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POPULATION ENERGETICS OF BACTERIAL-FEEDING NEMATODES: RESPIRATION AND METABOLIC RATES BASED ON CO_2 PRODUCTION

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Summary—By grazing on bacteria, bacterial-feeding nematodes participate in decomposition food webs and N mineralization to an extent determined by metabolic and behavioral attributes and by life history. We studied the respiratory and metabolic activities of 8 species of bacterial-feeding nematodes at 5 temperatures by measuring the rate of CO_2 evolution with an IR gas analyzer. We developed a method for determining size- or stage-specific respiration rates from measurements made on populations of varied stage-structure. Species from a single field site exhibited different thermal optima and temperature-niche breadths for respiratory and metabolic activity. Respiration rates of adults ranged between 1.25–8.80 nl O_2 h^{-1} at 20°C among the species. Metabolic rates of adults ranged from 1.15 nl O_2 μg(fresh wt)$^{-1}$ h^{-1} for *Rhabditis cucumeris* Andrassy to 4.43 nl O_2 μg(fresh wt)$^{-1}$ h^{-1} for *Bursilla labiata* Andrassy at 20°C. At each temperature, metabolic rates of nematodes of similar size varied with thermal adaptation of the species. Metabolic rates of *Cruznema tripartitum* Zullini and *Cephalobus persegnis* Bastian were more sensitive to temperature change than were those of *Acrobeloides bodenheimeri* Thorne, *A. buetschlii* Steiner and Buhrer and *Panagrolaimus detritophagus* Fuchs. *C. persegnis* exhibited the greatest total metabolic activity across a range of temperatures, and *P. detritophagus* the least. Observed differences in thermal adaptation may contribute to the predominance of species in the nematode community at different times during the year or at different depths in the soil.

INTRODUCTION

Metabolic and respiratory energetics of soil-inhabiting nematodes have been studied for populations of several species (e.g. Bair, 1956; Klekowski *et al.*, 1972, 1974, 1979; Nicholas, 1975; De Cuyper and Vanfleteren, 1982; Schiemer, 1982, 1983). In many cases, assessments have been based on O_2 consumption rates of adult nematodes using the Cartesian Diver method. Data are often based on one or a few individuals and usually at 20°C [e.g. as summarized in Klekowski *et al.* (1972) for 68 species].

Carbon budgets for soil nematodes have been developed by trophic level assignment of nematodes recovered from soil samples at the study site (Sohlenius, 1979; Persson *et al.*, 1980; Sohlenius *et al.*, 1988). In those studies, general relationships between temperature and respiration rate per unit weight of nematode are assumed to apply to all nematodes in the system. Also, C utilization in respiration is inferred from O_2 consumption, which requires assumptions of respiratory quotients. Although data are available for several nematode species (Klekowski *et al.*, 1972) identification of species of bacterial-feeding nematodes from field soil samples is an arduous task, so classification to trophic groups allows systems-level estimates of C utilization by nematodes. However, our preliminary data on individual nematode species from a single field site indicate differences in metabolic rates and in the response of respiration rates to temperature (Ferris and Lau, 1992). Since individual species predominate at different times of the year, higher resolution estimates of bacterial-feeding nematode participation in C flow may not be possible from trophic level groupings.

The extent to which an organism participates in energy flow in an ecosystem is a function of population size, individual and population growth rates, and metabolic activities of individuals. Our objectives were: (i) to measure the effect of temperature on metabolic activity of 8 species of bacterial-feeding nematodes, as indicated by CO_2 evolution rates; and (ii) to provide coefficients for the relationships between metabolic or respiration rates and temperature for juvenile and adult stages of these nematodes.

MATERIALS AND METHODS

The 8 nematode species studied are representatives of 3 families of the order Rhabditida: *Acrobeloides bodenheimeri* Thorne, *A. buetschlii* Steiner and Buhrer and *Cephalobus persegnis* Bastian (family Cephalobidae); *Bursilla labiata* Andrassy, *Caenorhabditis elegans* Dougherty, *Cruznema tripartitum* Zullini and *Rhabditis cucumeris* Andrassy (family Rhabditidae); and *Panagrolaimus detritophagus* Fuchs (family Panagrolaimidae). Other than *C. elegans*, all species were endemic in the field site of a long-term low-input sustainable agriculture experiment at the University of

California Davis campus in the Sacramento Valley of California (Temple *et al.*, 1994). Daily maximum soil temperatures at 10 cm depth range from 5 to 34 C under dry bare soil and 6 to 30 C under grass sod adjacent to the field site. Soil temperatures during the growing season for tomatoes (the primary economic crop in the experiment) ranged from 15 to 30 C at 10 cm depth under grass sod.

Nematodes were extracted from soil samples by elutriation and sugar centrifugation (Barker, 1985). They were hand-picked based on visual similarities, and placed on water agar. After about 1 wk, single gravid females were removed from the water agar and transferred to individual Petri dishes of nematode growth medium (NGM) (Sulston and Hodgkin, 1988). Bacteria associated with the nematodes flourished and provided a substrate. For some isolations, we transferred the nematodes with associated bacteria to Petri dishes with a lawn of *Escherichia coli* strain OP50 on NGM agar (Sulston and Hodgkin, 1988) in an attempt to standardize food sources. A laboratory culture of *C. elegans* was included as a comparative standard. Prior (1–2 wk) to use in respiration experiments, nematodes were transferred to NGM gel, in which 3.0 g l^{-1} gellan gum (Gelrite-Schweizerhall Inc., South Plainfield, NJ) and 720 mg l^{-1} $MgSO_4$ replace bactoagar as the solidifying agent (Eyre and Caswell, 1991).

Voucher specimens of the nematodes used in these experiments are deposited in the University of California Davis Nematode Collection with the following accession numbers: *A. bodenheimeri*—UCDNC 2908 and 2909; *A. buetschlii*—UCDNC 3030; *B. labiata*—UCDNC 3028 and 3029; *C. elegans*—UCDNC 3032; *C. persegnis*—UCDNC 3031; *C. tripartitum*—UCDNC 2910 and 2911; *P. detritophagus*—UCDNC 3034; and *R. cucumeris*—UCDNC 3033.

Rates of CO_2 evolution were measured at 15, 20, 25, 30 and 35 C for populations of each species. Thermal adaptation and cumulative depletion of C resources were avoided by exposing different individuals to each temperature. Minimum numbers of individuals of different species used in the experiments varied from 1500 to 18,000 to provide at least 1.1 mg(fresh wt) of nematode biomass. At least 15 CO_2 evolution measurements were made for each species at each temperature.

Respiration chambers consisted of 70 ml tissue culture flasks with a 5 ml layer of pH-buffered (pH 7.0) water agar. Seven respiration chambers were fitted with inlet and outlet tubes connected to manifolds. The manifolds, modified aquarium air valves that allowed control of gas flow to each chamber, were connected to a LiCor 6000 IR CO_2 gas analyzer. The respiration chambers were submerged in a water bath to allow temperature control. Water vapor was removed from the gas sample by passage through a magnesium perchlorate trap (Fig. 1).

The bacterial-feeding nematode species were washed from the surface of NGM gel plates into a graduated cylinder. An equal volume of 0.2 M EDTA

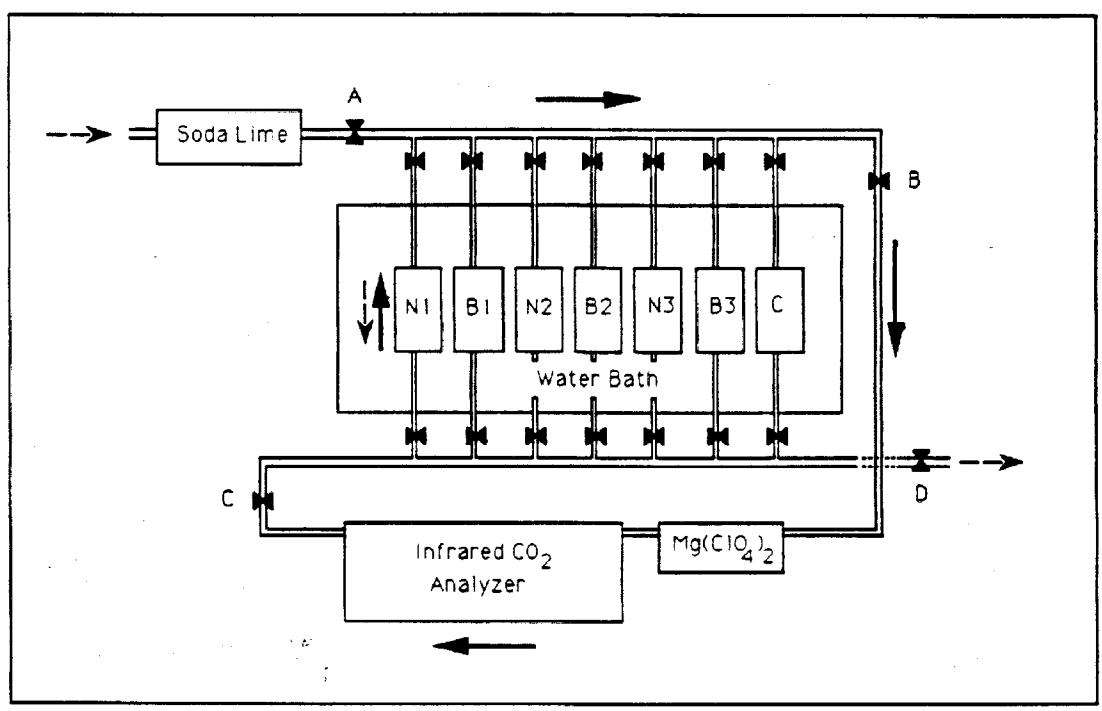

Fig. 1. Flow chart of apparatus for measuring CO_2 evolution of bacterial-feeding nematodes at constant temperatures.

was added to dissolve the gel (Eyre and Caswell, 1991). The suspension was centrifuged for 1 min at 740*g*, and the resulting pellet transferred to sterile deionized water. The nematode suspension was then divided into three 100-ml graduated cylinders and diluted to 80 ml. Five 1-ml samples drawn from each cylinder were used to determine the numbers of nematodes. Body lengths of 200–400 nematodes from each cylinder were also measured. The suspension in each cylinder was centrifuged (1 min at 740*g*), and the water was removed and replaced with degassed, deionized water. The suspension was again centrifuged and the supernatant drawn down to 1 ml. The upper 0.5 ml of the supernatant (mainly water and bacteria) was placed in the bacteria respiration chamber for that suspension. The remaining 0.5 ml (mainly nematodes and some associated bacteria) was placed in the nematode respiration chamber for that suspension. In individual trials, the number of nematodes in a respiration chamber ranged from 30,000 to 100,000. There were 3 replicates of the nematode and bacteria respiration chambers in each trial. The seventh chamber contained only degassed, deionized water as an absolute control.

CO_2-free air (passed through soda lime) was passed through all respiration chambers (Fig. 1: valves A and D open; valves B and C closed; all chamber inlet and outlet valves open) for over 1 h to remove background CO_2 from the system. This time period also allowed equilibration of the chambers (and nematodes) to the test temperature (Dusenbery *et al.*, 1978). Inlet and outlet valves for all chambers were then closed. After 30 min, CO_2-free air was passed through the infrared gas analyzer (Fig. 1: valves A–D open; chamber inlet and outlet valves closed), and the analyzer was calibrated to 0% CO_2. Then the air in one respiration chamber was circulated through the gas analyzer (Fig. 1: valves A and D closed; valves B and C open; inlet and outlet valves for one chamber open) and the concentration ($\mu g\ l^{-1}$) of CO_2 measured. After measurement of the CO_2 content of the air in each chamber, the analyzer, manifolds and tubing were flushed with CO_2-free air (Fig. 1: valves A–D open; chamber inlet and outlet valves closed) before measurements on the next chamber. The process was repeated for the 7 chambers. Measurements of CO_2 evolution in each chamber were made for 3–6 30-min periods in each trial.

Respiration rates of the nematodes were based on the difference between the $\mu g\ l^{-1}$ CO_2 evolved in respiration chambers containing nematodes (and associated bacteria) and those containing bacteria. The difference in CO_2 concentration in the bacterial and blank control chambers allowed determination of any potential for error resulting from lack of quantification of bacterial biomass. Metabolic rates were calculated, as ng CO_2 μg-nematode^{-1} h^{-1}, from the change in CO_2 concentration in each chamber in $\mu mol\ mol^{-1}$ during the incubation. Nematode weights are expressed as fresh wt throughout this study. The ideal-gas law was used to correct for temperature and pressure and to convert the CO_2 change from μmol to ng [D. McDermitt (LiCor Inc.), pers. commun.]. Bacterial CO_2 evolution (usually a minor quantity) was subtracted from CO_2 evolution in the nematode chambers and the calculated nematode biomass was used to express CO_2 evolution μg-nematode^{-1}.

Nematode biomass calculations

In preliminary studies, we developed regression models relating body widths to body lengths for each of the nematode species. Body lengths and widths (μm) of 200–367 individuals of each nematode species were measured with a calibrated ocular micrometer. In each case, body width as a dependent variable was regressed on body length using a cubic polynomial model. The models were checked visually for appropriateness of fit to the data and were only accepted if the r^2 value was >0.9. The resulting regression coefficients allowed calculation of body width from measured body lengths of a sample of nematodes. It resolved the problem of the time involved in measurement of body widths of many large samples of nematodes for biomass determinations.

The body lengths of a subsample of between 300–400 individuals of the nematodes introduced into each respiration chamber were measured. Body widths were estimated from the regression model. Nematode biomass was calculated using the Andrassy (1956) formula, i.e.

$$B = (D^2 \times L) \times (1.6 \times 10^6)^{-1},$$

where B is biomass per individual, in μg(fresh wt), L is the nematode length (μm) and D is the widest body diameter (μm). Individual biomass was calculated for each nematode in the subsample and an average obtained. Multiplying the average biomass from the subsample by the total number of nematodes in the respiration chamber provides an estimate of total nematode biomass in the chamber. Across all experiments, nematode biomass in the chambers ranged from 1.1 to 38.6 μg with an average value of 7.3 μg.

An anonymous reviewer of the manuscript suggested that by determining the relationship between B and L we could eliminate the step of calculating D. That did not occur to us at the time. Since D is calculated as a function of L, and B is a function of D^2, B is a function of L^3. Through subsequent analyses we find that the strong linear relationship between $B^{1/3}$ and L tends to underpredict the biomass of longer nematodes. A cubic polynomial model similar to the one we used to relate D to L would solve that problem.

Nematode survival at higher temperatures

Since we anticipated that higher temperatures may induce mortality or cryptobiotic survival states of some species, we tested survival and activity following

exposure to 35 °C. If there was reduced activity at 35°C, the test was also conducted at 30°C. Approximately 1000 individuals of each species were placed in respiration chambers and exposed to the test temperature for 6 h. Control chambers were placed on the laboratory bench at 24°C for the same period. Each treatment was replicated 3 times. At the end of the incubation, nematodes were washed from the flasks. A subsample was removed to determine numbers and biomass and the remainder of the suspension was placed on a Baermann funnel apparatus (Barker, 1985). The proportion of nematodes of each species exposed to the test temperature that moved through the funnel after 16 and 40 h was compared with that for nematodes maintained at room temperature. Nematodes passing through the funnel in 16 h were considered active and healthy, those passing through in 40 h were considered compromised or emerging from a survival state.

Determination of size-specific metabolic and respiration rates from population measurements

Previous studies on nematode respiration have used single age cohorts of nematodes; individuals of similar size have been selected and measured for Cartesian Diver respirometry (e.g. Klekowski *et al.*, 1979; Schiemer, 1982). Since we used large numbers of individuals in our experiments (1500–240,000), the populations were several generations removed from the initial cohort and included representatives of all life stages. We developed a procedure for determining size-specific metabolic and respiration rates from population measurements.

Nematode respiration rate per individual decreases with size of individuals according to the power dependence of basal metabolism and body weight observed in many organisms. The relationship is described by

$$R = aW^b,$$

where R is the respiration rate, W is the fresh wt of the individual and a and b are regression parameters, such that b is close to 0.75 for nematodes and other invertebrates (Klekowski *et al.*, 1974; Nicholas, 1975; Apple and Korostyshevskiy, 1980; Atkinson, 1980). From the CO_2 evolved for the nematodes in a chamber, we calculated the amount of CO_2 evolved (Y) by the 300–400 nematodes measured for biomass estimates for each chamber. Then, since

$$R_i = aW_i^{0.75},$$

Y is ΣR_i for the i individual nematodes measured. W_i for each nematode can be calculated from the width-to-length relationship and the formula of Andrassy (1956), so a, the constant of regression for that nematode species, is the only unknown. Then,

$$Y = \sum (aW^{0.75})$$

and

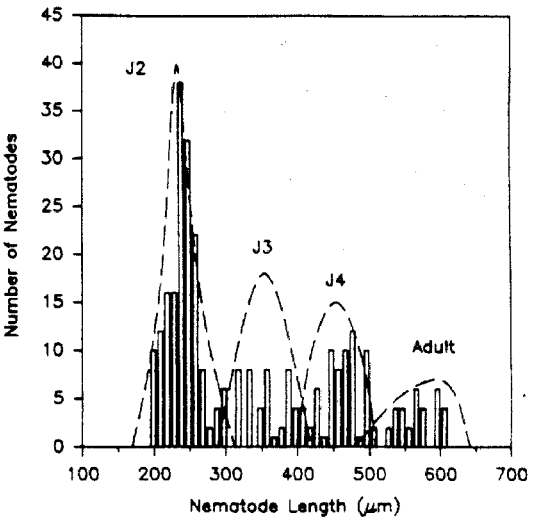

Fig. 2. Life-stage determinations for *C. persegnis* based on frequency distributions of lengths.

$$a = Y / \sum (W^{0.75}).$$

This calculated a value for each species at each temperature, and $b = 0.75$, enables calculation of the respiration and metabolic rates for individuals of any size.

The term *respiration rate* conventionally refers to O_2 consumption per individual h^{-1} and is usually calculated in nl h^{-1}. We measured CO_2 evolution in ng μg-nematode^{-1}. *Metabolic rate* is the O_2 consumption rate per unit body weight, measured in nl μg^{-1} h^{-1}. We used the derived a values to calculate stage-specific metabolic and respiration rates. Since a values are calculated at each temperature, we fitted a continuous model to allow interpolation and calculation of stage-specific respiration rates at any temperature.

We estimated size ranges for the life stages of each species and the average size of an individual in each life stage, based on size classes of individuals, in a process similar to that used by Anderson and Kirchner (1982). The body lengths of 300–500 individuals were arranged in rank order in a Lotus 1-2-3 spreadsheet, and graphed as a frequency distribution. Adults were distinguished by anatomical characters and their body lengths measured. Other life stages were distinguished by peaks in the frequency distribution of the body length dataset (e.g. Fig. 2). The smallest nematodes were considered to be J1 (where that stage was not completed in the egg) and J2. The size range for each life stage was determined as the length of individuals at the intersection points of frequency curves fitted empirically to the body length data for each putative life stage. The coincidence between body size and life stage is only inferred, since except for adults, it was not confirmed on the basis of anatomical characteristics. The median point of the body-length range for each stage was used as the representative length for that life stage.

Table 1. Coefficients of the cubic relationship $D = b_0 + b_1L + b_2L^2 + b_3L^3$ between body width (D, μm) and body length (L, μm) of 8 species of bacterial-feeding nematodes, and the upper (L_{max}) and lower (L_{min}) bounds of the relationships

Species	b_0	b_1	b_2	b_3	L_{min}	L_{max}	r^2	n
A. bodenheimeri	1.87E+1	−3.91E−2	1.08E−4	−3.28E−8	1.99E+2	1.32E+3	0.93	367
A. buetschlii	2.04E+1	−8.37E−2	3.43E−4	−2.42E−7	1.79E+2	7.75E+2	0.92	200
B. labiata	4.04E+0	4.06E−2	2.32E−5	−1.68E−8	1.79E+2	9.43E+2	0.92	302
C. elegans	3.17E+1	−1.41E−1	2.84E−4	−1.21E−7	2.98E+2	1.26E+3	0.90	354
C. persegnis	−1.47E+1	1.90E−1	−3.28E−4	2.52E−7	1.99E+2	6.06E+2	0.94	250
C. tripartitum	1.40E+1	2.00E−3	6.01E−5	−1.98E−8	1.79E+2	1.89E+3	0.95	322
P. detritophagus	6.51E+0	2.34E−2	2.44E−4	−6.34E−9	1.79E+2	1.24E+3	0.93	229
R. cucumeris	4.38E+1	−1.94E−1	3.68E−4	−1.54E−7	3.28E+2	1.26E+3	0.96	314

To allow comparisons among nematode species we standardized for nematode age structure across experiments. We used the relationship

$$R/W = aW^{(0.75-1)}$$

to calculate a standardized metabolic rate as the average rate for one J2, J3, J4 and an adult. The mean and standard deviation of the standardized metabolic rate for each nematode species was calculated for each trial at each temperature. The means for each trial were very similar, and final data were assembled as means and standard deviations of all observations across all trials for each nematode species at each temperature. The area under the curve fitted to standardized metabolic rates across temperature was used as a measure of the relative metabolic and respiratory activity for each species.

Conversion to O_2-based estimates

Our data on respiration and metabolic rates of nematode species based on CO_2 evolution can be converted to the equivalent rates expressed as O_2 consumption. Calculated respiratory quotients (RQ) for nematodes vary with the substrate metabolized. We used 0.95, following the observations of Cooper and Van Gundy (1970) for well-fed *Caenorhabditis* sp. as discussed by Nicholas (1975). We note that other researchers have used lower values; for example, Sohlenius *et al.* (1988) assumed 0.8. By using an RQ of 0.95 and the ideal-gas law, we calculated the O_2 consumption equivalent to the measured CO_2 evolution rates to allow comparison with previous reports. The respiration or metabolic rate based on O_2 consumption was determined by transforming the a value for CO_2 to an a value for O_2. That is accomplished by applying the principle that the units of a are in weight or volume of gas per weight of nematode raised to the exponent b. The procedure is:

1. Calculate the number of moles (m) of CO_2 equivalent to the $a_{(CO_2)}$ value as $m = a_{(CO_2)}/(44 \times 10^9)$.
2. Calculate the volume of CO_2, $V_{(CO_2)}$ in nl represented by that number of moles at that temperature (t) as $V_{(CO_2)} = m \times 0.0821 \times (273 + t) \times 10^9$.
3. Correct for the expected difference between CO_2 evolved and O_2 consumed using a respiratory quotient (RQ) of 0.95 by $a_{(O_2)} = V_{(CO_2)} \times (RQ)^{-1}$.

The respiration rate (R) in nl O_2 individual^{-1} h^{-1} is calculated using the weight of individuals (in μg) and the volumetric O_2-based a in the relationship $R = aW^{0.75}$. The metabolic rate (R/W) is calculated as $R/W = aW^{-0.25}$.

RESULTS

Nematode biomass calculations

The relationship between width and length for each nematode species was described by a cubic function within the bounds of minimum and maximum extremes of the datasets of length and width measurements (Table 1). When lengths of nematodes measured in the sample taken for individual respiration experiments were beyond the bounds set for the cubic functions, lengths and widths of a greater range of nematodes were measured, and coefficients of the relationship recalculated. The lengths and widths are used in the formula of Andrassy (1956) to calculate weight per individual of average length for each life stage (Table 2).

Sample stage-specific metabolic and respiration rates

Metabolic rates for each life stage expressed as CO_2 evolution [Fig. 3(A)], and respiration rates expressed

Table 2. Body-length range (L, μm) and weight (W, μg) of an average-sized individual of each putative life stage

	J1/J2		J3		J4		Adult	
Species	L	W	L	W	L	W	L	W
A. bodenheimeri	<266	0.031	266–500	0.076	500–999	0.249	>999	1.271
A. buetschlii	<266	0.034	266–350	0.078	350–533	0.240	>533	0.643
B. labiata	<233	0.021	233–300	0.044	300–466	0.116	>466	0.478
C. elegans	<333	0.024	333–466	0.042	466–666	0.156	>666	0.622
C. persegnis	<300	0.033	300–408	0.113	408–500	0.214	>500	0.475
C. tripartitum	<466	0.105	466–799	0.466	799–1332	2.436	>1332	7.708
P. detritophagus	<366	0.037	366–619	0.166	619–926	0.634	>926	1.661
R. cucumeris	<333	0.040	333–566	0.083	566–999	0.948	>999	4.273

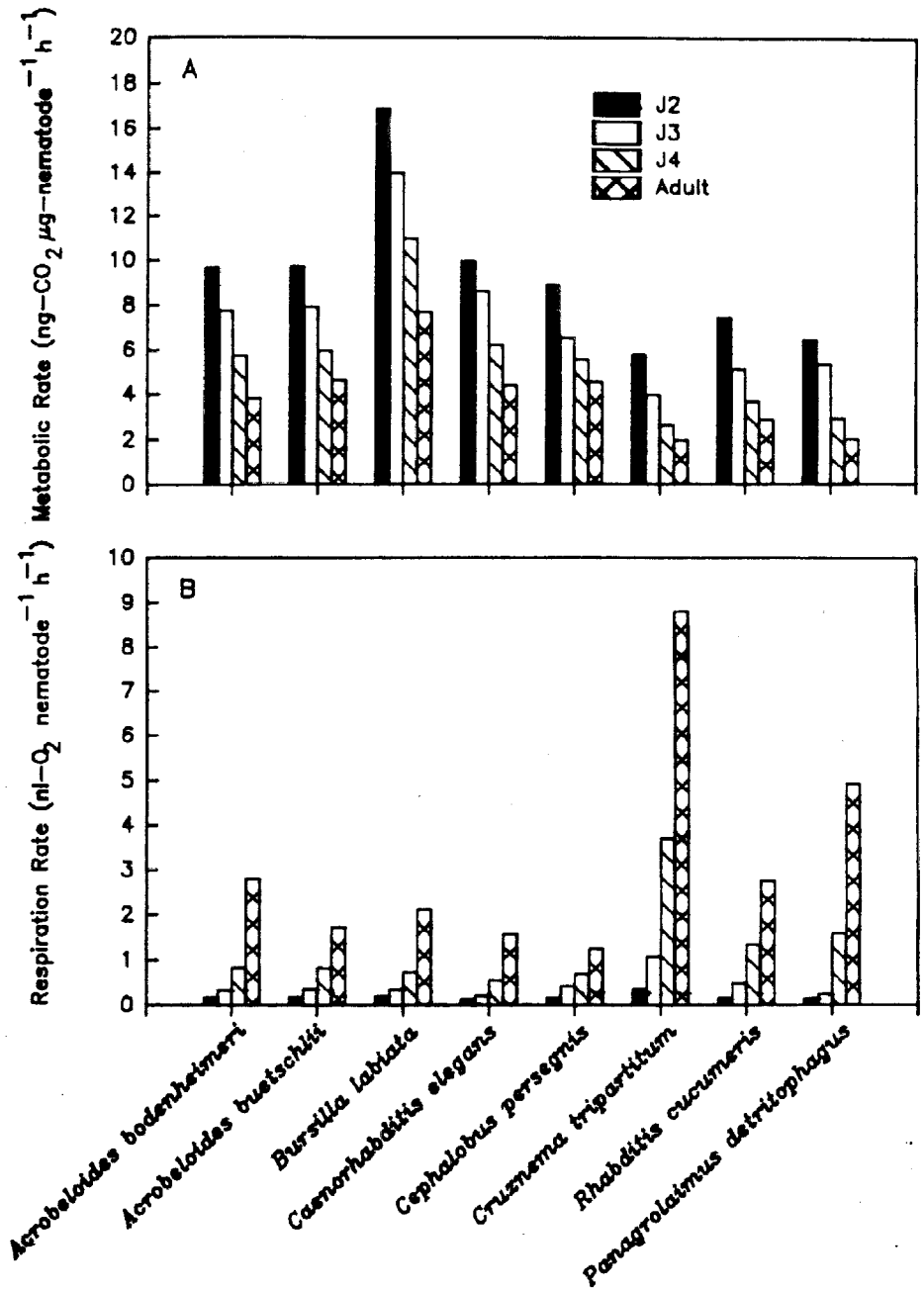

Fig. 3. (A)Stage-specific metabolic rates based on CO_2 evolution (ng CO_2 µg-nematode^{-1} h^{-1}) and $a_{(CO_2)}$ and (B) stage-specific respiration rates (O_2 consumption) (nl O_2 nematode^{-1} h^{-1}) for species of bacterial-feeding nematodes at 20°C.

as O_2 consumption (assuming an *RQ* of 0.95) [Fig. 3(B)], are calculated at 20°C. Many of the previous studies have measured rates at 20°C, so these data allow comparisons. There is considerable difference in the rates between life stages. For all species at 20°C, metabolic rates of J2 are 2- to 3-fold those for adults, while respiration rates for adults are often 10-fold those for J2.

Standardized metabolic and respiration rates

Standardized CO_2-based metabolic rates for the 8 nematode species ranged from 2.6 ng CO_2 µg-nematode^{-1} h^{-1} for *B. labiata* at 15°C to 19.3 ng CO_2 µg-nematode^{-1} h^{-1} for *C. persegnis* at 30°C (Fig. 4). Coefficients for the spline-fitted cubic functions describing these data are provided in Table 3.

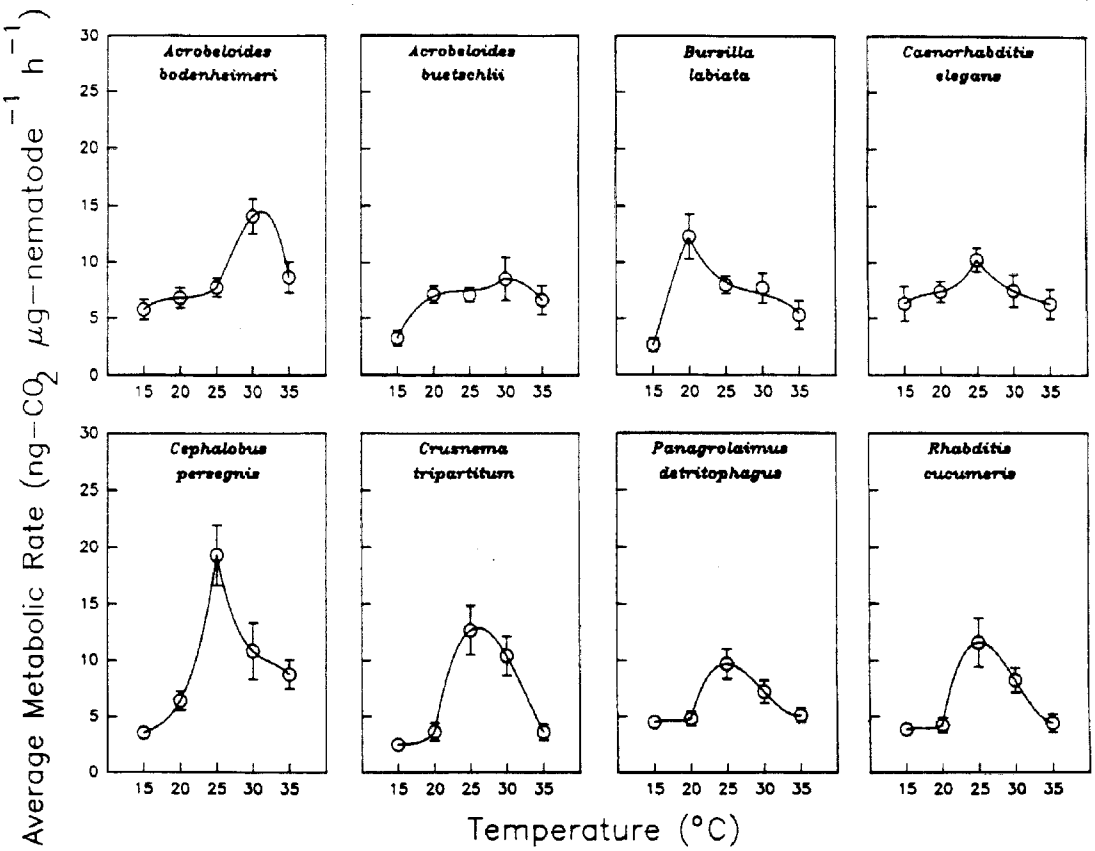

Fig. 4. Relationship between average metabolic rates, measured as ng CO_2 μg-nematode^{-1} h^{-1}, and temperature, for a standardized population of one individual of each life-stage. Bars represent mean $\pm$ 1 SD.

The species differed in the sensitivity of metabolic rates to temperature; *C. persegnis* exhibited a 5.46-fold increase in respiration rate between 15 and 25°C (i.e. for this species $Q_{10} = 5.46$), while *A. bodenheimeri* exhibited $Q_{10} = 1.35$ over the same temperature range (Fig. 4). Another measure of sensitivity to temperature is provided by the coefficient of variation of the points across the 5 temperatures at which observations were made. That indicates the degree of deviation from a no-change metabolic rate across temperatures (Table 4). Of the species from the field site, *C. tripartitum* and *C. persegnis* exhibited greatest sensitivity of metabolic rates to temperature and the two *Acrobeloides* spp and *P. detritophagus* the least. Metabolic rates of the

Table 3. Coefficients of the cubic relationships* $y = b_0 + b_1 t + b_2 t^2 + b_3 t^3$ between the standardized rate of CO_2 evolution (y, ng CO_2 μg-nematode^{-1} h^{-1} averaged across one individual of each life stage) and temperature (t, °C), for 8 species of bacterial-feeding nematodes (see Fig. 4)

Species	Range(°C)	b_0	b_1	b_2	b_3	r^2	n
A. bodenheimeri	15–25	−4.38E+1	7.45E+0	−3.68E−1	6.09E−3	0.93	98
	25–35	4.01E+2	−4.71E+1	1.83E+0	−2.29E−2	0.85	109
A. buetschlii	15–30	−6.51E+1	8.94E+0	−3.69E−1	5.09E−3	0.89	178
	30–35	1.77E+3	−6.06E+2	4.41E+1	−7.51E−1	0.83	135
B. labiata	15–20	1.00E+2	−2.13E+1	1.41E+0	−2.81E−2	0.94	110
	20–35	1.25E+2	−1.17E+1	3.96E−1	−4.52E−3	0.88	167
C. elegans	15–25	−5.60E+1	9.89E+0	−5.21E−1	9.28E−3	0.91	154
	25–35	1.24E+2	−1.00E+1	2.91E−1	−2.88E−3	0.91	129
C. persegnis	15–25	−5.16E+1	1.02E+1	−6.51E−1	1.42E−2	0.96	106
	25–35	6.11E+2	−5.50E+1	1.69E+0	−1.73E−2	0.90	126
C. tripartitum	15–20	−4.57E+1	9.13E+0	−5.79E−1	1.23E−2	0.93	137
	20–35	−2.83E+2	2.89E+1	−9.10E−1	9.10E−3	0.90	142
P. detritophagus	15–20	−5.64E+1	1.05E+1	−6.07E−1	1.16E−2	0.95	117
	20–35	−2.45E+2	2.69E+1	−9.29E−1	1.04E−2	0.82	127
R. cucumeris	15–20	−7.87E+1	1.45E+1	−8.46E−1	1.64E−2	0.87	105
	20–35	−3.37E+2	3.63E+1	−1.24E+0	1.36E−2	0.80	107

*Based on n observations.

Table 4. Coefficient of variation (CV) of observed metabolic rates across a range of temperatures, and relative area under the curves of metabolic and respiration rates in relation to temperature*

Species	CV(%)	Relative metabolic rate	Relative respiration rate
A. bodenheimeri	33.9	0.95	0.19
A. buetschlii	27.2	0.71	0.10
B. labiata	44.7	0.79	0.07
C. elegans	19.3	0.77	0.09
C. persegnis	54.7	1.00	0.13
C. tripartitum	63.7	0.78	1.00
P. detritophagus	31.5	0.68	0.22
R. cucumeris	46.8	0.74	0.40

*Data based on average CO_2 evolution across one individual of each life stage.

laboratory culture of the rhabditid nematode *C. elegans* were less sensitive to temperature than any of the other species.

A measure of the relative metabolic and respiratory activity of the different nematode species across a range of temperatures is provided by calculation of the area under the curves of metabolic or respiration rates in relation to temperature (Table 4). Of the nematodes studied, *C. persegnis* has the greatest metabolic activity across a range of temperatures, and *P. detritophagus* the least. The two *Acrobeloides* spp differed widely in their metabolic rates across temperatures, with *A. buetschlii* the least active. When respiration rates are calculated for a standardized population of each species across temperatures, the effect of body size on C utilization and CO_2 evolution is evident. The rate of CO_2 evolution from the large *C. tripartitum* is 10 times that of the small-bodied species (*A. buetschlii* and *B. labiata*) (Table 4).

Values of $a_{(CO_2)}$ for each species at each temperature

For each nematode species, the a value of the relationship $R = aW^b$, where $b = 0.75$, increases with temperature to maximum values between 20 and 30°C and declines at higher temperatures (Fig. 5). Since a is the determinant variable for calculating the respiration rate of each life stage in relation to temperature, we described the relationship as a continuous function. That allows calculation of respiration or metabolic rates of nematodes of any size at any temperature

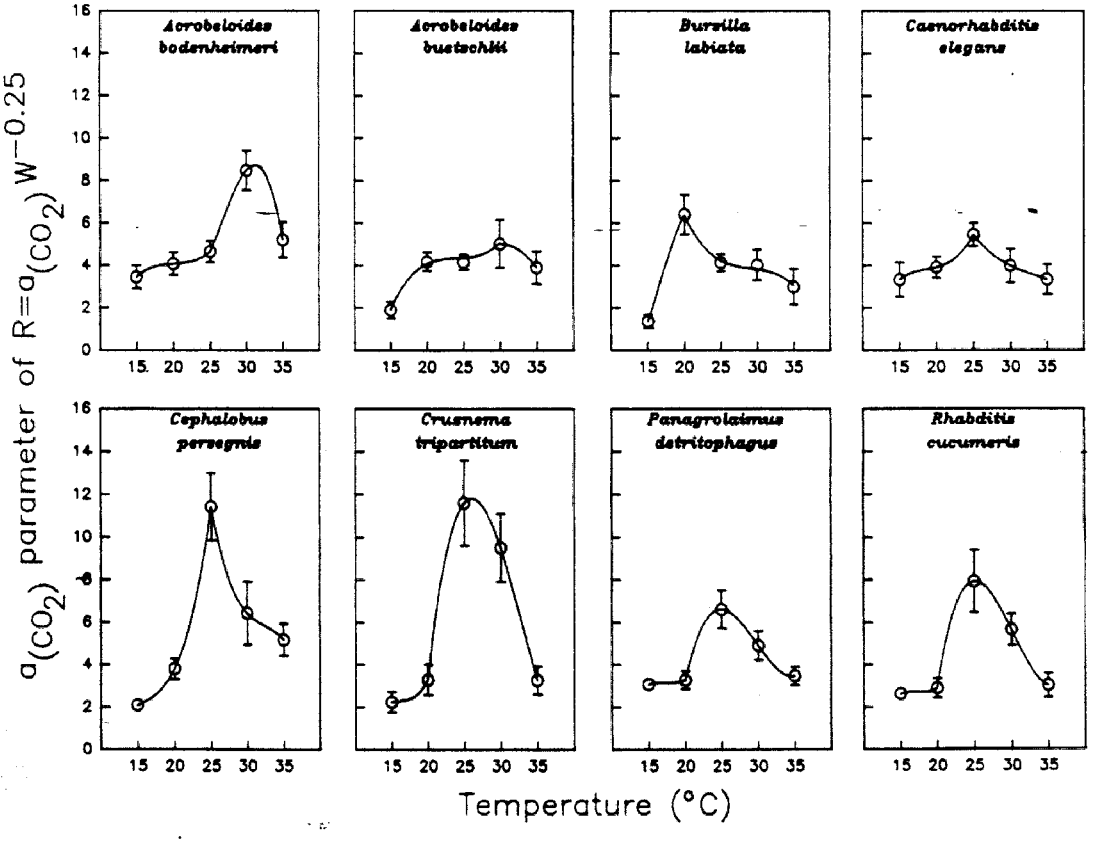

Fig. 5. Relationship between the a parameter of $R = aW^{-0.25}$ and temperature for 8 species of bacterial-feeding nematodes, where R is measured as ng CO_2 μg-nematode^{-1} h^{-1}. Bars represent mean ± 1 SD.

Table 5. Coefficients of the cubic relationships* $a = b_0 + b_1 t + b_2 t^2 + b_3 t^3$ between the a parameter of $R = aW^{0.75}$ and temperature (t, °C), where R is the rate of CO_2 evolution (ng CO_2 nematode^{-1} h^{-1}) and W is nematode body weight in μg, for 8 species of bacterial-feeding nematodes (see Fig. 5)

Species	Range(C)	b_0	b_1	b_2	b_3	r^2	n
A. bodenheimeri	15–25	−2.63E+1	4.48E+0	−2.21E−1	3.66E−3	0.93	98
	25–35	2.41E+2	−2.83E+1	1.10E+0	−1.38E−2	0.85	109
A. buetschlii	15–30	−3.90E+1	5.37E+0	−2.22E−1	3.08E−3	0.89	178
	30–35	−1.35E+0	−5.54E−1	5.81E−2	−1.09E−3	0.83	135
B. labiata	15–20	5.24E+1	−1.11E+1	7.35E−1	−1.47E−2	0.95	110
	20–35	7.29E+1	−7.00E+0	2.38E−1	−2.73E−3	0.86	167
C. elegans	15–25	−2.98E+1	5.27E+0	−2.78E−1	4.95E−3	0.91	154
	25–35	6.42E+1	−5.18E+0	1.51E−1	−1.49E−3	0.91	129
C. persegnis	15–25	−3.04E+1	6.05E+0	−3.84E−1	8.37E−3	0.96	106
	25–35	3.62E+2	−3.26E+1	9.98E−1	−1.03E−2	0.90	126
C. tripartitum	15–20	−4.18E+1	8.36E+0	−5.30E−1	1.13E−2	0.93	137
	20–35	−2.59E+2	2.65E+1	−8.34E−1	8.34E−1	0.90	142
P. detritophagus	15–20	−3.86E+1	7.23E+0	−4.16E−1	7.98E−3	0.94	117
	20–35	−1.67E+2	1.84E+1	−6.34E−1	7.11E−3	0.82	127
R. cucumeris	15–20	−5.74E+1	1.05E+1	−6.06E−1	1.17E−2	0.87	105
	20–35	−2.30E+2	2.48E+1	−8.44E−1	9.30E−3	0.80	107

*Based on n observations.

within the bounds of our datasets. Following Krogh (1916), we attempted to fit a generalized Poisson function to the data. However, we were unable adequately to describe the relationship of a to temperature for each species with that or any single generalized function. Instead we described each relationship as a spline of two cubic functions (Table 5).

Essentially the a value for each species represents the respiration or metabolic rate for a nematode of 1 μg. In all cases, minimum $a_{(CO_2)}$ or $a_{(O_2)}$ values were observed at 15°C. Maximum a values were observed at 20°C (*B. labiata*), 25°C (*C. elegans*, *C. persegnis*, *C. tripartitum*, *P. detritophagus*, *R. cucumeris*) or 30°C (*A. bodenheimeri*, *A. buetschlii*) (Figs 5 and 6). Maximum $a_{(CO_2)}$ values ranged from a low of 5.02 at 30°C for *A. buetschlii* to a high of 11.61 at 25°C for *C. tripartitum* (Fig. 5).

Conversion to O_2-based estimates

The $a_{(O_2)}$ values ranged from 0.77 for *B. labiata* at 15°C to 6.69 for *C. tripartitum* at 25°C. Maximum $a_{(O_2)}$ values ranged from a low of 2.98 for *A. buetschlii* at 30°C to a high of 6.69 for *C. tripartitum* (Fig. 6). Coefficients for the spline-fit curves relating $a_{(O_2)}$ values to temperature (Fig. 6) are provided in Table 6.

Nematode survival at higher temperatures

The numbers of *A. bodenheimeri*, *A. buetschlii*, *B. labiata*, *C. persegnis* and *P. detritophagus* passing through a Baermann funnel were not different following incubation for 6 h at 35 or 24°C (Table 7). The number of *C. elegans* incubated at 35°C for 6 h passing through the Baermann funnel in 16 h was only 56% of those incubated at 24°C, while none of the *R. cucumeris* survived 6 h at 35°C. The recovery after 40 h on the Baermann funnel suggested that neither species had entered a survival state. (Table 7). Activity of *C. elegans* and *R. cucumeris* after incubation for 6 h at 30°C was also tested; neither species was adversely affected (Table 7). The number of *C. tripartitum* passing through the funnel was greater after incubation at 35°C than at 24°C (Table 7). In all cases differences in biomass after exposure at higher temperatures mirrored the differences in numbers; consequently they are not presented.

DISCUSSION

These results allow calculation of the metabolic and respiration rates for each life stage of 8 species of bacterial-feeding nematodes over the range of temperatures between 15–35°C. The results were consistent with general patterns of response of metabolic rates to temperature in our preliminary report on *A. bodenheimeri* and *C. tripartitum* (Ferris and Lau, 1992). However, the magnitude of these metabolic rate estimates was lower than those in our previous report as earlier observations contained a calibration error. The general shape of the relationship between nematode respiratory and metabolic rates and temperature is that of Krogh's (1916) empirical normal curve as observed in previous studies on nematodes and other invertebrates. That is, the rate increases to a maximum level with relatively moderate increases in temperature and then decreases at higher temperatures (Nicholas, 1975). The shape of the curve, the maximum metabolic or respiratory activity, and the temperature at which the maximum is observed, varies with species (Fig. 4). Our survival experiments at higher temperatures indicate that all species survive at 30°C, so that where respiration rates declined at that temperature it was due to depressed or altered metabolism rather than mortality. At 35°C the depressed respiration rate of *R. cucumeris* was due to nematode mortality, while that of *C. elegans* was due to partial mortality of the population over the 6 h exposure. At the field site source for all species except *C. elegans* the maximum temperature at 10 cm depth in dry bare soil was 34°C during 1993. The temperature never exceeded 30°C in moist soil under grass sod. We did not correct the metabolic and respiration data of *C. elegans* for the reduction in respiring biomass across the series of readings taken during the incubation.

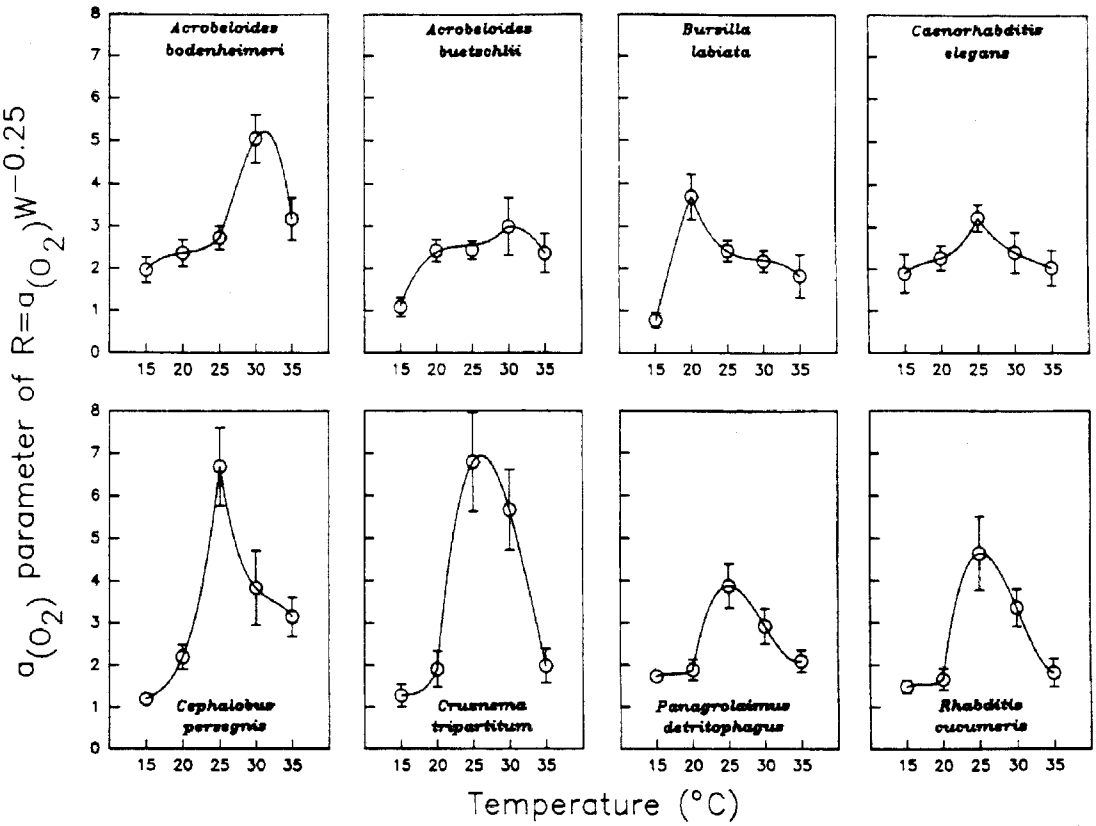

Fig. 6. Relationship between the a parameter of $R = aW^{-0.25}$ and temperature for 8 species of bacterial-feeding nematodes, where R is measured as nl O_2 μg-nematode^{-1} h^{-1}. Bars represent mean ± 1 SD.

Our estimates for O_2 consumption by individual adults range from 1.25 to 8.80 nl h^{-1} at 20°C. The rates equate to O_2-based metabolic rates ranging from 1.15 nl μg^{-1} h^{-1} for *R. cucumeris* to 4.43 nl μg^{-1} h^{-1} for *B. labiata* at 20°C (equivalent to 2.0 and 7.7 ng CO_2 μg^{-1} h^{-1}, respectively) [Fig. 3(A) and Table 2]. A large-bodied nematode will probably maintain a lower rate of metabolism per unit of body weight than a small-bodied nematode. That is consistent with the reduced surface-to-volume ratio of larger nematodes and the consequent reduced effectiveness of surface-dependent processes such as O_2 diffusion (Lee and Atkinson, 1977). However, the metabolic rate of nematodes of equal size may vary with life-history strategies, feeding habits and thermal adaptation of the individuals. *C. tripartitum* had a higher metabolic rate than *R. cucumeris* at 25°C, even though the adults are 1.8 times larger (Fig. 4), but a lower rate at 20°C

Table 6. Coefficients of the cubic relationships* $a = b_0 + b_1t + b_2t^2 + b_3t^3$ between the a parameter of $R = aW^{0.75}$ and temperature (t, °C), where R is the rate of O_2 consumption (nl O_2 nematode^{-1} h^{-1}) and W is the nematode body weight in μg, for 8 species of bacterial-feeding nematodes (see Fig. 6)

Species	Range(°C)	b_0	b_1	b_2	b_3	r^2	n
A. bodenheimeri	15–25	−1.47E+1	2.49E+0	−1.22E−1	2.03E−3	0.93	98
	25–35	1.44E+2	−1.70E+1	6.58E−1	−8.25E−3	0.86	109
A. buetschlii	15–30	−2.27E+1	3.13E+0	−1.30E−1	1.81E−3	0.89	178
	30–35	1.77E+0	−6.06E−1	4.41E−2	−7.51E−4	0.83	135
B. labiata	15–20	3.03E+1	−6.42E+0	4.23E−1	−8.46E−3	0.95	110
	20–35	4.46E+1	−4.30E+0	1.46E−1	−1.66E−3	0.87	167
C. elegans	15–25	−1.68E+1	2.96E+0	−1.57E−1	2.80E−3	0.92	154
	25–35	3.79E+1	−3.08E+0	9.03E−2	−9.01E−4	0.91	129
C. persegnis	15–25	−1.77E+1	3.52E+0	−2.25E−1	4.91E−3	0.96	106
	25–35	2.15E+2	−1.94E+1	5.98E−1	−6.16E−3	0.89	126
C. tripartitum	15–20	−2.40E+1	4.82E+0	−3.07E−1	6.53E−3	0.93	137
	20–35	−1.47E+2	1.50E+1	−4.68E−1	4.63E−3	0.90	142
P. detritophagus	15–20	−2.18E+1	4.09E+0	−2.36E−1	4.55E−3	0.95	117
	20–35	−9.70E+1	1.06E+1	−3.66E−1	4.10E−3	0.82	127
R. cucumeris	15–20	−3.27E+1	5.98E+0	−3.47E−1	6.72E−3	0.87	105
	20–35	−1.33E+2	1.43E+1	−4.86E−1	5.35E−3	0.80	107

*Based on n observations.

Table 7. Ability of 8 species of bacterial-feeding nematodes to move through Baermann funnels 0–16 and 16–40 h following 6 h exposure to 24, 30 or 35 C*

Species	Time after exposure (h)	Exposure temperature			Exposure temperature		
		30 C	24 C		35 C	24 C	
A. bodenheimeri	0–16				87.7	72.9	NS
	16–40				5.6	5.7	NS
A. buetschlii	0–16				**95.4**	**85.7**	**NS**
	16–40				**5.0**	**1.2**	**NS**
B. labiata	0–16				100.9	83.0	NS
	16–40				0.0	0.0	NS
C. elegans	0–16	71.7	84.2	NS	51.1	90.9	*
	16–40	15.3	9.9	NS	12.1	6.0	NS
C. persegnis	0–16				93.8	87.5	NS
	16–40				**0.0**	**0.0**	NS
C. tripartitum	0–16				**90.6**	**51.3**	*
	16–40				25.1	21.2	NS
P. detritophagus	0–16				114.9	99.4	NS
	16–40				13.5	11.1	NS
R. cucumeris	0–16	85.6	89.2	NS	0.0	114.2	*
	16–40	2.5	4.7	NS	0.0	0.0	NS

*Numbers of nematodes passing through funnels are expressed as percentages of those placed on the funnel.
Movement through funnels did not differ significantly (NS) or differed significantly (*$P < 0.05$) following exposure to each temperature.

[Fig. 3(A) and Table 2]. Adults of *B. labiata* and *C. persegnis* are of similar size (Table 2) but are adapted to different thermal ranges. At 20°C the metabolic rate of *B. labiata* is 1.7 times that of *C. persegnis* (4.43 and 2.63 nl O_2 μg^{-1} h^{-1} or 7.7 and 4.6 ng CO_2 μg^{-1} h^{-1}, respectively) [Fig. 3(A)]. The metabolic rate for *C. persegnis* adults at 20°C is close to the value of 2.72 nl O_2 μg^{-1} h^{-1} measured for a population in Poland (Klekowski *et al.*, 1972) [Fig. 3(B) and Table 2]. The maximum metabolic rate for our populations was at 20°C for *B. labiata* and at 25°C for *C. persegnis* (Fig. 4). At 25°C, the metabolic rates of adults of *B. labiata* and *C. persegnis* were 2.90 and 8.05 nl O_2 μg^{-1} h^{-1}, respectively (calculated from Table 2 and Fig. 6). The metabolic rate at 20°C for adults of *C. elegans* in these studies was 2.54 nl O_2 μg^{-1} h^{-1} or 4.4 ng CO_2 μg^{-1} h^{-1} [Fig. 3(A) and Table 2]. That is, in the same range as the 2.37 nl O_2 μg^{-1} h^{-1} measured by De Cuyper and Vanfleteren (1982) for young adults, although our nematodes were smaller (Table 2). However, similarities among such measurements for *C. elegans* are arguably coincidental considering the length of time those populations have been maintained in culture and the number of times that they have been subcultured.

Given the acceptance of b as 0.75 for most poikilothermic organisms, including nematodes (Lee and Atkinson, 1977; Atkinson, 1980), a determines the position of the respiration rate–temperature relationship relative to the abscissa. The expected respiration rate of an individual of any size can be calculated from its weight based on the value of a at that temperature. In our analyses, we maintained b constant across all temperatures. However, Storey (1984), using multiple regression analyses of respiration rates of field-derived nematodes, suggested that b values may be temperature-dependent. They ranged from 0.71 to 0.85 between 15–35°C, and are close to 0.75 at 20°C. If b values are temperature-dependent, our calculated a values are slightly underestimated at 15 C and overestimated at 25 and 30°C.

In our studies, and the preliminary experiments associated with them, we observed an effect of culture conditions on respiratory activity. Dusenbery *et al.* (1978) suggested that the effect of thermal adaptation during culture on O_2 consumption is minor. Rate of nematode metabolism and O_2 consumption are decreased by starvation as catabolized reserves shift from carbohydrates to lipids and protein (Cooper and Van Gundy, 1970; Nicholas, 1975). Sohlenius *et al.* (1988) speculated that soil nematodes are usually in a state of food deprivation. Periods of growth and unrestrained metabolic activity are determined by the availability of organic matter and associated bacterial populations. Our studies were conducted on nematode cultures maintained on an abundant bacterial food source and selected for their activity. Our measurements of respiratory activity undoubtedly overpredict respiration in a soil system where other constraints may exist. The measurements do, however, provide a basis for comparing respiratory and metabolic activity among species and at a range of temperatures.

We are intrigued that nematode species endemic, and apparently successful, in the same environment have different thermal optima. That finding coincides with observations of Anderson and Coleman (1982) that the range of temperatures favorable for population development differed for 4 coinhabiting species of bacterial-feeding nematodes. They suggested that the temperature-niche breadth reduced competition between species. We suggest that the species are adapted to predominance in the nematode community at different times during the year, or at different depths in the soil. That could determine their relative contribution to N mineralization in managed agricultural systems. N availability following incorporation of organic matter may be influenced by depth of incorporation and by thermal determinants of the activity of key nematode species.

Identification of species of free-living nematodes from soil samples is difficult and time-consuming.

Ecologists have categorized nematodes into trophic groups, based largely on oral structure and assumptions of feeding habits (e.g. Freckman and Mankau, 1986). Each trophic group consists of nematode species with very different biology, different metabolic and respiration rates, and different thermal adaptation of those rates. Estimates of nematode energetics based on trophic group aggregations are unlikely to provide accurate assessments of the contribution or importance of individual species in an ecosystem. Grouping of species of like biology into "trophic species" is an acceptable intermediate compromise (Cohen, 1989), with an attendant requirement of greater knowledge of the biology, metabolic rates and lifetable characteristics of the organisms to allow assembly of those categories.

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