CO₂ Release in Groups of Reticulitermes virginicus (Isoptera: Rhinotermitidae)¹

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Abstract: CO₂ release rates were measured from groups of 10 Reticulitermes virginicus Banks workers, soldiers, and nymphs. For workers, \( V_{CO₂} \) (\( \mu l \cdot mg^{-1} \cdot h^{-1} \)) increased linearly with temperature between 16.2 - 30.4°C. \( V_{CO₂} \) recorded at 20°C was 0.177 ± 0.005 \( \mu l \cdot mg^{-1} \cdot h^{-1} \) for soldiers and 0.219 ± 0.027 \( \mu l \cdot mg^{-1} \cdot h^{-1} \) for nymphs. Assuming a similar slope of temperature increase in \( V_{CO₂} \) for all castes, predicted mass-corrected CO₂ release values for grouped R. virginicus workers, soldiers and nymphs at 23.6°C were 27, 52 and 27% lower than literature values for the same castes of R. flavipes measured individually at that temperature. CO₂ release rate (\( \mu l \cdot mg^{-1} \cdot h^{-1} \)) for R. virginicus nearly doubled over the 20°C to 30°C temperature range (\( Q_{10} \approx 1.91 \)), similar to literature values for Zootermopsis nevadensis (Hagen) over the same range. For all temperatures except 25.2°C, CO₂ release rate (in \( \mu l \cdot h^{-1} \)) increased significantly with mass, with coefficients ranging from 0.123 (16.2°C) to 0.599 (30.4°C).

Key Words: respiration, CO₂, temperature, termite, Reticulitermes virginicus

Measurement of energy consumption by insects often uses respirometry as an indirect calorimetry method (Bartholomew 1968, Lighton 1991). Such measurements are easily made using individuals of large species such as blattid cockroaches (Kestler 1991) and acridid grasshoppers (Quinlan and Hadley 1993). Large subject size allows researchers to examine CO₂ release using fairly standard respirometry equipment. However, working with small species poses a problem for gas exchange measurement due to the very small ratios of signal to noise (background fluctuation).

Recently Shelton and Appel (2000a, b, 2001a, b) have provided descriptive information regarding the metabolic rates of lower termites, most commonly as carbon dioxide release rates (\( V_{CO₂} \)) rather than rates of oxygen consumption (\( V_O₂ \)). In these studies, individual termites were used to estimate \( V_{CO₂} \). Oxygen consumption (and often CO₂ release) rates are difficult to measure when animal mass is very low, which is the case for many lower termites. For example, Reticulitermes spp. "workers" (undifferentiated larvae of at least the third instar) are often <5 mg in size, whereas the largest termite from the references above, Zootermopsis nevadensis (Hagen), ranged in mass from 33.4 ± 2.5 - 46.2 ± 3.97 mg (Shelton and Appel 2000a).

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Other estimates of termite $\dot{V}_m$ and $\dot{V}$ appear in the literature (La Fage and Nutting 1979, Wheeler et al. 1996, Muradian et al. 1999), although only La Fage and Nutting (1979) and Wheeler et al. (1996) include lower termites based on data measuring multiple animals with Warburg-type manometer devices.

The most reasonable method of measuring a species' energy consumption is through the measurement of individuals. By measuring individuals, any energy consumption due to behavioral interactions is removed from consideration (although there is still the possibility of movement and other "non-social" behaviors). Whereas this is undoubtedly true for solitary or even subsocial species, can this statement be true for eusocial social insects like termites?

Colonies of Reticulitermes virginicus Banks naturally inhabit pine forests in the southeastern United States, where they aid in the breakdown of coarse woody debris, returning nutrients to the forest soil (Kofoid 1934). As with other subterranean termites (family Rhinotermitidae), R. virginicus colonies consist of thousands of individuals living in a system of tunnels and cavities within soil and wood (Kofoid 1934). Colony size for R. virginicus colonies has been anecdotally estimated as larger than that of R. flavipes (Kollar) (Howard et al. 1982), which can be a few hundred thousand (Howard et al. 1982) to millions (Grace et al. 1989, Su 1994) of individuals. However, the upper estimates should be interpreted with caution (Thorne et al. 1996). The tunnels and cavities inhabited by subterranean termites have microclimates which are hypercapnic (high $\text{CO}_2$) and hypoxic (low $\text{O}_2$) (Anderson and Ulbach 1987). Termites are never alone, except perhaps for the few that develop into their adult form (alates) which fly in swarms to new locations where they begin new colonies (Kofoid 1934). This high level of association with other termites makes it logical to work with $R$. virginicus (or possibly any subterranean termite) in groups. Work with the drywood termite Marginitermes hubbardi (Banks) indicated that group size (5 versus 10 individuals) did not affect $Q_10$ consumption (La Fage and Nutting 1979). Due to their small size (mean mass of workers in this study was $\sim$2.1 mg each), CO$_2$ release rates were measured using groups of 10 individuals.

For solitary animals, measurements include some unidentified level of stress from being in captivity, handling, and experimental conditions. This stress could very well inflate estimates of metabolic rate that are the goal of energy consumption studies. To some extent this stress may be unavoidable; the animals must be held in captivity and manipulated to make the measurements. However, when the animal in question is highly social, the level of stress may be even greater when isolated from nestmates. Estimates of metabolic rate may be flawed (over-estimated) when measuring social insects in isolation.

Environmental variables such as temperature and moisture impact fundamental physiological processes of poikilothermic animals, and for this reason, they help regulate insect life processes and population dynamics. The effects of these variables are well studied and modeled in insects (e.g., Curry and Feldman 1987, Goodenough and McKinion 1992). Temperature is a key environmental variable, and within limits, increases in temperature increase physiological rates, including metabolic rate. $Q_{10}$ is the variable used to describe these rate changes over 10°C intervals (Withers 1992). In termites, $\dot{V}_m$ increases linearly at temperatures from 20 - 40°C for Incisitermes minor (Hagen) and 10 - 36°C for Z. nevadensis. $Q_{10}$ values for these Kalotermitidae and Termitidae termites were 1.752 and 2.11, respectively (Shelton and Appel 2000a, b). We expect members of Rhinotermitidae to have a similar relationship between $\dot{V}_m$ and temperature.
The present study examines CO₂ release rates of groups of worker, nymph, and soldier *R. virginicus* at temperatures ranging from 16.2 - 30.4 °C. CO₂ release rates have not been described for this species. It is hypothesized that *R. virginicus* CO₂ release rates will increase linearly with temperature over this range. This work adds to the growing body of knowledge dealing with respiration of termites.

**Methods and Materials**

**Experimental procedures.** Termites originated from pine wood found in a mixed hardwood/pine woodlot in Oktibbeha Co., MS. The wood containing the termites was cut into smaller pieces and held in moistened plastic containers at room temperature. Individuals were collected from the host material as needed over a 3-d period beginning on the day of collection. Termites were identified as *R. virginicus* using a morphological key (Hostettler et al. 1995). In other *V.* studies on *R. flavipes* and *Coptotermes formosanus* Shiraki, colony origin did not significantly influence CO₂ release rates (Shelton and Appel 2001a).

Three termite castes were of interest: workers, soldiers, and nymphs (later instar individuals having wing buds and extended abdomens). Termites were separated into groups of 10 per caste and placed in 9-cm Petri dishes with moist filter paper. They were moved to a small temperature-controlled room to acclimate 60 - 90 min before use.

A Sable Systems International TR-3 Respirometer (Henderson, NV) was used to measure CO₂ emission rates of termites. The system contained 16 glass respirometry chambers (2-cm outside diam by 7.5-cm length, Model RC-M) connected to two 8-channel multiplexers (Model TR-RM). The first 4 chambers were used in this study. CO₂ levels were recorded every s for 3 min per chamber, with a 1-min delay in reading between chambers. Chamber 1 remained empty, whereas chambers 2 - 4 contained 10 termites each. The first chamber was recorded at the beginning and end of each test sequence and served to baseline the CO₂ readings acquired from the other chambers. Live termites from each chamber were weighed immediately after each test and preserved in 90% ethanol.

A diaphragm pump pulled air from outside the building through a glass filter and along 3.2-mm (inside diam.) polyethylene tubing. The air stream was pushed through an incubator set at 2 - 4 °C to remove excess moisture. Part of the air stream was pushed through a column of Drierite® and soda lime to remove moisture and CO₂ and then through all chambers except the one being monitored. The flow rate of this air stream was 20 - 30 ml·min⁻¹, continually flushing CO₂ from the waiting groups of termites and adding O₂. The remaining air stream was pulled sequentially through a single column of Drierite, Ascarite and Drierite, the active chamber containing the monitored group of termites, and the CO₂ analyzer (Model LI-6251, Li-Cor Inc., Lincoln, NE). The flow rate of the sampled airstream was 50 ml·min⁻¹ regulated by a Sable Systems pump (Model TR-SS1), electronics unit (Model TR-FC1), and mass flow controller valve (Model 840, Sierra Instruments Inc., Monterey, CA).

Each test sequence was repeated 5 times for workers (e.g., 15 groups of 10 termites) at 4 temperatures: 16.2 ± 0.05, 20.2 ± 0.12, 25.2 ± 0.04, and 30.4 ± 0.10 °C (reported as means ± SE). Because there were fewer nymphs and soldiers among the workers, only 7 groups of nymphs and 2 groups of soldiers were tested at 20.2 ± 0.06 and 20.3 ± 0.06 °C, respectively. Thus, there were 15 replicates for workers, 7 for nymphs and 2 for soldiers. Temperature was regulated using a heat pump and
supplemental electric heater in the respirometry room. An oscillating fan directed ambient air past the respirometry tubing, and 2 thermocouples recorded the air stream temperature in the tubing, positioned immediately before and after the active chamber. Temperatures were monitored every 5 sec, averaged, and recorded every min using a Campbell Scientific Inc. 21X Micrologger (Logan, UT).

**Analytical procedures.** CO₂ release rates of termites were measured in ppm every 5 over the 3-min sampling period resulting in 180 values per chamber. Data were corrected to baseline levels, converted to microliter CO₂ per mg live body weight per h (µl·mg⁻¹·h⁻¹), and averaged over the 3-min intervals using Sable Systems DATA CAN V® software. Air temperature of the active chamber was calculated as the average of 12 temperatures derived from the proximal and distal thermocouples for the 6-min period ending each test. Mean CO₂ rates (µl·mg⁻¹·h⁻¹) of worker termites (dependent variable) were plotted against mean air temperatures of active chambers (independent variable). A simple linear regression model was fitted to the worker CO₂ rate versus temperature data using PROC REG (SAS 2000). Proc GLM compared differences among regression slopes and intercepts by chamber. Simple linear regression also was used to examine the relationship between worker CO₂ rate (µl·h⁻¹) versus body mass (mg) grouped by temperature class. Only data from workers were examined for their response to temperature due to the lack of sufficient samples available for nymphs and soldiers. Observed values are reported as means ± SE.

**Results and Discussion**

Mean group masses (10 individuals) were 20.86 ± 0.15 mg for workers, 28.10 ± 0.10 mg for soldiers, and 46.50 ± 0.30 mg for nymphs. CO₂ release rates of *R. virginicus* workers increased with air temperature between 16 and 31°C (Fig. 1). Chamber did not significantly affect the rates based upon a comparison of regression slopes (P = 0.4983) and intercepts (P = 0.5174). Regression equations and statistics include µl·mg⁻¹·h⁻¹ for chamber 1 = 0.0279 ± 0.0016(°C)-0.2562 ± 0.0412 (df = 1, 19; F = 254; P < 0.0001; R² = 0.934); chamber 2 = 0.0280 ± 0.0014(°C)-0.2684 ± 0.0329 (df = 1, 19; F = 408; P < 0.0001; R² = 0.958); and chamber 3 = 0.0256 ± 0.0018(°C)-0.2089 ± 0.0414 (df = 1, 19; F = 215; P < 0.0001; R² = 0.923). The linear model was fitted to the combined data, yielding µl·mg⁻¹·h⁻¹ = 0.02715 ± 0.0009(°C)-0.2436 ± 0.0220 (df = 1, 59; F = 655; P < 0.0001; R² = 0.930). Based on the equation, respiration begins at about 9°C (y-intercept) and increases 0.027 ± 0.0009 µl CO₂·mg⁻¹·h⁻¹ per °C. Because the data do not extend into the temperature extremes, the typical nonlinear relationship between temperature and respiration was not observed (Sharpe and DeMichele 1977). For this reason, the linear model does not represent an accurate estimate at the temperature extremes. Whereas data do not exist for *R. virginicus*, low lethal temperatures for *R. flavipes* have been noted at 1.0-4.9°C (Hu and Appel 2004), -5 to -7°C (Davis and Kamble 1994), and -3.0 ± 0.114°C (Sponsler and Appel 1991).

*O₂* was calculated as 1.91 using predicted *Vₐₒₚ* values calculated at 20 and 30°C, similar to that for *Z. nevadensis* (2.11) over the same temperature range (Shelton and Appel 2000a). Temperature is known to have a marked effect on polikolthermic animals, and the relationship described here for *R. virginicus* indicates that the CO₂ release rate nearly doubles over this 10°C temperature range. Physiological rates commonly have *Q₁₀* values between 2 and 3 (Withers 1992). CO₂ emission averaged 0.177 ± 0.005 µl·mg⁻¹·h⁻¹ at 20.34 ± 0.055°C for soldiers (n = 2 groups of 10) and 0.219 ± 0.010 µl·mg⁻¹·h⁻¹ at 20.18 ± 0.056°C for nymphs.
Fig. 1. Temperature-dependent CO$_2$ release rates of R. virginicus workers (squares, triangles, and circles from chamber 1, 2, and 3, respectively). Solid and dotted lines represent linear regression model and 95% confidence intervals. Each data point represents the mean CO$_2$ release rate of three groups of 10 workers.

($n = 7$ groups of 10). Shelton and Appel (2001a) reported mean CO$_2$ rates for R. flavipes workers, soldiers, and nymphs of 0.544, 0.549, and 0.430 ml·g$^{-1}$·h$^{-1}$, respectively, at 23.6°C. A comparable predicted value at 23.6°C for R. virginicus workers is 0.397 ml·mg$^{-1}$·h$^{-1}$, 27% lower than that observed for R. flavipes. Assuming that soldiers and nymphs have the same regression slope as workers, the difference in temperature between studies would yield rates of 0.265 ml·mg$^{-1}$·h$^{-1}$ for R. virginicus soldiers and 0.312 for nymphs, about 52 and 27% lower than R. flavipes. Husby (1980) examined O$_2$ uptake and CO$_2$ release of 6 R. flavipes workers using a Warburg-type manometer finding an O$_2$ uptake rate of 0.86 ml·mg$^{-1}$·h$^{-1}$ and an RQ of 0.74 at 20°C. Husby (1980) did not report the CO$_2$ release data, but it can be calculated back from his RQ value to be 0.629 ml·mg$^{-1}$·h$^{-1}$, which is over twice the predicted value reported for CO$_2$ release at 20°C in this study (0.299 ml·mg$^{-1}$·h$^{-1}$). Husby’s (1980) value for RQ varied considerably from work with Prohynotermes simplex (Hagen) (Rhinitermidae) by Slama et al. (2007) who reported an RQ of 1.2 - 1.4 for all direct wood-feeding castes. RQ for soldiers of P. simplex was reported as 0.75 (Slama et al. 2007). Rasmussen and Khalil (1993) reported that subterranean termites had lower respiration rates compared with drywood (intermediate rates) and dampwood termites (highest rates), speculating on an association between respiration and apparent moisture requirements of each species.

Numerous investigators have reported a direct relationship between termite respiration rates and body mass (Gilmour 1940, Rasmussen and Khalil 1983, Shelton and Appel 2001a). This observation is common among arthropods (Wigglesworth 1939, Lighton and Fielden 1995). In the present study, a significant linear relationship was found between CO$_2$ release rates (μl·h$^{-1}$) and body mass (mg) except at 25.2°C ($P = 0.56$). Model coefficients of mass (e.g., slopes) for the remaining temperatures were:
Fig. 2. CO₂ release rate of *R. virginicus* workers at 16, 20, 25, and 30°C plotted against mass. Solid lines represent linear models describing the effect of mass on CO₂ release rate at each temperature. Each point represents the CO₂ release rate of a single group of 10 workers.

0.123 ± 0.05 at 16.2°C (df = 1, 14; F = 5.545; P = 0.0349; R² = 0.299), 0.502 ± 0.17 at 20.2°C (df = 1, 14; F = 8.433; P = 0.0123; R² = 0.394) and 0.599 ± 0.21 at 30.4°C (df = 1, 14; F = 7.9498; P = 0.0145; R² = 0.380). The coefficients (where a significant influence of mass was noted) increased with temperature (Fig. 2).

This study has provided descriptive information on CO₂ release rates of *R. virginicus* worker, soldier, and nymph groups. CO₂ release rates increased linearly with both temperature and mass. Values for mass-corrected CO₂ release rates in this study were smaller than those reported in the literature using individuals, suggesting a possible over-estimation of Vₑ values when using individuals. This may be due to the nearly continual movement of individual termites held under respirometry conditions (Shelton and Appel 2001a). Future research should examine the effect of using groups and individuals on CO₂ release rates.

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