolic Rates

Multiple regression analysis yielded the following equation relating adult firebrat $\dot{V}_{O_2}$ (ml h^{-1}) to temperature (°C) and mass (g):

$$\mathrm{Log}_{10}\dot{V}_{O_2} = -1.964(\pm 0.064) + 0.037(\pm 0.001)\ \mathrm{Temperature} - 0.793(\pm 0.036)\ \mathrm{Log}_{10}\mathrm{Mass}$$

($F_{2,147} = 1556.1$, $p < 0.0001$, $r^2 = 0.9549$). Coefficients for temperature and Log_{10}Mass were both highly significant ($p < 0.0001$). In comparison, silverfish $\dot{V}_{O_2}$ (ml h^{-1}) showed the following relationship to temperature (C) and mass (g):

$$\mathrm{Log}_{10}\dot{V}_{O_2} = -1.651(\pm 0.057) + 0.032(\pm 0.001)\ \mathrm{Temperature} - 0.899(\pm 0.032)\mathrm{Log}_{10}\mathrm{Mass}$$

($F_{2,193} = 1308.4$, $p < 0.0001$, $r^2 = 0.9313$). Coefficients for temperature and Log_{10}Mass were highly significant ($p < 0.0001$ for both). The interaction term (Temperature * Log_{10}Mass) was not included

Table 1
Mass data for adult and immature firebrats and silverfish. Also, a comparison of mean mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) for adult and immature firebrats and silverfish at 25 and 37 °C. Means within columns which differ significantly according to the LSD test are indicated by different letters ($p < 0.05$).

Species	Age	*n*	Mass (g) mean (±SE)	Minimum mass (g)	Maximum mass (g)	$\dot{V}_{O_2}$ at 25 °C mean (±SE)	$\dot{V}_{O_2}$ at 37 °C mean (±SE)
Firebrat	Adult	150	0.02154 (±0.00066)	0.00762	0.03647	0.204 (±0.004) A	0.562 (±0.010) A
	Immature	136	0.00252 (±0.00017)	0.00028	0.00647	0.236 (±0.005) B	0.831 (±0.016) C
Silverfish	Adult	196	0.02273 (±0.00067)	0.00713	0.04824	0.208 (±0.003) A	0.498 (±0.008) B
	Immature	142	0.00266 (±0.00017)	0.00029	0.00698	0.277 (±0.007) C	0.862 (±0.022) C

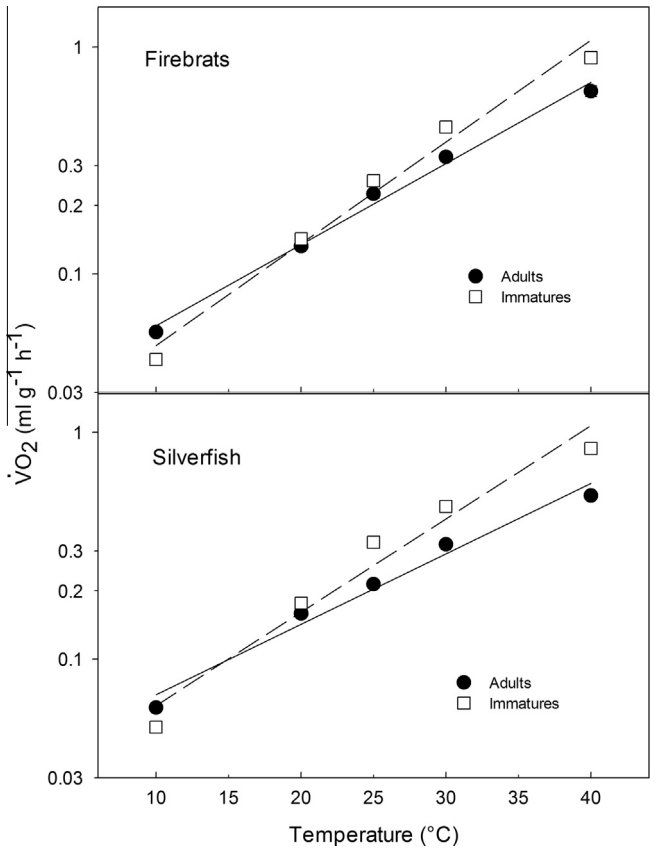

Fig. 1. Mass specific $\dot{V}_{O_2}$ for adult and immature firebrats and silverfish across a range of temperatures. See text for equations.

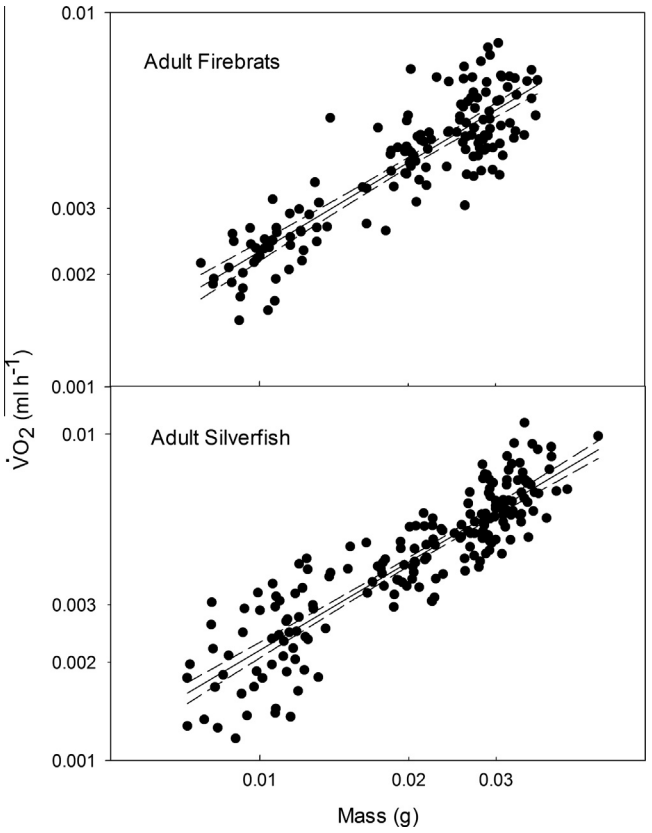

Fig. 2. Temperature adjusted $\dot{V}_{O_2}$ for a range of masses of adult firebrats and silverfish. See text for equations.

in either model because it only contributed a 0.2% (firebrats) to 0.3% (silverfish) increase in r^2. We also wanted to understand the effects of either temperature or mass alone. Therefore, we adjusted all adult $\dot{V}_{O_2}$ to 25 °C. After adjusting for temperature, the following equation was produced relating adult firebrat adjusted-$\dot{V}_{O_2}$ to mass (g):

$$\mathrm{Log}_{10}\dot{V}_{O_2@25^\circ C} = -1.052(\pm 0.062) + 0.793(\pm 0.036)\ \mathrm{Log}_{10}\mathrm{Mass}$$

($F_{1,148}$ = 479.6, $p < 0.0001$, r^2 = 0.7642) (Fig. 2). Adult silverfish adjusted-$\dot{V}_{O_2}$ had the following relationship with mass (g):

$$\mathrm{Log}_{10}\dot{V}_{O_2@25^\circ C} = -0.864(\pm 0.055) + 0.899(\pm 0.032)\ \mathrm{Log}_{10}\mathrm{Mass}$$

($F_{1,194}$ = 772.9, $p < 0.0001$, r^2 = 0.7994) (Fig. 2). Analysis of covariance (species as main effect, mass as covariate) reveled a significant interaction between species and mass, indicating that firebrat and silverfish $\dot{V}_{O_2}$ scale differently with mass ($F_{1,342}$ = 4.57, p = 0.0332). Mass can also be related to metabolic rate in μW, where 1 W is equal to 1 J s^{-1}, and 1 ml of O_2 is equal to 20.1 J (Lighton and Wehner, 1993). Because some studies have reported metabolic rates only in terms of μW, we have also reported the power equation relating adult firebrat metabolic rate (μW) to mass (g):

$$\mathrm{MR}\ [\mu W] = 433.5(\pm 84.8)\ \mathrm{Mass}^{0.753(\pm 0.053)}$$

($F_{1,148}$ = 290.3, $p < 0.0001$, r^2 = 0.6623). In adult silverfish, the following equation related metabolic rate (μW) to mass (g):

$$\mathrm{MR}\ [\mu W] = 808.6(\pm 125.5)\ \mathrm{Mass}^{0.909(\pm 0.043)}$$

($F_{1,194}$ = 648.9, $p < 0.0001$, r^2 = 0.7699).

In addition to looking at the effects of mass only, we also calculated the effects of temperature on mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) for adult firebrats:

$$\mathrm{Log}_{10}\dot{V}_{O_2} = -1.614(\pm 0.019) + 0.037(\pm 0.001)\ \mathrm{Temperature}$$

($F_{1,148}$ = 2244.9, $p < 0.0001$, r^2 = 0.9381) (Fig. 3). Adult silverfish $\dot{V}_{O_2}$ showed the following relationship with temperature:

$$\mathrm{Log}_{10}\dot{V}_{O_2} = -1.482(\pm 0.020) + 0.032(\pm 0.001)\ \mathrm{Temperature}$$

($F_{1,194}$ = 1834.2, $p < 0.0001$, r^2 = 0.9043) (Fig. 3). Analysis of covariance (species as main effect, temperature as covariate) revealed a significant interaction between species and temperature, indicating that mass specific $\dot{V}_{O_2}$ scales differently with temperature between both species ($F_{1,342}$ = 21.97, $p < 0.0001$).

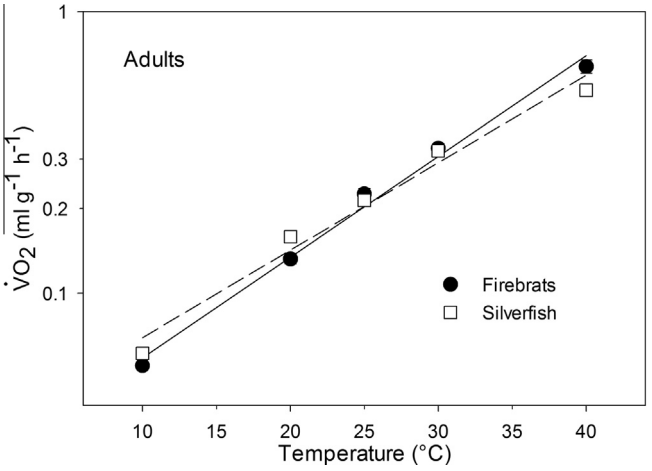

Fig. 3. Mass specific $\dot{V}_{O_2}$ for adult firebrats and adult silverfish across a range of temperatures. See text for equations.

3.4. Adult Q_{10} and respiratory quotient

Mean Q_{10} was calculated by multiplying the slope of the equation relating mass specific $\dot{V}_{O_2}$ by 10 and then taking the antilogarithm of the product (Lighton, 1989). This resulted in mean Q_{10} values of 2.32(±0.04) for adult firebrats and 2.07(±0.04) for adult silverfish. Because the linear relationship between mass specific $\dot{V}_{O_2}$ and temperature fits the data well for both species ($r^2 > 0.90$, Fig. 3), average Q_{10} can be assumed to be a constant across the measured temperature range.

Mean adult firebrat respiratory quotient (RQ) was plotted against temperature for both species (Fig. 4). Analysis of variance found RQ to be significantly different across the measured temperature range for both firebrats ($F_{4,145} = 14.3$, $p < 0.0001$) and silverfish ($F_{4,191} = 9.6$, $p < 0.0001$). The results of the LSD test were identical for firebrats and silverfish. The LSD test found RQ at 10 °C to be significantly lower than all other temperatures. It also found RQ at 30 °C to be significantly higher than all other temperatures. RQ at 20, 25, and 40 °C were found to be in the middle and not significantly different from each other (Fig. 4).

3.5. Immature metabolic rates

Multiple regression analysis yielded the following equation relating immature firebrat $\dot{V}_{O_2}$ (ml h^{-1}) to temperature (°C) and mass (g):

$$\mathrm{Log}_{10}\dot{V}_{O_2} = -1.781(\pm 0.061) + 0.046(\pm 0.001)\ \mathrm{Temperature} - 01.000(\pm 0.020)\ \mathrm{Log}_{10}\mathrm{Mass}$$

($F_{2,133} = 2294.4$, $p < 0.0001$, $r^2 = 0.9718$). Coefficients for temperature and Log_{10}Mass were highly significant ($p < 0.0001$). In comparison, immature silverfish $\dot{V}_{O_2}$ had the following relationship with temperature (°C) and mass (g):

$$\mathrm{Log}_{10}\dot{V}_{O_2} = -1.935(\pm 0.074) + 0.041(\pm 0.001)\ \mathrm{Temperature} - 0.877(\pm 0.025)\ \mathrm{Log}_{10}\mathrm{Mass}$$

($F_{2,139} = 1179.6$, $p < 0.0001$, $r^2 = 0.9444$). Coefficients for temperature and Log_{10}Mass were highly significant ($p < 0.0001$). Similar to the adults, the interaction term (Temperature * Log_{10}Mass) was not included because it contributed nothing to the models, generating no change in r^2 for firebrats and silverfish. In addition to understanding the effects of both predictors together, we also wanted to understand the effects of either temperature or mass alone. Therefore, we adjusted all immature $\dot{V}_{O_2}$ to 25 °C. After adjusting for temperature, the following equation related immature firebrat adjusted-$\dot{V}_{O_2}$ to mass (g):

$$\mathrm{Log}_{10}\dot{V}_{O_2\ @\ 25^\circ C} = -0.640(\pm 0.056) + 1.000(\pm 0.020)\mathrm{Log}_{10}\mathrm{Mass}$$

($F_{1,134} = 2539.3$, $p < 0.0001$, $r^2 = 0.9499$) (Fig. 5). Immature silverfish adjusted $\dot{V}_{O_2}$ was related to mass (g) by the following equation:

$$\mathrm{Log}_{10}\dot{V}_{O_2\ @\ 25^\circ C} = -0.914(\pm 0.068) + 0.877(\pm 0.025)\ \mathrm{Log}_{10}\mathrm{Mass}$$

($F_{1,140} = 1277.3$, $p < 0.0001$, $r^2 = 0.9012$) (Fig. 5). Analysis of covariance (species as main effect, mass as covariate) reveled a significant interaction between species and mass, indicating that firebrat and silverfish $\dot{V}_{O_2}$ scale differently with mass ($F_{1,274} = 3.89$, $p < 0.0001$). Some studies have reported metabolic rates only in terms of μW, therefore, we have also reported the power function relating immature firebrats metabolic rate (μW) to mass (g):

$$\mathrm{MR}\ [\mu W] = 1100.0(\pm 291.1)\ \mathrm{Mass}^{0.969(\pm 0.0490)}$$

($F_{1,134} = 959.9$, $p < 0.0001$, $r^2 = 0.8775$). For immature silverfish, the following equation related metabolic rate (μW) to mass (g):

$$\mathrm{MR}\ [\mu W] = 840.4(\pm 244.3)\ \mathrm{Mass}^{0.909(\pm 0.059)}$$

($F_{1,140} = 610.0$, $p < 0.0001$, $r^2 = 0.8133$).

In addition, we calculated the effects of temperature on mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) for immature firebrats:

$$\mathrm{Log}_{10}\dot{V}_{O_2} = -1.780(\pm 0.026) + 0.046(\pm 0.001)\ \mathrm{Temperature}$$

($F_{1,134} = 2181.9$, $p < 0.0001$, $r^2 = 0.9421$) (Fig. 6). Temperature had the following effect on immature silverfish mass specific $\dot{V}_{O_2}$:

$$\mathrm{Log}_{10}\dot{V}_{O_2} = -1.604(\pm 0.035) + 0.041(\pm 0.001)\ \mathrm{Temperature}$$

($F_{1,140} = 1012.8$, $p < 0.0001$, $r^2 = 0.8777$) (Fig. 6). Analysis of covariance (species as main effect, temperature as covariate) revealed a

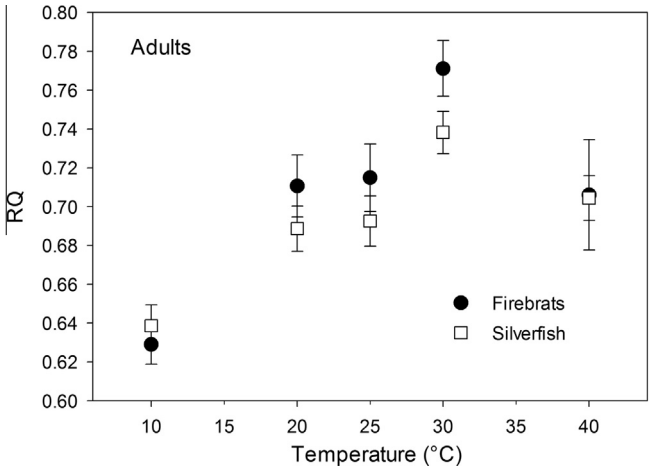

Fig. 4. RQ versus temperature for adult firebrats and adult silverfish.

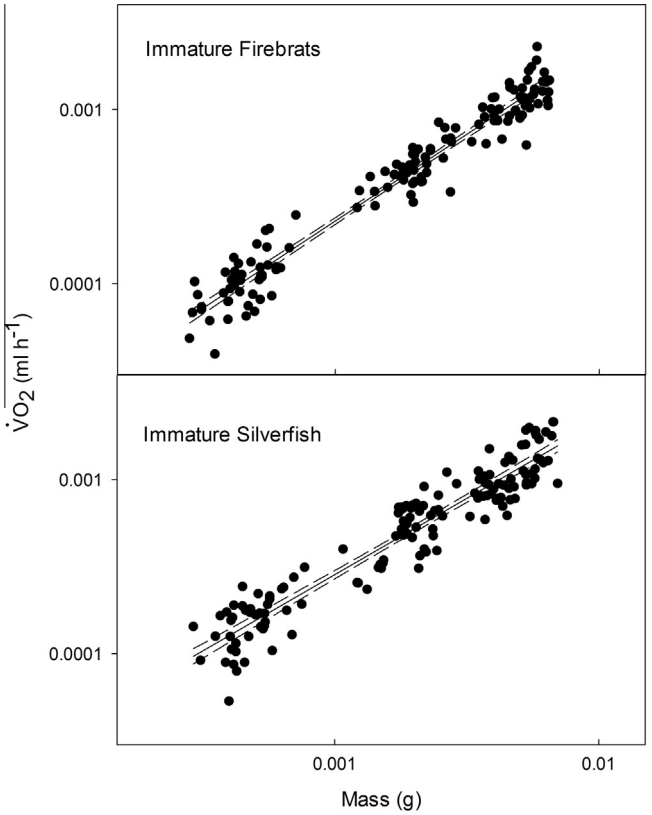

Fig. 5. Temperature adjusted $\dot{V}_{O_2}$ for a range of sizes of immature firebrats and silverfish. See text for equations.

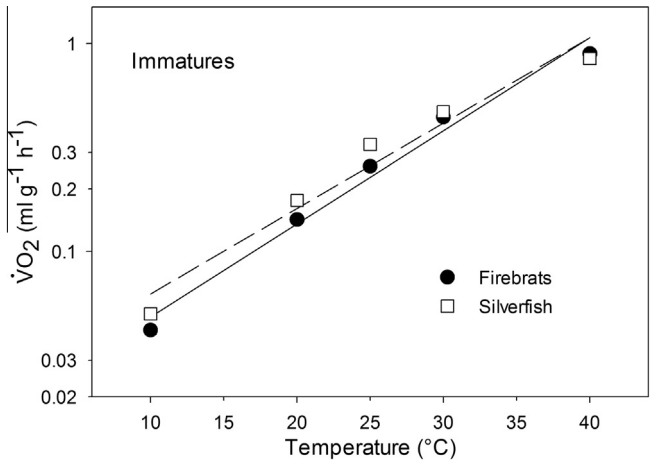

Fig. 6. Mass specific $\dot{V}_{O_2}$ for immature firebrats and immature silverfish across a range of temperatures. See text for equations.

significant interaction between species and temperature, indicating that firebrats and silverfish mass specific $\dot{V}_{O_2}$ scale differently with temperature ($F_{1,274} = 8.06$, $p < 0.0049$).

3.6. Immature Q_{10} and respiratory quotient

The same method used to calculate adult Q_{10} was also used to calculate immature Q_{10}. Immature firebrat and silverfish Q_{10}s were 2.86(±0.07) and 2.57(±0.08), respectively. Because the linear relationship between mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) fits the data well for both species ($r^2 > 0.87$, Fig. 6), average Q_{10}s can be assumed to be constant across the measured temperature range.

Mean immature respiratory quotient (RQ) was plotted against temperature for both species (Fig. 7). Analysis of variance found RQ to be significantly different across the measured temperature range for both firebrats ($F_{4,131} = 10.4$, $p < 0.0001$) and silverfish ($F_{4,137} = 14.4$, $p < 0.0001$). The results of the LSD test found RQ at 10 °C to be significantly lower than all other temperatures for both species. In addition, the other temperatures showed complex relationships best understood graphically (Fig. 7).

3.7. Comparison between ages and species

In addition to comparing mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) between adults and immatures for both species across a range of temperatures, we also compared all groups (species and age) at specific temperatures. We adjusted each mass specific $\dot{V}_{O_2}$ measurement to 25 °C (average preferred temperature of the silverfish, Sweetman, 1939), and 37 °C (average preferred temperature of firebrats, Adams, 1933) and used analysis of variance at each temperature to detect differences. There were significant differences in mass specific $\dot{V}_{O_2}$ between the species-age groups at 25 °C ($F_{3,620} = 49.6$, $p < 0.0001$) and 37 °C ($F_{3,620} = 174.8$, $p < 0.0001$), and the LSD test was used to compare means. Briefly, at 25 °C adult silverfish and adult firebrats did not differ from one another, but the immatures of both species had significantly greater $\dot{V}_{O_2}$ than adults and were significantly different from each other (Table 1). At 37 °C, immature silverfish and firebrats did not have significantly different $\dot{V}_{O_2}$; however, immatures had significantly greater $\dot{V}_{O_2}$ than both adult silverfish and firebrats which were significantly different from each other (Table 1).

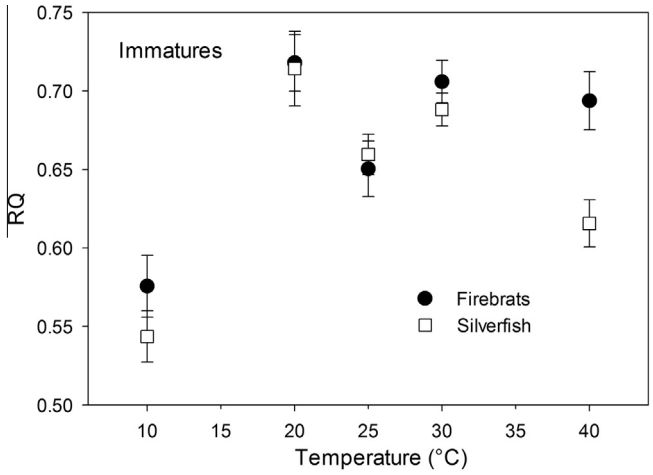

Fig. 7. RQ versus temperature for immature firebrats and immature silverfish.

3.8. Comparisons between Thysanura and other arthropods

To compare our results with those presented for other insects and arthropods, we adjusted all metabolic rates (μW) to 25 °C using the above equations which relate temperature to $\dot{V}_{O_2}$. Despite the differences in $\dot{V}_{O_2}$ we detected earlier, we also pooled all of our data for adults and immatures of both species into a combined set we called Thysanura. In this way we could determine if Thysanura as a group showed any differences with values reported for ticks and other arthropods (ants, beetles, spiders) by Lighton and Fielden (1995). Mass (g) related to the metabolic rate (μW) of Thysanura by the following equation:

$$\text{MR } [\mu W] = 699.4(\pm 49.8)\ \text{Mass}^{0.875(\pm 0.019)}$$

($F_{1,622} = 6094.0$, $p < 0.0001$, $r^2 = 0.9074$) (Fig. 8). After log-transforming the above equation, we used analysis of covariance to compare the slope (mass scaling exponent) with ticks and other arthropods as reported by Lighton and Fielden (1995). When compared with ticks, analysis of covariance revealed a significant interaction between the group (Thysanura versus ticks) and mass, indicating that thysanurans and ticks scale differently with mass, with thysanurans scaling approximately 1.59 times more with mass ($F_{1,628} = 14.64$, $p = 0.0001$) (Fig. 8). Similarly, when compared with other arthropods, analysis of covariance revealed a significant interaction between the group (Thysanura versus other arthropods) and mass, indicating that thysanurans and other arthropods scale differently with mass, with thysanurans scaling approximately 1.12 times more with mass ($F_{1,703} = 19.74$, $p < 0.0001$) (Fig. 8). Due to the

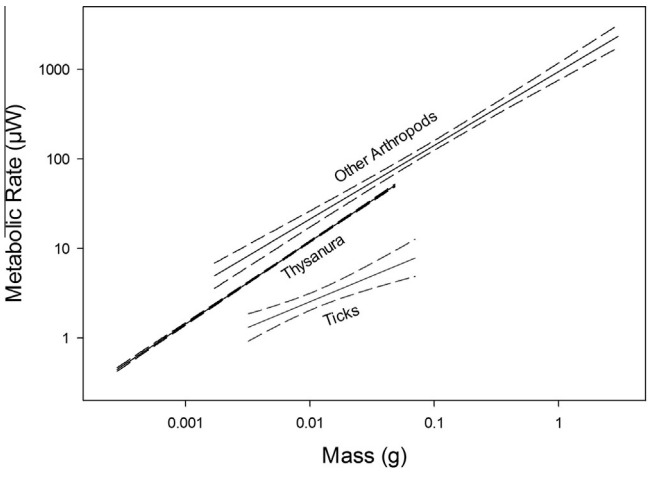

Fig. 8. Metabolic rate (μW) versus mass (g) for Thysanura, ticks and other arthropods (ants, beetles, spiders). See text for Thysanura equation, see Lighton and Fielden (1995) for other arthropod and tick equations.

differences in scaling factors, comparisons among the groups are difficult to make. However, visual examination of the plot of metabolic rate (μW) versus mass (g) reveals no overlap in the 95% confidence intervals of Thysanura with ticks or other arthropods for the range of masses measured, with ticks having the lowest metabolic rates, followed by thysanurans, and then other arthropods (Fig. 8).

4. Discussion

One complication that is typically encountered when measuring the standard metabolic rate of any ectothermic animal is how to prevent and/or control for activity/movement. Movement can cause significant changes in rates of O_2 consumption, making comparisons among individuals and species nearly impossible (Bartholomew et al., 1985; Lighton and Duncan, 1995; Lighton and Feener, 1989). In this study, we were able to negate the effects of movement, by videotaping all individuals during incubation and only including those whose movement did not significantly affect $\dot{V}_{O_2}$. Unfortunately, few authors have adequately addressed and accounted for movement in their studies, making comparisons between data sets very difficult. Edwards and Nutting (1950) reported a mass specific $\dot{V}_{O_2}$ for firebrats approximately twice as great as the values we report here across a similar range of temperatures. Differences between their findings and ours are likely due to a large amount of movement during their experiments, as indicated by the wide range of $\dot{V}_{O_2}$ values they reported (see Edwards and Nutting, 1950). In addition, we also compared individuals who were discarded from our experiments due to movement with the range of $\dot{V}_{O_2}$ values reported by Edwards and Nutting (1950). This comparison revealed all individuals we discarded to fall within the range of $\dot{V}_{O_2}$ values reported by Edwards and Nutting (1950), indicating that there was probably considerable movement during their experiments.

Another complication we encountered was how to measure and compare different life stages of an ametabolous insect. For insects with a determinant number of life stages or different castes, it is much easier to know where to divide and form groups for comparative purposes. Despite this problem, our data suggested two groups (adults and immatures) which were modeled independently. The biology of the firebrat also supported this division. Adams (1933) noted that eggs were first reported in a colony with an average mass of 0.00920 g. Because sexual maturation can lead to changes in metabolic rates in holometabolous insects (Vogt and Appel, 1999), we divided our animals into adults and immatures at a similar mass of 0.00700 g (Table 1). We chose this mass to be sure that we not only captured sexually mature individuals, but we would also capture those individuals beginning sexual maturation. The two groups were significantly different, which led us to model both groups (adults and immatures) independently for each species.

The relationship between mass-specific $\dot{V}_{O_2}$ ($ml\ g^{-1}\ h^{-1}$) and temperature was strong ($r^2 > 0.87$) for immature and adult silverfish and firebrats. In addition, each age-species combination was affected in a significantly different way by temperature. Specifically, temperature had a 23–24% greater effect on immature mass-specific $\dot{V}_{O_2}$ when compared with adults for both species (Fig. 1). The difference observed is supported by Lindsay's (1940) findings that adult silverfish could survive several months at 2 °C while immatures could only survive 2 days at the same temperature. This suggests that immatures are more sensitive to temperature, possibly because of the difference in energy expenditure between immatures (growth and development) and adults (maintenance and reproduction). Comparisons between adult and immature firebrats and silverfish were simplified by adjusting all mass specific $\dot{V}_{O_2}$ values to 25 °C (optimum temperature of silverfish) and 37 °C (optimum temperature of firebrat) and using analysis of variance to compare (Table 1). At both temperatures immatures had significantly higher mass specific $\dot{V}_{O_2}$. We believe this could be due to different selective factors on different ages. Adults were likely selected for survival and longevity (lower metabolism) whereas immatures were likely selected to rapidly grow and develop (higher metabolism).

In addition, firebrat mass specific $\dot{V}_{O_2}$ showed an 11–12% stronger relationship with temperature than silverfish mass specific $\dot{V}_{O_2}$. For adults, this is illustrated in Fig. 3 and the respective Q_{10} values of 2.32 for firebrats and 2.07 for silverfish. For immatures, this is illustrated in Fig. 6 and the respective Q_{10} values of 2.86 for firebrats and 2.57 for silverfish. One possible explanation for the higher Q_{10} values seen in firebrats in comparison to silverfish could be the ability of firebrats to actively absorb water from the air (Beament et al., 1964; Noble-Nesbit, 1969, 1970, 1975). Active water absorption is likely energy expensive, especially at higher temperatures where water loss would be the greatest and the partial pressure gradient between outside air and air inside the insect rectum the greatest (Hadley, 1994). Although ineffective at the relative humidity of this study (0%), this behavior would likely lead to elevated metabolic rates at higher temperatures, explaining the higher Q_{10} values seen in firebrats.

Metabolic rates of all animals tested were also adjusted to 25 °C so the effects of mass could be compared (Figs. 2 and 5). Log-mass scaling factors ranged from 0.7932 (firebrat adults) to 0.9999 (firebrat immatures). However, no patterns were detected when adults and immatures of firebrats and silverfish were compared to one another. When $\dot{V}_{O_2}$ values were converted to μW, mass scaling factors for adult silverfish and immature firebrats and silverfish were greater than those reported by Lighton and Fielden (1995) for arthropods ($mass^{0.825}$), while adult firebrats were lower. The different mass scaling coefficients for different species-age groups (ranging from 0.753 to 0.969) offer some insight into the ongoing mass-scaling debate. The inter- and intra-specific variation along with the deviation from a 0.75 mass scaling coefficient (except adult firebrats) provide support for the cell-size model of mass scaling (Chown et al., 2007).

Respiratory quotients of both firebrats and silverfish displayed rather complex relationships with temperature (Figs. 4 and 7). In all ages and species, RQ was lowest at 10 °C (0.544–0.639), where a higher percentage of fat or protein was likely being metabolized. At all other temperatures measured, it is likely that metabolism shifts to a higher combination of protein and carbohydrates. The one exception is in immature silverfish, which show a sharp decline in RQ at 40 °C (Fig. 7). This might be explained due to the long incubation period at such a high temperature for this stage. This extended incubation period could have led to the depletion of usable carbohydrate energy, forcing smaller silverfish to rely on fat or protein for energy. Our results show that the substrate being metabolized (carbohydrate, protein, fat) for all thysanurans changes with temperature. However, our study only showed a change in substrate metabolism, not in diet. Therefore, long term exposure to different temperatures should be investigated to determine if diet changes to accommodate the observed shift in substrate metabolism.

In addition to comparisons made between ages and species, we also compared the pooled data for all thysanurans with data reported for ticks and other arthropods (ants, beetles and spiders) by Lighton and Fielden (1995). The relationships that we observed agree with the biology of each of the groups measured and support the idea that metabolic rates are good predictors of survivorship during starvation (Rixon and Stevenson, 1957). Ticks are capable of surviving extended periods of starvation and are known to live for years without feeding and therefore would be expected to have the lowest metabolic rates at any given mass (Needham and Teel,

1991). Other arthropods including spiders, beetles, and ants generally do not have the same capacity to survive extended periods of starvation and would therefore be expected to have the highest metabolic rates of the groups considered at any given mass (Klotz, 2008; Mallis, 2011). Finally, thysanurans would be expected to be in between both of these groups. Although they do not have the ability to survive periods of starvation for as long as ticks, they can survive longer without feeding than a majority of the other arthropods reported (Adams, 1933; Lindsay, 1940; Meek, 2011; Sweetman, 1938, 1939).

In conclusion, firebrat and silverfish $\dot{V}_{O_2}$ were significantly affected by both temperature and mass. The strength of these relationships differed between species and ages. This study represents the first use of modern technology to measure the metabolic rate of this basal order of insects, which includes monitoring and accounting for movement. In addition, this study also provides a guide for the division of insects which proceed through an indeterminate number of instars. Our data also provide support for findings by Chown et al. (2007) who suggest that the 3/4-power law is not necessarily accurate across intraspecific groups. Finally, the results presented here should aid in the understanding of metabolism in other species with the capacity to survive extended periods of starvation.

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